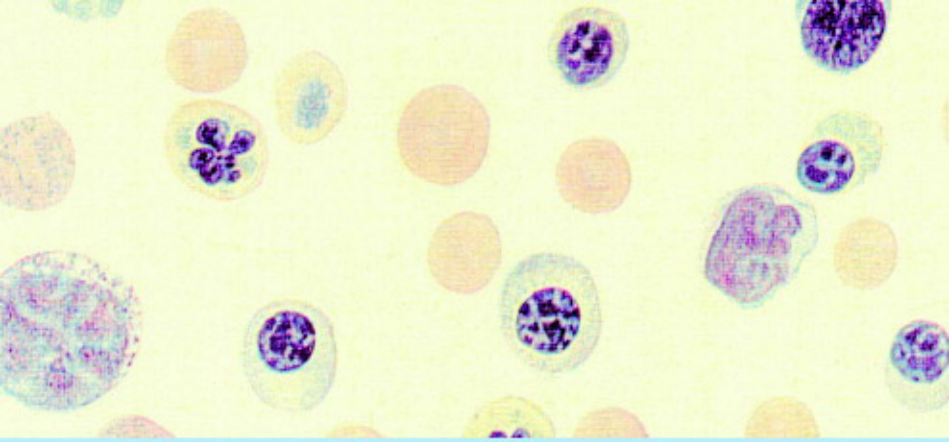

Edited by Pedro de Alarcón and Eric Werner

Neonatal Hematology

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Neonatal Hematology

Written by practicing physicians specializing in pediatric hematology, neonatology, immunology, pediatric infectious disease, and transfusion medicine, this is a practical guide to the pathogenesis, recognition, and management of hematologic problems in the neonate. The focus is on clinical issues encountered by pediatric specialists. There are chapters devoted to disorders of leukocytes, platelets, procoagulant and anticoagulant proteins, and disorders of red blood cells. Neonatal transfusion, malignant disorders in the newborn, neonatal hemoglobinopathy screening, and harvesting and storage of umbilical-cord stem cells are also covered, and practical approaches to diagnosis and treatment are given.

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Neonatal Hematology

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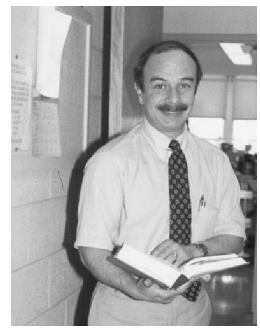
Dedication



Dedication to Dr Frank A. Oski

Our journey to the creation of this book in neonatal hematology began with a challenge from Dr Oski to update the text that he and Dr Naiman last produced in 1982. A hematologist and a consummate pediatrician, Dr Oski's special love was neonatal hematology. We both were attracted to Syracuse, New York, not because of the wonderful weather in that sunny city of eternal snow but because of the program that Dr Oski had built both in pediatrics and in pediatric hematology. As Fellows, we had the privilege and unusual experience of making neonatal hematology rounds once a week. Dr Oski attended in the neonatal intensive care unit once a year. A MEDLINE search yields 80 publications by Dr Oski in the field of neonatal hematology. Three editions of *Hematologic Problems in the Newborn*, co-edited with his friend and colleague Dr Laurie Naiman, helped many of us maintain an interest in neonatal hematology. Inspired by Dr Diamond's contributions, Drs Oski and Naiman established neonatal hematology as a field worth devoting a career to. Dr Oski contributed basic information to the field of neonatal red-cell enzymes, the neonatal red cell as it differed from the characteristic red cell in children and adults. Oxygen delivery and the hemoglobin dissociation curve were a natural sequence of study in an attempt to understand why newborns became "anemic" at birth. The role of iron, transfusions of red cells, and vitamin E

in the anemia of the newborn and the premature followed suit, culminating with Dr Oski's logical next step, nature's solution, breast milk, became areas to which Dr Oski contributed throughout his career. It is with respect and a deep felt thanks that we dedicate this book to our mentor Dr Oski. We also want to thank Dr Naiman for writing the foreword to this book. He also deserves credit and gratitude for his contributions to the field of neonatal hematology and his role in establishing this discipline.

PEDRO DE ALARCÓN AND ERIC WERNER

Dedication to Dr Maureen E. Andrew

Dr Maureen Andrew (1952–2001) died suddenly during the preparation of this chapter. Dr Andrew was one of the most influential pediatric researchers/clinicians of our time. A past president of the Society for Paediatric Research, she worked actively in research until her death, introducing the concept of developmental hemostasis and leading the field of thromboembolic disease in children with an evidence-based approach. As founder of the 1-800-NO-CLOTS service, she directly helped thousands of babies as a source of clinical expertise. Dr Andrew trained numerous pediatricians in the art of pediatric haematology. She will be remembered by many as a brilliant scientist, a caring doctor, a thoughtful mentor, and, for those of us lucky enough to know her well, as a warm and wonderful friend.

PAUL MONAGLE

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Foreword

It is an honor to be invited to write a foreword to this book. I know my friend and late colleague Dr Frank A. Oski, with whom I coauthored three editions of the monograph *Hematology of the Newborn* from 1966 to 1982, would echo this sentiment. And he would be delighted that his former fellows Drs Werner and de Alarcón shared our interest in the importance of this subject sufficiently to bring it up to date in an expanded textbook rich with information of great scientific and practical value.

As expected, there have been many important advances in the field of neonatal hematology in the past 20 years – new diseases, new ways of diagnosing, treating, and preventing old diseases. These are covered thoroughly in the chapters written by the authors of this book, each chosen carefully by the editors for his or her expert knowledge and experience.

With progress, diseases that virtually established neonatal hematology as a distinct discipline have largely come under control, reducing the space needs for describing them. There is no greater example of this than in the section devoted here to hemolytic disease of the fetus and newborn (formerly referred to as erythroblastosis fetalis), one that represented the largest chapter in our earlier monograph. All this resulted from the successful implementation in 1968 of Rh immunoglobulin to prevent Rh alloimmunization and hemolytic disease of the newborn. In its place, we now see chapters devoted to subjects hardly known then,

such as hemoglobinopathy screening, immunology, malignancy, thromboembolic disorders, transfusion practices, and umbilical-cord stem-cell harvest and transfusion.

What started as a practical monograph to assist clinicians dealing with hematologic problems encountered in the newborn has grown into a comprehensive reference source for everyone interested in the unique aspects of blood and neoplastic disorders seen at this age – and a useful guide to those directly responsible for care of these patients.

Books such as the present one and that by Dr Oski and myself serve also to stimulate others to investigate unsolved problems and develop new therapies. I was reminded of this by a chance meeting several years ago with Dr Pablo Rubinstein, who developed the first public cord blood bank (Placental Blood Program) at the New York Blood Center and made these products available for hematopoietic stem-cell transplant programs worldwide. At this, our first meeting together, he attributed his interest in the potential of cord blood for transplantation to statements in our book about cord blood being a rich source of blood-cell precursors. At the time we wrote our book, we had no idea that statements like that might have led to a major development such as the use of cord blood for transplantation. But it encourages me to predict that similar material in the present book by Drs Werner and de Alarcón will provide the seed for advances by others that were not at all conceived at the time this text went to press. And this is how the tree of knowledge grows.

J. Lawrence Naiman, M.D.

Preface

There is no time in life when human physiology changes more rapidly than in the neonatal period. The blood is very much affected by the transition from the intrauterine to the extrauterine environment. During this time, the normal range becomes a moving target, making it difficult to distinguish many abnormalities from physiologic variations. Furthermore, remarkable advances in perinatal/neonatal medicine have led to dramatic improvements in infant survival – now extending to the extremely low-birthweight infant. Many previously fatal congenital disorders are no longer universally so, due both to advances in basic and clinical research and to the hard work of perinatologists, neonatologists, pediatricians, pediatric subspecialists, and surgeons.

Melissa Warfield, M.D., a pediatric hematologist of great experience, would refer to textbooks as either "How come?" books or "How to" books. It is the goal of this textbook to be a "How to" book, with some discussion of the pathophysiology of the hematologic problems while focussing on practical aspects for the clinician. While there is some overlap between each of the areas covered in this book, as there is with most of the hematologic disorders of the newborn, we have chosen to be inclusive of the discussions prepared by each of the contributors.

The contributors to this text bring a wealth of knowledge and expertise to each of the chapters. We are so fortunate to have readily acknowledged

experts with a wide range of backgrounds, including neonatologists, pediatric hematologists, pediatric immunologists, pediatric transfusion medicine specialists, and pediatric infectious diseases physicians. These authors took time from their very busy activities to review the state of the art in their fields, often dealing with repeated questions and requests from the editors. In particular, we, and the entire medical world, will greatly miss Dr Maureen Andrew. In addition to her extensive research into hematologic problems of the newborn, especially in the area of thromboembolic disease, she could always be called upon for her wisdom and experience in the management of difficult clinical problems.

We thank Dr J. Lawrence Naiman for his continuous support through the production of this text and Drs James A. Stockman III and Jack Widness for their insightful comments and criticisms. Lastly, but certainly not the least, we wish to thank our wives (Jill and Alice), our children (Alessandro, Tessa, Jacob, Abby, and Andrew), and the patient spouses and children of all of our contributors whose family activities were limited by their dedication to completing this text.

Neonatal hematology: a historical overview

Howard A. Pearson, M.D.

University School of Medicine, Yale New Haven Hospital, New Haven, CT, USA

Ancient concepts of the blood were described by Hippocrates and Galen 2000 years ago in their doctrine of "humors." It was believed that the body was made up of four humors – blood, phlegm, black bile, and yellow bile – and that these four components had the qualities of heat ("hot-blooded!"), cold, moist, and dry. The Galenic concept of the blood prevailed through the Middle Ages. Health or disease was a result of a balance or imbalance, respectively, between these humors, and this was the basis of the practice of therapeutic blood-letting (which, fortunately, was performed infrequently in children) through the mid nineteenth century as a way to rid the body of the abnormal humors believed to cause a wide variety of diseases.

The hematology of the fetus and newborn is a relatively recent area of study whose development depended upon the evolution of the science of hematology and, especially, upon methods to study the blood and its elements. As Wintrobe has pointed out, the development of the field of hematology has been driven by technology. He divided the evolution of hematology into two general areas: *morphology*, which relied on the development of microscopy, and *quantitation of the elements of the blood*, which came later [1].

The invention of the microscope enabled identification of the blood cells. Antonj van Leeuwenhoek, working in Delft, Holland, constructed a primitive microscope from a minute biconcave lens mounted between two metal plates attached to a screw that permitted focussing. Leeuwenhoek's publication in 1674 contained the first accurate description of the red blood corpuscles [2]:

> The blood is composed of exceedingly small particles, named globules, which in most animals are red in color . . . These particles are so minute that 100 of them placed side by side would not equal the diameter of a common grain of sand.

In the centuries following, the development of compound microscopes with two lenses greatly increased magnification and minimized spherical aberration, permitting more accurate descriptions of the blood cells. William Hewson, who has been designated as one of the "fathers of hematology," noted that the red cells were flat rather than globular and also described the leukocytes for the first time [3]. The last of the formed elements of the blood, the platelet, was recognized independently by several investigators. The most definitive early work on the platelet was done by Julius Bizzozero. His monograph, published in 1882, clearly recognized these cells as being distinct from red and white blood cells, and suggested that they should be called "*Plättchen*." He also assigned a hemostatic function to the platelet [4]. William Osler, early in his illustrious career, also described platelets accurately, although he believed that they might be infectious agents, perhaps analogous to bacteria [5].

With improvements in microscopy, the morphology of the fixed blood cells began to be examined using thin films of blood, spread and dried on glass slides, which were then stained with analine dyes that stained differentially the nuclei and granules of

the leukocytes. Staining of peripheral blood smears was developed by Paul Ehrlich in 1877, while he was still a medical student, and became practical in the early twentieth century by the work of James Homer Wright of Boston, who formulated the polychromatic Wright stain that is still used today for morphologic examination of the blood and bone marrow [6]. The development of supravital dyes provided a method for assessment of erythropoiesis by reticulocyte counts. These techniques permitted the flowering of morphologic hematology, and many blood diseases such as the leukemias and the various types of anemia were described on the basis of typical morphological findings.

Hematology as a quantitative discipline began with the development of practical and reliable methods to quantify accurately the numbers of the various blood cells. These methods used gridded chambers of uniform depth (hematocytometers) into which precisely diluted suspensions of blood were placed. The numbers of cells in the chamber were counted and, when combined with the known dilutions, the actual numbers of cells per cubic milliliter in the patient's blood could be calculated. Hemoglobin levels were estimated by comparing the density of color in fixed dilutions of hemolyzed blood with colorometric standards and, later, by spectroscopy. For many years, hemoglobin values were reported as "% of normal;" because the definition of "normal" was often different, however, there was considerable variability from study to study. In 1929, Maxwell Wintrobe described his method for obtaining the hematocrit or packed red-cell volume (PCV) by centrifugation of blood in a glass tube [7]. He then defined so-called red-cell indices, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), which proved of enormous value in classifying the various forms of anemia [8]. The latest advance in blood-cell quantitation began in the 1950s with the introduction of increasingly more complicated and sophisticated computer-driven electronic instruments that measure very accurately hemoglobin, the numbers of all the blood cells, as well as the red-cell indices and the red cell distribution width (RDW). Some instruments now also provide automated differential counts of the leukocytes.

Most of the pre-twentieth century American pediatric textbooks gave scant attention to hematologic problems of the neonate. Dewees's 1825 *A Treatise on the Physical and Medical Treatment of Children*, arguably the first American pediatric textbook, and Job Lewis Smith's 1869 *A Treatise on the Diseases of Infancy and Childhood* gave only passing notice to blood conditions of the neonate, such as neonatal jaundice and hemorrhage from improper ligature of the umbilical cord [9, 10]. However, the monumental text of L. Emmett Holt, *The Diseases of Infancy and Childhood*, first published in 1897, contained a section on "the Diseases of the Newly-Born," including the hemorrhagic disease, and a 17-page section on "the Diseases of the Blood," which included the normal blood findings in the newborn [11]. Holt was obviously familiar with the many studies published in the German literature, and his descriptions are reasonably consistent with modern findings:

> The percentage of haemoglobin is highest in the blood of the newly born . . . At this time the number of red blood corpuscles is from 4,350,000 to 6,500,000 in each cubic millimeter . . . In size, a much greater variation is seen in the red cells of the neonate. In the blood of the foetus there are present nucleated red corpuscles or erythroblasts. These diminish in number toward the end of pregnancy. These are always found in the blood of prematures, but in infants born at term, they are seen only in small numbers. The number of leukocytes in the blood of the newly born is three or four times that of the adult, being on the average 18,000 per cubic millimetre.

In 1921, W. P. Lucas and associates from the University of California Medical School in San Francisco described their extensive studies of the blood of 150 infants at birth and during the first two months of life [12]. Their samples were obtained from serial punctures of the longitudinal sinus! The polycythemia of the newborn and changes in the leukocytes were defined clearly.

In 1924, H. S. Lippman from the University of Minnesota published detailed studies of the blood

of newborn infants [13]. He noted (without further details) that "Denis published the first observations on the subject in 1831." Lippman's review of the literature cited 70 previous articles on the hematology of the newborn. Most of these studies were published in European, especially German, journals. Although there was considerable variability because of different methods and standards, the consensus of these early studies was that "hemoglobin values at birth are higher than at any other period in the child's life." Some of these studies described reticulocytosis and normoblastemia in the first day of life, which declined rapidly in the first week of life. Lippman conducted serial studies of capillary blood over the first 48 hours of life in 71 normal newborns as well as changes in the leukocytes during this period.

It has been known for 100 years that the red blood cells of the fetus and newborn are large compared with those of adults, as determined by microscopic measurement of red-cell diameter. Newer electronic cell-sizing techniques have demonstrated that the mean MCV of the neonate's red blood cells averages 110 fl, compared with the 90 fl of adults. The red cells of midgestational fetuses are even larger [14].

In 1856, Korber, in an inaugural dissertation, is reported to have described his experiments that showed that solutions of the hemoglobin of newborn infants resisted denaturation by strong alkaline solutions and maintained a red color, while hemoglobin solutions from adults treated in the same way were rapidly denatured and decolorized [15]. The property of alkali resistance became the basis of the Singer one-minute alkali denaturation test for quantitation of fetal hemoglobin (Hb F), as well as the Apt test, used to differentiate fetal from swallowed maternal blood in infants with gross blood in the gastrointestinal tract [16, 17]. Fetal hemoglobin is also resistant to acid denaturation, which is the basis for the red-cell acid elution staining procedure of Kleihauer and Betke that is used widely to quantitate the magnitude of fetomaternal transfusions [18].

The understanding of the protein structure of hemoglobin advanced rapidly in the 1950s when it was shown that adult hemoglobin, Hb A, ($\alpha_2\beta_2$) is a tetramer of alpha (α) and beta (β) polypeptide chains and that Hb F ($\alpha_2\gamma_2$) contains a different pair of polypeptide chains designated as gamma (γ) chains [19, 20]. During fetal development, synthesis of γ chains predominates, but with approaching term there is a fall-off of γ-chain synthesis and a simultaneous reciprocal increase in β-chain synthesis. The regulatory mechanisms that govern this "β/γ switch" remain to be elucidated. The blood of the newborn contains large amounts of Hb F, averaging 60–80%. The affinity of Hb F for oxygen is greater than that of Hb A, because of poor binding of 2-3-diphosphoglycerate. This results in a shift of the oxygen dissociation curve to the left, which is favorable for oxygen transport to the fetus in the relative hypoxia of intrauterine existence but which may be disadvantageous after birth [21]. The high level of Hb F at birth offers temporary protection from hemoglobinopathies such as sickle cell anemia and may hamper their diagnosis in the newborn. Roland Scott, using the classical "sickle cell prep," demonstrated a much lower frequency of "sicklemia" in black newborns than was found in older children from the same community [22]. The development of techniques such as acid agar gel electrophoresis has permitted genotypic diagnosis of most hemoglobinopathies at birth, and neonatal testing for hemoglobinopathies is now performed routinely in 47 states of the USA [23].

The only somewhat common hemoglobinopathy that produces symptoms in the newborn is homozygous α-thalassemia resulting from deletion of four α-globin genes [24]. In parts of Southeast Asia, fetal hydrops is caused much more frequently by α-thalassemia than by Rh immunization. The recent immigrations of large numbers of Southeastern Asian people into the USA have resulted in increasing numbers of affected infants. Some of these have survived after intrauterine transfusions but are transfusion-dependent [25].

Since the turn of the twentieth century, a large number of studies of the hematology and blood diseases of the newborn have been reported. Much of this information has been incorporated into textbooks of hematology. Maxwell Wintrobe's

monumental *Clinical Hematology*, which was first published in 1943, contained sections on normal blood values, anemias, and hemorrhagic disease of the newborn. Neonatal thrombocytopenia in infants born of mothers with ITP was also mentioned briefly. In subsequent editions of Wintrobe's text, many more neonatal hematological conditions were described. In 1960, Carl Smith published *Blood Diseases of Infancy and Childhood*, the first American textbook of pediatric hematology/oncology, which had several chapters devoted to normal values and hematologic problems in the neonatal period.

In 1966, Frank Oski and Laurie Naiman published the first textbook devoted solely to the hematology and hematological problems of the newborn [26]. The authors' stated purpose was

> . . . to provide in a single source much of what is known concerning both the abnormal and abnormal hematologic processes of the first month of life and the effects of prenatal factors on them . . . And to provide a useful guide to all who care for the newborn infant – those who are continually confronted with infants who are bleeding, anemic or jaundiced.

The Oski–Naiman text had two subsequent re-editions in 1972 and 1982. Subsequently, there have been a plethora of texts and handbooks on pediatric hematology, most of which devote chapters to the newborn.

The history of neonatal hematology and the process of understanding hematologic diseases based on clinical and laboratory observations that stimulate investigation of basic mechanisms and then therapeutic interventions are illustrated well by two quintessential neonatal blood diseases: *erythroblastosis fetalis* and *hemorrhagic disease of the newborn*.

Erythroblastosis fetalis

As recently as 1946, erythroblastosis fetalis, or hemolytic disease of the newborn, affected between 0.5% and 1.0% of fetuses and newborns in the USA. It had a 50% mortality as well as significant neurologic morbidity in many survivors [27]. Prior to 1936, four seemingly distinct neonatal syndromes had been identified: *fetal hydrops*; *fetal erythroblastosis* with massive red-cell proliferation in fetal organs; *icterus gravis familiaris*, a severe neonatal jaundice that often affected subsequent infants; and severe anemia in surviving infants who had not had edema or striking jaundice, which was simply called *anemia of the newborn*. Based on histological and hematological similarities and the familial occurrence, Diamond, Blackfan, and Baty put forth their unifying hypothesis that these four syndromes were all manifestations of an unknown single underlying disease process. They designated all of these neonatal syndromes "erythroblastosis fetalis" [28].

In 1938, Ruth Darrow, a pathologist, several children of whom had died of erythroblastosis, advanced a brilliant inductive hypothesis about its cause. Assembling all of the available information, as well as drawing on her personal experience, she noted the usual sparing of the first child and the involvement of most subsequently born children. She also recognized that the hematologic and histopathologic findings in these infants could be best explained by severe hemolysis. She concluded that the disease resulted because:

> the mother is actively immunized against fetal red cells or some component of them . . . The antibodies formed in the maternal organism may then pass to the child through the placenta [29].

The elusive offending antibody and its red-cell antigen were discovered in 1940 by Karl Landsteiner and Alexander Weiner. It was given the name Rh (rhesus factor) because the antibody was produced by injection of red blood cells of rhesus monkeys into rabbits. This antibody agglutinated the red cells of 85% of normal individuals [30]. Interestingly, Landsteiner's discovery of the Rh blood group was accomplished almost 50 years after he had discovered the ABO blood groups [31]. Philip Levine described a transfusion reaction in a postpartum woman who was given a transfusion of her husband's blood shortly after delivering a stillborn baby with hydrops fetalis. Levine was able to demonstrate Rh antibodies in the mother's circulation, defining clearly the pathophysiology of erythroblastosis fetalis [32, 33].

Effective treatment for erythroblastosis fetalis progressed slowly. The treatment of icterus gravis by "exsanguination transfusion" was first reported in 1925 by A. P. Hart at Toronto's Hospital for Sick Children [34]. With the discovery of the Rh factor, exchange transfusion evolved rapidly as a way to remove circulating antibody, sensitized red blood cells, and bilirubin. This treatment was spearheaded by Harry Wallerstein and Alexander Weiner in New York and Louis K. Diamond in Boston. Wallerstein's method involved aspiration of blood from the sagittal sinus and infusion of Rh negative blood into a peripheral vein [35]. Weiner's method employed heparinization and surgical cannulation of the radial artery and saphenous vein. Interestingly, at a time long before institutional review boards for research, he first evaluated the technique in a non-erythroblastotic "mongolian idiot" [36]. Diamond's much more practical method utilized the umbilical vein to alternately remove and infuse blood, and this rapidly became the accepted method around the world [37]. Diamond developed practical guidelines for the prenatal and postnatal management of Rh-sensitized mothers and their erythroblastotic newborns. These reduced neonatal mortality from 50% to 5% and intrauterine death from 20% to less than 10%, and kernicterus associated with severe hyperbilirubinemia was virtually eliminated [38].

Implicit in the pathogenesis of Rh erythroblastosis is that small numbers of fetal erythrocytes gain entrance into the maternal circulation, where they evoke maternal immunization and Rh antibody formation. The possibility of large fetomaternal transfusion was first hypothesized by Weiner and later proven definitively by Bruce Chown, who used differential agglutination to demonstrate fetal red cells in the maternal circulation in a case of neonatal anemia [39, 40]. It is now recognized that acute, massive fetomaternal transfusion results in neonatal pallor and hypovolemic shock resembling asphyxia pallida, while chronic hemorrhage may be associated with well-compensated congenital microcytic hypochromic anemia due to iron deficiency [41].

The penultimate important developments in erythroblastosis fetalis were provided by A. W. Liley of New Zealand, who devised a method of spectroscopic analysis of amniotic fluid. This identified immunized fetuses at high risk of intrauterine death and who could be given intrauterine intraperitoneal blood transfusion to carry them to delivery [42, 43]. Development of percutaneous umbilical blood sampling under ultrasonographic guidance has enabled perinatologists to directly diagnose and assess the severity of anemia in immunized fetuses and to treat them with simple or exchange transfusions in utero. Finally, Clark in Liverpool and Freda and associates in New York showed independently that primary isoimmunization of Rh-negative mothers by the Rh-positive red cells of their fetuses could be largely prevented by immediate postnatal administration of potent anti-Rh gamma globulin to the mother [44, 45]. In most of the developed world, erythroblastosis fetalis has become a rare disease of largely historical interest, and exchange transfusions have become a lost skill.

Hemorrhagic disease of the newborn

Newborn infants may bleed seriously from several causes. More than 2000 years ago, the familial occurrence of severe bleeding following ritual circumcision of boys, who doubtless had hemophilia, was recognized:

> It has been reported of four sisters at Sapphoris; the first one circumcised her son, and he died; the second, and he died; the third, and he died. Then the fourth came before Rabban Simeon be Ganalied who said to her; abstain from circumcision . . . for there are families whose blood is loose; while in others it coagulates. (Babylonian Talmud, tractate Yehamot, fol. 64, p. 2 [46])

Armand Quick, in a 1942 review of the history of coagulation, noted that possible cases of a neonatal hemorrhagic disease, distinct from hemophilia, had been reported from time to time as far back as 1682. Quick also postulated that the delay of ritual circumcision by Jews until the eighth day of life may have been based on their empirical observations that neonatal bleeding symptoms have largely waned by that time [47].

However, the first definitive description of "the haemorrhagic disease of the newborn" was provided by C. W. Townsend in Boston in 1894. Townsend described a generalized, not local, bleeding disorder, beginning on the second or third day of life. About 0.6% of newborns were affected with clinical hemorrhage, chiefly into the skin, gastrointestinal tract, and central nervous system. There was a 62% mortality rate, but if not fatal, the disease was self-limited, with most cases recovering within five days. The sexes were affected equally [48]. The onset of transient bleeding only in the first few days of life, as well as the involvement of girls, clearly differentiated hemorrhagic disease of the newborn from hemophilia.

Lucas and associates performed clotting times, a measure of the entire coagulation mechanism, and showed that during the first four days of life, "there is a definite and fairly consistent prolongation of the coagulation time which favors the so called hemorrhagic condition of the new born" [12]. Whipple in 1912 found that the plasma of a newborn with hemorrhagic disease was deficient in prothrombin; this deficiency was proven definitively by Brinkhous and colleagues in 1937 [49, 50].

Treatment of hemorrhagic disease of the newborn was essentially limited to supportive measures, including local compression when possible [11]. More than half of affected babies died of intracranial hemorrhage or hemorrhagic shock. Lambert in 1908 was able to rapidly reverse the bleeding of an affected baby by a transfusion in which the father's radial artery was anastomosed to the baby's popliteal vein [51]. In 1923, J. B. Sidbury, a practicing pediatrician in North Carolina, treated successfully the hypovolemic shock and bleeding disorder of an affected newborn by giving a blood transfusion through the umbilical vein. Sidbury stated that "human whole blood has acted as a *specific* in this condition" [52]. In the 1920s, and continuing into the 1940s, the standard treatment of hemorrhagic disease of the newborn, and in some centers the prophylaxis of the condition, was the intramuscular injection of adult blood, often obtained from the father. This was before the discovery of the Rh factor, and led to the Rh immunization of some girls and erythroblastosis in their offspring [53].

Understanding of the pathogenesis of hemorrhagic disease of the newborn was made possible in 1929, when Dam and associates showed that chicks fed an ether-extracted diet developed a severe bleeding tendency that could be prevented by feeding material extracted and purified from cereals or seeds. They named the correcting factor "Koagulationsvitamin," or vitamin K [54, 55]. The nature of the bleeding defect in vitamin K-deficient chicks was soon localized to a deficiency of prothrombin and defined clearly by Brinkhaus and colleagues and Dam and colleagues in normal babies and those with hemorrhagic disease [50, 56]. Waddell and associates showed that vitamin K administration could prevent coagulation abnormalities in newborns [57]. Synthesis of vitamin K was accomplished in 1939 [58]. Routine vitamin K prophylaxis (0.5–1.0 mg) for all newborns was recommended by the Committee on Nutrition of the American Academy of Pediatrics in 1961, and vitamin K-associated hemorrhagic disease of the newborn has virtually disappeared in the developed world [59]. It should be mentioned, however, that the incidence of hemorrhagic disease of the newborn had decreased markedly in the USA even before vitamin K prophylaxis became a routine; this decrease was probably a consequence of the declining incidence of breast feeding from the 1930s through the 1960s. The vitamin K content of breast milk is much lower than that of cows' milk, and hemorrhagic disease of the newborn occurs almost exclusively in breast-fed infants who, deliberately or inadvertently, do not receive prophylactic vitamin K [60, 61]. The biochemical basis of the action of vitamin K has been shown to relate to the gamma-carboxylation of glutamic acid residues in the vitamin K-dependent coagulation factors, including prothrombin [62].

Epilog

This review of the history of neonatal hematology makes clear that study of the blood of the fetus and

newborn has captured the attention of many pediatricians and hematologists over many years. It is surprising how large their contributions were and that "there is little new under the sun." The sagas of erythroblastosis fetalis and hemorrhagic disease of the newborn illustrate well the progress from clinical recognition and description, to definition of pathogenesis, to empiric and then specific therapy, and finally to prevention.

The majority of work and investigation in neonatal hematology has been performed by pediatric hematologists. However, we are now seeing a generation of hematologists who have been trained in neonatology, who work in newborn special care units, and who have made hematology their clinical and research focus.

As we examine neonatal hematology today, the morphological and quantitative earlier eras of hematology have been succeeded by modern eras of biochemical and genetic investigation of the processes that regulate fetal and neonatal blood and are causes of many of the blood diseases that affect the newborn. Discoveries in these areas will revolutionize neonatal hematology and will make a wonderful story for another historical overview in the twenty-second century, or perhaps even sooner.

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2

Disorders of the fetomaternal unit

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In health, the placenta functions marvelously well as the interface between the maternal and fetal circulations, providing nutrition, oxygen, fluid, and electrolytes and removing fetal waste and carbon dioxide. Disorders that disrupt placental physiology, and the presence of pathogens or toxins that can cross the placental barrier, can adversely affect the fetus. This chapter will discuss disorders of the maternal–fetal unit that result in hematologic abnormalities in the fetus and/or newborn infant.

Hemorrhagic disorders of the fetoplacental unit

The average blood volume of the fetoplacental circulation is roughly 110 ml/kg [1], and hence a relatively small amount of blood loss can be a sizable proportion of the fetal blood volume. Placental abnormalities causing fetal blood loss are shown in Table 2.1. Such blood loss can be visible, as with placenta previa, or occult, as with fetomaternal bleeds or twin–twin transfusion syndrome. The clinician must suspect fetal blood loss if the neonate presents with shock and pallor. The placenta and cord should be inspected for pallor, a hematoma, or other anomalies. As discussed below, maternal blood should be studied for the presence of fetal cells.

Abruptio placenta and placenta previa

While the majority of blood loss with placenta previa or abruptio placenta is maternal, fetal blood loss can also occur [2, 3]. The frequency of neonatal anemia requiring transfusion increases with the severity of maternal bleeding [4]. Vaginal blood can be tested for fetal erythrocytes using the Kleihauer–Betke stain, which identifies cells containing hemoglobin F.

Placental or umbilical-cord damage can cause neonatal anemia. For instance, umbilical cord-blood rupture may occur, especially with traumatic delivery, the presence of velomentous cord insertion, or a multilobed placenta. Surgical laceration of the placenta, as may occur with Cesarian section, may cause significant fetal blood loss.

Infants who have experienced acute hemorrhage during fetal life present with clinical features of acute anemia; pallor, hypovolemia, and hypotension. Unlike patients with hemolytic disorders, these infants do not typically develop hyperbilirubinemia. A hemoglobin measurement immediately following birth often does not reflect accurately the severity of the bleeding. Furthermore, because capillary blood counts are generally higher than central measurements, especially in the acidotic infant, they may underestimate anemia [5]. The management of such infants initially consists of volume replacement, usually with volume expanders such as Ringer's lactate or normal saline. Red-blood-cell transfusion may be indicated for large fetal blood loss.

Fetomaternal bleeds

Small quantities of fetal erythrocytes pass into the maternal circulation in the majority of pregnancies [6]. In approximately 98% of pregnancies, less than 2 ml of fetal cells are found in the maternal blood

Table 2.1 Causes of fetal blood loss

Placental
Abruptio placenta
Placenta previa (if fetal vessels are torn)
Placental laceration at operative delivery
Umbilical-vessel injury during amniocentesis
Umbilical-cord rupture
Transfusion syndromes
Fetomaternal transfusion
Fetofetal transfusion (twin–twin transfusion)

[7]. However, in about 0.3% of pregnancies, this volume exceeds 30 ml, roughly equivalent to 10% of the blood volume of a 3-kg infant. The presence of pregnancy complications, abortion, pre-eclampsia, Cesarian section, or complicated delivery increases the risk of significant fetomaternal bleeding [6]. Massive fetomaternal hemorrhage, e.g. over 30–50 ml, can cause symptomatic anemia or even fetal demise [8, 9]. Chronic fetomaternal bleeding can cause nonimmune hydrops [10]. The presence of symptomatic anemia is dependent upon both the amount of and the time course of the hemorrhage, i.e. a large acute bleed presents with hypovolemic shock, whereas chronic bleeding may present with pallor or congestive heart failure.

Diagnosis

The usual approach is to examine maternal blood for fetal erythrocytes using the Kleihauer–Betke stain or flow-cytometric techniques [11, 12]. Because fetal cells may be cleared quickly from the maternal circulation, delay in assessment may prevent the identification of, or underestimate, the volume of fetal blood lost into the maternal circulation, especially if there is a blood-group incompatibility between the infant and mother. False-positive results can occur if the mother has an increased percentage of hemoglobin F in her own cells, as may occur with the hereditary persistence of fetal hemoglobin, aplastic anemia, or use of cancer chemotherapy [13]. Fetal ultrasound abnormalities and/or non-stress test may not be reliably sensitive to the identification of fetal anemia but, as discussed in Chapter 6, Doppler studies of fetal middle cerebral artery-flow velocity may be a useful technique to identify fetal anemia [14, 15]. Elevated maternal serum alfa-fetoprotein levels may indicate fetomaternal transfusion [10].

Management

As with acute hemorrhage, the initial management for acute severe fetomaternal bleeding is volume expansion using isotonic crystalloid solutions and possibly red-blood-cell transfusion. Chronic bleeding, not resulting in severe or symptomatic anemia, can be managed with iron supplementation.

Twin–twin transfusion syndrome

Twin–twin transfusion syndrome (TTS) is the result of blood traversing from the donor monozygotic twin to the recipient twin. TTS occurs in roughly 8–30% of monozygotic, monochorionic twins. Dickinson and Evans [16] estimated the incidence of TTS to be about 1 per 4000 births and 1 per 58 twin births in Western Australia. Vascular anastomoses can be arterio-arteriolar, veno-venous, or arterio-venous. The incidence of TTS is higher if there is a velamentous cord insertion [17]. Postnatally, the diagnosis can be confirmed by injecting milk or other fluids into the placenta [18].

TTS may be acute or chronic. With chronic twin–twin transfusion, the recipient twin becomes hypervolemic, stimulating increased fetal urination and polyhydramnios. The donor twin becomes anemic and develops oligohydramnios. Acute TTS usually occurs at delivery. The donor twin typically has symptoms of hypovolemia while the recipient twin is at risk for polycythemia/hyperviscosity syndrome.

In the past, the diagnosis of TTS was usually made by documenting a >5-g/dl difference in hemoglobin concentration and a >20% difference in birth weight between the twins. These criteria have recently been called into question. In a recent study of 97 pairs of monochorionic twins, Wenstrom and colleagues [19] identified 35 pairs with this size discordance.

Half were concordant for hemoglobin concentration. In 18%, the smaller twin had the higher hemoglobin, while in 32%, the smaller twin had the lower hemoglobin. Furthermore, 36% of the size-concordant twins were discordant for hemoglobin concentration. In a study of 178 consecutive twin pregnancies, hemoglobin levels in the first few hours of life differed by more than 5 g/dl in six monochorionic twins and in seven dichorionic twins. Only one of five twins with both a >5-g/dl hemoglobin and a 20% birthweight difference was monochorionic [20]. Diagnosis of TTS is now often made by prenatal ultrasonography. Criteria include discordant fetal abdominal circumference, polyhydramnios in one twin and oligohydamnios in the other, and an increased intertwin difference of systolic/diastolic ratio [18].

Clinical manifestations

Most infants with TTS are born early and suffer complications of prematurity. In addition to plethora in the recipient twin, a number of other systems may be involved in the recipient. Cardiac findings include biventricular hypertrophy and tricuspid regurgitation [21]. Hyperviscosity secondary to polycythemia can result in vascular occlusion [22, 23]. Cutaneous erythropoiesis (manifested as blueberry-muffin rash) [24, 25] or neutropenia [26] may be present in the donor twin. The donor twin may also suffer renal failure [27, 28].

Management

Many advances have come about recently in the obstetric management of TTS. Transplacental digoxin therapy for hydrops fetalis, amnioreduction, and endoscopic laser coagulation of placental vascular anastomoses may improve survival [29–31]. Postnatally, the donor twin may require volume expansion. If the twin is severely hypovolemic and/or anemic, then red-blood-cell transfusion is indicated. Glucose infusions may be necessary for hypoglycemia in the donor twin. Long-term iron supplementation should be administered to the donor twin. The recipient twin should be evaluated for complications of the polycythemia/hyperviscosity syndrome, such as respiratory distress, jitteriness, seizures, hypocalcemia, hypoglycemia, and hyperbilirubinemia. The affected twin may require partial exchange transfusion. The complications and management of the polycythemia/hyperviscosity syndrome are discussed in Chapter 9.

Hematologic consequences to the fetus of maternal diseases

Diabetes

Maternal diabetes can have profound effects on fetal development. Maternal diabetes may predate the pregnancy or can develop during pregnancy (gestational diabetes). It is estimated that 0.2–0.3% of pregnancies are affected by pre-existing diabetes mellitus and 1–5% are affected by gestational diabetes [32]. Reported problems in infants of diabetic mothers (IDM) include decreased survival and increased rates of prematurity and frequency of both small for gestational age (SGA) and large for gestational age (LGA), although the LGA group predominates. Insulin does not cross the placenta, and hence maternal hyperglycemia causes fetal hyperglycemia and resultant fetal hyperinsulinemia. This increased fetal insulin leads to accelerated fat deposition and macrosomia. There is an increased rate of congenital anomalies in infants of mothers with pre-existing diabetes but not in infants of mothers with gestational diabetes. Tight control of maternal glucose levels decreases the rates of congenital malformations and fetal macrosomia. There are several hematologic complications in the IDM, most notably increased rates of polycythemia and thrombosis.

Polycythemia in infants of diabetic mothers

IDMs have an increased incidence of polycythemia [33]. Mimouni and colleagues [34] found that the

incidence of polycythemia (venous hematocrit (Hct) ≥65% at two hours of age) in IDMs was 29% versus 6% for infants matched for gestational age, mode of delivery, and Apgar scores. The hematocrit of the infant did not correlate with maternal glycosylated hemoglobin levels but did correlate with neonatal hypoglycemia. In contrast, Green and colleagues [35] found that the hematocrit of IDMs correlated with maternal glycosylated hemoglobin levels at term (average gestation 38 weeks) but not at 36 weeks gestational age. This may suggest that the maternal glucose control in late gestation has the greatest influence on the incidence of polycythemia.

Several factors may contribute to polycythemia in the IDM. Insulin itself may promote erythropoiesis. Insulin infusion causes increased red cell mass in fetal sheep [36]. Insulin, at levels found in IDMs, can stimulate the growth of late erythroid progenitors in tissue culture [37]. Widness and colleagues [38] found that the umbilical-vein erythropoietin concentrations were elevated in IDMs and correlated with maternal HbA1c levels taken the month before delivery. Fetal erythropoietin concentrations correlate with fetal insulin levels [39]. Shannon and colleagues [40] did not find increased erythropoietin concentrations in IDMs whose mothers had tight glycemic control throughout pregnancy. Nucleated red-blood-cell (RBC) levels, which may be a marker of fetal hypoxia, are also elevated in the IDM [41, 42]. There is a delayed switch from gamma- to beta-chain production in the IDM [43, 44].

The complications of the polycythemia/hyperviscosity syndrome such as hypocalcemia, hypoglycemia, and hyperbilirubinemia should be expected and managed (see Chapter 9).

Thrombosis in infants of diabetic mothers

The incidence of thrombosis, especially renal-vein thrombosis, is increased in IDMs [33, 45]. Clinical manifestations of renal vein thrombosis may include shock, vomiting, hematuria, and a palpable kidney. Both venous and arterial thrombosis can occur in the IDM. While reported cases of peripartum gangrene of the limb are rare, 22% of one series were reported to be in IDMs [46].

It is likely that the increased incidence of thrombosis in the IDM is multifactorial. Polycythemia causes increased blood viscosity. Birth trauma, in part caused by macrosomia, may lead to vessel damage. Both platelet and plasma factors place the IDM at increased risk for thrombosis. Hathaway and colleagues suggested that there is increased platelet consumption [47]. Stuart and colleagues [48] documented increased platelet reactivity and platelet endoperoxide formation in diabetic mothers and transiently in their infants. Umbilical arteries from IDMs born to mothers with elevated HbA1c values produce significantly less prostacyclin, a potent inhibitor of platelet aggregation, than those obtained from control infants or IDMs of mothers with normal values for HbA1c [49]. Easa and Coen [50] failed to find a difference in the prothrombin time, activated partial thromboplastin time, fibrinogen, factors V, X, or XII, or von Willebrand antigen. Ironically, they found slightly lower levels for factor VIII and increased levels of antithrombin. Antiplasmin concentrations are increased in mothers with diabetes and their infants [51]. Recently, elevated levels of homocysteine, a known risk factor for thrombosis, have been reported in IDM [52].

Thrombocytopenia in infants of diabetic mothers

Mild thrombocytopenia is seen in IDM [47, 50]. The mean platelet count in IDMs was shown to be lower than in matched controls and did not correlate with maternal glycemic control [42]. Usually, no specific therapy is indicated.

Hypertension

Hypertension is a well-recognized and potentially serious complication of pregnancy. Several predisposing maternal factors, including age (young or old), parity, twin gestation, lower socioeconomic

status, genetic factors, and diabetes, have been described [53]. The HELLP (hemolysis, elevated liver function tests, and low platelets) syndrome develops in some women.

Neutropenia in infants of hypertensive mothers

In their classic paper defining the normal neonatal neutrophil count, Manroe and colleagues [54] demonstrated a high incidence of neutropenia in the infant of the hypertensive mother (IHM). Since that time, other studies have demonstrated a 40–50% incidence of neutropenia in the IHM, using Manroe's data for the normal range [55–57]. Doron and colleagues [58] found an incidence of 48% using normative data developed for premature infants. In contrast, Gray and Rodwell [59] failed to find an increased incidence of neutropenia in premature IHMs compared with matched controls also using normative data developed for premature infants. Smaller (<1500 g), more premature (under 30 weeks' gestational age), and/or Cesarian-section-delivered IHMs are more likely to have neutropenia. The incidence of neutropenia increases with the severity of maternal hypertension [56, 58]. Koenig and Christensen [57] identified decreased neutrophil production, perhaps due to an inhibitor of myelopoiesis, as the cause of neutropenia in the IHM. Some studies have shown this neutropenia to last for less than 72 hours [55, 57], while another study reported more prolonged neutropenia [59]. Compared with infants with sepsis-induced neutropenia, the neutropenia in IHMs occurs earlier in life and does not have an increased ratio of immature to total neutrophils [56]. The neutropenia in the IHM may result in an increased risk of infection. Doron and colleagues [58] reported an increased rate of bacterial infection in the first 48 hours of life in neutropenic compared with non-neutropenic IHMs. Other studies have shown an increased incidence of late-onset infection, usually after the neutropenia had resolved [56, 57]. Neutropenia in IHMs may respond to granulocyte colony stimulating factor injections [60]. However, the need for routine use of this medication in the neutropenic IHM is not clear.

Thrombocytopenia in infants of hypertensive mothers

While thrombocytopenia is seen in 15–36% of IHMs [55–57, 61], it is generally mild. Thrombocytopenia appears to be more common in infants of mothers with more severe hypertension or HELLP syndrome [57, 61]. Disseminated intravascular coagulation (DIC) has been reported in IHMs whose mothers' platelet counts were less than 50 000 per μl [61].

Polycythemia in infants of hypertensive mothers

An increased incidence of polycythemia in IHMs has been reported [62]. Brazy and colleagues [61] found the mean hemoglobin level to be 5% higher in IHMs. The recommendations regarding the management of polycythemia are outlined in Chapter 9.

Maternal malignancy

Cancer complicates approximately 1 in 1000 pregnancies. The most common cancers in pregnant women are of the uterus, breast, lymphatic system and ovaries [63]. The potential effect of treatment on the fetus raises serious questions for the management of the mother [64]. While malignant melanoma can also occur in pregnant women, the usual surgical treatment does not typically affect either the pregnancy or the fetus.

Antineoplastic agents used to treat cancer can cross the placenta [65]. The ability of the fetal liver and kidney to metabolize these agents is not well studied. There are differences in both the fetal and maternal physiology that might affect the toxicity of these agents [66, 67]. An increase in fetal malformations has been attributed to chemotherapy in the first trimester [68]. Congenital anomalies are produced by alkylating agents in animal models [69]. Zemlickis and colleagues [70] reported that of 13 women

receiving chemotherapy in the first trimester, four underwent elective abortion, four had a spontaneous abortion, and two gave birth to infants with major malformations. The relative risk of chemotherapy-induced malformation decreases after the first trimester. The incidence of stillbirth after intensive chemotherapy for leukemia is 25% in the first trimester [66] and falls to about 13% in the second and third trimesters [71]. Infants exposed to chemotherapy during the second and third trimesters may be at increased risk for intrauterine growth retardation [72]. Idarubicin-induced fetal cardiotoxicity has been reported [73]. Myelosuppressive chemotherapy administered near to the time of delivery may cause neonatal pancytopenia [74], and hence some authors have recommended trying to avoid myelosuppressive chemotherapy within three weeks of delivery [75].

There is limited long-term follow-up of children whose mothers were treated with chemotherapy. One study found no long-term hematologic, immunologic, or cytogenetic problems among 43 children aged 3–19 years whose mothers received chemotherapy for malignancies during pregnancy [76]. A national registry has been established to follow the long-term effects of maternal chemotherapy [72, 77].

Each year, approximately 4000 women undergo radiation therapy during pregnancy. The topic of radiation administered during pregnancy has recently been reviewed [78]. Intrauterine growth retardation, microcephaly, eye, and central nervous system (CNS) abnormalities are the predominant complications of intrauterine exposure to ionizing radiation in humans [79]. Hematopoietic, hepatic, renal, and cutaneous effects of radiation therapy have been reported when the fetus is exposed to radiation late in gestation.

Maternal malignancy rarely spreads to the fetus or the placenta. Malignant melanoma is the most common malignancy to metastasize to products of conception [63]. Dildy and colleagues [80] reviewed 53 cases of maternal cancer metastatic to the products of conception. Twelve involved the fetus, of which seven were malignant melanoma and four were leukemia or lymphoma. This topic is discussed further in Chapter 16.

Autoimmune syndromes

Neonatal lupus erythematosus

The neonatal lupus erythematosus (NLE) syndrome is believed to be due to the transplacental passage of autoantibodies, usually anti-Ro (SS-A) or anti-La (SS-B). Irreversible congenital heart block arising in the second trimester is the most devastating complication of the NLE syndrome. Cutaneous manifestations of NLE often begin after birth. Other complications include hepatitis and occasionally neurologic manifestations, including seizures.

Transient thrombocytopenia has been noted in 10% of infants with NLE [81]. Antiplatelet antibody studies were negative in two infants with NLE and thrombocytopenia [82]. Hariharan and colleagues reported a case of NLE-associated microangiopathic anemia with severe thrombocytopenia that responded to intravenous gamma globulin and corticosteroid treatment [83].

Antiphospholipid antibody syndrome

An association between the presence of anticardiolipin antibodies and recurrent fetal loss has been well described. The presence of anticardiolipin antibody and/or the lupus anticoagulant is associated with an increased risk for venous and arterial thrombosis. The cause of the increased thrombosis is not certain, but it appears to be related to platelet activation. The IgG isotype of the anticardiolipin antibody can cross the placenta [84]. The presence of these antibodies in the mother may increase the risk for intrauterine growth retardation [85]. There have been numerous case reports of fetal and neonatal thrombosis, including the CNS vessels [86], renal vasculature, vena cava, and aorta [87]. The presence of the anticardiolipin antibody in the newborn is transient, and thrombosis should be managed as described in Chapter 13.

Hematologic effects of maternal medications/toxins

Smoking

Despite extensive publicity about the medical complications of smoking, maternal cigarette usage remains common worldwide [88]. In 1994, approximately 15% of pregnant women, as opposed to 23% of all women, smoked cigarettes. The prevalence of cigarette usage during pregnancy varies with ethnicity and maternal education [89]. In Ottawa, approximately 25% of pregnant women used cigarettes, but a decreasing percentage reported using greater than one pack/day as the pregnancy progressed [88]. Although cigarette smoke contains numerous toxins, the majority of the literature has addressed the impact of carbon monoxide and nicotine. Measurement of fetal or maternal levels of the nicotine metabolite cotinine is a better marker of maternal cigarette use than the mother's smoking history [90].

Infants of smoking mothers have lower birth weights [91]. In Finland, infants exposed to nicotine *in utero* weighed 188 g less and measured 10 mm less than unexposed infants [90]. Maternal cigarette use is associated with increased rates of placenta previa, abruptio placenta, ectopic pregnancy, and premature rupture of membranes (PROM) but with decreased rates of pre-eclampsia [92].

Polycythemia with maternal tobacco usage

Cigarette usage increases the concentration of carbon monoxide in the mother's blood. Hemoglobin has a 200-fold greater affinity for carbon monoxide than oxygen. In addition, the presence of carbon monoxide increases the hemoglobin oxygen affinity. Hence, in the presence of carbon monoxide, there is a substantial decrease in oxygen delivery to the tissues. When the mother smokes cigarettes, the level of carboxyhemoglobin is significantly higher in the newborn than in the mother [93]. Bureau and colleagues [94] found that, compared with control infants, infants of smoking mothers have significantly higher concentrations of total hemoglobin and hemoglobin F and that their hemoglobin has an increased oxygen affinity. Other studies have confirmed increased hemoglobin concentrations in infants of smoking mothers [95–98]. While Mercelina-Roumans and colleagues failed to show this relationship [99], the degree of maternal tobacco exposure was relatively low in their study.

Erythropoietin concentrations are higher in infants of smoking mothers [95]. As erythropoietin does not cross the placenta, the increased levels are likely a response to decreased oxygen delivery to fetal tissues. Cord-blood erythropoietin levels increase in proportion to maternal cigarette usage [96, 100, 101] and to fetal cotinine levels [100]. In a large consecutive series, there was no correlation between the number of neonatal nucleated RBCs and maternal smoking history [102], but, ironically, Dollberg and colleagues [103] found that infants whose mothers were exposed to passive smoking had increased numbers of nucleated RBCs in their cord blood. Hence, multiple factors place the infants of mothers who smoke cigarettes at increased risk for polycythemia and tissue hypoxia.

Bleeding with maternal tobacco usage

While platelet counts are similar in infants of smoking and non-smoking mothers [97, 99], Spinillo and colleagues [104] noted an epidemiologic association between heavy maternal smoking and intraventricular hemorrhage, especially grade I–II. However, one study did not show an excessive activation of the coagulation or fibrinolytic pathways in the infants of smoking mothers [105].

Maternal medications

Effects on hemostasis

Vitamin K deficiency is seen in 10–66% of newborns whose mothers have been treated with anticonvulsants, including phenobarbital, phenytoin, and carbamazepam [106–108]. Hemorrhagic disease of the newborn with intraventricular hemorrhage has been reported. The coagulopathy can be reversed with

vitamin K administration. Vitamin K deficiency with severe bleeding can also be caused by maternal warfarin ingestion.

Aspirin irreversibly acetylates platelet cyclooxygenase, leading to platelet dysfunction. Prepartum maternal aspirin ingestion within five days of delivery can cause platelet function abnormalities in the newborn [109] and has been associated with clinical bleeding in the fetus and neonate, including intracranial hemorrhage [110]. Antenatal exposure to indomethacin is also associated with an increased rate of intraventricular hemorrhage in infants delivered at under 30 weeks gestational age [111].

As described above, cancer chemotherapeutic agents can cause neonatal thrombocytopenia. Thrombocytopenia has been noted in women treated with thiazides for hypertension, but it is unclear whether the reduction in platelets is due to the medication or the underlying pregnancy-induced hypertension [112]. Heparin-induced thrombocytopenia (HIT) occurs in a small percentage of individuals treated with heparin. It is manifested by mild thrombocytopenia and, paradoxically, an increased risk for thrombosis. HIT is caused by an antibody directed against the heparin–platelet factor 4 complex. HIT occurs in pregnant women; since the HIT antibody is an IgG antibody that is transported transplacentally, it has been found in cord blood [113].

Effects on red blood cells

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is by far the most prevalent genetically transmitted erythrocyte enzymopathy, with millions of individuals affected. As the gene is transmitted in an X-linked recessive manner, the disorder is far more common in males than females, but females may be affected if they are homozygous or by the inactivation of a disproportionate number of normal X chromosomes, as per the Lyon hypothesis. Fetal hemolysis in G-6-PD deficient fetuses can be triggered by maternal ingestion of compounds known to induce hemolysis in deficient RBCs, including naphthalene, fava beans (broad beans), and the medications as listed in Table 7.2 [112, 114]. The neonatal manifestations of G-6-PD deficiency are described in Chapter 7. Cancer chemotherapy administered to pregnant women can interfere with erythropoiesis in the newborn.

Effects on leukocytes

Barak and colleagues [115] reported that infants whose mothers received antenatal administration of betamethasone within 36 hours of delivery had higher leukocyte and neutrophil counts than control infants, and that this effect lasted for up to seven days. Leukemoid reactions have been noted in a small group of infants whose mothers received antenatal dexamethasone [116]. In a small trial, granulocyte colony stimulating factor was administered to mothers with a pregnancy of <30 weeks' gestational age and imminent delivery. Two infants who were delivered more than 30 hours after the mothers received the medication had a prolonged increase in their absolute neutrophil counts while no increase in neonatal neutrophil counts was noted for infants delivered <30 hours after maternal administration of this agent [117].

Maternal nutritional deficiencies

Iron

Iron deficiency is the most common cause of anemia worldwide, with billions of people affected by iron deficiency and iron deficiency anemia [118]. Iron deficiency may exist without anemia. In the USA, iron deficiency and iron-deficiency anemia were found in 11% and 5%, respectively, of non-pregnant women aged 20–49 years [119], with nearly identical rates in Northern Ireland [120]. In the USA, iron deficiency is more prevalent in non-white, poor, and multiparous women [119]. In the developing world, estimates of anemia in pregnancy range from 39% to 80%, with at least half being due to iron deficiency [121].

The diagnosis of iron deficiency can be difficult, because many standard diagnostic measures can be affected by pregnancy. Ferritin is an acute-phase

reactant whose levels may increase with many disorders. The ratio of serum iron to iron-binding capacity (iron-saturation ratio) can be affected by inflammation, diurnal variation, and acute iron ingestion. The most reliable test is the presence or absence of stainable iron in the bone marrow, but marrow sampling is neither practical nor necessary in most instances. Some studies have used a hemoglobin concentration or hematocrit less than two standard deviations below the World Health Organization (WHO) criteria for the diagnosis of anemia and factors such as a serum ferritin <12 ng/ml or an iron saturation ratio of <15% in adult women to identify iron deficiency. The serum transferrin receptor level is elevated in iron deficiency as well as in the presence of increased erythropoiesis [122]. The reticulocyte hemoglobin content is reduced in iron deficiency, but this has not been well studied in the newborn [123]. Measurements of iron sufficiency and nutrition in the neonate are discussed in greater detail in Chapter 4.

The increased blood volume of pregnancy and the needs of the growing fetus place an exceptional burden on the iron stores of the mother. The average daily requirement for absorbed iron during pregnancy is 4.4 mg/day and increases from 0.8 mg/day in the first ten weeks to 7.5 mg/day in the last ten weeks [124]. The fetal iron endowment is 75 mg/kg [125]. The total iron requirement of an average pregnancy is over 1000 mg to cover the needs of the fetus and the increased maternal red cell volume. Iron deficiency may be caused by inadequate nutritional intake, excessive blood losses, including those of preceding pregnancies, or a combination of these problems.

The effect of maternal iron deficiency on maternal and fetal health has been reviewed [126]. The fetus is remarkably effective at extracting iron from its mother, even when her iron stores are depleted. There is no correlation between the maternal and neonatal hemoglobin concentrations at birth [126]. Studies of the effect of maternal iron stores during pregnancy on neonatal iron stores at birth (as estimated by ferritin levels) have yielded conflicting results. For instance, Choi found that ferritin concentrations were similar in neonates of mothers with iron deficiency (as determined by a ferritin concentration below 12 μg/l), as compared with offspring of mothers with normal ferritin levels, but that neonatal ferritin concentrations were low if the mother was anemic [122]. However, serum transferrin receptor levels were increased significantly in the infants of iron-deficient mothers. Using elevated zinc protoporphyrin as an indicator of maternal iron deficiency in non-anemic mothers, Harthroon-Lasthuizen and colleagues [127] failed to find a significant effect on neonatal hemoglobin, ferritin, or zinc protoporphyrin levels. Iron supplementation administered to non-anemic mothers improved measures of iron stores and lowered erythropoietin levels in the mother but did not affect measures of iron sufficiency in cord blood [128]. Mild maternal anemia likely has little impact on fetal growth or measurable outcomes. For instance, Steer [129] found that a maternal hemoglobin concentration of 9.6–11.5 g/dl was associated with the lowest incidence of low-birth-weight infants. Severe anemia, however, can increase the risk of an adverse outcome, such as low birth weight or premature delivery [126, 130].

While the newborn's hemoglobin concentration may not be affected unless the mother is very anemic, maternal iron supplementation during pregnancy may improve the infant's hemoglobin levels later in life. In a population at high risk for iron deficiency, Preziosi and colleagues [131] found that infants of mothers supplemented with iron late in pregnancy had higher serum ferritin levels at three months of age.

Folate

Folate is a required cofactor in many one-carbon-transfer reactions. Such reactions are components of the purine and thymidine synthetic pathways and, hence, affect DNA synthesis. Because this step is critical for hematopoiesis, folate deficiency can be a cause of anemia, thrombocytopenia, and neutropenia. Folates are also important in the degradation of histidine and homocysteine. Folate is found in many foods, especially leafy vegetables, fruits, and yeast. It is also found in human and cows' milk, but

it is absent in goats' milk. In addition to diet, several other factors can affect maternal folate levels. Excessive cooking inactivates folate. Methotrexate, pyrimethamine, and trimethoprim inhibit dihydrofolate reductase and interfere with the production of 5,6,7,8-tetrahydrofolic acid, the active form of folate in one-carbon transfers. Phenytoin and sulfasalazine decrease folate absorption from the intestine [132]. Ethanol, aspirin, smoking, and oral contraceptives can reduce folate concentrations in the blood. Medical conditions in the mother, such as hemolysis, anticonvulsant use, and twin pregnancy increase the risk of maternal folate deficiency.

Folate deficiency in unsupplemented pregnant women is common but may be masked by concomitant iron deficiency. Megaloblastic changes are seen in the bone marrows of 25% of pregnant women [133]. Folate supplementation will improve birth weight in deficient but not sufficient populations [133]. Maternal folate deficiency is associated with an increased risk for neural-tube defects, prematurity, and growth retardation to the fetus [134]. Current recommended daily intake for women of childbearing age for folate is 400 μg/day [135]. Folate is transported against a gradient in the placenta [134]. Ek [136] compared serum and RBC folate levels in unsupplemented mothers and their infants. At all gestational ages studied (22–43 weeks), the infants had higher RBC and plasma folate levels. The infant RBC folate concentrations were similar from 22 to 37 weeks but then increased significantly while maternal levels decreased near term, suggesting transfer to the infant. A high-affinity folate-binding protein is present in umbilical-cord serum [137].

Because of the preferential transport of folate to the fetus, deficiency in the immediate newborn period is unlikely. Neonates of mothers with even severe megaloblastic anemia have normal hemoglobin concentrations [138]. Postnatal events such as hemolysis, infection, malnutrition, diarrhea, and liver disease increase the likelihood of folate deficiency in the neonate. The diagnosis should be suspected in the malnourished infant with persistent anemia, especially when associated with thrombocytopenia and/or neutropenia. Bone-marrow examination may reveal megaloblastic hematopoiesis, and hypersegmented neutrophils may be seen on the peripheral blood smear. The diagnosis is confirmed by reduced serum or erythrocyte folate concentrations. Erythrocyte folate levels would be expected to decline more slowly than serum folate levels, although whole-blood folate levels have been shown to decline more rapidly over the first six weeks of age in term newborn infants of mothers with an omnivorous diet [139]. Total plasma homocysteine levels may be elevated in folate deficiency. Cobalamin deficiency should also be considered in megaloblastic anemia in infants, as cobalamin deficiency causes reduced erythrocyte folate concentrations with normal to elevated serum folate levels [140].

Treatment

Infants with folate deficiency will respond to folate supplementation. Often doses as low as 200–500 μg may be sufficient, but higher doses may be necessary in cases of intestinal malabsorption syndromes. Doses of 100 μg/kg have been recommended for children with intestinal disease [140]. Folate may also be added to hyperalimentation solutions.

Cobalamin

Cobalamin, or vitamin B12, is a cofactor in the conversion of homocysteine to methionine and methylmalonyl coenzyme A to succinyl coenzyme A. The former reaction is critical for creation of intracellular polyglutamated tetrahydrofolate, the functional metabolite of folic acid. Hence, cobalamin deficiency impairs DNA synthesis. In addition to megaloblastic anemia, a peripheral neuropathy with degeneration of the lateral and posterior columns of the spinal cord develops. Cobalamin is present only in foods of animal origin. A complex series of reactions leads to the absorption and distribution of cobalamin in humans. Intrinsic factor, produced in saliva and gastric juice, combines in the gastrointestinal tract with cobalamin and facilitates binding to a specific receptor in the ileum. The complex is then absorbed,

metabolized, and transported through the blood by transcobalamin II.

In adults, most cases of cobalamin deficiency are caused by pernicious anemia (i.e. an absence or defect in intrinsic factor) or as a result of a strict vegan diet. Deficiency in pregnancy is rare, as pernicious anemia is usually a disorder of older adults and cobalamin deficiency causes infertility [133]. In infants, congenital disorders of transport proteins such as transcobalamine II or of cobalamine metabolism are more common than nutritional disorders.

Adult stores of cobalamin total approximately 3000 μg [133]. Since the daily cobalamine requirement is 3 μg/day, it should take years for a cobalamin-sufficient individual to become depleted. There is preferential transport of cobalamin from the mother to the fetus. While this causes a decline in maternal serum cobalamine levels during pregnancy, the fetal cobalamine content of 50 μg is but a small fraction of the usual maternal stores. Neonatal serum cobalamin levels are higher than maternal levels [133] but fall over the first six weeks of age [139]. Maternal cobalamin levels correlate with neonatal levels in infants of mothers with omnivorous diets [139]. Vitamin B12 deficiency is rare in infants, but cases caused by maternal deficiency have been reported in breast-feeding infants of strict vegans or mothers with pernicious anemia [134, 141].

Diagnosis of cobalamin deficiency may be made by documentation of a low serum vitamin B12 level, although this does not explain the etiology of the deficiency. Significant cobalamin deficiency results in macrocytic anemia, often accompanied by thrombocytopenia and/or neutropenia. The peripheral blood smear may reveal macrocytes and hypersegmented neutrophils. Bone-marrow findings of megaloblastic hematopoiesis may precede the presence of anemia. Nonspecific findings of cobalamin deficiency include elevation of the serum lactate dehydrogenase (LDH), hyperbilirubinemia, and an elevated transferrin saturation ratio. Evidence of functional cobalamin deficiency, such as increased plasma levels of methylmalonic acid or total homocysteine, may precede anemia [140].

Treatment

The treatment of the infant with cobalamin deficiency depends in part on the degree of anemia. Severe anemia should be treated with slow transfusion, as rapid response to cobalamin is unlikely. Rapid transfusion in the severely anemic child may precipitate or aggravate congestive heart failure. Low initial doses, e.g. 0.2 μg/kg, of cyanocobalamin may be given subcutaneously [140]. Serum potassium levels should be monitored carefully, and potassium treatment initiated if indicated, as hypokalemia may develop, especially if large doses of cobalamin are administered. Unless there is documentation of a nutritional etiology for the cobalamin deficiency, the infant should be studied for a defect in cobalamine absorption, transport, or metabolism [140].

Intrauterine infection

On occasion, the fetus can acquire bacterial, viral, or protozoal infections transplacentally from the mother (Table 2.2). This section will focus on the diagnosis and hematologic manifestations of perinatal infections. Anemia, thrombocytopenia, leukocyte abnormalities, and coagulation abnormalities are common in the fetus with intrauterine infection. As it is not yet possible to rapidly differentiate viral from bacterial infection in the newborn, the initial therapy for most of these patients will usually include empirical antibiotics. Unless specifically stated, the management of the hematologic problems found in these infants is outlined in other chapters. The reader is referred to other references, such as the *Red Book: 2003 Report of the Committee on Infectious Diseases* from the American Academy of Pediatrics, for specific recommendations regarding the treatment of infants with congenital infections [142].

Toxoplasmosis

Toxoplasma gondii is a protozoal feline parasite that can infect humans and other animals. In almost all cases of fetal infection, the susceptible mother has

Table 2.2 Intrauterine infections

Protozoal
Toxoplasmosis
Bacterial
Syphilis
Viral
Cytomegalovirus
Enterovirus
Herpes simplex
Human immunodeficiency virus
Parvovirus B19
Rubella

experienced a primary infection that goes unnoticed. Immunocompromised mothers can transmit reactivated disease to the fetus. Maternal primary infection late in pregnancy increases the likelihood of fetal infection, but the consequences of late-gestation fetal infection are fewer [143]. Antibiotic treatment of the mother decreases the likelihood of fetal infection [144]. Toxoplasma infection in the newborn has been reviewed [144, 145].

The frequency of seropositivity in pregnant women varies from 22% and 32% in New York and London, respectively, to 84% in Paris, where ingestion of uncooked meat is believed to increase the infection rate [144]. In France, from 1970–1980 the prevalence of congenital infection was estimated to be 1.9 to 3.2 per 1000 newborns. In contrast, congenital toxoplasmosis was found by neonatal screening in 52 of 635 000 infants in two US states [146].

Clinical manifestations in symptomatic patients include seizures, hydrocephalus, chorioretinitis, fever, hepatomegaly, splenomegaly, and jaundice. Neonatal disease can be generalized or limited to the CNS. Generalized disease appears to be limited to those infants infected in the first two trimesters. The majority of infants with congenital toxoplasmosis have asymptomatic or subclinical disease [144, 147].

Diagnosis

The diagnosis of congenital toxoplasmosis can be based on demonstration of the organism, serologic studies, or antigen testing. Histologic demonstration and/or tissue culture of the organism can be accomplished on several tissues or fluids, including the placenta and amniotic fluid [144]. Serologic tests include the Sabin–Feldman dye test, indirect hemagglutination, the indirect fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA) tests, and others. The sensitivity and availability of these assays vary significantly between laboratories. There are several issues to consider when using humoral responses to diagnose congenital toxoplasmosis. Specific IgG in neonatal serum can be acquired transplacentally from the mother; hence, in the first few months of life, a single positive test for IgG does not confirm neonatal infection. Persistent or rising IgG titers in the infant are considered diagnostic but require serial testing over the first several months of life. Immunoglobulin A (IgA) may be more sensitive than IgM [148]. While a positive immunoglobulin M (IgM) or IgA serologic test for *Toxoplasma* in the newborn should be considered diagnostic for congenital toxoplasmosis, the fetus does not begin to make specific antibody until after the fifteenth to twentieth week of gestation. More recently, the polymerase chain reaction (PCR) has been shown to have very high sensitivity and specificity for *Toxoplasma* [143]. PCR testing of amniotic fluid was shown to have a sensitivity of 64% and a specificity of 100% for the prenatal diagnosis of congenital toxoplasmosis [149]. Peripheral blood leukocytes and cerebrospinal fluid (CSF) can also be tested by PCR for *Toxoplasma* [150]. Often, a combination of tests is necessary to confirm infection.

Hematologic manifestations of congenital toxoplasmosis

Anemia has been reported in 4–64% of infants with congenital toxoplasmosis and is present in most symptomatic infants [112, 151, 152]. The incidence of anemia reflects the presence of other manifestations. For instance, 77% of a referral population with symptomatic generalized disease had anemia, as opposed to only 10% of infants identified through serologic screening [144, 152]. The presence of increased nucleated RBCs and reticulocytosis indicate that the

anemia is due to hemolysis [112]. Congenital toxoplasmosis can cause non-immune hydrops [153].

Thrombocytopenia can be seen in symptomatic infection. Alford and colleagues found thrombocytopenia in 10% of prospectively identified infants [152]. Hohlfeld and colleagues found thrombocytopenia in 26% of fetuses with congenital toxoplasmosis studied by fetal-blood sampling [154].

Leukocyte abnormalities have been described in congenital toxoplasmosis. Eighteen percent of infants with generalized disease had eosinophilia [144]. Hohlfeld and colleagues found leukocytosis and eosinophilia in 7% and 9%, respectively, of infected fetuses [143]. Infected infants had lower levels of CD4 lymphocytes and a lower ratio of CD4 to CD8 lymphocytes than uninfected infants of mothers with gestational toxoplasmosis [155].

Syphilis

Congenital syphilis caused by the spirochete *Treponema pallidum* was one of the first recognized perinatal infections. In the USA, risk factors for congenital syphilis include young maternal age, low socioeconomic status, parental drug use, sexual promiscuity, and inadequate prenatal care [156]. There was a transient increase in the incidence of congenital syphilis in the late 1980s and early 1990s in the USA. In the UK, low maternal infection rates were noted in the mid 1990s. Risk factors identified in the UK included being born abroad, nonwhite ethnicity, and living near London [157]. A high incidence has been noted in the newly independent states of the former Soviet Union [158]. High rates of gestational syphilis have also been reported from Bolivia (4.3%) [159], Cameroon (15.9%) [160], and aboriginal Australia (28%) [161].

Clinical manifestations

In endemic areas, the incidence of maternal syphilis infection in mothers of stillbirths is significantly greater than in mothers of live births. Syphilis is a cause of stillbirth, non-immune hydrops [153] and intrauterine growth retardation. Many organs can be involved with congenital syphilis. Mucoid rhinitis (snuffles) can be profuse and, occasionally, bloody. Other features include a maculopapular skin rash, nephrotic syndrome, pneumonitis, jaundice, lymphadenopathy, hepatomegaly, splenomegaly, and other skin rashes. X-rays may reveal osteochondritis, osteomyelitis, and/or periostitis of the long bones. Congenital syphilis has been reviewed [156, 162].

Diagnosis

The diagnosis of congenital syphilis may require a high index of suspicion in the absence of obvious clinical stigmata of the disease. Maternal infection is often asymptomatic. Even if the characteristic chancres appear, they may be non-painful and hidden from sight. Maternal screening serologies performed early in pregnancy will miss infection later in gestation. Placental abnormalities, such as the presence of plasma cells, may suggest the diagnosis of maternal infection. *The Red Book: 2003 Report of the Committee on Infectious Diseases* from the American Academy of Pediatrics notes that no newborn should be discharged from the hospital without determination of the mother's serologic status for syphilis and that testing of cord blood or infant sera is inadequate for screening [163].

Diagnostic tests for the infant include X-ray examinations, tests for the organism, serologic tests, and, recently, PCR assays. Non-treponemal tests have long been used as screening tests for syphilis. The venereal disease research lab (VDRL) and rapid plasma regain (RPR) identify antibody against cardiolipin. The RPR may be the more sensitive test for serum, but only the VDRL is recommended for detection in the CSF [156]. If the infant's serum antibody titer is higher than the mother's, a presumptive diagnosis of congenital infection is made, but it is important to use the same assay performed in the same laboratory. Problems with non-treponemal assays include a relatively high false-negative rate in early, latent, and tertiary syphilis, a potential for a false-negative reaction with very high titers of antibody, and false-positive results caused by autoimmune

disorders, other infections, hepatitis, and lymphoma. Wharton jelly contamination can cause a false-positive non-treponemal antibody test from cord blood [163]. Testing cord-blood sera may be less sensitive than testing neonatal serum at two to three days of age [164]. Non-treponemal antibody titers decrease over time with successful treatment [163].

Specific treponemal antibody tests are also used; these include the fluorescent treponemal antibody absorption test (FTA-ABS) and the microhemagglutination *T. pallidum* (MHA-TP) test. These tests have a sensitivity of 90% in early syphilis and 100% for later diagnosis [156]. Because these tests usually remain positive, even in treated patients, the presence of a positive test does not prove active disease and thus cannot be used to monitor response to therapy. Unless the disease is early, a negative test excludes the diagnosis. Because false-positive tests can be caused by infection with non-pallidum treponemes, treponemal antibody tests should be combined with a non-treponemal test.

Several tests for the organism or antigen have been used. Organisms can be seen on darkfield microscopy of placenta and discharge from snuffles and skin lesions. In the past, the rabbit infectivity test was the gold standard for identification of the organism, although this is a technically difficult test. Use of the PCR for identification of *T. palladum* DNA has compared well to the rabbit infectivity test [156].

Hematologic features of congenital syphilis

Whitaker and colleagues [165] described nine infants with congenital syphilis. Eight had anemia. They observed that infants with the largest spleens had the most severe anemia, but splenectomy failed to resolve the anemia in one of these infants. Infants presenting in the first week of life have typical features of hemolysis, i.e. hyperbilirubinemia, increased reticulocyte counts, and peripheral blood-smear findings of polychromasia and increased numbers of nucleated RBCs. The hemolysis appeared to be most rapid in the first week of life. Hemoglobin levels generally became normal after three months of age. While hyperbilirubinemia is common, most infants have elevation of both the direct and indirect fractions of bilirubin.

Thrombocytopenia was found in 28% of South African infants with congenital syphilis [166]. Whitaker and colleagues [165] documented thrombocytopenia in five of seven infants in whom platelet counts were performed. Platelet counts as low as 17 000–20 000/μl were seen in both series. Neutrophilia with a left shift in the differential count was seen in 33% of Whitaker's series. One infant had both vacuolization of the granulocytes and 5% peripheral blast forms that resolved in the first week of life [165]. Hemophagocytosis has also been described [167].

Congenital viral infections

Cytomegalovirus

Approximately 1% (range 0.2–2.2%) of newborns are infected by cytomegalovirus (CMV) [168–170]. Most symptomatic intrauterine infections occur with primary infection in the mother; however, congenital infection occasionally may occur in infants of seropositive mothers [171]. Maternal seropositivity rates vary with location and socioeconomic status. Females in developing countries or of lower socioeconomic status are more likely to become infected with CMV early in life and to be seropositive before pregnancy [169]. Despite this lower susceptibility rate for primary infection, women of low socioeconomic status are just as likely to have a primary infection during pregnancy [170]. Stagno and colleagues found that 52% of infants born to mothers with primary CMV contracted the infection as opposed to <1% of infants born to mothers who were seropositive before pregnancy [170].

Infants can also be infected during delivery from exposure to maternal secretions, and postnatally from breast milk of infected mothers, via blood transfusion, or from exposure to infected individuals [172]. The symptoms of postnatally acquired CMV can be similar to those of congenital CMV [170].

At birth, the majority of infants infected with CMV as fetuses are asymptomatic. In a study of 197 infants

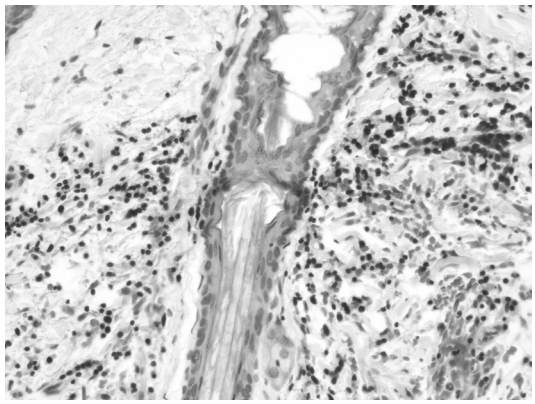

Fig. 2.1 Dermal hematopoiesis in a newborn. Courtesy of Dr Evan Farmer, with permission.

with congenital CMV, only 18% of infants showed signs of the disorder, and all of these were born to mothers with primary infection [173]. Symptomatic congenital CMV infection has been described in infants of mothers who were seropositive for CMV before pregnancy [174]. Babies infected in utero with CMV may be born prematurely or have intrauterine growth retardation. Approximately 10% of infants with congenital CMV have profound involvement, with findings that include petechiae, hepatomegaly, splenomegaly, and jaundice [175]. Neurologic problems are often prominent and include microcephaly, lethargy, poor suck, and seizures [173, 174]. Intracerebral calcifications are a sequel of congenital infection. Ophthalmologic findings include chorioretinitis, optic neuritis, cataracts, and colobomas. Multiple other organs, including the kidneys, liver, gastrointestinal tract, lungs, thyroid, and pituitary, can be affected [170]. Infants may present with the classic "blueberry-muffin rash" due to dermal hematopoiesis (Fig. 2.1) [176]. Survival and/or long-term outcome of these infants is very poor.

Most infants with congenital CMV infection are asymptomatic at birth, but sequelae may appear in later life. Approximately 15% of infants with congenital CMV infection who are asymptomatic at birth develop significant mental retardation or hearing deficit within the first five years of life [177].

Diagnosis

The recent demonstration of symptomatic CMV in infants of seropositive mothers indicates that maternal IgG cannot be used to exclude perinatal CMV infection [171]. Diagnosis in the fetus can be challenging. While ultrasound abnormalities consistent with CMV combined with positive maternal serologies strongly support the diagnosis of intrauterine infection, a normal ultrasound does not exclude either congenital infection or sequelae [178]. Early in gestation, infants are incapable of producing IgM [179]. Furthermore, if the infected fetus does produce specific CMV IgM, then it may disappear before delivery. The most sensitive techniques for prenatal diagnosis are viral culture from amniotic fluid and detection of CMV DNA via PCR, although this may be less sensitive in fetuses under 21 weeks' gestation [179]. Culture of CMV and PCR from amniotic fluid have similar sensitivity, specificity, and positive predictive value [180]. Postnatally, techniques for diagnosing congenital infection include culturing of the virus from blood or urine and detecting CMV antigen in the urine or peripheral blood [181].

Hematologic manifestations of congenital cytomegalovirus

Thrombocytopenia and petechiae are common in symptomatic infants [181]. Hohlfeld and colleagues found that 36% of infants with congenital CMV had thrombocytopenia, and that 38% of these had a platelet count below 50 000/μl [154]. Several observations suggest that there is accelerated platelet destruction with congenital CMV. Consumptive coagulopathy and thrombosis have been noted in infants with congenital CMV [182, 183]. Apparent immune thrombocytopenia following congenital CMV infection has been reported [184]. Hypersplenism may also contribute to the shortened platelet lifespan. However, human CMV infection can also impair megakaryocyte viability [185]. Hence, decreased platelet production may contribute to the thrombocytopenia.

Hemolytic anemia is a common finding in infants with symptomatic CMV [170]. Erythroblastosis, polychromasia, and abnormal RBC morphology may be seen in the peripheral blood of infants with congenital CMV [170, 186]. Contributing factors include RBC membrane damage and hypersplenism [187]. Autoimmune hemolytic anemia is a complication of infantile CMV [188]. CMV has been shown to infect hematopoietic precursors, and hence decreased erythrocyte production may play a role [189].

Leukocyte abnormalities are common. Atypical lymphocytosis can be seen [190]. Prolonged neutropenia following congenital CMV infection has been reported [191].

Rubella

Rubella is a member of the togavirus family. It has a worldwide distribution, but vaccination has dramatically decreased the prevalence of viral infection in the developed world. Maternal infection is usually confirmed serologically [192]. Positive rubella-specific IgM is a good indication of a recent maternal infection, but false-positive tests may be seen with rheumatoid factor. Maternal reinfection in immune-compromised women may occur, but the risk of damage to the fetus from such reinfections is small [193]. Still, rare cases of congenital rubella syndrome have been reported in women who have been vaccinated or who have serologic documentation of immunity before pregnancy [194, 195]. The transmission rate from an infected mother to the fetus is highest in the first and last trimesters; however, the development of fetal embryopathic changes is limited to infection in the first 15 weeks of gestation [193].

The diagnosis in the newborn is often suspected on clinical grounds. The common manifestations of congenital rubella syndrome include intrauterine growth retardation (IUGR), hepatosplenomegaly, congenital heart disease (such as patent ductus arteriosus and peripheral pulmonary artery stenosis), meningoencephalitis, mental retardation, bony lucencies on X-ray, sensorineural hearing loss, interstitial pneumonitis, and ophthalmologic findings, including cataracts, retinopathy, and/or cloudy corneas [192, 196]. These infants may also present with the blueberry-muffin rash of dermal erythropoiesis (Fig. 2.1) [197].

Diagnosis

Viral culture should be attempted from urine and from throat swabs. Viral shedding may persist for months, and virus may be cultured from the CNS of infants with encephalitis [192]. Virus can also be cultured from blood, urine, throat, and nasal specimens [196]. Rubella-specific IgM from the infant is a strong indicator of congenital rubella, but false-positive tests can occur with rheumatic factor or incomplete removal of IgG in the preparation of the specimen [192]. Rubella-specific IgM persists for a longer time in the infant with congenital rubella syndrome [198]. Stable or increasing rubella-specific IgG over the course of several months also indicates congenital infection [196]. PCR has been used to diagnose rubella in the fetus of infected mothers [199].

Hematologic manifestations of congenital rubella

Decreased platelet counts are commonly seen in the congenital rubella syndrome (CRS) [200, 201]. Petechiae and purpura are noted in 29–100% of such infants [202, 203]. Cooper and colleagues reported that 17% of infants with congenital rubella had platelet counts below 20 000/μl [204]. Bone marrow studies from thrombocytopenic infants with congenital rubella showed decreased megakaryopoiesis with a shift to more juvenile megakaryocytes [202, 205]. Splenic sequestration may be a contributing factor. DIC has been reported [183].

Anemia is common and may be present at birth or develop over the first month [202, 204]. There are several features that suggest hemolysis. Peripheral blood smears show abnormal red-cell morphology and increased numbers of normoblasts. The reticulocyte count is increased. The bone marrow usually shows accelerated erythropoiesis with an increased erythroid : myeloid ratio [205]. Decreased RBC survival has been documented [202]. One

report, however, described a transient bone marrow (BM) hypoplasia in an infant with congenital rubella [206].

Leukopenia and leukocytosis have been noted in patients with congenital rubella [202]. Lymphadenopathy has been noted in approximately 20% of patients. Hepatomegaly and/or splenomegaly are common in symptomatic infants [202, 204].

Herpes simplex

Herpes simplex virus (HSV), like most of the herpesvirus family, has the ability to remain latent after primary infection for many years and then re-emerge with symptoms. There are two distinct antigenic types of HSV. HSV 1 usually infects the oral region while HSV 2 typically involves the genital region. Acquisition generally occurs through intimate contact. Seropositivity to HSV 2 parallels the onset of sexual activity. The commercially available serologic tests do not distinguish reliably between HSV 1 and HSV 2. Unless the exposure was recent, a negative serologic test should exclude prior exposure in the mother [207]. PCR of active lesions can be used for diagnosis. Viral culture has been the gold standard but is limited to times of active viral shedding [207]. Most infants with congenital HSV are born to asymptomatic mothers [208]. Factors that increase the risk of neonatal infection include primary maternal infection during gestation, infection late in gestation (preventing the transmission of maternal HSV specific antibody to the fetus), and prolonged rupture of membranes [208].

The incidence of perinatal HSV in the USA is approximately 1 in 3500–5000 deliveries [209]. Infection of the neonate can occur in utero, during delivery, or postnatally. Intrauterine transmission is uncommon but may cause signs and symptoms similar to those of other congenital infections, such as toxoplasmosis and CMV. More commonly, the newborn is exposed to virus from maternal genital fluids during delivery [208]. Perinatal HSV presents in the first month of life, with two-thirds of cases presenting in the first week [210]. HSV 2 is responsible for 70% of neonatal HSV infection [209]. Infection may also occur with exposure to infected caregivers, including mothers, fathers [211], and other providers. Perinatal infections are classified as localized disease to the eyes, mouth, and/or skin, CNS disease with or without involvement of the eye, mouth, and skin, and disseminated disease [208]. Disseminated disease is often fatal and may involve the liver, lungs, heart, CNS, skin, and other organs.

Diagnosis

In the absence of skin vesicles, the diagnosis of disseminated neonatal HSV is clinically indistinguishable from many other congenital infections. Rapid diagnosis can be attempted with direct fluorescent antibody staining of scrapings from viral lesions. The gold standard is viral culture, which may be obtained from the infant's CSF, stool, conjunctivae, urine, and/or oropharynx. Recently, the PCR has been shown to be effective in the diagnosis of CNS HSV in the newborn [212].

Hematologic manifestations of congenital herpes simplex

DIC has been reported repeatedly in congenital HSV infection [183, 211, 213–215]. In 1970, Miller and colleagues [215] reviewed the available literature on fatal neonatal HSV. Abnormal bleeding was noted in 22 of 54 cases and abnormal coagulation studies were reported in seven of ten studied cases. Hepatic disease can contribute to the coagulopathy. The presence of DIC increases the infant's risk of dying from neonatal HSV [210]. Neutropenia may also be present [208].

Enterovirus

The enterovirus family, including coxsackie virus, echovirus, poliovirus, and enterovirus, consists of single-stranded RNA viruses [216]. They are common causes of illness in humans. In the immunocompetent host, infections are generally self-limited and benign. However, serious sequelae, such as paralytic

polio, may occur. Enterovirus is most prevalent in the summer and the fall.

While most cases of enteroviral infection in the newborn are self-limited, serious infection may occur [217]. Infection can occur through transplacental passage, contact with the virus in the passage through the birth canal, and postnatal exposure. Several nursery epidemics have been reported with different enteroviruses [216].

Symptoms of neonatal enterovirus infection vary widely. An extensive review of these potential symptoms by organ system and by virus has been written [216]. Abzug *et al.* [218] identified 29 infants with culture-proven enterovirus in the first 14 days of life. Fever, irritability, lethargy, and anorexia were noted in over half, while decreased perfusion, respiratory abnormalities, jaundice, and rash were also common. CNS involvement was found in 53% of the infants in whom CSF cultures were obtained. Severe multisystem disease, including hepatitis, coagulopathy, meningitis, and pneumonitis, may occur. These infants were more likely to present early in life. While comparative data are lacking, echovirus 11 has been reported frequently to present with severe disease [216].

Diagnosis

Virus isolation has been the gold standard for diagnosis of enteroviral infections. Proper handling of the specimen is critical. It is recommended that specimens be taken from multiple sites, including CSF, blood, urine, and other body fluids. Swabs from mucosal surfaces need to be sent in transport medium [216]. The large number of potential serotypes makes antibody detection impractical. Recently, PCR studies on serum and CSF of newborns infected with enterovirus have been shown to be more sensitive and with a shorter turnaround time than viral culture [219].

Hematologic manifestations of congenital enterovirus

DIC is a common feature of enteroviral hepatitis with multiorgan involvement. In a series of patients who, by definition, had hepatitis and coagulopathy, thrombocytopenia and increased partial thromboplastin and thrombin times were seen in 100%, and prolonged prothrombin times, elevated fibrin split products, and decreased fibrinogen concentrations were seen in over 85%. Two-thirds had anemia and 60% had peripheral leukocytosis [220]. In another series of infants diagnosed with enterovirus in the first 14 days of life but not limited to those with hepatitis and coagulopathy, 17% were noted to have thrombocytopenia [218]. Bleeding is often a cause of death in infants with enterovirus hepatitis and coagulopathy [221]. Intraventricular hemorrhage may result [222]. Leukocytosis, neutrophilia, and increased numbers of band forms are often noted [217].

Treatment

From the hematologic perspective, supportive care consists of platelet, RBC and plasma transfusion. The management of DIC is outlined in Chapter 13.

Human parvovirus B19

Human parvovirus B19 is a small DNA nonenveloped virus that propagates in the human erythrocyte precursor. It enters the cell through the P antigen [223], hence individuals whose RBCs lack the P antigen are not susceptible to infection. The common childhood illness erythema infectiosum, also known as fifth disease, is caused by parvovirus B19.

Parvovirus B19 is transmitted mainly by respiratory droplets, although percutaneous exposure to blood or blood products and vertical maternal-fetal transmission have also been documented [224]. Infection is often asymptomatic. Common symptoms include fever, rash, and arthralgia. Symptoms of the joints may be difficult to distinguish from rheumatoid arthritis, especially in adults. In rare instances, vasculitis, myocarditis, and neurologic disease have been reported [225]. In immunocompromised individuals, pancytopenia or chronic anemia may result.

In immunocompetent individuals, acute parvovirus B19 infection inhibits erythropoiesis for several days. Because the usual erythrocyte lifespan is 120 days, this should not cause symptomatic anemia. However, in individuals with hemolytic disorders, e.g. sickle cell disease or hereditary spherocytosis, severe anemia may result.

Susceptibility rates in pregnant women range from 35% to 81% [226–228]. In England, the risk of acquiring parvovirus during pregnancy is estimated to be 1 in 400 [229]. Despite multiple case reports of non-immune hydrops and other complications, most prospective studies have shown a low rate of complications in the offspring of mothers with primary parvovirus B19 infection during pregnancy. For instance, Rodis found 37 of 39 infants of infected mothers were healthy, with no cases of hydrops [230]. In Spain, a prospective study identified 1 of 60 infants with perinatal parvovirus infection to have parvovirus-related fetal loss [227]. In contast, Miller and colleagues [231] described an excess rate of fetal loss of 9% for women infected with parvovirus in the first 20 weeks of pregnancy. In this group, there were seven cases of fetal hydrops, all in weeks 11–18 of gestation. There was no increased risk of adverse outcomes for women infected after the twentieth week. Thus, the likelihood of a fetus developing non-immune hydrops as a result of maternal parvovirus infection is small, especially after 20 weeks of gestation. There do not appear to be adverse long-term effects in surviving infants [231, 232].

Diagnosis

Diagnosis of parvovirus B19 infection can be made serologically through a four-fold increase in IgG titers or by the presence of parvovirus-specific IgM. Parvovirus-specific IgM is available commercially and has a sensitivity of 90–97% with a specificity of 88–96% in adults [233]. Alternatively, PCR can be used to identify parvovirus DNA [233]. IgM may be absent, especially if the infant is infected early in gestation. Koch and colleagues [234] studied infants from 43 women with primary parvovirus B19 infection during pregnancy. Using a combination of IgG, IgM, IgA, and PCR, they identified 22 infected infants. Of these, 10 were positive for IgA, 11 were positive for IgM, and 11 were positive by PCR. Only 22% of the infants infected in the first trimester were positive for IgM. Giant pronormoblasts may be identified in the marrow of individuals infected with parvovirus B19 [235].

Hematologic manifestations of congenital parvovirus B19 infection

While it is uncommon, fetal hydrops has been reported as a complication of maternal infection with parvovirus B19 [236–239]. Fetal demise may occur up to 14 weeks after infection. The management of non-immune hydrops in such fetuses is unclear. While intrauterine transfusions have been utilized to treat affected fetuses [240]; spontaneous resolution has also been noted [241, 242]. In a retrospective study, Fairley and colleagues found a statistical survival advantage for infants with B19-induced hydrops fetalis treated with intrauterine transfusion [243]. There are no prospective controlled trials to answer this question. Forestier and colleagues [244] found that 11 of 13 infants with hydrops secondary to parvovirus infection were thrombocytopenic, two having platelet counts below 50 000/μl.

The long-term outlook, however, is quite good. In a long-term follow-up study of 129 congenitally infected infants, Miller and colleagues found two instances of transient iron deficiency, one case of transient idiopathic thrombocytopenic purpura, and one case of transient eosinophilia [231]. While there was not a control group, there did not appear to be an increase in non-hematologic long-term issues. Long-term transfusion-dependent anemia has been described in three infants from whom parvovirus DNA was recovered from the bone marrow [245].

Treatment

Red blood cell transfusions may be necessary in the newborn with parvovirus-induced anemia. Chronic hypoproliferative anemia may respond to intravenous gammaglobulin [246].

Human immunodeficiency virus

Despite intensive research, education, and the development of new effective therapies, human immunodeficiency virus (HIV) infection remains a problem of massive worldwide proportions. Perinatal transmission rates from infected mothers range from 13% to 40% and appear to be higher in Africa than in Europe or the USA [247]. The transmission rate can be decreased by over 50% with maternal zidovudine treatment.

Hematologic manifestations of human immunodeficiency virus

HIV infection affects virtually every aspect of hematology in the infected host. While hepatomegaly and/or splenomegaly may be present, most neonates are asymptomatic and the clinical manifestations occur later in infancy and childhood. The hematologic aspects of HIV in older infants and children have been reviewed elsewhere [248]. The clinical and immunologic effects of congenital HIV infection are discussed in Chapter 12. The majority of infants infected with HIV are asymptomatic, but the current use of antiretroviral therapy in the mother can affect the hematologic status of the infant. In a randomized trial of zidovudine in pregnant HIV-infected women, Connor and colleagues [249] reported lower initial hemoglobin levels in infants of treated mothers, although the mean hemoglobin at birth was still 16 g/dl. The maximal difference between the infants of zidovudine-treated versus untreated mothers was 1 g/dl at three weeks of age. The mean hemoglobin nadir at six weeks of age was 10 g/dl, which was still slightly lower than that in infants of untreated mothers. By 12 weeks of age, both groups were similar, at about 11 g/dl. Sperling and colleagues [250] followed 30 infants whose mothers were treated with zidovudine during pregnancy. The mean hemoglobin at birth was 15.0 ± 2.3 g/dl. Seven infants had anemia, three with hemoglobin values below 12 g/dl. Of these seven, three were under 28 weeks' gestational age. A 28-week gestational age infant (one of twins) had thrombocytopenia but also congenital sepsis and CMV. One infant also exposed to aciclovir and trimethoprim-sulfamethoxazole had neutropenia. Mandelbrot and colleagues [251] evaluated infants of 29 thrombocytopenic women with HIV who received different antiretroviral regimens during pregnancy. Of 28 infants, only two had mildly reduced platelet counts. One had concomitant group B streptococcus (GBS) sepsis and a platelet count above 100 000/μl while the other had a platelet count of 74 000/μl. Combined antiretroviral therapy may have a greater effect on neonatal hematopoiesis. Postnatally zidovudine treatment in the infant can cause anemia and neutropenia [252].

Management

The general and antiviral management of the infant with congenital HIV is complex and beyond the scope of this chapter; it is discussed in Chapter 12. A bone-marrow examination should be considered in HIV-infected infants with depressed hematopoiesis to rule out marrow invasion or infection by opportunistic organisms.

Erythropoietin may help increase hemoglobin in children treated with zidovudine. Iron supplementation of 6 mg/kg/day of elemental iron is usually necessary to see an optimal effect from erythropoietin. Red blood cell transfusion may be necessary occasionally for severe or symptomatic anemia. Anemia due to chronic infection may be ameliorated with appropriate antimicrobial therapy. Intravenous gamma globulin has been beneficial for HIV-infected patients with chronic human parvovirus B19 infection [246]. Relatively low doses of granulocyte colony-stimulating factor can increase the neutrophil count to normal in children with HIV-induced neutropenia (253).

Severe thrombocytopenia can cause clinical bleeding. Microangiopathic changes on the peripheral blood smear suggest the diagnosis of hemolytic uremic syndrome/thrombotic thrombocytopenic purpura or DIC. The management of DIC begins with appropriate therapy for the underlying cause.

Platelet and/or plasma transfusion may be necessary to treat severe bleeding. The majority of cases of thrombocytopenia in patients with HIV are immune in nature. Intravenous gamma globulin, intravenous anti-D, and corticosteroids have all been shown to improve the platelet count in thrombocytopenic children with HIV [248].

Epstein–Barr virus

Goldberg and colleagues [254] described a term infant with multiple congenital anomalies, including hypotonia, micronathia, cataracts, cryptorchidism, and metaphyseal bone changes. Over the next 20 months, the infant had hypotonia, lymphadenopathy, and hepatosplenomegaly. The hematologic picture at birth revealed thrombocytopenia and a large number of atypical lymphocytes. The infant had serologic and viral culture evidence for perinatal Epstein–Barr virus (EBV) infection. Another infant with hepatosplenomegaly, petechiae, thrombocytopenia, and intraventricular calcifications had seroconversion to both CMV and EBV in the first three months of life [255]. Horwitz and colleagues [256] described an infant with recurrent emesis, diarrhea, and failure to thrive. The infant died on day 57, despite aggressive therapy. Serologic studies suggested acute EBV infection, and DNA hybridization studies demonstrated EBV in the lymphocytes. Fleisher and Bologonese followed three women with primary EBV in the first trimester of pregnancy. All three products of these pregnancies were normal without physical abnormalities or serologic evidence of intrauterine infection [257]. Despite these case reports, one of which may have been attributed to congenital CMV, the incidence of perinatal EBV appears to be very low. The susceptibility rate for EBV is low. Le and colleagues [258] found only 58 of 1729 pregnant women to be susceptible to primary EBV infection, and none of these developed infection during pregnancy. Fleisher identified three seroconversions to EBV in 4063 pregnant women [259].

There were no readily identified hematologic effects in these infants. Two infants had thrombocytopenia: one had classic pathologic and clinical features of congenital CMV [255] while the other infant's mother had significant hypertension [254]. None of the three infants described in Fleisher's series had petechiae, jaundice, or hepatosplenomegaly, but CBC results were not noted [248].

Summary

Many maternal conditions have profound effects on the hematologic status of their newborns. As put succinctly by Dr Frank Oski: "It is apparent that disease in the newborn infant can only be interpreted after careful questioning and study of the mother. Apparently insoluble diagnostic problems often become clear after a few well spent minutes with the mother" [260].

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3

Erythropoiesis, red cells, and the approach to anemia

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Neonatal erythropoiesis

Neonatal erythropoiesis differs significantly from that in older children and adults. The birthing process with the rapid changes in oxygen concentration precipitates drastic changes in the newborn's erythroid system. To understand neonatal erythropoiesis, one needs to understand the ontogeny of erythropoiesis, from the embryo through the fetus to the newborn.

The current hypothesis of hematopoiesis is that there is a pleuripotent hematopoietic stem cell that gives rise to all hematopoietic lineages (Fig. 3.1). The ability of bone-marrow cells to reconstitute the hematopoietic system of lethally irradiated mice documented the existence of the stem cell [1]. In vitro clonogenic culture assays of bone-marrow cells documented the existence of a cascade of pluripotent and committed progenitor cells [2–6]. The commitment of the stem cell to differentiation may be a stochastic (random) or deterministic event, or a combination of both [7]. As stem cells differentiate, they lose their ability for self-renewal. Proliferation, differentiation, and survival of erythroid progenitors are dependent on the hormone erythropoietin [8]. The cascade of differentiation from the stem cell proceeds through a multipotent progenitor cell identified in vitro as colony-forming unit-granulocyte erythroid macrophage megakaryocyte (CFU-GEMM). The first recognizable pure erythroid progenitor is the burst-forming unit-erythroid (BFU-E), which then matures into the colony-forming unit-erythroid (CFU-E). The hormone erythropoietin is necessary for terminal maturation of the CFU-E. It also has an antiapoptotic effect on progenitor cells. Early progenitors depend on several cytokine mixtures for their proliferation and maturation. In utero hematopoiesis is primarily erythroid. The blood and bone marrow of fetuses and newborns is rich in stem cells as well as erythroid progenitors [9]. Their response to erythropoietin is normal [10]. However, at different stages of embryonic and fetal development, erythroid cells behave differently. As we will discuss in this chapter, embryonic erythropoiesis gives rise to large nucleated red cells endowed with embryonic hemoglobin. Through gestation, erythropoiesis switches from this primitive erythropoiesis to definitive or mature erythropoiesis with smaller, anuclear cells that contain adult-type hemoglobins.

The ability to isolate progenitor cells and identify single cells in embryonic tissue has facilitated the understanding of the ontogeny of hematopoiesis. Avian and murine hematopoiesis have been studied extensively and have been reviewed [11–17]. In humans, these elegant studies have not been carried out and our understanding comes from the excellent histological studies of Kelemen, Fukuda, Emura, Zamboni, and Gilmour 16a [18, 19–24].

There are three phases of hematopoiesis: mesoblastic, hepatic, and myeloid. In the human, at about 16 days of gestation (day 7 in the mouse), blood islands appear in the yolk-sac membrane [13]. This primitive hematopoiesis consists of pale cells known as hemocytoblasts. Each of these cells has a nucleus with wrinkled contours and homogeneous

chromatin and a high nuclear-to-cytoplasmic ratio. The majority of these cells differentiate into primitive nucleated erythrocytes [22]. The simultaneous development of blood cells and endothelial cells in mouse embryos has led to the hypothesis that both come from the same cell or hemangioblast. Both embryonic stem-cell studies and avian hematopoiesis studies have lent support to this hypothesis [25]. In human electron-microscopic studies, Takashina demonstrated that blood cells arise in the yolk-sac endoderm [26]. Unlike the murine yolk sac that functions throughout gestation, the human yolk sac begins regression by the tenth week of gestation and ceases function by the sixteenth week. As the yolk sac regresses, the vitellin blood system joins with the allantoic vascular system and portal system, and fetal circulation is established. Hepatic hematopoiesis begins during the second month of gestation (about the fifth week) and, like primitive erythropoiesis, is predominantly erythropoietic [24, 27]. The matter of which cells establish hepatic and definitive erythropoiesis is an area of controversy [17]. In the mouse, hemocytoblast and hematopoietic stem cells circulate and seed the liver [13]. These primitive erythroid progenitors (EryP-CFC) produce nucleated erythroblasts that are much larger than enucleated primitive erythroblasts or definitive erythroblasts (about 465–530 fl) [13]. Studies by Moore and Metcalf documented that the yolk sac was capable of seeding hepatic hematopoiesis but that the liver was incapable of developing its own hematopoiesis, establishing the extraembryonic origin of hematopoiesis [28]. Avian and murine studies have suggested an alternative hypothesis. Hematopoietic stem cells have been isolated from the aortic-gonadal-mesonephric (AGM) region [29]. It is unclear whether hematopoiesis begins de novo in this area or is seeded from the yolk sac. Initial studies of hematopoietic reconstitution suggested that only the AGM-region stem cells were able to reconstitute definitive hematopoiesis, but the development of a neonatal model of hematopoietic reconstitution has documented the ability of stem cells from both the AGM region and the yolk sac

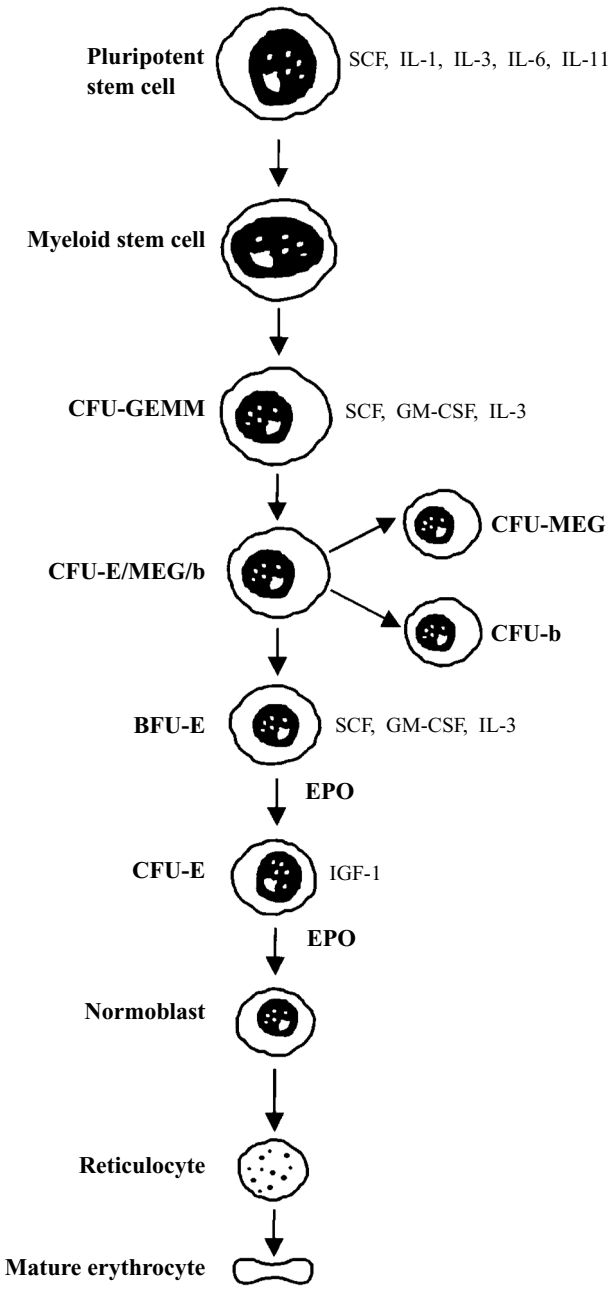

Fig. 3.1 Erythropoietic development and the growth factors that influence erythropoiesis. Reproduced with permission from Fisher [8]. BFU, burst-forming unit; CFU, colony-forming unit; E, erythroid; EPO, erythropoietin.

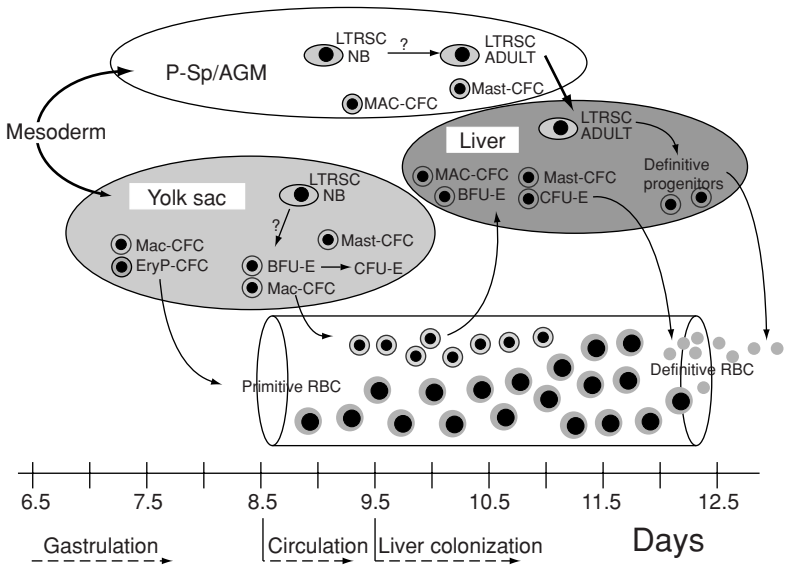

Fig. 3.2 Model of early hematopoietic ontogeny in the mouse embryo. BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; EryP-CFC, primitive erythroid progenitors; LTRSC ADULT, long-term adult-repopulating stem cells; LTRSC NB, long-term newborn-repopulating stem cells; Mac-CFC, macrophage progenitors; Mast-CFC, mast-cell progenitors; P-Sp/AGM, para-aortic splanchnopleura/aorta-gonad-mesonephros region; RBC, red blood cells. Reproduced with permission from Palis *et al.* [11].

to reconstitute definitive hematopoiesis [30, 31]. Palis and colleagues have suggested a hypothesis of the ontogeny of hematopoiesis based on mouse and avian experiments (Fig. 3.2) [11]. Yolk-sac hematopoietic cells seed the liver and provide primitive hematopoiesis. They also may seed the AGM region, or the AGM may develop its own endogenous stem cells that then seed the liver with definitive hematopoiesis. Whether yolk-sac hematopoiesis in the microenvironment of the fetal liver transforms into definitive hematopoiesis or is replaced by AGM-region hematopoiesis is still a matter of debate. In the human, the third phase of hematopoiesis begins in utero. Bone-marrow hematopoiesis begins around the thirteenth week of gestation to become the predominant hematopoietic organ in the last trimester of gestation. Concomitant with the changes of erythropoiesis from yolk-sac (primitive) to hepatic and then to myeloid erythropoiesis, red cells switch hemoglobin content as well as the type of hemoglobin. Synthesis of embryonic hemoglobins characterizes yolk-sac erythropoiesis; fetal hemoglobin synthesis predominates during hepatic erythropoiesis; and adult hemoglobin characterizes definitive erythropoiesis (Figs. 3.3, 3.5 and Table 3.1).

Although in utero hematopoiesis is predominantly erythropoietic, macrophages, megakaryocytes, and platelets have been identified in the yolk sac [13, 22]. Progenitor cells of all lineages can be grown from yolk-sac cells [28].

The erythrocyte

Ninety-five percent of the protein in the erythrocyte is hemoglobin. The remaining proteins protect and sustain viability of the erythrocyte. Maintaining the viability of the erythrocyte is critical, as free hemoglobin is catabolized and excreted renally within minutes. Therefore, the function of the red blood cell is to protect hemoglobin and the function of hemoglobin is to transport oxygen from the lungs to the tissues and to facilitate the return of carbon dioxide.

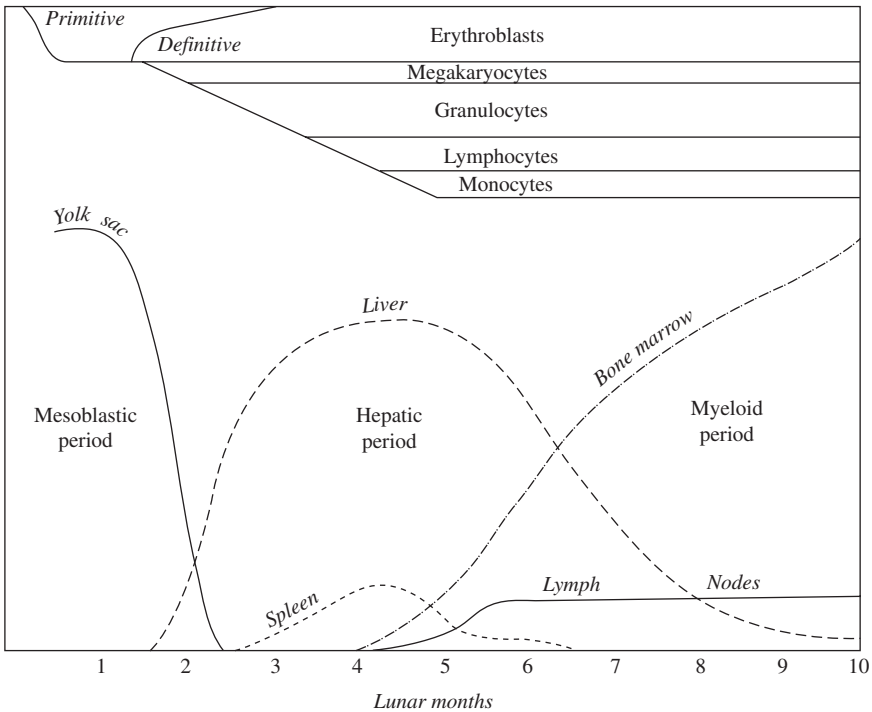

Fig. 3.3 The stages of hematopoiesis in the developing embryo and fetus, indicating the time of appearance and the comparative participation of the chief centers of hematopoiesis. Reproduced with permission from Stockman, J. A. III and de Alarcón, P. A. Hematopoiesis and granulopoiesis. In Polin, R. A. and Fox, W. W., eds. *Fetal and Neonatal Physiology*. W. B. Saunders Co.

Hemoglobin

Each 100 ml of red blood cells contains 32–36 g of hemoglobin. Each gram of hemoglobin has the potential to hold 1.34 ml of O_2 [33]. Hemoglobin is a tetramer of two pairs of, usually unlike, globin polypeptide chains, each associated with a heme group. Developmentally, there are embryonic, fetal, and adult hemoglobins (Table 3.1). There are six known normal globins in humans: alpha (α), beta (β), epsilon (ε), delta (δ), gamma (γ), and theta (ζ). The α-like globins are α and ζ, and their encoding genes are found on chromosome 16. The β-like globins are β, ε, δ, and γ, and their encoding genes are found on chromosome 11 (Fig. 3.4). Hemoglobin synthesis proceeds in an organized fashion from 5′ to 3′ in a process referred to as "hemoglobin switching" (Fig. 3.5). The first globin produced is the ε chain, which when formed into tetramers and combined with heme, forms hemoglobin (Hb) Gower 1. This is followed by production of α and ζ chains, forming Hb Gower 2 $\alpha_2\varepsilon_2$ and more Gower 1 in the $\zeta_2\varepsilon_2$ form. Early γ globin results in the presence of Hb Portland $\zeta_2\gamma_2$ and fetal hemoglobin $\alpha_2\gamma_2$. With the appearance of small quantities of the β chain at six

Table 3.1 Globin-chain development

Stage	Hemoglobin	Composition
Embryo	Gower 1	ε_4 or $\zeta_2\varepsilon_2$
Embryo	Gower 2	$\alpha_2\varepsilon_2$
Embryo	Portland	$\zeta_2\gamma_2$
Embryo	Fetal	$\zeta_2\gamma_2$
Fetus	Fetal	$\zeta_2\gamma_2$
Fetus	A	$\alpha_2\beta_2$
Adult	A	$\alpha_2\beta_2$
Adult	A2	$\alpha_2\delta_2$
Adult	F	$\alpha_2\gamma_2$

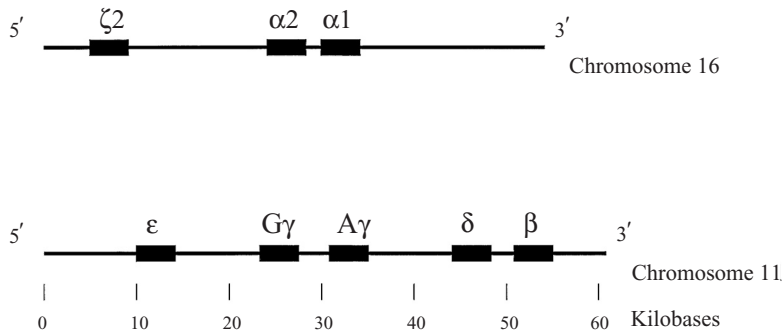

Fig. 3.4

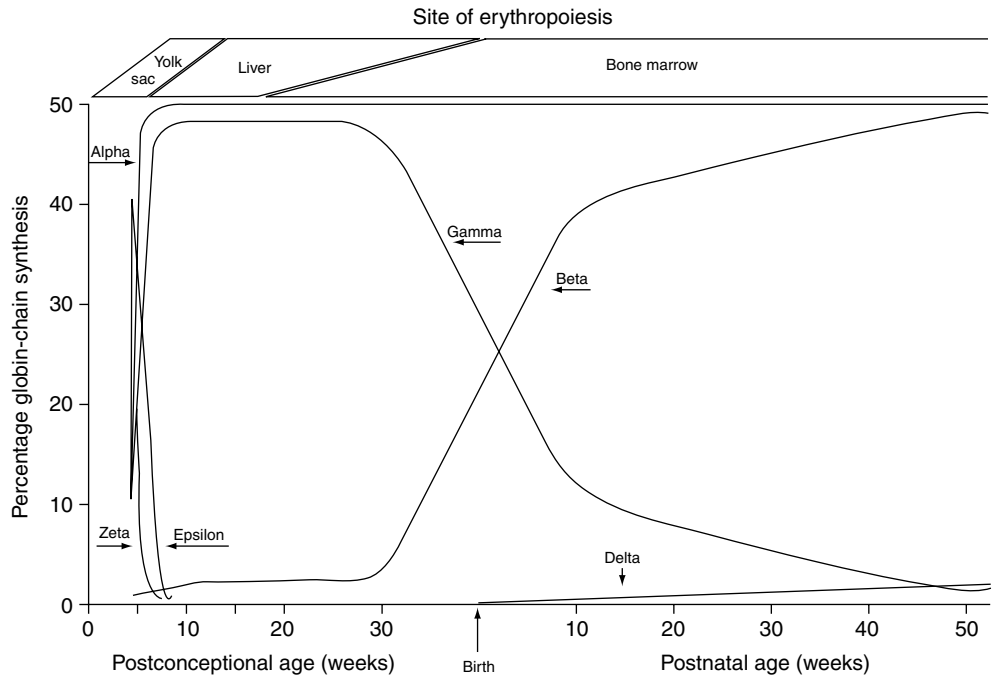

Fig. 3.5 Hemoglobin switching. Changes in globin subunits during human development from embryo to early infancy. The top of the figure represents the changes in sites of erythropoiesis. Reproduced with permission from Stockman & Pochedly [32].

to eight weeks' gestation, small quantities of Hb A appear (Fig. 3.5). At about 37 days' gestation, Gower 1 and Gower 2 constitute 42% and 24%, respectively, of the total hemoglobin, with fetal Hb representing the remainder. By 12 weeks' gestation, Hb F accounts for the majority of hemoglobin. Over the following 22 weeks, it gradually decreases from 100% to 85% by 34 weeks' gestation. A parallel increase in Hb A occurs throughout the decrease in Hb F. Hb A_2 is present in trace amounts at birth and increases over the first year of life to a maximum of about 2.5%. Hemoglobin switching facilitates oxygen delivery [34].

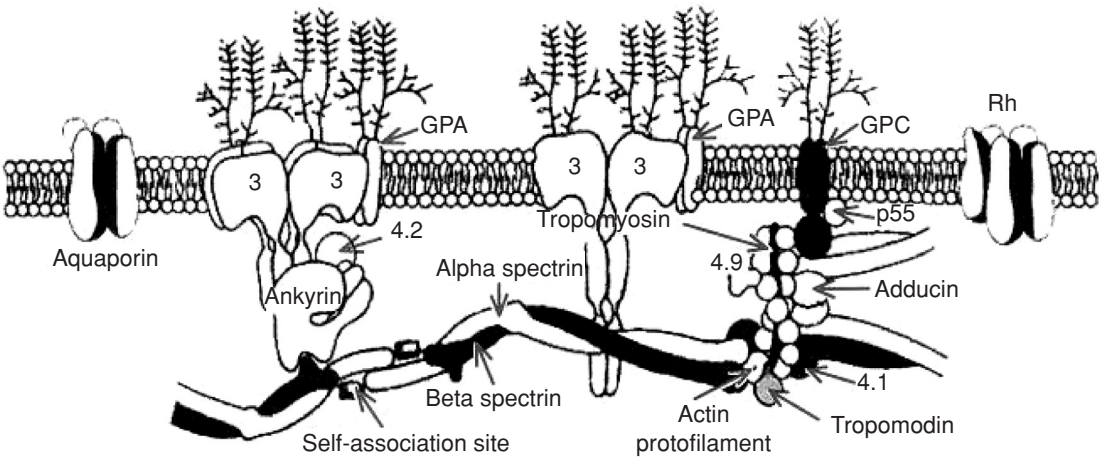

Fig. 3.6 Schematic diagram of the erythrocyte cell-membrane organization. Reproduced with permission from Hoffman [42].

Erythrocyte cell membrane

The erythrocyte membrane is only 1% of the total weight of the red cell (31). Lipids compose 50% of the weight of the erythrocyte membrane. The protein cytoskeleton that maintains the erythrocyte integrity has been well-described and is shown in Fig. 3.6 [32].

Neonatal erythrocyte membranes differ from adult membranes in several ways that make them more resistant to osmotic lysis. At the time of birth, some cells have increased osmotic fragility, but they are selectively destroyed within the first few days.

Newborn erythrocytes are less filterable, especially in the presence of acidosis and hypoxemia [35–37]. They also have lower intracellular and membrane viscosity, with a more tightly linked cytoskeleton [38].

Cord erythrocytes contain increased lipid, lipid phosphorous, and cholesterol per cell [39]. They also have more sphingomyelin and less lecithin [40, 41].

Biochemistry of the red cell

Erythrocytes metabolize glucose using the Embden–Meyerhof pathway. Fetal and newborn erythrocytes consume more glucose and galactose than do those of adults [43, 44]. Galactokinase activity is three times greater in the newborn erythrocyte. Phosphoglycerate kinase and enolase activity is higher, while phosphofructokinase activity is lower in erythrocytes from newborn infants [45, 46]. The activities of these three enzymes approach normal adult values during the first year of life and are apparently independent of red-cell age. The changes in activity of these enzymes reflect development from fetal to adult erythropoiesis [47, 48]. Accelerated decay of unstable phosphofructokinase may account for its decreased activity. This deficiency may alter glucose metabolism and could have functional significance. Adult erythrocytes have lower levels of adenosine triphosphate (ATP) than infant erythrocytes, with the highest ATP levels being found in premature infants [49, 50].

The pentose phosphate pathway in the newborn erythrocyte is more susceptible to oxidant-induced injury. This leads to glutathione instability, Heinz body formation, and the development of methemoglobinemia [51, 52]. Other etiologies of this susceptibility include a decreased number of membrane sulfhydrol groups resulting in Heinz body formation [52], and decreased antioxidant capacity of the newborn plasma [53]. Newborn erythrocytes have diminished glutathione peroxidase, rendering them more vulnerable to hydrogen peroxide-induced oxidant injury. Additionally, newborns have less

capacity for handling singlet oxygen and superoxide radicals [54]. Superoxide dismutase converts superoxide radicals to hydrogen peroxide. Its level varies widely between infants; this could result in accumulation of the superoxide radical. Free radicals are detoxified by antioxidants; however, if superoxide dismutase levels are increased, the hydrogen peroxide presented to reduced glutathione may not be detoxified adequately [55]. When an imbalance occurs between enzymes involved in production and detoxification of free radicals and oxidative intermediates, oxidant-induced injury may result.

Postnatal changes in oxygen delivery

In addition to the increased oxygen content of the blood in the extrauterine environment, changes in the hemoglobin itself contribute to the physiologic anemia of infancy and the anemia of prematurity. Synthesis of Hb A begins in the third trimester, but the majority of hemoglobin at birth is still Hb F. Hb F has a higher oxygen affinity than Hb A, principally because it is relatively insensitive to the effects of 2,3-diphoglycerate (2,3-DPG). In fact, in the absence of phosphates, Hb A and Hb F have similar oxygen affinities [56]. However, 2,3-DPG interacts with Hb A to shift the oxygen dissociation curve to the right, decreasing oxygen affinity and increasing oxygen delivery to the tissues at physiologic arteriovenous oxygen gradients. Levels of 2,3-DPG increase postnatally, which may also shift the oxygen dissociation curve to the right by decreasing intracellular pH. Levels of Hb F are higher while those of 2,3-DPG are lower in the premature infant [57].

The transition from Hb F to Hb A begins in the third trimester and continues postnatally. This transition is accelerated by red blood cell transfusion and is markedly affected by exchange transfusion with adult blood. The levels of erythropoietin are higher in infants who have higher proportions of Hb F, suggesting that the increased oxygen affinity in these infants leads to decreased oxygen delivery to the tissues, stimulating erythropoietin production [58]. As shown in Fig. 3.8, because of the increased oxygen delivery, the oxygen-releasing capacity of blood in the well-oxygenated premature infant is actually greater at weeks 9–10 than at birth, despite a dramatic decrease in blood oxygen content caused by decreased hemoglobin concentration [59]. While some newborns appear to have respiratory symptoms (increased oxygen requirement and apnea), Bifano and colleagues were unable to find a direct link between measures of oxygen delivery and apnea [60]. However, others have noted increased lactic-acid concentrations, indicating anaerobic metabolism [61] and decreased growth [62] that resolve with transfusion. Exchange transfusion with adult blood was shown to decrease mortality in premature infants with severe respiratory distress syndrome (RDS) in the era before surfactant [63]. Hence, the transition from Hb F to Hb A and other factors influencing the oxygen dissociation curve

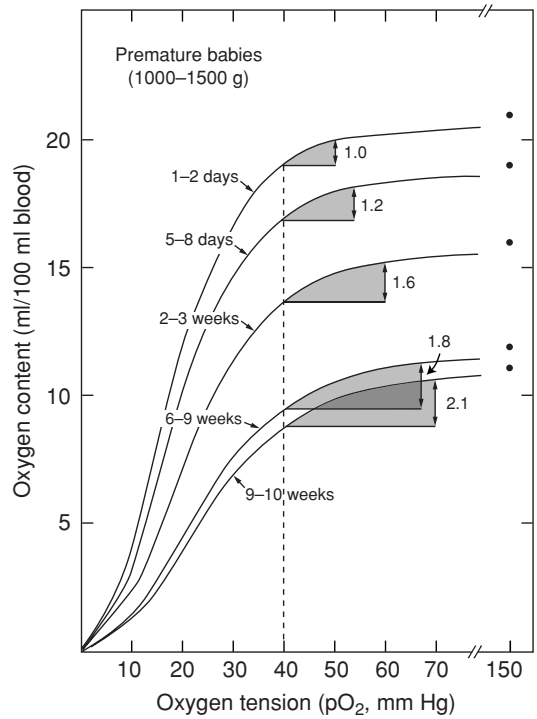

Fig. 3.7 Oxygen equilibrium curves from premature infants with birth weights of 1000–1500 g at different postnatal age. Double arrows represent the oxygen-unloading capacity between a given arterial and venous pO_2. From Delivoria-Papadopoulos M., Roncevic, N. P., Oski, F. A. [63] with permission.

are likely contributing factors to the physiologic anemia of infancy and the anemia of prematurity.

Differential diagnosis of anemia in the newborn

Introduction

At birth with exposure to higher concentrations of oxygen, the newborn undergoes critical changes in erythropoiesis. The birthing process also presents specific and serious challenges to erythropoiesis. The newborn can undergo rapid changes in hemoglobin concentration, making the evaluation of anemia difficult. The different obstetric complications and malformations of the placenta that may cause anemia in the newborn are discussed in Chapter 2. Chapter 6 provides a comprehensive discussion of the clinical presentation and pathophysiology of alloimmune red-cell sensitization and hemolytic disease of the fetus and newborn. The bone-marrow-failure causes of anemia of the newborn are discussed in Chapters 4 and 5. Hemolytic anemia due to defects in hemoglobin, red-cell membranes, and red-cell enzymes are discussed in Chapter 6. We present here an overview and a practical approach to the anemic newborn.

Normal hematological values for the fetus are now available due to the use of umbilical-cord blood sampling in the prenatal diagnosis and therapy of hemolytic disease of the newborn [64]. The fetal sonogram diagnostic criteria for both immune and non-immune hydrops fetalis, with and without anemia, are well established [65]. The diagnosis of hydrops fetalis with anemia requires intrauterine red-blood-cell transfusions. This is found most commonly in the setting of fetomaternal red-blood-cell incompatibility. The diagnosis, follow-up, and therapy of immune hydrops fetalis are discussed in Chapter 6. Normal values for cord-blood hemoglobin in the newborn vary slightly with gestational age (Table 3.2) [66]. The hemoglobin concentration is higher in samples collected from capillary blood rather than venous blood because of hemoconcentration and stasis, a phenomenon that is more significant in the newborn [67, 68]. Hemoglobin concentration may increase in the first few days of life, but then it will begin to fall steadily, reaching a nadir at 6–12 weeks of age, or so-called "physiologic anemia of the newborn." A normal full-term newborn's hemoglobin should not drop below 9.9 g/dl [69, 70]. A premature infant may have a more significant drop of hemoglobin, to the point of becoming symptomatic [71]. This hemoglobin nadir in the premature infant is known as "anemia of prematurity." Both physiologic anemia and anemia of prematurity are discussed in Chapter 4.

Table 3.2 Normal cord-blood hemoglobin values

	Hemoglobin (g/dl)	
Gestational age (weeks)	Male	Female
28–29	15.00 ± 2.45	13.60 ± 2.16
30–31	15.91 ± 1.34	14.73 ± 1.07
32–33	16.29 ± 1.86	15.21 ± 2.64
34–35	16.29 ± 2.05	15.82 ± 2.43
36–37	16.20 ± 2.20	15.88 ± 2.45
38–39	16.22 ± 2.24	16.68 ± 2.23
39–40		16.56 ± 1.65

Adapted from Burman and Morris [66].

Initial assessment

Anemia can be an acute life-threatening event or an incidental finding in a newborn, particularly at the time of delivery. The approach to these two circumstances is different. In one instance, the primary priority is to stabilize the infant, while in the second case, the clinician has time to develop a diagnostic plan before there is a need for a therapeutic intervention. A thorough maternal family history and history of pregnancy are paramount in the work-up of a newborn with anemia. The family history should include a history of hematological abnormalities and autoimmune disorders in the family. It also should include a history of prenatal exposure to infections, medications, drugs, and environmental toxins. A peripartum history is also important, particularly with respect to bleeding or infection. A careful physical examination of the infant, with attention to congenital abnormalities, signs of intrauterine infection, hepatosplenomegaly, signs

of occult hemorrhage, and signs of cardiac failure, must be done. If at all possible, the placenta should be examined, looking for signs of infection, malformations, and hemorrhage. Basic laboratory data that need to be obtained include a complete blood count (CBC) including red-cell indices, white-cell differential count, platelet count, peripheral blood smear, maternal and infant blood type, Coombs' test, serum bilirubin, and reticulocyte count. The differential diagnosis is extensive, but a simple approach based on the reticulocyte count, Coombs' test, red-cell indices, and peripheral smear is illustrated in Fig. 3.8.

The examination of the peripheral blood smear is a key component of the evaluation of anemia and other blood disorders in the neonate. An adequate preparation for evaluation is necessary; attempts to diagnose red-cell disorders should not be made from a poorly prepared smear. A properly prepared smear will have a visible feathered edge. One should begin the examination at 100 × power, noting the white- and red-cell morphology. The red blood cells should be examined in a location where they are roughly adjacent to each other. The examination of too thick an area can cause morphologic abnormalities to be disguised, while in excessively thin areas artifactual spherocytes are common. The red blood cells are then examined under 1000 × power. The red blood cells should be studied for abnormal shapes (spherocytes, elliptocytes, stomatocytes, sickle cells, target cells, byte cells, etc.). An increased number of cells with a bluish hue, called polychromatophilic cells, implies an increased reticulocyte percentage. Red-cell inclusions such as Heinz bodies, Howell–Jolly bodies, Pappenheimer bodies, or intracellular parasites such as malaria or babesiosis may be identified. While in the adult and older child Howell–Jolly bodies indicate inadequate splenic function, they are seen commonly in the normal newborn.

Blood loss

The most common cause of newborn anemia is blood loss. In particular, an otherwise healthy full-term infant with anemia is likely to have had occult blood loss due to intrapartum infant-to-mother transfusion, accidental rupture of cord, or laceration of the placenta. The anemia may occur at birth or may develop later during the neonatal period. Blood loss can be acute or chronic. Pallor, which is present in both chronic and acute blood loss, is not a specific sign. In the ill newborn, pallor can be a sign of asphyxia, sepsis, or cardiac failure, just to mention a few of the circumstances leading to pallor in the newborn. It is the constellation of symptoms and the supporting laboratory data that help direct the differential diagnosis towards blood loss. The causes of blood loss can be due to various underlying conditions (Table 3.3).

Table 3.3 Causes of blood loss in the newborn infant

Obstetric complications
Cord hematoma
Ruptured cord
Placental laceration
Placenta previa
Abruptio placentae
Malformations
Aberrant placental vessels
Placental chorangioma
Velamentous insertion of cord
Communicating vessels in multilobed placenta
Cavernous hemangioma
Occult prenatal hemorrhage
Fetomaternal transfusion
Twin-to-twin transfusion
Internal hemorrhage
Intracranial
Retroperitoneal
Intrathoracic
Rupture or hemorrhage of liver, spleen, or other organ
Iatrogenic
Blood loss from venopuncture or surgery in an infant with hemostatic problems
Blood-letting in a normal infant

Obstetric complications that may lead to blood loss include injury to the umbilical cord, such as hematomas and rupture of the cord. Placental

complications may include laceration of the placenta during Cesarian delivery, placenta previa, and abruptio placentae. Malformations of the placenta can also cause hemorrhage. These include aberrant vessels, velamentous insertion of the cord, and communicating vessels in multilobed placenta, which can rupture. Occult hemorrhage in utero can be due to fetomaternal transfusion or twin-to-twin transfusion. Internal hemorrhage, be it occult or obvious, can be due to intracranial, retroperitoneal, or intrathoracic hemorrhage, rupture of the liver or the spleen, or a large cephalohematoma. An important cause of anemia, particularly in very small premature infants, is iatrogenic from blood-letting [72]. Most intensive-care nurseries have a policy of blood replacement for the necessary blood samples. However, this policy should include not only clear guidelines for the amount of blood to be transfused as replacement, but also limits for the amount of blood letting including the discard volume after drawing blood, as well as the use of transfusion. These issues are discussed further in Chapter 14.

Hemolysis

Hemolysis represents an important differential diagnosis of anemia in this age group. The etiology of hemolysis may be immune, intrinsic to the red blood cell, or mechanical. Table 3.4 lists the different causes of hemolytic anemia in the newborn. Hemolytic disease of the newborn is discussed extensively in Chapter 6. This disorder is due to incompatibility between the maternal and the fetal blood types. Historically, anti-D hemolytic disease was the most important. However, because of the prevalent use of preventive measures with anti-D globulin, ABO incompatibility and other Rh-group incompatibilities and minor group incompatibilities have become more prominent in countries where anti-D globulin is used routinely (73). In developing countries, anti-D hemolytic disease of the newborn continues to be the predominant disorder. Blood type of mother and fetus to determine whether there is a set-up for incompatibility, and a positive direct Coombs' test (or direct antiglobulin test (DAT)), confirm the diagnosis in a suspected infant. The direct Coombs' test may not be positive in ABO incompatibility, and an antibody elution test to detect the antibody on the red cells of the infant may be needed. The direct Coombs' test is always positive when the other Rh group antigens (c, C, e, E) or other minor antigens are involved in hemolytic disease of the newborn.

Table 3.4 Hemolytic causes of anemia in the newborn infant

Immune
Alloimmune
ABO incompatibility
Rh incompatibility
Other
Isoimmune
IAIHA
Lupus erythematosus
Evan's syndrome
Other
Red-cell membrane
Hereditary spherocytosis
Hereditary eliptocytosis
Hereditary pyropoikilocytosis
Hereditary schistocytosis
Other
Hemoglobin disorders
Thalassemias, α and γ
Unstable hemoglobins
Red-cell enzyme deficiencies
G6PD deficiency
Other
Acquired red-blood-cell defects
Drug-induced
Toxin
Infection
DIC
Microangiopathic
Hemangioma
Congenital heart disease
Extra-corporeal membrane oxygenation
Thrombotic thrombocytopenia purpura

DIC, disseminated intravascular coagulation; G6PD, glucose-6-phosphate dehydrogenase; IAIHA, idiopathic autoimmune hemolytic anemia.

Rarely, autoimmune disorders such as idiopathic autoimmune hemolytic anemia (IAIHA) and lupus erythematosus can cause neonatal hemolytic anemia due to passive transfer of antibodies [74–80].

Congenital red-blood-cell defects can cause severe hemolytic anemia in the newborn. Hydrops fetalis has been reported in several of the red-cell enzyme deficiencies [81–83], membrane defects [84–86], and hemoglobin defects. These disorders are discussed extensively in Chapter 7. Delhommeau and colleagues reviewed the natural history of the first year of life of infants with hereditary spherocytosis. The hemoglobin concentrations, in general, were normal at birth but in most infants decreased during the first 20 days of life. The majority of patients required transfusion [27–35, 87]. These infants need to be followed closely, since their anemia develops postnatally in most cases.

The common hemoglobinopathies, such as sickle cell disease and β thalassemia, involve the β chain of hemoglobin and therefore do not present during the neonatal period. As discussed in Chapter 7, α and γ thalassemia, as well as the unstable hemoglobins that affect either the α or the γ chain of hemoglobin, present at birth. Worldwide, the most common red-cell enzyme deficiency is glucose-6-phosphate dehydrogenase (G6PD) deficiency. In heterozygous males, jaundice is the most prevalent manifestation of hemolysis in the newborn [88, 89]. Different phenotypes of this enzyme deficiency present differently in the newborn period. Severe neonatal jaundice is a common presentation in Israel [90], Taiwan [91], and Greece [92]. Other genetic factors may influence the degree of jaundice [93]. However, severe jaundice and its complications in G6PD-deficient infants may be more widespread [89, 94] and may be present even in heterozygous females [95].

Hemolysis can be secondary to drugs, toxin, mechanical destruction, infections, and microangiopathic processes. Infection can cause anemia by suppression as well as by increased destruction; Chapters 2 and 5 cover this topic. In addition to the coagulation disturbance, anemia is a prominent component of diffuse intravascular coagulation (DIC) in the newborn.

Hemangiomas can cause local destruction of red blood cells and lead to anemia [96]. Extracorporeal circulation used for therapy of neonatal cardiac or pulmonary disease can cause a mechanical damage of red cell type of anemia [97]. Rarely, hereditary thrombotic thrombocytopenic purpura and hemolytic uremic syndrome can cause a microangiopathic hemolytic anemia in the newborn [98].

Failure of production

Bone marrow failure disorders are covered extensively in Chapter 5. Table 3.5 lists the causes of anemia due to bone-marrow failure seen in the newborn. The most common and prominent cause of anemia due to marrow failure in the newborn occurs at the nadir of the physiological decrease in hemoglobin concentration. This nadir can be severe, particularly in small premature infants [71]. The mechanism and therapy of this anemia are discussed in Chapter 4. These infants have a blunted erythropoietin response to their anemia [99]. Another marrow-failure anemia of the newborn with a blunted erythropoietin response is the late anemia seen in both Rh and ABO hemolytic disease of the newborn [100–102]. Bronchopulmonary dysplasia (BPD) is also associated with a bone-marrow-failure type of anemia [103, 104].

Other causes of marrow failure in the newborn include constitutional syndromes such as Diamond–Blackfan anemia, Fanconi anemia, congenital dyserythropoietic anemia, thrombocytopenia and absent radii (TAR) syndrome, and other rare marrow-failure disorders. Sideroblastic anemia is a rare cause of anemia in the newborn. Nutritional deficiencies in the newborn include vitamin B12 deficiency, which is rare. If it occurs, it tends to be secondary to transcobalamin deficiency rather than the more common causes of vitamin B12 deficiency. Iron deficiency can occur due to intrauterine blood loss. The very small premature infant does not receive its full complement of iron

Table 3.5 Bone-marrow-failure causes of anemia in the newborn infant

Physiological
Anemia of the newborn
Anemia of prematurity
Late anemia of hemolytic disease of the newborn
Constitutional
Diamond–Blackfan anemia
Fanconi anemia
Congenital dyserythropoietic anemia
Pearson syndrome
TAR syndrome
Other rare syndromes
Acquired
Aplastic anemia
Infection
Drugs
Anemia associated with bronchopulmonary dysplasia
Nutritional
Iron deficiency
Folate deficiency
Vitamin B12
Copper
Vitamin E
Infiltrative disorders
Leukemia
Neuroblastoma
Histiocytoses

TAR, thrombocytopenia and absent radii.

during intrauterine life and requires iron supplementation. Maternal iron deficiency does not cause neonatal anemia due to iron deficiency [105]. Small premature infants can also become deficient in other required minerals such as copper and zinc, particularly if they are dependent on central venous nutrition [106–109]. These infants require supplementation with these minerals. Valproic acid, other anticonvulsants, and chloramphenicol have been reported to cause anemia in the newborn [110–112]. Drugs that cause anemia in older children and adults can also cause anemia in the newborn. The anemia can be hemolytic or hypoplastic. Anticonvulsants, antibiotics, and sulfa drugs are the most common drugs to cause anemia.

Leukemia, neuroblastoma, histiocytic disorders, and the transient myeloproliferative syndrome seen in Down's syndrome, are some of the malignant or marrow-infiltrative disorders that can lead to anemia in the newborn. Often, these disorders cause pancytopenia (discussed in Chapter (16)).

Therapy

The therapy for neonatal anemia is dependent on the cause of the anemia. In general, therapy is directed to the replacement of intravascular space, the replacement of hemoglobin, and the correction of the underlying cause of the anemia. In the delivery room, transfusion therapy for the acutely symptomatic newborn can be life-saving. A dose of 5 ml of packed red blood cells per kilogram of body weight is sufficient to stabilize most infants. Hydrops fetalis may require a single volume-exchange transfusion in addition to the initial transfusion. The techniques and indications for exchange transfusion in the neonate are discussed in Chapter 14, and its use for hemolytic disease of the newborn is discussed in Chapter 6. Transfusion of red blood cells is the therapy for most of the severe anemias seen in the newborn. The use of transfusions is an evolving field, with criteria for transfusion in constant flux as we balance the benefits of transfusion with the risks [113]; these topics are discussed in Chapter 14. The usual dose for a newborn that needs a transfusion is 10–15 ml per kilogram of body weight. The anemia of prematurity can be treated with transfusions when the infant is symptomatic. Therapy with erythropoietin for anemia of prematurity is controversial [114]. Some studies show benefit for the very small premature infant, but other studies have not. For a further discussion of this topic, see Chapter 4. Supplementation of the lacking mineral or vitamin is the therapy for nutritional deficiencies. The therapy for the constitutional syndromes varies with the particular syndrome. For further discussion of these syndromes, see Chapter 5.

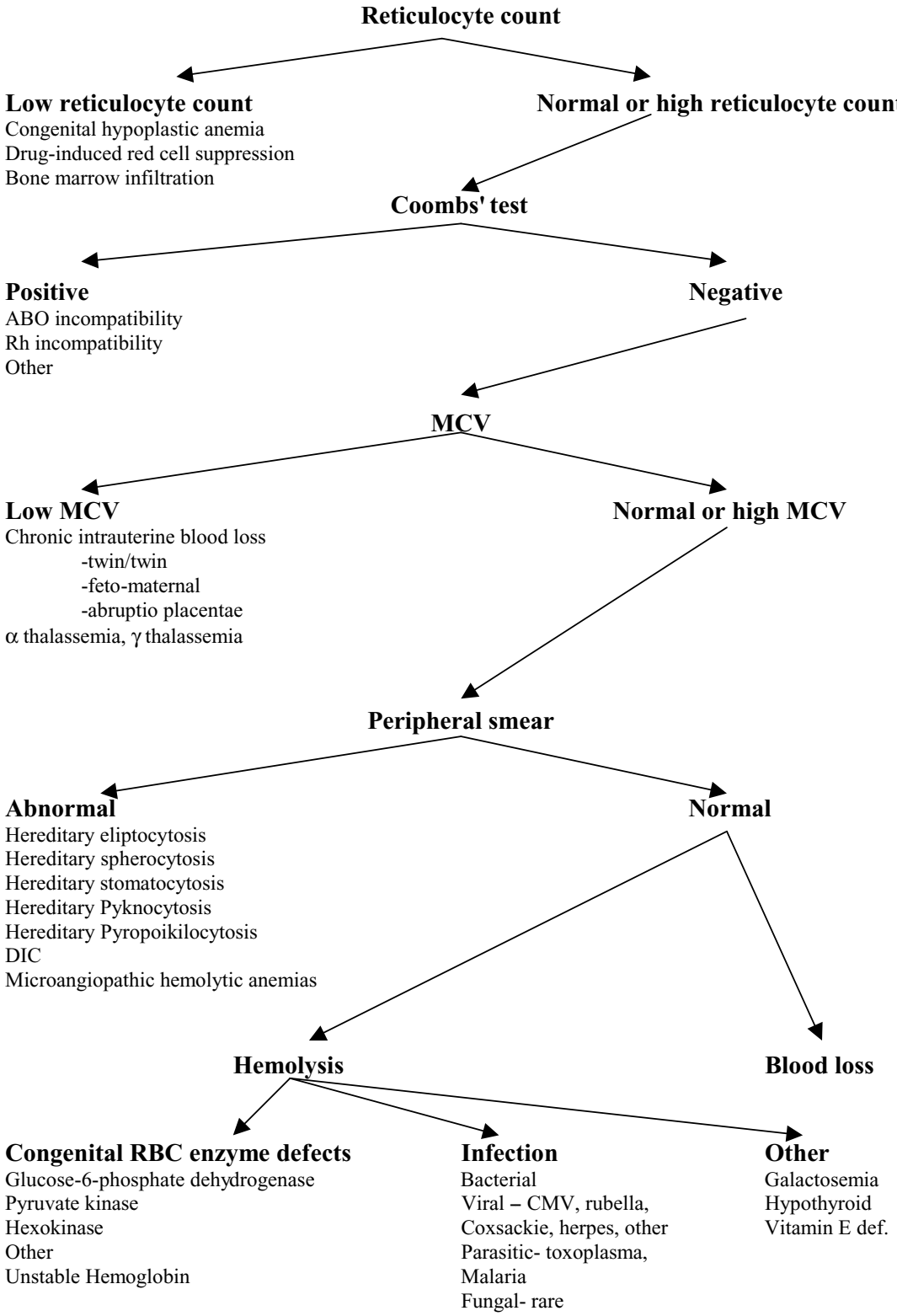

Fig. 3.8 Diagnostic approach to anemia in the newborn based on reticulocyte count. Adapted from Stockman, J. A., III. [53]

Algorithm

The algorithm that follows, adapted from Stockman, J. A., III [115] (Fig. 3.8), outlines a logical approach to anemia in the newborn based on the reticulocyte count. A low reticulocyte count suggests marrow failure. If the reticulocyte count is normal or elevated, then hemolytic disease of the newborn needs to be ruled out; a direct antiglobulin test or Coombs' test can either rule in or out this disorder. With a negative test, the red-cell indices become important. A low MCV suggests chronic blood loss or thalassemia. A normal MCV suggests either a hemolytic process due to red-cell-membrane, enzyme, or hemoglobin abnormalities. Most of these disorders have a characteristic morphological abnormality, and examination of the peripheral blood smear can be helpful in differentiating these disorders. A normal smear does not rule out red-cell-enzyme defects, but blood loss, infection, and metabolic disorders must be considered.

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4

Anemia of prematurity and indications for erythropoietin therapy

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Introduction

Anemia of prematurity is a multifactorial anemia characterized by relatively low plasma erythropoietin (EPO) levels, iatrogenic blood loss, low circulating blood volumes, and insufficient erythropoiesis. This anemia has been long characterized as nutritionally insensitive, but nutrition may influence its clinical course. Anemia of prematurity is treated with erythrocyte transfusions, and many published studies have examined the potential of recombinant human erythropoietin (rhEPO) therapy. Although rhEPO therapy is associated with a statistically lower number of transfusions, it does not eliminate transfusions in most premature infants. In addition, optimal dosage, route of administration, and timing of rhEPO therapy in prematurity remain under study. Of concern, rhEPO therapy is associated with both functional iron deficiency and depleted iron stores in other populations. In prematurity, rhEPO is given in conjunction with supplemental iron, but long-term iron status of premature infants after rhEPO therapy has been studied poorly.

Physiology of anemia of prematurity

EPO, the primary hormone regulating erythropoiesis, is measurable throughout fetal gestation [1]. In the fetus and newborn, EPO is produced primarily by the liver, which may be relatively insensitive to hypoxia compared with the kidney [1, 2]. After term birth, erythropoiesis is suppressed by markedly improved postnatal oxygen delivery and a relatively depressed plasma EPO level; consequently, a fall in hemoglobin occurs, which reaches physiologic nadir in the first months postpartum [3]. This response is exaggerated in premature infants [4]. The anemia of prematurity reflects not only insufficient EPO production [4] but also small circulating blood volume, iatrogenic blood loss, hemorrhage, hemolysis, and shortened red-blood-cell survival (summarized by Ohls [5]). Anemia of prematurity is traditionally described as nutritionally insensitive, in that it does not improve with addition of iron, although iron contributes to the recovery of hemoglobin [6, 7]. Iron status is critical, in that insufficient iron supplementation may inhibit the efficacy of rhEPO in prematurity [7, 8].

Therapy for anemia of prematurity

Minimizing blood loss

Blood loss affects the clinical course of the anemia of prematurity. In a typical 800-g birth-weight premature infant, with red cell mass at birth of 27 ml, avoidance of blood transfusions depends on avoidance of blood loss [9]. In the absence or presence of rhEPO therapy, phlebotomy clearly correlates to erythrocyte volumes transfused [9–15]. This is most evident when precise transfusion criteria are employed [13]. Some centers report relatively lower phlebotomy loss on even the smallest children [16, 17]. Using inline and microsampling point-of-care testing may contribute

Table 4.1 Transfusion guidelines

1 Transfuse if hematocrit ≤20% or hemoglobin <70 g/l and reticulocyte count ≤100 000/mm^3 (or ≤4%)
2 Transfuse if hematocrit ≤25% or hemoglobin <8 g/l and any of the following is/are present (other causes ruled out):
   (a) *Increased severity of AB episodes*
   ≥10 episodes in 24 h or
   ≥2 episodes requiring bag and mask over previous 24 h
   (b) Sustained tachycardia of ≥180 beats/min for 24 h or sustained tachypnea of ≥80 breaths/minute for 24 h by averaging monitor data
   (c) Cessation of previously adequate weight gain (≥10 g/day) over previous 4 days
   (d) Mild respiratory disease: infants on 1/8 to 1/4 l O_2/min nasal cannula or 0.25 FIO_2 by hood, NCPAP, or ventilator
3 Transfuse if hematocrit ≤30% or hemoglobin <100 g/l with moderate respiratory distress on >1/4 l O_2/min nasal cannula or >0.35 FIO_2 on hood, NPCPAP, or ventilator
4 Transfuse if hematocrit ≤35% or hemoglobin <120 g/l in infants with "severe" respiratory disease requiring mechanical ventilation and FiO_2 ≥50%
5 Acute blood loss with shock: blood replacement to re-establish adequate blood volume and hematocrit of 40%
6 Transfuse if hematocrit ≤30% and undergoing surgery [12]

FiO_2, fraction of inspired oxygen; NCPAP, nasal continuous positive airway pressure; AB apnea/bradycardia.

to less blood loss in unstable premature infants [18, 19]. In a recent editorial, Ohls challenged all neonatologists to minimize blood draws in order to effectively treat the anemia of prematurity [9].

Erythrocyte transfusions

Premature neonates are among the most frequently transfused group of hospitalized patients [20]. A desire to limit blood-donor exposure has led to lower hematocrit (Hct) levels, which trigger transfusions [21, 22]. In 1989, premature infants with birth weight 1500 g received eight to ten transfusions during initial hospitalization, while currently two transfusions per infant are reported [11, 12]. Many centers now follow the conservative consensus transfusion guidelines for premature infants presented initially by the 1995 US Multicenter rhEPO trial (Table 4.1) [7, 10, 22, 23]. Conservative transfusion criteria are effective tools in limiting transfusions [13].

The goal of eliminating allogeneic transfusions in premature infants has not been met. First, despite much effort, clinically accessible tests indicating physiologic transfusion needs in premature infants are not available [24–30]. Thus, the clinically available, but imprecise, hemoglobin/hematocrit measures are generally used to assess tissue oxygenation and to trigger transfusions [31]. Second, it is logical that advances in perinatal care resulting in lower patient acuity could decrease blood administration [11], but transfusion practices on infants with similar acuity vary widely [13–15]. Third, procedures for collection and storage of cord blood for later autologous transfusion are technically challenging [32–34]. Lastly, delayed cord clamping at premature delivery, i.e. autologous transfusion at birth, reduces transfusions in premature infants [35–37] but is not practiced widely in the USA. The role of transfusions in the therapy of anemia in the newborn including the anemia of prematurity and the criteria for transfusion are discussed further in Chapter 14.

rhEPO therapy for anemia of prematurity

Since 1990, over 50 studies have been published reporting use of rhEPO in prematurity. Primary and secondary endpoints to these studies include avoidance of transfusion, number of transfusions, transfusion volume, rise in reticulocytes, and rise in hematocrit. The studies are diverse with respect to the degree of stability and birth weight of infants. These studies vary according to timing of initiation,

rhEPO dose, dosing interval, duration of therapy, and route of administration. Data support the idea that rhEPO might be more efficacious if initiated earlier (before four days postnatal age) [23, 38, 39]. Data in premature infants clearly support a greater per-kilogram dosage of subcutaneous or intravenous rhEPO, secondary to greater plasma clearance, greater distribution volume, and shorter mean residence time in premature neonates, compared with older individuals [40, 41]. Various subcutaneous dosing intervals and continuous intravenous infusion have been examined. More frequent subcutaneous dosing may be more efficacious [42]. Additionally, continuous rhEPO infusion in total parenteral nutrition (TPN) results in similar plasma EPO levels, clearance, and reticulocyte counts as daily subcutaneous administration of the same dose [43].

Data also support the suggestion that optimal caloric and protein intake may optimize the effect of rhEPO [44–46]. Nearly all rhEPO trials in prematurity administered supplemental iron. In published studies, oral iron supplementation during rhEPO therapy ranges from 2 to 36 mg/kg daily; parenteral iron administration ranges from 1 to 3 mg/kg daily and from 6 to 20 mg/kg as a weekly injection [23, 39, 43, 47–50]. Optimal iron supplementation during rhEPO therapy remains under investigation.

The trials of rhEPO in prematurity are diverse (Table 4.2). A recent meta-analysis of these trials had significant limitations [51]: of 21 eligible studies, only four met the acceptable design criteria [10, 23, 52–54]. From these four studies, authors of the meta-analysis concluded that rhEPO reduced erythrocyte transfusion by 11.0 ml/kg, that the studies were extremely variable, and that rhEPO is not yet standard therapy [51].

Side effects of rhEPO therapy are relatively uncommon in premature neonates. Side effects seen in adults, including hypertension, thrombosis, bone pain, rash, and seizures, are generally not seen. Pure red-cell aplasia, which has been reported in adults on chronic therapy, has not been reported in infants [55]. Several studies show decreased absolute neutrophil counts but no increase in infections [53, 56–58]. Studies also show increased platelet numbers [57–59]. Poor weight gain has been cited [55]. A major concern of rhEPO in prematurity is the extension of functional iron deficiency into tissue iron deficiency; this is discussed later.

Table 4.2 rhEPO trials in prematurity [10, 17, 23, 44, 45, 47, 52, 53, 56, 59, 61, 62, 76, 77, 80, 107–114]

Timing of initiation of therapy	Day of life 2–28
rhEPO dose (U/kg/week)	150–1500
Dosing interval	Twice weekly to daily
Duration of therapy	Day of life 10 until 6 weeks, or until hospital discharge
Route of administration	IV bolus, IV continuous drip, subcutaneous
Nutritional supplementation	
Oral iron	2–36 mg/kg daily
Parenteral iron	0–3 mg/kg daily or 20 mg/kg weekly
Folate	40–1000 μg daily
Vitamin E	5–25 IU daily

Several studies show that rhEPO therapy in neonates is probably not cost-effective [60–63], but many centers dose multiple infants with aliquots from the same drug vial, decreasing the cost [64]. In light of worldwide blood shortages and blood-safety issues, cost-effectiveness may be a less important consideration. However, studies of premature infants cared for in developing countries with limited resources showed that rhEPO treatment did not decrease transfusions [65, 66].

Summary of rhEPO in Prematurity 2004

A 2003 editorial cautions that rhEPO therapy in premature neonates is rarely indicated, secondary to minimal efficacy and unclear safety [55]. The meta-analysis warns that rhEPO is not standard treatment for the anemia of prematurity [51]. Although more than 50 studies examining rhEPO in prematurity have been published, unanswered questions remain.

Iron status of premature infants

Perinatal iron acquisition

Iron stores in premature infants increase in proportion to gestational age and birth weight. The infant born at term has a relatively constant total body iron content of 75 mg/kg body weight [67, 68]. Normally, fetal iron is accrued at 1.6–2.0 mg/kg daily, even with poor maternal iron stores [69, 70]. As much as 80% of body iron is contained in hemoglobin [68]. Although utilized in all body tissues, the greatest demand for iron in the premature infant is erythropoiesis. As neonatal blood volume expands with rapid growth, premature infants produce massive amounts of hemoglobin. For each gram of hemoglobin synthesized, 3.47 mg of elemental iron is required. This red-blood-cell expansion is much greater for premature infants than for term infants, secondary to the relatively faster growth rate. Postnatal iron status for infants (and older individuals) is controlled through enteral iron absorption. Although the mechanisms behind enteral iron absorption in human infants are understood incompletely, stable isotope studies show that infants are very efficient at intestinal iron absorption [71–74].

Measuring iron status in premature infants

In premature infants treated with rhEPO, the potential for extending a functional iron deficiency into tissue iron deficiency is a pressing concern. However, clinical measures of tissue iron deficiency are not available. Normal values for the battery of tests used to measure iron status in older individuals are not available in early postnatal life. These tests poorly reflect iron stores in early development. However, investigators examining rhEPO in prematurity have attempted to measure iron status. Although an imprecise index of iron status in the first months of life [6, 71, 75], plasma ferritin levels are reported in many controlled rhEPO studies in premature infants [10, 48, 62, 76–80]. Plasma ferritin levels generally fall with increasing postnatal age in the premature infant [6]. The lower cut-off value for normal plasma ferritin in premature infants is unknown, but it is likely to be much higher than textbook normals of 12 μg/l [68]. In children and adults with rhEPO-stimulated erythropoiesis, clinicians use cut-off values of plasma ferritin as high as 60–100 μg/l [81].

Erythroid maturation and proliferation rely on the transferrin receptor (TfR) pathway for iron delivery [82]. Soluble TfR (sTfR) levels are reported in premature infants receiving rhEPO [49, 83, 84]. In adults, the sTfR is shed from reticulocytes entering the circulation and measures either erythropoiesis or iron status [85, 86]. An early rise in sTfR has been shown to predict erythropoietic response in adults treated with rhEPO [87]. Although sTfR levels have been reported to reflect iron status in newborns [88] and premature infants [83], we have observed that sTfR levels correlate to stimulation of erythropoiesis, but not iron status, in both term and premature infants [89, 90]. Further evaluation of this measurement as a reflection of iron status is required.

Several other studies utilize different markers of iron status during rhEPO treatment in prematurity. Bechensteen and colleagues recommend a cut-off value of serum iron below 90 μg/100 ml to indicate iron deficiency [44]. In adults receiving rhEPO, hypochromic erythrocytes over 6% are abnormal [91] and may also be abnormal in neonates [48, 80, 84]. Zinc protoporphyrin/heme (ZnPP) ratios also measure incomplete erythrocyte iron incorporation seen accompanying iron deficiency in older term infants [92]. One report shows that ZnPP ratios in rhEPO-treated premature infants rise, and using the adult cut-off value of ZnPP ratio greater than 100 μM/M potentially could measure incomplete iron incorporation in premature infants [93, 94]. More study is necessary.

Postnatal iron sources

The anemia of prematurity traditionally was treated with frequent packed erythrocyte transfusions, but this practice is decreasing. The effect of conservative transfusion on the long-term iron status of premature infants has been studied poorly. Because of the high hematocrit of packed erythrocytes, as

much as 1 mg of iron can be administered in each milliliter transfused. This iron "administration" may be clinically significant. In premature infants, iron stores and plasma ferritin levels rise with erythrocyte transfusions [95–97]. rhEPO treatment does not seem to alter this pattern, as rhEPO-treated infants given transfusions exhibited higher ferritin levels compared with rhEPO-treated infants without transfusion [8]. In studies of unstable premature patients, rhEPO-treated infants who experienced fewer transfusions than control infants exhibited lower plasma ferritin levels than control infants [17, 52, 53, 59, 76, 77, 80, 98]. In rhEPO studies whose enrollees were relatively larger or more stable, both experimental and control groups experienced infrequent and similar transfusion frequency and exhibited similar plasma ferritin levels [46, 99].

Although ZnPP ratios measure incomplete iron incorporation into erythrocytes, and not necessarily iron stores, we observed lower ZnPP ratios in non-rhEPO-treated premature infants given erythrocyte transfusions compared with those not transfused [100]. Our findings support the idea that iron availability for incorporation into erythrocytes may be greater in premature infants transfused. As the number of erythrocyte transfusions per infant declines, the allotment of iron from transfusions will decrease.

Enteral iron supplementation

Data in adults support the hypothesis that iron supplementation is necessary for effective treatment, and studies of rhEPO in prematurity have given oral iron supplements. Most oral iron supplementation has been between 2 and 6 mg/kg daily. Several studies have found hypochromic erythrocytes, which are evidence of iron-deficient erythropoiesis, during therapy in rhEPO-treated, oral iron-supplemented premature infants compared with oral iron-supplemented controls [43, 48, 49]. High-dose oral iron (18–36 mg/kg/day of ferrous fumarate) is used routinely in Norway and may be associated with less severe anemia of prematurity [44, 46].

Several studies report using markers of iron status during rhEPO treatment to guide increases in oral iron supplementation. As when treating older patients with rhEPO, plasma ferritin levels much higher than 12 μg/l are used for cut-offs to increase iron dosage. Maier and colleagues recommend less than 100 μg/l as a cut-off, while Meyer and colleagues recommend less than 65 μg/l as a cut-off to increase oral iron supplementation [62, 80]. Bechensteen and colleagues recommend a serum iron below 90 μg/100 ml as a cut-off to double oral iron supplementation [44]. Others recommend increasing oral iron supplementation in premature infants when hypochromic reticulocytes are above 8–20% [48, 80, 84].

Parenteral iron supplementation

Studies in adults on rhEPO have shown that parenteral iron increases rhEPO effectiveness and decreases the functional iron deficiency [101]. Although several neonatal studies have examined parenteral iron (intramuscular, intravenous) supplementation with rhEPO [23, 39, 43, 47], only one study has compared rhEPO treatment with parenteral iron to a group without any iron [39]. Carnielli and colleagues showed that hematocrit, reticulocytes, and plasma ferritin levels were higher in rhEPO plus parenteral iron compared with rhEPO without iron [39]. Three studies have addressed the question of whether parenteral iron versus enteral iron supplementation is more effective in premature infants [48–50]. Two studies have showed similar hematocrit and reticulocytes in oral versus parenteral iron supplemented infants given rhEPO [48, 49]. Pollak and colleagues showed similar hematocrit between groups but higher reticulocytes in the rhEPO plus parenteral group compared with the rhEPO plus oral iron group [50]. Meyer and colleagues observed higher hypochromic reticulocytes in the oral iron group, while Pollak and colleagues observed elevated hypochromic erythrocytes in both groups compared with controls [50]. rhEPO plus parenteral iron treatment may result in higher plasma ferritin levels than rhEPO plus oral iron [50].

Parenteral iron therapy may have toxicity. Because of deficient iron-transport capabilities and the deficient antioxidant status of premature infants, iron-induced free-radical diseases, such as bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis, are common in premature infants [102–104]. In the parenteral iron/rhEPO studies listed above, no clinical evidence of iron toxicity was reported. However, only one study investigated specific measures of lipid peroxidation. Pollak and colleagues studied plasma malondialdehyde levels in premature infants treated with rhEPO and intravenous iron [50]. These investigators observed elevated serum lipid peroxides immediately after the two-hour intravenous infusion of 2 mg iron sucrose but found no clinical complications [50]. Because significant hemolysis is observed after erythrocyte transfusion [105], Kling and colleagues studied and found elevated serum malondialdehyde levels in premature infants after packed erythrocyte transfusion [106]. Because of concern for oxidant stress, administration of parenteral iron in conjunction with erythrocyte transfusions may be more problematic than either given alone. Further examination of the toxicity of rhEPO, parenteral iron, and transfusions is necessary.

Long-term iron status of premature infants

Long-term iron status is of major concern in rapidly growing premature infants. Only three studies have followed iron status of rhEPO and oral iron-treated infants after hospital discharge. Although lower plasma ferritin levels are seen in rhEPO versus control infants during study, it is reassuring that both treatment and control infants have similar plasma ferritin levels at 4–12 months of age [76, 98, 99]. Less reassuring are the extremely low ferritin levels seen (mean levels 21–40 μg/l), with several individual plasma ferritin levels below the 12-μg/l cut-off for iron deficiency [76, 98, 99]. It is possible that the avoidance of transfusions has endangered the iron stores of all premature infants, regardless of whether they receive rhEPO. More long-term studies of iron status after rhEPO therapy are needed.

Conclusion

The anemia of prematurity is multifactorial, but it is less severe if blood loss is minimized. Conservative transfusion criteria may minimize exogenous erythrocyte transfusions, but rhEPO does not completely eliminate transfusions in most premature infants. Many unanswered, but testable, questions remain regarding the anemia of prematurity and its treatment. These questions include: (i) can more transfusions be avoided with decreased iatrogenic blood draws and strict transfusion criteria? (ii) Is there a subset of premature infants who are the best candidates for rhEPO? (iii) Does earlier rhEPO treatment improve efficacy? (iv) Does nutritional supplementation optimize rhEPO efficacy? (v) Does rhEPO therapy precipitate tissue iron deficiency later in life? (vi) Is it safe to supplement with parenteral iron? More study is necessary to answer these questions.

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5

Hypoplastic anemia

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When a neonate presents with anemia, the first diagnostic thought of the clinician is not of a congenital aplastic disorder. Indeed, compared with adults, all newborns normally are relatively "anemic" during a window of one to two months postpartum, the so-called physiologic anemia of the newborn. Thereafter, erythropoiesis will be stimulated, and higher hemoglobin levels are achieved. However, persistence of anemia beyond this period signals a pathologic condition. When one encounters an anemic baby whose work-up reveals no evidence of hemolysis, a negative Coombs' test, and a low reticulocyte count, with or without additional cell lines depressed, then a work-up that considers the common infectious etiologies, as well as the less common genetic causes of hypoplastic anemia, must be undertaken.

Causes of cytopenias

Aside from the physiological anemias discussed in Chapter 4, any or all of the blood-cell lines in the neonate may be affected by primary or secondary causes. The clinical picture, including historical and physical findings, will provide clues as to the etiology of the cytopenias (as discussed in Chapter 3), but often only time will allow identification of the ultimate cause of the cytopenias. Table 5.1 lists the causes of hypoplastic anemia in the newborn.

Infections

Typically, congenital infections in the neonate cause hemolytic anemias. However, some viruses can have a suppressive effect on the bone marrow. This can occur through (i) a direct cytotoxic effect against specific progenitors; (ii) progenitor infection, which may lead to suppression through the activation of an immune reaction against the progenitor cells; or (iii) bone-marrow stroma infection, leading to a lack of the supportive environment critical for proper hematopoiesis. Intrauterine infections and their hematological manifestations in the newborn are discussed in Chapter 2. The focus of this section of the chapter will be the effect of infections on erythropoiesis and the resultant anemia.

Parvovirus B19

Parvovirus B19, the cause of erythema infectiosum, Fifth's disease, in the general population, is a known cause of early hydrops fetalis [1]. In spite of the seemingly benign course in the normal child, in utero infection nonetheless may result in hydrops fetalis and an approximately 5% risk of fetal loss; however, the majority of infants born to infected mothers are born normal. It is in utero second trimester infections that are associated with hydrops fetalis. Parvovirus B19 does not appear to be directly teratogenic. Congenital infection can also be associated with a Diamond–Blackfan anemia (DBA)-like picture (pure red-cell aplasia at birth). Parvovirus B19 may also cause significant marrow suppression in the setting of the newborn that has a shortened red-cell lifespan, where an underlying red-blood-cell molecular defect, such as hereditary spherocytosis, exists or where there is an ongoing immune hemolytic

Table 5.1 Bone-marrow failure in neonates

Infection
Parvovirus
Cytomegalovirus
Human immunodeficiency virus
Adenovirus
Epstein–Barr virus
Rubella
Hepatitides-flavivirus, human herpes virus 6
Drugs
Chloramphenicol
Antiepileptics
Clastogenic agents cyclophosphamide, vinculin alkaloids, benzene
Nonsteroidal anti-inflammatory agents
Inherited disorders
Diamond–Blackfan anemia
Fanconi anemia
Dyskeratosis congenita
Pearson's syndrome
Cartilage hair hypoplasia
Schwachman–Diamond syndrome
Kostmann's syndrome
Reticular dysgenesis
Amegakaryocytic thrombocytopenia
Osteopetrosis
Thrombocytopenia and absent radii (TAR) syndrome
Dubowitz' syndrome
Noonan's syndrome
Seckel's syndrome
Down's syndrome
Familial marrow syndromes

process. The effect on erythropoiesis in normal patients with normal immune systems is typically short-lived. However, the immunocompromised infant may suffer chronic anemia and persistent parvovirus antigenemia [2–11].

Parvovirus B19 is the prototypical virus that causes direct toxicity by infection of erythroid progenitors. This is clinically evident by the presence of giant erythroid pronormoblasts in the bone marrow of infected infants, in the absence of further erythroid precursor maturation or peripheral blood reticulocytes. Direct cytotoxicity has been demonstrated by infection of erythroid progenitor cell cultures in vitro. The target of infection is the mature erythroid progenitor cell. Infection of these progenitors prevents their maturation and causes erythroid arrest [12–16]. Although erythroid suppression is the predominant manifestation of parvovirus B19 infection, suppression of all cell lines also can occur [17, 18].

In the clinical setting of suspected parvovirus infection, maternal anti-parvovirus immunoglobulin M (IgM) titer followed by elevation of immunoglobulin G (IgG) titer can be helpful in the diagnosis. The polymerase chain reaction (PCR) test of viral antigen is now the state of the art for direct detection of virus.

Severe anemia in the neonate may necessitate the employment of transfusion. However, the indications for fetal and perinatal transfusions in parvovirus B19 infection are unclear. While their use has ameliorated the effects of hydrops fetalis, they have also caused measurable morbidity and mortality [19, 20]. Also, spontaneous in utero resolution of hydrops may occur, which suggests that careful evaluation is necessary before initiating in utero transfusions [21]. The reported use of intravenous immunoglobulin therapy for intrauterine infection in three patients provides anecdotal evidence for its use. However, its efficacy is not clear [22].

Other viruses

While other viruses can cause bone-marrow suppression, recognition of this effect can be difficult. Viral infections often occur in the setting of antimicrobial administration or sepsis, both of which can cause marrow suppression.

Cytomegalovirus (CMV) is a double-stranded DNA virus with systemic effects on the developing fetus. While thrombocytopenia caused by splenic sequestration and hemolytic anemia are hallmarks of the hematological manifestations, animal models reveal that direct megakaryocytic infection by CMV, which

causes decreased platelet production (see Chapter 10), can also occur [23]. Human immunodeficiency virus (HIV) is associated with bone-marrow suppression of all blood-cell lines. However, the direct effect of HIV is difficult to determine because of the use of antiviral agents and concomitant infection by opportunistic organisms that can cause pancytopenia [24–27]. There is also evidence that the suppression of hematopoiesis seen in HIV infection is caused by the effect of an activated immune system on the bone-marrow microenvironment [28]. Other viruses have been implicated in bone-marrow suppression, including adenovirus, rubella, and Epstein–Barr virus (EBV) [29].

Hepatitis

Hepatitis is an acknowledged cause of aplastic anemia in all ages, including infants. Most cases don't involve hepatitis virus A, B or C and are likely to include a wide array of viruses such as flaviviruses, EBV, CMV, and human herpes virus (HHV) 6. Aplasia due to giant-cell hepatitis has also been reported. CMV in particular is noted for causing toxicity to bone marrow stroma [30–34].

Drugs

The phenomenon of drug-induced aplasia is difficult to separate from the aplasia caused by the underlying clinical condition in the ill neonate. Drugs or toxins may induce marrow suppression or toxicity in a dose-dependent or idiosyncratic fashion.

Chloramphenicol is the classic drug associated with aplasia. It may induce aplasia in two different ways. The first is the "gray-baby syndrome," whereby the lack of detoxification activity in the neonatal liver enhances marrow toxicity. This effect is chiefly one of erythropoietic suppression and reticulocytopenia. Chloramphenicol is conjugated to glucuronide for urinary excretion. The neonatal liver has decreased conjugating capacity. Neonatal use of chloramphenicol became extremely rare as a result of awareness of neonatal liver immaturity and, thus, neonatal aplasia is now unheard of [35–38]. In the second mechanism, an idiosyncratic drug reaction occurs, inducing irreversible marrow toxicity some time (weeks to months) from the administration of the drug. This effect occurs across all cell lines, causing severe, potentially lethal aplasia.

Other drugs and toxins associated with aplasia include antiepileptics, nonsteroidals, pesticides, and clastogenic agents, such as chemotherapeutics, benzene, and antitubulins [39–41].

Inherited diseases of marrow failure

In spite of the rarity of bone-marrow-failure syndromes, they represent important diseases both for the management of the few affected families and for the insight into the genetics of developmental hematopoiesis that they provide. Thus, study and presentation of the details of their molecular biology and biochemistry are warranted. The sections that follow present a more detailed analysis of several diseases for which this information is known.

Diamond–Blackfan anemia

DBA is the most common disease of isolated red-cell aplasia in the neonate. Most patients are diagnosed in the first year of life. While DBA can present in the older patient, at least 10% in one series presented at birth and 25% within the first month of life [42–46].

DBA is a genetic disease with evidence of mixed inheritance, although sporadic cases comprise the majority of all cases (up to 85%). A significant portion of inherited cases has an autosomal dominant pattern of inheritance, with equal sex frequency. These patients appear to have fewer physical anomalies. Another portion of cases is autosomal recessive, with disproportionately more males than females, implying an X-linked form of the disease [47–49].

DBA patients have a host of physical abnormalities, including typical dysmorphic facies, short stature (unrelated to steroid therapy), and eye, kidney, and hand abnormalities [50–52] (Table 5.2). However, it is the clinical signs of anemia that usually lead to the diagnosis. Patients first present with pallor, macrocytic anemia, and reticulocytopenia. The anemia is often associated with failure to thrive.

Table 5.2 Diamond–Blackfan anemia presentation

Failure to thrive
Pallor
Malformed thumbs
Flattened nasal bridge, upturned nose
Urogenital malformations
Short stature
Usually isolated, macrocytic anemia; occasionally pancytopenia
Leukemia

However, the diagnosis may be obscured by the normal physiologic red-cell changes in the newborn. Indeed, the mean cell volume often does not exceed the normal range. Thus, most commonly, patients present between two and four months of age. The diagnosis is confirmed by the finding of reticulocytopenia and decreased erythroid activity in the marrow [53, 54].

Mechanism

A gene for DBA has been cloned. An X:19 translocation was identified in a patient with DBA. Since then, 25% of DBA patients have been found to have heterozygous S19 mutations for the gene for the ribosomal protein S19 mapped to chromosome 19. This implies that the allele acts in a dominant negative fashion and that the presence of both mutated alleles may be lethal. The translocation in the proband above, while involving the X-linked chromosome, does not represent the X-linked form of the disease. Other potential genes causing DBA have been linked not only to the X chromosome but also to chromosome 8, but these genes have not yet been cloned [55–59].

The cloning of this gene alone does not answer the basic question as to how DBA occurs. Presumably, the mechanism involves ribosomal function and protein expression, but this is hardly helpful. Previous theories include circulating red-cell progenitor inhibitors, erythropoietin inhibitors, and defects in the erythroid progenitor. Reduced erythroid progenitors, measured by in vitro erythroid colony-forming activity, colony-forming unit-erythroid (CFU-E) and burst-forming unit-erythroid (BFU-E), in DBA patients have been observed. However, this has not proven to be a reliable predictor of DBA [60–63]. The measured decrease in erythroid progenitors may be due to accelerated apoptosis [64]. Defects in the microenvironment of the marrow have also been proposed because of inhibition of erythropoiesis by bone fragments from a DBA patient. Other proposed mechanisms include affected function of transcription factors, growth factors, and growth factor receptors [65].

Diagnosis

While the differential diagnosis of DBA includes all causes of hypoplastic anemia listed in Table 5.1, special consideration should be given to Fanconi anemia, transient erythroblastopenia of childhood (TEC), hypothyroidism, and parvovirus B19 infection. Studies to rule out parvovirus and Fanconi anemia, as discussed elsewhere in this chapter, are necessary. Neonatal screening results may rule out hypothyroidism. TEC is uncommon in the newborn and should resolve within a few weeks. The presence of macrocytic anemia and reticulocytopenia beyond four months of age should alert the clinician to the possibility of a diagnosis of DBA. A bone-marrow examination is performed to confirm the diagnosis of DBA. The bone-marrow examination reveals adequate cellularity, with present neutrophil and platelet precursors, but with absent or marked decrease erythroid precursors. However, a significant minority of patients present with neutropenia, thrombocytosis, or thrombocytopenia, even though DBA is thought of as a pure red-cell aplasia [66]. Other nonspecific abnormalities include elevations of fetal hemoglobin, red cell antigen i, serum iron, serum or red cell folate, and levels of vitamin B12 [53, 54].

Therapy

Over 50% of patients with DBA respond to systemic corticosteroid treatment with recovery of erythropoiesis. Corticosteroids are indicated for anemia that causes cardiovascular and/or developmental

compromise. Most patients begin therapy with a regimen of prednisone at a dose of 2 mg/kg/day. In responding patients, a hemoglobin of 10 g/dl and reticulocytosis are the usual parameters desired before beginning to taper prednisone therapy. Within the group of responders, a sizable minority of patients will be able to discontinue prednisone therapy or require very little prednisone to maintain an adequate hemoglobin [52, 67, 68]. Corticosteroids may become problematic with chronic use, leading to, among other problems, growth retardation, weight gain, gastritis, and decreased bone mineralization.

Roughly half of the cases of DBA become transfusion-dependent because corticosteroids cause intolerable side effects or the patient is resistant to corticosteroid therapy. In the absence of adequate iron chelation, chronic transfusion therapy results in iron overload, which leads to cardiac, endocrine, and pulmonary disease. Chronic iron chelation using desferoxamine has mitigated many of these problems.

Any discussion of transfusion and iron overload in the therapy of DBA must be balanced against the proposed use of hematopoietic stem-cell transplantation (HSCT) as a curative modality. The side effects of HSCT include graft-versus-host disease (GVHD), infections, and chemotherapeutic toxicity involved with conditioning. With matched-sibling donors, the risk of GVHD has become lower, with long-term survival of over 80%. The timing of HSCT in DBA is controversial. The younger patient is more apt to have a lower degree of GVHD and better long-term survival. However, the younger patient is also more likely to have greater late effects, and some authors argue to withhold therapy to allow for maximal growth and to ensure that no late remission will occur [69, 70].

Growth factors have also been used in DBA. Erythropoietin, the logical choice, is not effective in the therapy of DBA [71]. Interleukin 3 (IL-3) has shown some modest benefit amongst transfusion-dependent patients; however, it is associated with multiple side effects, including deep venous thromboses and constitutional symptoms such as fever, malaise, and flu-like symptoms [72, 73].

Outcome

Patients with DBA have a reduced life expectancy from causes alluded to above. In the modern era of transfusion, iron overload has become a chief cause of death. In addition, hematological malignancy is a major cause of death. DBA patients have an increased risk of developing acute myeloid leukemia (AML). The lifelong risk of developing AML is up to 25% [74]. Acute lymphoblastic leukemia (ALL), Hodgkin's disease, and myelodysplastic syndrome (MDS) have all been reported in DBA, but a clear association has not been established [75–78]. Reports of an increased risk of osteogenic sarcoma and hepatocellular carcinoma, the latter of which is likely associated with concomitant viral hepatitis infection, have also been published [79, 80].

Pearson syndrome

Pearson syndrome is a rare sideroblastic anemia with associated exocrine pancreatic dysfunction [81]. Often, patients are diagnosed in the neonatal period, and Pearson syndrome has been reported as a cause of hydrops fetalis. Rarely, these patients can have physical anomalies, but often they present with failure to thrive or poor growth along with persistent macrocytic anemia. These cases, numbering no more than 40 reported in the literature, have been diagnosed with a bone-marrow examination showing the characteristic histological finding of vacuolization of bone-marrow erythroid precursors [67, 82]. In the 1990s, the molecular defect of Pearson syndrome was characterized. It involves deletions and duplications of regions of mitochondrial DNA [83, 84]. As a result of the mitochondrial defect, the enzymes of the oxidative respiration cascade are compromised, leading to acidosis, which is a major component of the disease. In patients with the disease who survive the neonatal period, the anemia becomes less of an issue, and increases in hemoglobin levels have been observed.

Growth factors have been used in the therapy of Pearson syndrome but have not been effective.

Transfusions remain the mainstay of therapy, but mortality is high in early childhood from acidosis, sepsis, or liver and kidney failure. Malignancy has not been associated with the disease [85].

Cartilage hair hypoplasia

Cartilage hair hypoplasia (CHH) is a recessive disease with a constellation of skeletal abnormalities consisting of short stature due to dwarfism, lordosis, scoliosis, and chest-wall deformities. Sparse fine hair is a consistent feature of the disorder. CHH was first described among the Old Order Amish [86, 87]. Growth failure occurs prenatally, with shortness of limbs or stature. CHH patients also have increased risk of Hirschsprung disease [88]. Mutations in the RNA component of Rnase mitochondrial processing riboendoribonuclease (MPR) involved in mitochondrial and nucleolar RNA processing have been reported to be responsible for CHH [89].

Clinically, CHH presents with anemia and macrocytosis, with variable pancytopenia [90]. Lymphopenia occurs, along with defective cellular immunity [91]. Corticosteroids and transfusion have been used temporarily, with patients outgrowing marrow failure [92]. However, an increased malignancy risk throughout infancy, childhood, and adulthood has been reported [86, 93].

Fanconi anemia

Fanconi anemia is a rare disorder with classic features of congenital skeletal abnormalities, bone-marrow failure, and propensity to malignancy (Table 5.3). While bone-marrow failure is not evident in the neonate, quite often the existence of birth defects can lead to the diagnosis of Fanconi anemia. In a review of a Fanconi anemia registry, only 4% of patients were diagnosed in the first year of life [94]. Early management and follow-up of patients suspected of having Fanconi anemia can both improve outcome of affected children and allow the parents to receive genetic counseling.

Table 5.3 Fanconi anemia presentation

Café-au-lait spots
Short stature
Absent radii
Absent/malformed thumbs
Hypogonadism male > female
Microcephaly
Hypertelorism
Renal and ureter malformations
Gastrointestinal malformations
No physical anomalies
Usually pancytopenia; sometimes single lineage temporarily
Aplastic anemia in first decade of life
Leukemia in childhood after aplastic anemia
Solid tumors if patient reaches adulthood

Clinical features

The clinical features of Fanconi anemia are numerous and heterogeneous, making the diagnosis a challenge [67, 95–97]. Classically, Fanconi anemia patients exhibit bony abnormalities, growth retardation, and café-au-lait spots. Gonadal failure is a frequent manifestation. Its presence implies that Fanconi anemia genes are involved in gonadal development and/or gamete production. At least a third of all patients exhibit none of the classic features of the disease and look completely normal. This suggests the possibility that there are undiagnosed patients with Fanconi anemia and that the Fanconi anemia genes may play a larger role in cancer than is currently appreciated [94, 98–100].

Fanconi anemia patients present most commonly with aplastic anemia. Patients develop pancytopenia in the first decade of life. In the past, complications of pancytopenia were the leading cause of death of Fanconi anemia patients. However, in the face of adequate blood-banking and bone-marrow transplant, survival has been extended and malignancy has become the predominant cause of death [97, 101]. All pediatric patients with pancytopenia should be tested for Fanconi anemia.

Fanconi anemia patients are at high risk of developing malignancies. While myeloid malignancies predominate, numerous other cancers, including

squamous cell carcinomas of the reproductive tract, skin, and gastrointestinal system, occur at increased frequency in individuals affected by Fanconi anemia. Liver tumors have been described, although these have been associated with the use of androgens for treatment of bone-marrow failure, as has been noted generally in other bone-marrow-failure syndromes. Patients surviving aplastic anemia or AML of childhood will often go on to develop solid tumors as adults [67, 95, 102].

Biology

Fanconi anemia traditionally has been defined by clinical criteria. We can now define it more on a molecular and biochemical basis. The most important clue to the nature of this disorder came from the observation that Fanconi anemia patients being treated for AML exhibited an untoward degree of toxicity. This phenomenon can be reproduced in cell culture. Cells derived from Fanconi anemia subjects show a one-log increase in sensitivity to DNA bifunctional alkylating agents, such as diepozybutane (DEB) and mitomycin C (MMC), demonstrated as increased chromosomal breakage, above that seen in cells from normal individuals [95, 103]. This hypersensitivity of Fanconi anemia cells to bifunctional alkylating agents, captured in a cell-based assay, is the most commonly used diagnostic tool for Fanconi anemia [104, 105]. Hypersensitivity of Fanconi anemia cells is restricted to bifunctional alkylating agents that result in cross-linked DNA, including agents such as DEB and MMC as well as chemotherapeutic agents such as cisplatin, cyclophosphamide, and nitrogen mustard. These agents, while chemically diverse, are unique in their ability to form adducts with two adjacent guanidine residues on DNA, leading to both intra- and interstrand cross-links. Although it is unclear which type of lesion is the lethal one, what is clear is that monofunctional agents (one alkylating moiety per molecule) do not confer hypersensitivity in Fanconi anemia cells [106, 107]. Recent evidence has raised the possibility of radiation sensitivity being part of the Fanconi anemia phenotype, but this has remained controversial. Patients with Fanconi anemia and undergoing radiation therapy require a decrease in dose due to increased toxicity. However, the reason for this increased toxicity is not clear [108, 109].

Other investigators have pointed to oxygen and free radicals as the primary agents to which Fanconi anemia cells are hypersensitive [110–112]. This observation comes from the fact that Fanconi anemia cells reportedly grow best under low-oxygen conditions. Other authors have noted that oxygen is not the only variable. When Fanconi anemia cells are immortalized into cell lines by simian virus (SV) 40 infection, the effect of oxygen is mitigated [113]. Another striking phenotype of Fanconi anemia cells is accumulation in the G2/M phase of the cell cycle after DNA damage, such as that induced by MMC [114–116]. Mutant cells display an excessive percentage of G2/M cells after MMC treatment compared with wild-type cells. This phenomenon appears to be agent-specific and limited to bifunctional alkylating agents. Even untreated Fanconi anemia cells have been shown to have increased G2 transit time.

Genetics

Fanconi anemia is an autosomal recessive disorder. However, it has been shown that overexpression of some mutants can cause a dominant negative effect, which, if true in vivo, would lead to a dominant inheritance pattern. Somatic cell hybridization has led to significant insight into the genetics of Fanconi anemia. In these studies, Fanconi anemia cells are fused either with a cell from a normal individual (wild-type) or with another Fanconi anemia cell from a different patient, and the MMC sensitivity is analyzed. Fusion between a wild-type (normal) cell and a Fanconi anemia cell results in maintenance of MMC resistance [116–119]. The fusion of two different Fanconi anemia cells, both being MMC-sensitive, may result in MMC resistance, suggesting that more than one gene is involved in the development of clinical Fanconi anemia. These types of experiment have enabled the distinction of at least eight Fanconi anemia complementation (FANC) groups, A, B, C, D1, D2, E, F, and G.

Most information on Fanconi anemia proteins is known concerning FANCC, FANCA, and FANCG. FANCC was the first complementation group gene to be cloned. Strathdee and coworkers took advantage of the MMC-sensitivity phenotype to expression clone FANCC [120]. FANCC is a 63-kDa protein encoded by an 80-kb genomic clone, which includes 14 exons [121]. This gene maps to 9q22, which is the site of frequent loss of heterozygosity (LOH) amongst AML in non-Fanconi anemia patients [122]. The FANCA gene was cloned by positional as well as expression-based strategies [123, 124]. FANCA is a 160-kDa protein containing a nuclear localization signal and a partial leucine zipper. The FANCA gene localizes to chromosome 16p, also a site of LOH associated with numerous cancers. FANCG protein is a 70-kDa protein whose gene was also cloned by expression [125]. The FANCG protein is homologous to XRCC9, a gene that was cloned originally from a Chinese hamster ovary (CHO) cell line with radiation hypersensitivity. Other Fanconi anemia genes that have been cloned include the FANCE and FANCF genes. The FANCE (60 kDa) and FANCF (40 kDa) proteins are ill-described and contain no distinct homology to known proteins [126, 127].

The biochemical understanding of Fanconi anemia is advancing rapidly with the cloning of the Fanconi anemia genes. A Fanconi anemia complex has been described in which FANCA, FANCC, FANCG, FANCE, and FANCF proteins bind together [128–130]. In fact, in all the complementation groups, except FANCD2, the complex fails to form, indicating that it is important in Fanconi anemia pathogenesis.

Most recently, the FANCD2 gene has been cloned. FANCD2 is a 160-kDa protein. Uniquely, the FANCD2 mutant cells contain an intact Fanconi anemia nuclear complex, but the FANCD2 is not a binding member of the complex. However, the FANCD2 protein is nonetheless dependent on the complex as it becomes modified by a monoubiquitin upon DNA damage and during S phase. Furthermore, the FANCD2 protein binds and colocalizes with BRCA1, a breast-cancer-susceptibility gene [109]. This finding is especially interesting in light of the fact that BRCA1 associates in a super-complex with numerous DNA repair proteins [109, 131]. BRCA2, another breast-cancer-susceptibility gene, has close association with BRCA1. While gene abnormalities were not found in BRCA1 in Fanconi anemia patients, five patients with the Fanconi anemia phenotype were found to have biallelic defects in BRCA2. It thus appears likely that the Fanconi anemia complex, FANCD2 protein, and BRCA1 and BRCA2 are all involved in the process of DNA repair [132].

Treatment

Until recently, most children with Fanconi anemia died in the first decade of life as a result of the complications of pancytopenia. Modern hematopoietic and clinical support and chemotherapy have lengthened the lifespan so that many children reach adulthood. Because HSCT can replace the defective hematopoietic progenitor cell, it is curative for aplastic anemia. However, HSCT solves only the hematopoietic defect, and the potential for increased risk of solid-tumor formation remains in transplanted patients with Fanconi anemia. The optimal time to proceed to HSCT is unclear.

In the past, Fanconi anemia patients have had increased morbidity and mortality from the preparative regimens needed for allogeneic HSCT to engraft. In addition, Fanconi anemia patients have exhibited a marked degree of graft failure, perhaps due to the modifications needed to decrease toxicity. Post-HSCT GVHD and infection can cause significant morbidity and mortality. Recent advances in the techniques for HSCT for Fanconi anemia patients have produced conditioning regimens with diminished toxicity, and the recent addition of fludaribine has shown promise in decreasing GVHD. In addition, the use of antithymocyte gamma globulin (ATG) and cyclosporin have also reduced GVHD notably [133–136].

The optimal donor for HSCT for Fanconi anemia is a human leukocyte antigen (HLA)-matched sibling donor who has been proven not to have Fanconi anemia. Proceeding without an HLA-matched sibling donor is a much murkier issue. More traditional approaches to Fanconi anemia treatment involve

the use of androgens [137, 138] and hematopoietic growth factors [139, 140]. Androgens are not a long-term solution, because of the likelihood of the patient developing toxicities, including secondary liver tumors, and the precocious development of secondary sexual characteristics. Growth-factor administration in the setting of bone-marrow stress raises the specter of leukemia stimulation. Recent trials to use unrelated HSCT donors are under way, but initial results remain mixed [134].

Shwachman–Diamond syndrome

Shwachman–Diamond syndrome (SDS) is a syndrome of neutropenia, pancreatic exocrine function insufficiency, and metaphyseal dysostosis. The neutropenia can be relatively mild, intermittent, or cyclical. Neutrophil functional defects have been described in these patients. Anemia and thrombocytopenia can also accompany the neutropenia in about 33% and 20% of cases, respectively. Pancytopenia develops over time in 20% of patients, with a median age of onset of three years. SDS patients present in the neonatal period with signs of failure to thrive, including short stature, low birth weight, and abdominal distension. SDS patients can have additional physical findings, including skin rashes and macules, teeth and palatal defects, and syndactyly [44, 141–144].

SDS is an autosomal recessive disorder, and a candidate gene has been cloned. The SBDS (Shwachman–Bodian–Diamond syndrome) gene encodes a 250-amino-acid protein with predicted functions in RNA metabolism. Its widespread expression may be the basis of the multiple organ involvement of SDS [145].

No definitive therapy exists for SDS neutropenia, except for HSCT. Pancreatic insufficiency can be improved with the oral administration of exogenous pancreatic enzymes. Some efficacy has been reported with the use of granulocyte-colony-stimulating factor (G-CSF), with six out of seven treated patients responding to G-CSF. Supportive care is indicated with transfusions in the 20% of SDS patients who develop pancytopenia. In patients with SDS, there is an increased risk of developing leukemia, including ALL, chronic myelogenous leukemia (CML), and AML, with reports putting the risk at about 5%. The increased risk of developing leukemia makes the decision to use growth factors more difficult for fear of stimulating a clonal expansion [146–150].

Dyskeratosis congenita

Dyskeratosis congenita is another rare disorder of marrow failure. It is comprised of physical findings including hyperpigmented skin, dystrophic nails, leukoplakia, and eye abnormalities. Dyskeratosis congenita appears to be usually an X-linked disease, with a minority of cases presenting a pattern of autosomal dominant or recessive inheritance. Fifty percent of patients go on to develop bone-marrow failure, with a median age of onset at 13–15 years. Dyskeratosis congenita is rarely diagnosed in the neonatal period. While not typically diagnosed in the neonatal period, the outward skin and nail signs are seen at a wide range of ages, including infancy. A particularly severe variant, Hoyeraal–Hreidarsson syndrome, is a systemic dyskeratosis congenita with cerebellar and developmental dysfunction that begins prenatally. Reported cases have included probands with intrauterine growth retardation and microcephaly. Dyskeratosis congenita patients clearly have a predisposition to myelodysplasia, myeloid leukemia, and various solid tumors [67, 151–155].

Cells from patients with dyskeratosis congenita are similarly hypersensitive to DNA-damaging agents as Fanconi anemia cells, but they display no chromosomal breakage [156, 157, 158]. At least one putative gene responsible for dyskeratosis congenita, DKC1, has been cloned. The coded protein, dyskerin, is a 57-kDa nucleolar protein involved in ribosome RNA synthesis [159–161].

Treatment for dyskeratosis congenita, as in the other marrow-failure syndromes, is initially supportive in nature, including transfusions as needed. The potential long-term treatment includes HSCT. HSCT in dyskeratosis congenita has an increased

risk of pulmonary complications because of the association of pulmonary disease with dyskeratosis congenita [156, 162–165]. The use of growth factors has achieved some temporary success, but the worry of malignant transformation in stressed marrow remains a concern [166, 167].

Reticular dysgenesis

This extremely rare disease presents almost exclusively in the neonatal period with leukopenia with both neutropenia and lymphopenia. Anemia is also common but thrombocytopenia is not. The bone-marrow examination reveals absence of myeloid and lymphoid precursors but usually not complete marrow aplasia [168–171]. Lymphoid tissue is generally absent, with an absent thymus in the chest radiograph. Early progenitors are probably the defective cells, since two distinct lineages are affected.

As reticular dysgenesis patients act clinically like severe combined immune deficiency patients, HSCT has been attempted, with mixed results [172, 173].

Amegakaryocytic thrombocytopenia

Amegakaryocytic thrombocytopenia (AMT) is discussed in greater detail in Chapter 10. It is mentioned here because it presents in the neonatal period and a large percentage of patients develop bone-marrow failure. These patients present with isolated hypoplastic thrombocytopenia and either no physical abnormalities or findings distinct from Fanconi anemia or thrombocytopenia and absent radii (TAR) syndrome. Such developmental defects include microcephaly, low birth weight, developmental delay, cardiac defects, central nervous system defects, and orthopedic anomalies. A familial pattern has emerged in these patients that fits both autosomal recessive and X-linked inheritance. Forty percent of patients develop pancytopenia along a variable timeline. These patients are also at an increased risk of developing leukemia. Corticosteroids have been used with limited success, and little HSCT experience exists in this disease [44, 67, 174–176].

Kostmann syndrome

Severe congenital neutropenia

Classical Kostmann syndrome is an autosomal recessive disease with severe neutropenia presenting in infancy. Severe congenital neutropenia (SCN), which has the same clinical features as classic Kostmann syndrome, can present also as a sporadic disorder or an autosomal dominant disorder. Patients present with recurrent life-threatening infections in early infancy. Multiple organisms may be involved. Increases in the monocyte and eosinophil counts are common. While anemia may be present, it is thought to be secondary to chronic infection as opposed to failure of erythropoiesis. Untreated, the disease is usually fatal. Myeloid precursors are notably lacking or decreased in the bone marrow. No cases of pancytopenia have been reported [44, 67].

Mutations in the G-CSF receptor have been found in Kostmann syndrome patients and have been associated with progression to leukemia [184, 185]. Neutrophil elastase deficiency does not appear to be associated with classical autosomal recessive Kostmann syndrome, while mutations have been found in cases of sporadic and autosomal dominant congenital neutropenia [177]. At present, there is no candidate gene for classical autosomal recessive Kostmann syndrome. Therapy with G-CSF has dramatically altered the outcome for patients with Kostmann syndrome: the great majority of patients respond to G-CSF therapy with an increase in the neutrophil count and a decrease in the rate of infections [44, 67]. Several case reports suggested an increased risk of developing leukemia in patients with SCN treated with G-CSF. The analysis of the Severe Congenital Neutropenia International Registry shows that 12% of treated patients have developed leukemia to date [178]. However, terminal leukemia in SCN had been reported before the use of G-CSF therapy [179–182]. It is unclear whether the therapy increases the risk of leukemia or whether the increased survival allows the development of leukemia in a cancer-prone disorder. Increased somatic mutagenesis has been demonstrated in SCN. The first of these mutations were mutations

in the *ras* gene [183]. This increased risk of developing leukemia and its possible association with G-CSF therapy have tempered the enthusiasm for the use of G-CSF therapy in SCN. HSCT has been curative in some cases [186, 187] and offers a potentially curative therapy for those few patients who do not respond to G-CSF therapy or who develop leukemia.

Osteopetrosis

Osteopetrosis is a collection of genetic diseases marked by abnormal bone remodeling. Of the existing variants, the infant form is an autosomal recessive disease that is associated uniquely with hematological sequelae. This infant form is marked by abnormal bone resorption, resulting in macrocephaly, blindness, deafness, organomegaly, and sclerotic bones. The resulting abnormal marrow architecture results in a myelophthisic picture, with hypocellularity, leukocytosis, and signs of stress erythropoiesis [67, 188]. The anemia is typically macrocytic and the fetal hemoglobin is increased. The cause of infantile osteopetrosis appears to be a defect in the osteoclast precursor cell, which leads to the resulting abnormal marrow architecture. Infantile osteopetrosis appears to be multigenic. At least two genes have been associated with this form of the disease [189, 190].

These patients require transfusion therapy support until definitive therapy can be given. Only HSCT offers the hope of long-term cure, as donor cells replace hematopoietic precursors as well as osteoclasts, thought to be the agent of the primary disease [191]. A variety of agents, including calcitrol, erythropoietin, and prednisone, have been used, with variable success [192–195]. Interferon gamma has been the most efficacious in promoting bone-area decrease and improvement in blood cell counts [196].

Congenital dyserythropoietic anemia

Congenital dyserythropoietic anemia (CDA) represents a collection of rare genetic disorders accompanied by ineffective and dysplastic erythropoiesis with little or no abnormality in the other blood cell lines [197].

Type I

CDA type I is an autosomal recessive disorder. Its phenotype is characterized by the microscopic appearance of red cell precursors in the bone marrow. Erythroblasts are macrocytic and megaloblastic and have intranuclear changes, including chromatin strands, Swiss-cheese appearance, and bi- and tetranucleated normoblasts (<10%). Often, cytoplasmic organelles can be invaginated into the nucleus [198, 199].

The clinical picture of CDA type I includes mild macrocytic anemia with signs of hemolysis such as pallor and jaundice. Splenomegaly develops over time. This subtype has been reported in neonates and as a cause of in utero fetal loss. As a result, the neonate may have accompanying features of cardiovascular insufficiency and persistent fetal circulation and require transfusions, although this is not necessarily a permanent clinical requirement. Ineffective erythropoiesis and hemolysis may contribute to variable degrees of jaundice and subsequent cholelithiasis.

Children have been described with a number of associated congenital defects, including syndactyly, digit hypoplasia, short stature, ptosis, and deafness. These are often the first clues to the diagnosis, although they are not specific for CDA type I [200].

Iron overload is often a problem for these patients as they age as a result of increased iron absorption. Other measures of increased red-cell turnover are present, such as elevated lactose dehydrogenase (LDH) and bilirubin [201].

The gene for CDA I has been cloned and localized to chromosome 15q15.1–15.3. It encodes a 1226-amino-acid protein termed codanin-1. Although no firm function has been established for codanin-l, its homology to microtubule attachment proteins and the nuclear phenotype suggest a function in the maintenance of nuclear-envelope integrity [202]. Several other subjects have not had mutations of this gene, suggesting genetic heterogeneity in this disorder [203].

Treatment for these patients is supportive, with transfusion therapy when needed and phlebotomy or chelation therapy for iron overload. Interferon alfa-2 therapy has produced increased hemoglobin in some patients, although the basic morphological phenotype persists. No defect in the interferon signaling pathway has been noted in these patients [197, 204, 205].

Type II

CDA type II is an autosomal recessive disorder and is the most common of the three types of CDA. The characteristic bone-marrow finding is the presence of more than 10% binucleated erythroid precursors. In contrast to CDA type I, very few patients with CDA type II are diagnosed as infants, because the degree of anemia is often mild, but it can vary. Severe anemia requiring transfusions is seen in 15% of patients. The anemia is normocytic but macrocytosis has been observed. The peripheral blood smear often reveals poikilocyosis and tear-drop cells. These patients also have accompanying hyperbilirubinemia and splenomegaly [197, 206].

Under electron microscopy, the erythroblasts contain cisternae, double-membraned structures thought to be derived from endoplasmic reticulum. Classically, CDA type II erythrocytes lyze in 30% of acidified donor sera that contain IgM antibody against CDA type II-specific antigens not present in normal red cells. Hence, the term hereditary erythroblastic multinuclearity with a positive acidified serum lysis test (HEMPAS) is a synonym for CDA II. Often, multiple donors must be used to identify the lysis. CDA type II erythrocytes show increased agglutination with anti-i serum.

Thus far, the gene for CDA type II has not been cloned, although the genetic locus is thought to be at chromosome 20q11.2. Analysis of membrane proteins such as band 3 and band 4.5 suggest decreased glycosylation, but recent studies have excluded several galactosyl transferases from consideration as the CDA type II causative gene [207–209].

Type III

This rarest CDA type exists both as an autosomal dominant form and as a sporadic form. The clinical features overlap with the other CDA types, and at least one case has been described associated with in utero fetal loss. No gene responsible for CDA type III has been cloned, but the locus has been mapped to 15q22 [197, 210].

Morphologically, CDA III is distinctive because of the presence of giant multinucleate (up to 12 nuclei) and polychromatic erythroblasts in the bone marrow. Nuclear lobulation, intranuclear clefts, and cytoplasmic inclusions of beta globin are also unique features [211].

Other types

Additional subtypes of CDA (total of seven) have been described. CDA type IV is worth mentioning since it appears most often in infancy; this type is marked by erythroid hyperplasia, some nuclear irregularity, and lack of cytoplasmic inclusions. Only scanty data exist for most of these additional categories [197].

Treatment

Aside from supportive care, utilizing transfusions and iron chelation therapy, treatment options have been limited. Interferon has not been effective in types other than CDA I. Erythropoietin has not been efficacious. Extension of red-cell survival through splenectomy has been somewhat successful in decreasing transfusion requirements [197].

Sideroblastic anemia

Sideroblastic anemia is a dyserythropoietic anemia with erythroid hyperplasia. Its name comes from the pathognomonic finding in bone-marrow-aspirate slides stained for iron. This finding consists of rings of dots around the nucleus of bone-marrow erythroid precursors, composed of iron-laden mitochondria [212, 213].

Patients with sideroblastic anemia may have accompanying thrombocytopenia and neutropenia [214]. The disorder may be either X-linked or autosomal (both dominant and recessive). It also may occur sporadically, and is associated with a variety of drugs, including alkylating agents, ethanol, isoniazid, and chloramphenicol. Copper and pyridoxine deficiency have also been implicated in the onset of acquired sideroblastic anemia [213, 215].

Biochemically, patients present with elevated protoporphyrins, implying a defect in hemoglobin synthesis. An enzyme in that pathway, aminolevulinic acid synthase (ALAS-2), has been implicated as a cause of SA. Several probands have mutations in this gene, which is located on the X chromosome [216, 217]. The disorder may be multigenic. An additional gene with mutations associated with sideroblastic anemia on the X chromosome is the membrane transporter hABC7 [218]. Other, as yet unidentified, genes are thought to be involved in the autosomal recessive and dominant inherited cases [213, 215].

Idiopathic aplastic anemia

By definition, idiopathic cases of bone-marrow failure involve those without any ascribable etiology. In this era of ever-expanding diagnostic capabilities, the number of bone-marrow failure cases classified as "idiopathic" continues to shrink. For example, 2 of 17 cases classified previously as idiopathic were found to contain mutations in telomerase RNA [220] and no longer belong to the group of idiopathic cases. In addition, several cases of idiopathic aplastic anemia may be due to variable penetrance of the bone-marrow failure syndromes. In Fanconi anemia, a sizable minority of patients exhibit no stigmata and may be diagnosed erroneously as having idiopathic aplastic anemia. Therefore, the number of cases that can be termed idiopathic is real but probably cannot be estimated adequately. Aplastic anemia in the general population is a rare disorder, with a frequency in the order of a few cases per million [219]. The frequency in infants is even lower.

Thrombocytopenia with absent radii syndrome

TAR syndrome is an autosomal recessive disease. Contrary to Fanconi anemia patients, TAR patients have absent radii but the thumbs are preserved. Thrombocytopenia is usually evident from birth and almost certainly by four months of age and is accompanied by significant hemorrhagic events. Megakaryocytes are markedly decreased or absent in the bone marrow, classifying the syndrome as a megakaryocyte progenitor disorder. Some patients exhibit a leukemoid reaction, but this subsides in infancy. Because the disorder does not have an increased risk of developing malignancy and does not have genomic instability, and there have been no reports of Fanconi anemia mutations, TAR syndrome is not likely to be an overlap syndrome with Fanconi anemia [67, 221–223].

Platelet counts improve after infancy; thus, patients do relatively well after the first year of life, requiring platelet transfusions usually only during infancy. TAR syndrome is discussed further in Chapter 10.

Other rare disorders involving marrow failure in infancy

A number of inherited disorders can present in infancy with one or more cytopenias. While some are well-described syndromes, others may have overlapping signs and symptoms with other diseases.

Dubowitz syndrome is an autosomal recessive disease with growth failure, developmental delay, and pancytopenia. Intrauterine growth retardation may also be seen. In some patients, pancytopenia develops within the first few years of life. This disorder also has an increased risk of developing malignancy. It has been lumped with Fanconi anemia from time to time by virtue of increased chromosomal breakage induced by DNA intercalating agents [224–228].

Seckel syndrome is an autosomal recessive disease marked by growth retardation, developmental delay, microcephaly, and characteristic facies. Some patients have developed pancytopenia. As in Dubowitz syndrome, some variable degree of

chromosomal breakage can occur, which is suggestive of Fanconi anemia. However, other patients with Seckel syndrome have normal results with the Fanconia anemia chromosomal fragility test. The risk of developing malignancy appears to be increased [229–231].

Noonan syndrome consists of a constellation of physical findings, including typical facies, growth failure, and heart defects, which resemble Turner syndrome. Pancytopenia or isolated amegakaryocytic thrombocytopenia occur to a variable degree. A myeloproliferative disorder of infancy, with or without spontaneous remission, can occur, further supporting the classification of Noonan's disorder as a bone-marrow-failure disorder [232–234]. Mutations in the gene for protein tyrosine phosphatase 2 (SHP2), localized to chromosome 12q24.1, appear to be responsible for Noonan's syndrome [235–237].

Down syndrome is associated with a multitude of hematological abnormalities. The most prominent neonatal manifestation is the transient myeloproliferative syndrome. However, at least one case report exists of a neonate with Down syndrome developing aplastic anemia [67, 238].

Familial marrow syndromes represent a miscellaneous grouping of marrow-dysfunction diseases with variable genetic transmission. Clinical features often overlap with other known more defined diseases. The reported autosomal dominant variants are phenotypically similar to Fanconi anemia, with characteristic radial and thumb abnormalities, but the degree of cytopenia is variable and increased sensitivity to DEB or MMC is absent [239, 240]. Other autosomal recessive forms may resemble Shwachman–Diamond syndrome or Diamond–Blackfan syndrome and have even been associated with xeroderma pigmentosum. Still other X-linked or sporadic cases have been associated with lymphoproliferative disease, Friedreich's ataxia, Brachmann–de Lange syndrome, and multiple medications [67].

Transcobalamine II deficiency

Transcobalamine II (TCII) deficiency is a disease of deficiency of transport of vitamin B12 into the bloodstream [241–243]. Normally, TCII ferries cobalamine in the circulation after absorption from the gut. In TCII deficiency, severe anemia, pancytopenia, and isolated diminished erythropoiesis may result, usually at earlier times than in patients with nutritional deficiency of vitamin B12. As is the case with B12 deficiency, neurological abnormalities may accompany the hematological abnormalities, particularly if the patient is treated with folate only [244].

Table 5.4 Aplastic anemia work-up

Complete blood count
Manual examination of peripheral smear
Bone-marrow aspiration and biopsy
Diepoxybutane chromosome fragility test
Genotype analysis
Cytogenetics
Flow cytometry
Viral serologies
Viral cultures

Work-up of a neonate with aplastic anemia

The clinical signs of pancytopenia include infection, bruising, and pallor. The initial work-up begins with a complete blood cell count and careful review of the peripheral blood smear (Table 5.4). Isolated cytopenias and pancytopenia are frequent manifestations of severe illness in the neonate, particularly manifestations of sepsis. The presence of vacuolated immature myeloid precursors with Dohle bodies and toxic granulation suggests sepsis. The approaches to isolated cytopenias in the neonate are discussed in Chapters 3, 10, and 11. For persistent or otherwise unexplained pancytopenia or evidence of hypoproductive disorder of a single blood cell line, a bone-marrow aspiration and biopsy should be performed. A technique for performing bone-marrow aspirates and biopsies in the newborn has been described [245]. It is mandatory that a careful microscopic analysis be performed by an expert in neonatal hematology. In addition to histological examination of

the bone marrow, studies to consider include the following:

- *Cytogenetics:* Culturing of mononuclear cells and examination of chromosomes for cytogenetic abnormalities is critical in the evaluation of aplasia. An abnormality indicative of malignancy, myelodysplasia, genetic syndrome (e.g. Down syndrome), or clonal process would alter therapeutic interventions.
- *Diepoxybutane chromosome fragility assay:* This assay involves the incubation of a mononuclear fraction of blood cells in the presence of a bifunctional alkylating agent, i.e. an agent that can cause an inter- or intrastrand DNA cross-link. The cells are then treated with colchicine to arrest them in mitosis and are dropped on to a slide for a karyotype-like analysis. Increased chromosome breaks in this setting are pathognomonic for Fanconi anemia. The DEB test is crucial because many Fanconi anemia patients bear none of the classic physical findings of the disorder.
- *Flow cytometry:* Analysis of mononuclear cells by flow cytometry to detect the presence of particular antigens, e.g. CD55/OD59 to rule out paroxysmal nocturnal hemoglobinuria, can be very helpful in the work-up of aplasia in older children with marrow failure. Its role in neonatal aplasia is unclear.
- *Genotype analysis:* Our understanding of the genetic causes of inherited bone-marrow-failure disorders is expanding. Genotype analysis of infants with these syndromes can be helpful for genetic counseling and to improve understanding of the disorder. For example, six Fanconi anemia genes have been cloned, and retroviral gene correction is available. In vitro, these techniques can establish the genetic complementation subgroup for Fanconi anemia as well as focus the search for mutations. Once the mutation is described, effective genetic counseling may be provided for the family. While in most instances this analysis is used for research purposes, the clinician might wish to include affected infants in available studies. As stated above, this may be helpful both in the understanding of the disease process and, if a genetic mutation of a disorder is found, in genetic counseling.
- *Infectious etiologies:* A multitude of diagnostic tests for infectious agents associated with bone-marrow suppression are available. Those associated with intrauterine infection are discussed in Chapter 2. These include antibody titers for suspected etiologies (both IgM and IgG), viral/bacterial cultures, and polymerase chain reaction (PCR) detection of pathogens.
- *Research:* Given the rarity of bone-marrow-failure syndromes in infants, the physician should consider contacting centers or physicians specialized in particular disorders, such as the International Fanconi Anemia Registry, the Diamond–Blackfan Anemia Registry, or the Severe Congenital Neutropenia International Registry, when working up a neonate for these disorders. The doctor should also consider the collection of additional blood or bone-marrow samples for research purposes (after obtaining appropriate approved consent) in consultation with a recognized expert in the disease under consideration.

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6

Hemolytic disease of the fetus and newborn

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Introduction

The fetus has a special immunological relationship with the mother that prevents rejection despite it being an allogeneic tissue [1–4]. If fetal blood cells enter the maternal circulation, the peaceful coexistence may be disrupted. The clinical problem of maternal antibody-mediated fetal red-blood-cell (RBC) destruction was a powerful stimulus to the acquisition of knowledge of the RBC antigen systems [5]. The triumphs of Landsteiner, Wiener, Levine, Darrow, Diamond, Chown, Liley, Clarke, Freda, Bowman and many others were among the great accomplishments of twentieth-century medicine [6]. Additionally, the assessment and management of hemolytic disease of the newborn (HDN) was a stimulus to progress in perinatal care in the second half of the twentieth century, including advances in fetal monitoring, in utero intervention, Cesarian section, neonatal resuscitation, and neonatal intensive care [7, 8]. A study of the interactions of RBC antigens with the maternal immune system and the fetal response to anemia can provide tremendous insight into systems of fundamental importance to neonatal health and disease.

The term "hemolytic disease of the newborn" was chosen to replace the term "erythroblastosis fetalis" when the mechanism of fetal anemia and neonatal jaundice was determined [9]. It was intended to name the maternal antibody-mediated fetal hemolytic disease, which, in these investigators' subjects, was the dominant etiology of fetal hemolysis and is a major cause of HDN worldwide. RhD continues to be the most commonly identified antigenic stimulus to HDN [10]. RhD-negative mothers give birth to RhD-positive fetuses in about 9% of European-ancestry pregnancies. In a first pregnancy, without prophylaxis, 15% of these at-risk mothers would become immunized against RhD and 0.7% of the infants will have HDN. Second and later pregnancies with the same maternal fetal antigen status have a higher frequency of affected infants. Overall, among women with established anti-D, 50% of RhD-positive babies with HDN will have minimal disease requiring no intervention, 25% will have disease requiring intervention after birth to prevent kernicterus and the effects of significant anemia, and 25% will require in utero intervention to prevent severe morbidity or mortality [11]. The case fatality rate for RhD HDN in 1986 in the USA was 2.6% [12]. In 1998, the death rate attributed to HDN in the USA was reported to be nine infants per million live newborns [13]. During the period from 1970 through the late 1990s, the use of prophylactic anti-D antibody increased and the frequency of RhD HDN decreased dramatically [10, 14, 15]. Unfortunately, at the same time, there has been a gradual increase in the frequency in HDN due to other antigens [10, 16]. The principal antibodies in maternal serum that are evaluated during pregnancy as possible agents of HDN are listed in Table 6.1. This chapter will review the current understanding of the biochemistry and genetics of the RBC antigens that have some frequency of involvement with HDN, the process of maternal antibody development and fetal RBC destruction, management of the affected fetus and neonate,

Table 6.1 Maternal antibodies

Associated with severe HDN
RhD, Rhc, RhC, RhE,
Kell 1 [K], Kell2 [k], Js^b, Fy^a, M, S, U
Usually associated with mild to moderate HDN
ABO, Kp^a, Kp^b, LW, Jk^a, Jk^b, Jk^3, Js^a, Ula (Kel 10], Fy^3
Not associated with HDN
*Fy^b, *N, *s, P1, Le^a, Le^b, Lu^a, Lu^b

*One reported case of mild or moderate HDN.
Js^a mild only [359]; Js^b moderate [335] and severe [334, 360].
HDN, hemolytic disease of the newborn.
See text under the headings:
Kell system for Kell 1, 2, Jsa, Jsb, Ula.
Duffy system for Fy.
Kidd system for Jk.

and clinical issues associated with specific antibodies producing hemolysis.

Red-blood-cell antigen systems: biochemistry, genetics, and expression during erythropoiesis

The Rh antigens

The five principal Rh antigens are RhD, C/c, and E/e. The antigens are polypeptides that are encoded by two homologous genetic loci, the *RHD* gene and the *RHCE* gene. The protein products of the *RHD* and *RHCE* loci share 92% sequence identity [17]. Chimpanzee and gorilla have both *RHD* and *RHC* loci, but lower primates and rodents have only an *RHC* homologous locus [18]. Thus, the *RHD* locus appears to have arisen from gene duplication of an ancestral *RHC* gene. Only humans have the Rh E/e antigenic component on the RhC homologous protein. The structures of the Rh proteins are shown in Fig. 6.1 [19]. During development of erythroid progenitors from undifferentiated precursors, the RHCE antigen is expressed before the RhD antigen but after the Kell antigen (Fig. 6.2) [20]. The Rh proteins are expressed only in erythroid cells [21]. In fetal development, the RhD antigen can be detected on RBCs as early as the seventh week of gestation [22].

An individual who is described on the basis of the antibody–antigen reaction (serologically) as RhD negative lacks the D protein on their RBC surface. For clarity, the notation "RhD-negative" will be used in this chapter to describe this state. For many years, it was thought that a protein antithetical to RhD was present but beyond the current technology to detect it. The finding in 1991 that individuals who were serologically RhD-negative lacked the gene *RHD* clarified the picture [22, 23]. Thus, in contrast to the RhC and E antigens, there is no antithetical or d version of the D antigen, only an absence of D expression. In Caucasian populations, the lack of RhD expression is due consistently to gene deletion and occurs in 11–35% of Caucasian ethnic groups [23]. The RhD-negative phenotype is also found in Indoeurasians, native North Americans, and African-Americans with the same genotype as in Caucasians and is presumed to be on the basis of mating with the latter. However, the genetic basis for the RhD-negative phenotype is polymorphic in other ethnic groups. Among the 0.5–7% eastern Asian and southern African people who are serologically RhD-negative, as many as 66% have a grossly intact RHD gene [22, 24–29]. This is essential information for accurate DNA-based determination of RhD status.

RhD is a complex antigen that a current model describes as containing 37 epitopes, or antibody stimulating and binding sites [30]. A special situation is the RhD variant formerly termed D^u and now included in the group called weak D. Although the weak D phenotype was thought to be due principally to a decreased expression of a normal protein, molecular studies have shown that essentially all weak D variants have amino-acid differences from the normal RhD protein in addition to decreased protein expression [31]. Weak D is a highly polymorphic group with variation in both antigen number per RBC and in the proportion of the RhD epitopes that are included in their variant RhD protein. Routine serologic testing may identify these people as RhD-negative. The number of RhD antigen sites on their red cells that can be detected by flow cytometry is about 10% of the normal number [32, 33]. Weak D can be detected with high-titer antisera [34], but

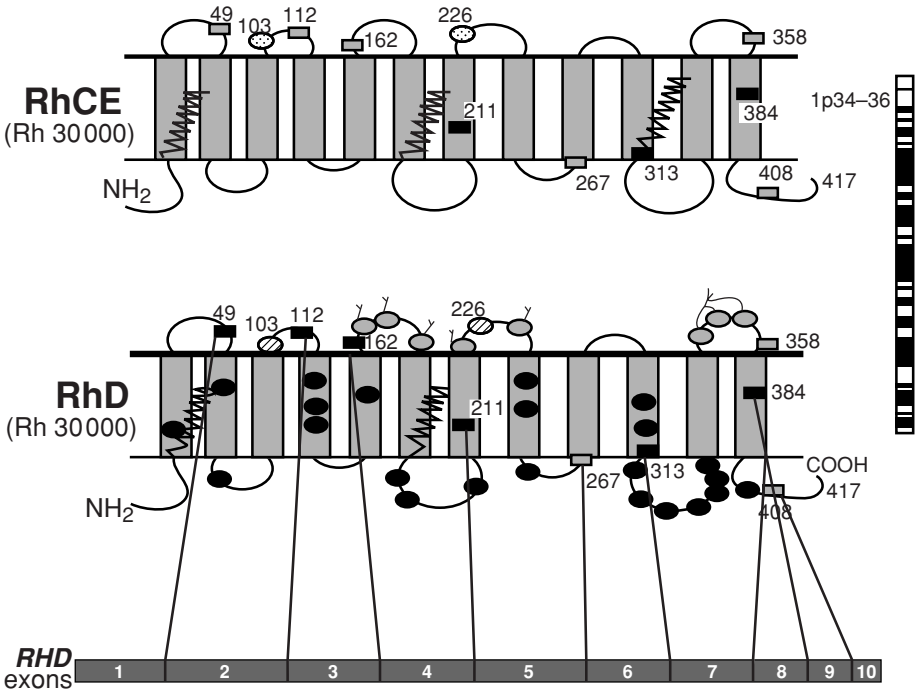

Fig. 6.1 Model of topology for RhCE and RhD. RhCE and RhD (M_r 30 000) are encoded by the genes *RHCE* and *RHD*, which are adjacent on chromosome 1p34–p36. The domain of the RhD protein encoded by each exon is depicted by numbered boxes, which represent the start and finish of each exon. Of the D-specific amino acids, 8 are on the external surface (gray filled ovals) and 24 are predicted to reside in the transmembrane and cytoplasmic domains (black ovals). Dotted ovals represent amino acids that are critical for C/c (Ser103Pro) and E/e (Pro226Ala) antigens; hatched ovals represent Ser103 and Ala226 on RhD. The zigzag lines represent the Cys–Leu–Pro motifs that are probably involved in the palmitoylation sites. Reprinted from Avent, N. D., Reed, M. E. [19] with permission.

the use of a reagent to detect weak D expression is considered optional in prenatal screening [35]. Most individuals with weak D will not form anti-D antibodies if transfused with RhD blood, and their RBC will not elicit an antibody response if transfused into RhD-negative recipients [33]. However, there is at least one case of a mother with weak D having a fetus severely affected by RhD HDN [36].

The E/e antigens are encoded by the *RHCE* locus. The antigens encoded by the antithetical E/e alleles differ by a single amino acid at position 226 in exon 5 with an alanine for [e] and a proline for [E]. The C/c antigen is encoded by the same locus. It was thought originally to be carried on an alternatively spliced, truncated protein, but subsequent studies showed that the full-length RhCE protein also encodes the C/c antigen [37–40]. The C/c antigens diverge by four amino acids located in the first two exons of the RhCE protein, but it appears that only one of the four amino acids is a part of the antigenic surface. The other amino acids may affect the folding of the molecule and maintenance of the antigenic conformation [19]. RhC^w, an antigen associated with HDN, is a mutated form of RhC, differing by a single amino acid [41].

The RhD and RhCE proteins are part of a membrane complex that includes the Rh-associated glycoprotein [RhAG], CD47, the LW antigen, and glycophorin B [19]. The RhAG component of this complex has been shown to function as a cellular

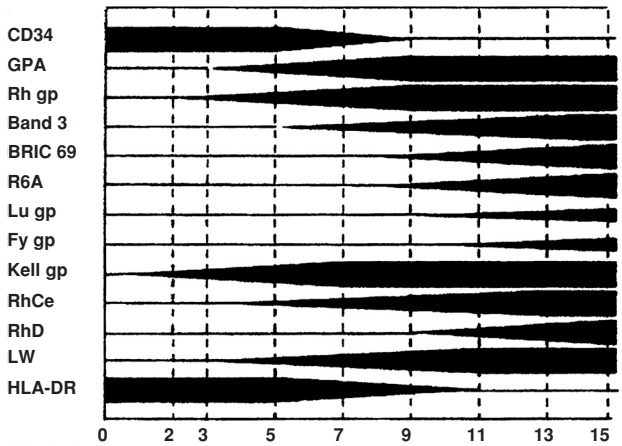

Fig. 6.2 Diagrammatic representation of red-blood-cell antigen expression of umbilical-cord blood cells in culture, by day of sampling. The thickness of the bar represents the percentage of positive cells for that marker, with full thickness representing 100%. Reprinted from Southcott, M. J., Tanner, M. J., Anstee, D. J., with permission [20].

transporter of ammonium in experimental systems [42]. Each of the Rh antigens [D, C, c, E, e], the Rh variants C^w, C^x, E^w, Ce^s, G, and the Rh-related antigen LW have been implicated as causes of significant HDN [43]. The Rh null phenotype appears serologically the same as Rhcde but is characterized hematologically by normochromic, hemolytic anemia and complete lack of the Rh antigens in the RBC membrane [44].

The Rh genes *RHD* and *RHCE* are separated by a very short span on chromosome 1, such that recombination between these genes is extremely rare [45–47]. The RBC antigen genes, including the Rh genes, are expressed in a codominant fashion. Thus, although individuals who inherit the heterozygous genetic endowment of RhD-negative/RhD-positive are serologically "Rh positive," they have about half the antigen density of individuals who are RhD/D or homozygous for RhD [48, 49].

ABO

The ABO antigen system, which was the first RBC antigen group to be identified, is comprised of carbohydrate molecules. Historically, these antigens were the greatest barrier to safe transfusion, and they remain a potential cause of major hemolytic transfusion reactions. Type A is due to inheritance of the ABO gene which codes for the enzyme *N*-acetylgalactosaminyl transferase (A transferase); type B is due to inheritance of the ABO gene coding for the enzyme galactosyl transferase (B transferase); and type AB individuals have inherited a copy of each allele. The A and B transferases are alleles that differ by four amino acids. Type O individuals are homozygous for an ABO gene that has a single nucleotide deletion resulting in a frameshift mutation and coding for a protein without enzymatic activity [50]. The A antigens (poly-N-acetyl-galactosaminyl) and B antigens (poly-galactosyl) are added enzymatically to a substrate, the H antigen, which is comprised of L-fucose. Since the H antigen is required for A or B polysaccharide chain attachment, the rare patients who lack the enzyme L-fucosyltransferase (H transferase) also lack the A and B antigens [50]. Those individuals who have no H transferase activity will make antibodies against the A and B antigens but will also produce antibody to the H antigen. Such people are described as having the Bombay red-cell phenotype and can be transfused safely only with scarce H antigen-negative blood. The ABO antigens are expressed on many tissues, including placenta, salivary glands, vascular endothelium, pancreas, kidney, and epithelium of the gastrointestinal tract, and as secreted antigens [51]. Individuals who do not express A or B antigens and are immune-competent develop antibody against the unexpressed antigen by the end of the first year of life [52].

Kell

The Kell antigen system was named after the woman in whom the antibody was first identified [53]. The 15 Kell antigens and their antithetical variants are on a single glycoprotein that contains multiple antigenic faces [54]. Although the majority of these antigens are without clinical relevance to newborns, antibody to Kel 1 (K), the antithetical variant of the common Kel 2 (k), is a significant cause of HDN [55, 56]. The difference between these two forms is a single base

pair, which yields both an amino-acid change from methionine (k or Kel 2) to threonine (K or Kel 1) and loss of an *N*-glycosylation site [57]. Two other sets of antithetical antigens on this glycoprotein also vary by a single amino acid. The variants Kel 4 (Kp^b), Kel 3 (Kp^a), and Kel 21 (Kp^c) differ only at amino acid 281, and Js^a (Kel 6) and Js^b (Kel 7) antigens have an amino-acid difference at number 597 [58]. The Kell antigens Kel 1, Kel 2, Kel 3, Kel 4, Kel 6, Kel 7, Kel 10 [Ula], and K_o [null] have been reported to be involved in mild to severe HDN (see Table 6.1). The Kell glycoprotein is the first erythroid-specific antigen known to be expressed during erythroid development from multilineage precursors (Fig. 6.2) [20]. It is expressed by these precursor cells at a stage of high proliferative potential. Thus, antibody targeted against this antigen can profoundly affect RBC production. This observation helps to explain the manifestations of anti-Kell HDN in which anemia and reticulocytopenia may be more severe, and hyperbilirubinemia may be less severe, than when other antigens are involved [59–61].

Duffy (FY)

The Duffy antigen is a red-cell membrane glycoprotein that has stimulated great interest due to its function as an attachment site for the malaria agent *Plasmodium vivax* [62, 63] and as a receptor for multiple chemokines [64, 65]. There are two principal forms, Fy^a and Fy^b, which are codominant and differ by a single amino acid [66]. The null variant genotype *FY/FY* and phenotype Fy (a−b−) is important because it is found in 95% of West Africans and 68% of African-Americans [64]. The lack of erythroid expression of Fy^a and Fy^b in *FY/FY* individuals is considered to be among the many RBC adaptations to the environmental pressure of malaria [67]. The molecular basis for the phenotype Fy (a−b−) (lack of RBC Fy a and Fy b) is a single base-pair change in the promoter region at the binding site for the hematopoietic transcription factor GATA-1 [68, 69]. This is an example of tissue-specific gene regulation, as the Duffy antigen is expressed in nonerythroid tissues, including those with the *FY/FY* genotype, apparently under control of other transcription factors [70]. The Fy antigen is expressed very late in erythroid development (Fig. 6.2) [20]. Antibody to Fy^a has been implicated in significant HDN [71, 72]. A single case of anti-Fy^b-associated HDN has been reported [73]. Another distinct antigenic site, Fy3, is absent in individuals who are Fy (a−b−) but detectable on those who are Fy (a+b−) and those who are Fy (a−b+). Anti-Fy3 has been associated with alloimmunization and, very rarely, with moderate HDN [74].

Kidd (Jk)

Kidd antigen is a glycoprotein that functions as a constitutive urea transporter in RBCs and kidney [75, 76]. The common antithetical variants are Jk^a and Jk^b, which differ by a single base pair and amino acid [77]. The Kidd glycoprotein carries an additional antigen, Jk^3, which appears to generate an antibody response only in Kidd null Jk (a−b−) recipients. Anti-Jk^3 will react with all Jk^a- or Jk^b-positive cells. Jk (a−b−) is uncommon, except among people of Polynesian ancestry [78]. Each of these three epitopes has been associated with HDN requiring intervention in a small number of cases [79–81].

MN Ss

The antigens of the MN and Ss blood group system are glycoproteins carried by the glycophorins A and B, respectively [82]. Glycophorin A (GPA) expression is limited to erythroid cells. There is considerable homology between the two proteins, and the first 26 amino acids of the glycophorin B (GPB) and the N variant of GPA are identical [83, 84]. The function of GPA remains speculative, but it may be related to two observations. Ligand binding by GPA results in as much as an 18-fold increase in RBC membrane rigidity. It is hypothesized that bacterial antigen binding by GPA may result in clearance of the stiff RBC during transit through the reticular endothelial system. In addition, GPA is the principal carrier of membrane negative charge [85]. There is no known function of GPB. The M and N antigens are antithetical and codominant, as are the S and s antigens.

The 26-amino-acid identity at the N-terminal region of GPA and GPB explains the finding that individuals who are M+N− react weakly with anti-N unless they are also negative for S and s. The GPB molecule also carries the U antigen, which may be present even among those lacking both the S and s antigens. During erythroid maturation, GPA is detectable shortly after the Kell antigenic group (Fig. 6.2) [20]. In rare cases, antibodies to M [86, 87], N [88], S [89], and U [90] have been associated with significant HDN.

The process of fetal hemolysis by maternal antibody

Maternal exposure to fetal red-blood-cell antigens

Once immunoglobulin G (IgG) antibody production is established against the highly immunogenic molecules commonly involved in HDN, it is irreversible [91, 92]. Thus, prevention of HDN requires avoidance of maternal exposure to nonshared fetal RBC antigens. Fetal-maternal hemorrhage (FMH) is the most important mechanism of maternal immunization to fetal RBC antigens, but exposure by prior maternal RBC transfusion has a significant role. More effective medical care for a range of life-threatening conditions in childhood, including congenital disorders, severe trauma, and cancer [93], has resulted in improved survival of multiply transfused children. RBC antigen-matching for routine transfusion typically is limited to ABO and RhD. Thus, women with a history of transfusion have an increased risk of developing a high-titer, non-RhD antibody during a second antigen exposure with pregnancy [94–97]. Transfused women of childbearing age do not comprise a large proportion of the population, but they comprise 50% or more of mothers of infants with HDN due to non-RhD antibodies. This has led authors to recommend RBC matching for Rh Cc and Ee antigens and for Kel 1 before RBC transfusions to all premenopausal females [10, 98]. Extended cross-matching would clearly result in increased initial costs, but no analysis of whether this would be cost-effective when considering the cost of managing sensitized pregnancies has been published [16, 96].

Maternal exposure to fetal red cells, in the absence of protection by antibody against nonshared fetal RBC antigens, continues to be the predominant mechanism of maternal sensitization [99]. Assays of fetal cells in maternal circulation or of maternal antibody response to fetal alloantigens have showed that FMH increases with gestational age. FMH can occur in the first trimester, but it is infrequent before the start of the third trimester [99–101]. Among primigravida women, less than 15% of all new anti-RhD antibodies detected before term are detected before 28 weeks' gestation [14, 99]. The association between week of gestation and increasing frequency of FMH is likely a direct effect of increasing fetal mass exerting greater force on placental tissue with normal maternal movement as well as increasing fetal blood volume. Events during pregnancy that are associated with an increased risk of FMH are described in Table 6.2 [102]. Knowledge of the association of FMH with these events during pregnancy is used to apply additional doses of anti-RhD for prevention of maternal immunization. FMH occurs most frequently at delivery [14]. Cesarian delivery had been considered an increased risk for FMH, but two studies have found no significant difference in the frequency of FMH between groups of mothers following Cesarian delivery compared with vaginal delivery [103, 104].

The maternal antibody response

The capacity of a RBC antigen to elicit an antibody response is dependent on the antigen composition [105] and the amount and frequency of antigen exposure [94, 106] and may be affected by the genotype of the recipient's immune-response molecules [107] and the genotype of the donor/recipient antigenic pair [108]. The ability of an antigen to elicit antibody formation in a host is referred to as immunogenicity. An antigen that elicits a high-titer antibody response with a minimal exposure in a high

Table 6.2 Maternal risk factors associated with hemolytic disease of the newborn (HDN)

Known previous transfusions
Major medical events associated with transfusions in a subject who is not aware of prior transfusion
Prolonged hospital stay as a newborn
Major surgical procedure including repair of craniosynostosis, scoliosis, congenital heart disease, abdominal trauma, splenectomy for medical reason with possible associated chronic hemolytic disease
Childhood cancer diagnosis
Return to operating room within seven days after previous pregnancy
In RhD-negative mothers without anti-D administration, and in mothers with other antigen-negative set-up, the following obstetric complications: ectopic pregnancy, spontaneous or induced abortion, amniocentesis, chorionic villus sampling, cordocentesis, fetal version maneuvers, maternal abdominal trauma, placental abruption, fetal surgery, prenatal fetal demise
Prior pregnancy history
In utero transfusion
Early delivery for HDN
Maternal interventions, including intravenous immunoglobulin, plasma exchange
Prior infant requiring an exchange transfusion or phototherapy

proportion of exposed individuals is considered to be highly immunogenic. The molecular basis for relative immunogenicity of RBC-associated antigens is not understood well. Based on data of the frequency of antibody formation with exposure to antigen, RhD is the most immunogenic of the RBC antigens [109]. The importance of chemical structure to antibody–antigen interaction is demonstrated by rare individuals who have a single amino-acid change in their RhD protein and diminished interaction with standard antisera [110].

The antibody response to an initial exposure to antigen is formation of immunoglobulin M (IgM). This antibody production, however, is low-titer, short-lived, and of no consequence to the fetus, due to the inability of IgM to cross the placenta. With second exposure to a peptide antigen, or a large dose of an initial exposure, the IgM response will convert to an IgG response. The IgG response can be sustained, high-titer, and have the potential for further increase in titer and avidity with additional antigenic stimuli [111]. The sensitivity of the immune system to RhD is illustrated by the fact that multiple 0.01-ml doses of whole blood can be sufficient to immunize [112]. During pregnancy, a second maternal exposure to alloantigens may be from a previous pregnancy, multiple FMHs during a first pregnancy, or FMH preceded by antigen exposure from transfusion.

Without anti-RhD immunoprophylaxis, 1–2% of RhD-negative mothers of RhD-positive, ABO-compatible fetuses will have anti-RhD detectable at the end of a first pregnancy [113, 114]. At three to six months postpartum, 9% of these mothers will have a positive indirect antiglobulin test (IAT), with the increase attributable to immunization by FMH at delivery. During a second RhD-positive, ABO-compatible pregnancy, an additional 8% of RhD-negative mothers will have anti-RhD detected, consistent with secondary immunization following undetected primary immunization during the first pregnancy. Thus, 17% of mothers will be immunized following a first ABO-compatible, RhD-incompatible pregnancy without immunoprophylaxis [115]. Twenty percent of RhD-negative individuals will not make anti-RhD, even after high-volume multiple exposures. These people have been called nonresponders. The basis for this lack of response is not known. Although a genetic basis seems likely, no association with a specific human leukocyte antigen (HLA) type has been found [116].

The AB blood-group antigens are carbohydrates and a special case in the immunogenicity hierarchy. The antibody response to carbohydrate antigens

differs from the response to peptide antigens, in that it is T-cell-independent, principally of the IgM class, and usually not associated with an anamnestic response or development of higher antibody affinity with repeat exposure [117]. These concepts are familiar to healthcare providers to children from their experience with the evolution of carbohydrate antigens as immunogens for *Haemophilus influenza* and *Streptococcus pneumoniae* to the use of the more effective peptide conjugate vaccines for prevention of diseases [118]. Although high-titer antibody to AB antigens is present in all immune-competent AB antigen-negative individuals after 24 months of age, it is predominantly IgM [119]. The presence of the high-titer antibody to a carbohydrate antigen appears to be due primarily to the continuous antigenic stimulus via exposure to the AB blood-group epitopes that are also present on intestinal flora [51]. The A/B RBC antigens are an uncommon cause of HDN; when it does occur, it is exclusively among type O mothers. This is postulated to be due to the near identity of the A and B antigens preventing an IgG anti-A or -B response among types B or A mothers, respectively [120].

An important early observation regarding maternal antibody response was that type O RhD-negative mothers of type A or B RhD fetuses had a lower frequency of anti-RhD immunization [121]. This was subsequently confirmed by others [122–125]. Initially, there was controversy regarding the mechanism that decreased the frequency of immunization [126]. It is now known that the anti-A or -B agglutinating substance is an IgM antibody that can fix complement and lyse an RBC. Because of this ability, IgM is termed a complete antibody. Thus, the preformed maternal anti-A or anti-B, present in the sera of type O individuals, was in some way preventing anti-RhD antibody formation. The phenomenon of protection against RhD immunization by anti-A or -B also can occur for type A or B mothers, but the preventive effect is less. The observation that an agglutinating substance from blood could prevent immunization by a RBC antigen was a major component in the development of the use of anti-D therapeutically to prevent HDN [98].

The method of measuring antibody titer has changed considerably over time. The principal characteristics that determine antibody titer are the concentration of the antibody and the avidity of the antibody for the antigen. Originally, RBCs were suspended in saline and the test serum was added to the cell suspension in serial dilutions, with each test tube having half the serum concentration of the preceding tube. The single-cell suspension was then observed for a change to clumps of red cells due to the antibody in the test serum forming links between the individual cells. However, since the RBC negative charge tends to oppose the effect of the suspended IgG antibody to agglutinate the cells, this method detected only those sera with IgM as a component of the anti-RBC antibody. Due to the poor correlation of this technique with clinical outcome of transfusion or pregnancy, alternative methods for antibody detection were sought. Two commonly used alternatives are the albumin suspension and enzyme treatment of the RBC with bromelin or papain. Both of these have the effect of decreasing the effective negative charge repulsion by RBC and increasing the ability to detect IgG anti-RBC antibody. A modification to these assays is the addition of the reducing agents dithiothreitol or 2-mercaptoethanol to the serum to disrupt the disulfide bonds of the IgM molecules and, thus, to destroy their affinity for antigen. The resulting titer after addition of reducing agents is due solely to IgG. The approach to antibody titration recommended by the American Association of Blood Banks is the indirect antibody test (IAT), also known as the indirect Coombs' test [35]. Following the incubation of the test serum, or dilution of the serum, to RBCs, the cells are washed repeatedly with saline to remove nonspecifically bound antibody. The RBCs are then incubated with an antibody specific for the constant portion (Fc) of the IgG molecule and observed for agglutination. The titer determined by the IAT is typically two- to three-fold greater than the saline-, albumin-, or enzyme-treated methods [127]. Therefore, a titer of 1 : 8 by the albumin method would be expected to yield a titer of 1 : 32 or 1 : 64 by IAT. Due to the differences in titer with differences in technique, it is essential that the method for antibody titration

be specified when using or comparing results from different laboratories.

In addition to the amount and avidity of antibody produced, the IgG subclass that is produced can also affect hemolysis [116]. IgG1 and IgG3 can cause hemolysis secondary to their ability to be bound by Fc receptors, but IgG2 subclass binds only weakly to its Fc receptor, FcγRII, and neither IgG subclass 2 nor 4 causes hemolysis [128]. Almost all women with pregnancy-induced anti-RhD antibodies make IgG1. IgG3 also is found often, but usually with IgG1. IgG2 and IgG4 are found infrequently as anti-RhD and very rarely as the sole IgG subclass [129]. IgG2 is frequently produced as a response to T-cell-independent antigens, which include carbohydrates, as is found in the response to the ABO blood-group antigens [111]. IgG3 appears to be a more potent effector of hemolysis, since fewer bound molecules are required for red-cell lysis than with IgG1 [130].

Transport of antibody across the placenta

Immunoglobulin is transported across the placenta by an energy-dependent process that uses an Fc receptor, FcRn [131]. Immunoglobulin A (IgA) and IgM are not transported, since they are not bound by FcRn and there is no other transfer mechanism. Maternal IgG transfer appears to begin in the fifteenth week, but it is minimal to the twenty-second week of gestation in normal pregnancies. After the twenty-second week, IgG transport accelerates rapidly, such that the IgG concentration in term neonates equals or exceeds the maternal level [132]. This transport system, which is important as a part of the neonatal defense against infection, also moves maternal antibodies against blood-group antigens into the fetal circulation. Fetal IgG1 concentrations and the fetal maternal IgG1 ratio increase at a greater rate than that of IgG2, 3, or 4 [133]. At term, the IgG subclasses in the fetus are distributed as follows: IgG1, 66%; IgG2, 25%; IgG3, 6%; and IgG4, 3% [134]. The importance of the relative concentrations of IgG subclasses is in their varying ability to effect hemolysis, as described below. Rarely, decreased placental IgG transport may diminish the severity of, or eliminate, HDN [134].

Interactions of antibody with red-blood-cell antigens and the process of red-blood-cell destruction

Once antibody enters the fetal circulation, it is available for binding to RBC surface antigens. However, the AB, Duffy, and Kidd antigens are expressed on nonerythroid tissue. Adherence of antibody to these nonerythroid sites can act to decrease the effective hemolytic antibody titer to which the fetal RBC is exposed. The Rh, MN, and Ss antigens are expressed only on erythroid cells, and the Kell protein is expressed only on myeloid and erythroid cells [21, 135]. The exclusive hematopoietic expression of these antigens may be a factor in the observation of the greater proportion of immunized mothers having affected infants when antibodies to Rh or Kell are produced compared with those mothers with antibody to ABO or Duffy antigens. The timing during erythroid development of antigen expression has an effect on the manifestations of the hemolytic process, as described below for the Kell antigen system (Fig. 6.2) [20].

The placental barrier and decreased density of some fetal RBC antigens, compared with the adult, makes fetal hemolysis a special case of the general phenomenon of antibody-mediated RBC destruction. Antibody-mediated RBC destruction is achieved by the RBC binding of an antibody that is capable of initiating the complement cascade or of promoting cell-mediated cytotoxicity. A single IgM molecule can initiate the complement cascade. However, IgM does not cross the placenta and, therefore, is not involved in HDN. Although IgG can fix complement, this appears to be a rare event in the fetus. This is due to the requirement that two bound antibody molecules be bound by the C1q complement-initiating protein to initiate activation of the complement cascade [108]. The RhD antigen density is about 5% the density of adult RBC AB antigens [116], and the AB antigen density on fetal RBCs is 20–30% of that in the adult [136]. Therefore,

the necessary antigen proximity occurs rarely for RhD, and complement activation on fetal RBCs is an unlikely event. An additional modifier of anti-A/B IgG antibody-mediated hemolysis is the fact that of the total anti-A/B IgG transferred into the fetus, IgG2 comprises 25% by weight. However, IgG2 binds only weakly to its Fc receptor, FcγRII, and is ineffective in assays of hemolysis [128]. Finally, investigators using two types of transgenic mice, either lacking the ability to activate complement or lacking the Fc receptors for IgG1 (FcγRI) and IgG3 (FcγRIII), showed definitively that IgG-induced RBC destruction is dependent on Fc receptor activity, not complement [137, 138].

Fc receptor-mediated RBC destruction in the fetus typically occurs intracellularly, as indicated by the finding of anemia and hyperbilirubinemia in the absence of hemoglobinemia or hemoglobinuria, as would be expected with extracellular/intravascular hemolysis. In vitro data suggest that the dominance of extracellular destruction is due to the density of bound antibody and to the subclass of IgG bound. Increased bound antibody density and increased amount of bound IgG3 promote extracellular hemolysis, but lower density of bound antibody on the RBC and a higher proportion of the bound antibody as IgG1 promote intracellular RBC destruction [130, 139, 140]. In the rare reports of hemoglobinemia and hemoglobinuria accompanying HDN, the associated clinical problems include transient renal insufficiency, apparently secondary to a toxic effect of high plasma hemoglobin concentration at the renal distal collecting tubules, and disseminated intravascular coagulation (DIC) initiated by red-cell membrane fragments [141–143].

An additional factor associated with modulation of fetal RBC destruction by maternal antibodies was identified when a discrepancy was noted between the observed and predicted outcomes of a group of neonates at high risk for severe HDN. The mothers of these infants were found to have a high frequency of antibody against the paternal HLA class II, HLA-DR [144, 145]. Subsequent studies reported an association between decreased RhD HDN severity and maternal antibodies against class I or class II paternal HLA antigens, as well as a proposal to use this phenomenon therapeutically [146, 147]. A rabbit model of this approach has shown some efficacy [148]. Whether the inhibiting effect of the anti-HLA antibody is due to a steric effect on effector cell Fc receptors, or to another mechanism, is not known.

Interventions to prevent morbidity due to hemolytic disease of the newborn

Prenatal interventions

Prevention of maternal isoimmunization

When antibody immune suppression was first attempted, an impressive variety of schemes was considered to prevent maternal isoimmunization. The clear winner was the use of a single postnatal dose of intramuscular IgG anti-D [6, 149–152]. The mechanism of this protective effect has not been determined [153]. Hypotheses to explain the phenomenon include clearance of fetal RBC before antigen-presenting cell interaction, anti-RhD antibody blocking maternal immune system access to antigens, and the antibody–antigen complex having antigen-specific immunosuppression of the primary immune response [154, 155]. Any explanation for the prevention of maternal anti-RhD development by intramuscular injection of anti-RhD must account for the observations that the effective dose of anti-RhD will bind only about 20% of the RhD antigenic sites following an average FMH [154]; that there is a dose–response relationship between the amount of anti-RhD given, the RBC immunizing dose, and protection from development of anti-RhD [106]; that the Fc portion of the IgG molecule is necessary for its immunosuppressive effect [156]; and that one-third of a group of women given an appropriate amount of anti-RhD postnatally had half of the originally detected number of fetal cells present at five to six days after delivery but did not develop anti-RhD [157].

The application of the strategy of postnatal injection of anti-RhD resulted in a steady decline in

the frequency of anti-D in pregnant women and a concomitant decrease in the frequency of perinatal death due to anti-D HDN [12, 158, 159]. During the period from its introduction for widespread use from 1968 to 1983, the reduction in maternal immunization and perinatal death was about 90% for each. After 10–15 years of routine administration of postnatal intramuscular anti-D to RhD-negative and anti-RhD antibody-negative mothers, the immunization rate of at-risk mothers had decreased from 16% to 2%, and deaths due to HDN were decreased from 22–45 per 100 000 live births to 3.9–7.5 per 100 000 live births [14, 114, 159, 160]. This decrease was attained largely by the application of postnatal anti-RhD prophylaxis, but it was aided during that time by the birth pattern of a smaller number of second and subsequent pregnancies with the accompanying risk for repeat antigen exposure [161].

The persistence of new anti-D immunization following the initial success of postnatal prophylaxis was met with a re-examination of events that may result in FMH and maternal immunization [14, 113]. These efforts identified failure of application of the postnatal prophylaxis policy, failure of administered prophylaxis due to high-volume FMH, immunization in the early third trimester, and prior immunization as causes of ongoing identification of anti-RhD in the age of immunoprophylaxis. Clinical trials showed that the addition of a dose of anti-RhD at 28 weeks could further decrease the frequency of immunization ten-fold from approximately 2% with consistent postnatal prophylaxis to 0.2% with pre- and postnatal anti-RhD [113, 114, 162]. In addition, specific events during pregnancy, including abortion [163, 164], amniocentesis [165, 166], cordocentesis [167, 168], chorionic villus sampling [169, 170], antepartum hemorrhage, and blunt abdominal trauma [159, 171], were identified as increasing the risk of immunization to RhD. Subsequent studies showed a decrease in immunization when anti-D was given following amniocentesis [172] and pregnancy termination [173]. Policies were developed for additional immune prophylaxis to prevent immunization following the above specific high-risk events for FMH [102].

The consensus recommendation is for pregnant women to have an antibody screen and red-cell antigen typing for ABO and RhD performed at the first prenatal visit. Mothers without a significant antibody, but who are RhD-negative, may have a repeat antibody screen at 24–28 weeks and, if they remain anti-RhD negative, are recommended to receive a dose of anti-RhD as prophylaxis against prenatal sensitization [155, 174]. In 2001, the American Association of Blood Banks' (AABB) recommendation to repeat the antibody screen at 24–28 weeks in routine pregnancies was abandoned due to the low yield [35]. High-risk groups, including women with a history of transfusion, prior obstetric complications associated with a high risk of FMH, obstetric complications or procedures not treated with anti-RhD, and known IV drug users (due to sharing of needles and associated RBCs), were recommended to continue receiving a screening test for antibodies at the cusp of the second and third trimester. The Scientific Advisory Committee of the Royal College of Obstetricians and Gynaecologists and the Canadian Task Force on the Periodic Health Examination have not revised their guidelines regarding repeat antibody screen at 24–28 weeks. Additional anti-RhD is recommended in the event of obstetric complications associated with an increased risk of FMH [102]. Finally, a dose of anti-RhD is given following delivery [35, 175, 176]. The original immune prophylaxis, which was a single postnatal dose for RhD-negative women delivering RhD-positive infants, continues as the foundation of this approach. In countries where the supply of anti-RhD is limited, clearly the application of the postnatal dose will have the greatest impact. Table 6.3 gives a summary of recommendations for RhD prophylaxis from representative countries. The half-life of anti-RhD is 24 days; therefore, a repeat dose of anti-RhD is not given for a high-risk obstetric event if it occurs less than three weeks from a dose and it is not a high-volume FMH. If there is concern that the FMH is high-volume, then testing of maternal blood for fetal cells is indicated [174]. The prolonged circulation of anti-RhD may produce a weakly positive IAT in babies delivered within six weeks of a dose of anti-RhD. There is no apparent hemolytic effect of

Table 6.3 Anti-D dosing recommendations by country and timing

Reference	Country	Postnatal dose	Routine prenatal dose	Prenatal event dose
[361]	Canada	1500 iu with testing for volume of FMH	1500 iu at 28 weeks	250 iu with first-trimester events; 1500 iu for later events
[362]	Mexico	750 iu and 1500 iu for known FMH or multiple births	None	None
[363]	UK	500 iu with testing for volume of FMH	500 iu at 28 weeks and at 34 weeks	250 iu before 20th week; 500 iu all others with testing for amount of FMH
[364]	USA	1500 iu with testing for volume of FMH	1500 iu at 28 weeks	250 iu with first trimester events; 1500 iu for later events

1 μm = 5 iu anti-D. 500 iu given intramuscularly is capable of suppressing maternal anti-D development by a stimulus of up to 5 ml of RhD-positive FMH.
FMH, fetal-maternal hemorrhage.

maternally administered anti-RhD on fetal red cells [177]. To date, the preventive approach of maternal injection of antibody directed against an RBC antigen has been applied only to the RhD antigen due to practical considerations, but a similar strategy should be effective with other antigens.

The basis for the anti-RhD dose was the finding that 20 μg of anti-RhD is sufficient to protect from immunization when 1 ml of packed RBCs, equivalent to about 2 ml of whole adult blood, is introduced into the circulation [106]. Thus, the standard postpartum dose used in the USA of 300 μg is expected to prevent immunization when FMH is as much as 15 ml of newborn blood with a mean packed cell volume (hematocrit) of 61%, which is similar to the packed cell volume of transfused RBCs. This will prevent immunization in 99.8% of deliveries [178]. Higher volumes of FMH at delivery are associated with manual removal of the placenta, fetal distress in labor [87], abruptio placenta, placenta previa, and intrauterine fetal manipulation, as with fetal version or vaginal delivery of twins. However, using these events alone as screening criteria, a risk remains that a large-volume FMH, in excess of the amount covered by the standard dose, will be missed [103]. Thus, the recommendation from the AABB is that all RhD-negative mothers who deliver RhD-positive infants should be screened for FMH with a qualitative test, and that mothers with positive assays should have the quantity of the FMH determined [35, 175]. Knowledge of the quantity of FMH will then allow determination of the dose of anti-RhD required to prevent maternal immunization. Modification of the Kleihauer–Bethke technique for determination of cells with fetal hemoglobin continues to be used widely for this purpose [179]. Flow cytometry using an antibody against fetal hemoglobin is an alternative method, but it is not widely available [180].

A hypothesis, sometimes referred to as the grandmother theory, proposed that RBCs of RhD-positive mothers might sensitize their daughters who are RhD-negative in the perinatal period [181]. The finding that maternal cells enter the fetal circulation, just as fetal cells enter the maternal circulation, supported this concept [182]. An analysis of RhD-negative women who became immunized during their first pregnancy did not show an increased frequency of RhD-positive antigen status among their mothers compared with the general population [183]. Hence, this means of sensitization is considered to be, at best, very rare, and prophylactic anti-D is not given to RhD-negative female newborns of RhD-positive mothers.

Lastly, there is a disease-prevention approach that does not fit neatly into any other area: in vitro fertilization with preimplantation diagnosis and embryo selection. Although this is an extreme approach, for a sensitized woman with a history of severely affected fetuses, and a partner who is heterozygous for an antigen, it can be considered. The feasibility of this approach for RhD has been reported [184].

The decision to perform invasive monitoring and intervention

Women with a history of infants with significant HDN, as well as women with a newly detected antibody known to cause HDN, require close monitoring during pregnancy. Significant HDN is most often described as fetal hydrops or infants requiring in utero or exchange transfusion. In the context of a prenatal history to evaluate the potential for HDN, however, hyperbilirubinemia attributable to immune-mediated hemolysis should be included, since subsequent fetuses are at risk for more severe disease.

The goal of monitoring for HDN, as in all perinatal care, is a healthy newborn and mother. The challenge of monitoring is to balance the possible risk to the fetus of the hemolytic disease with the risk to the fetus and mother of intervention for diagnosis and treatment. Available monitoring techniques include serial testing of maternal antibody titer by the IAT or other techniques; functional assays of the maternal antibody; fetal biophysical profile, and Doppler blood-velocity studies by ultrasound; amniocentesis using optical density change of the amniotic fluid as an indicator of hemolytic activity in the fetus, and direct determination of fetal hemoglobin level by percutaneous umbilical-cord blood sampling. The approach to monitoring is determined by the antigen involved, the mother's history with prior pregnancies, the father's genotype, the location of the placenta within the uterus, and the gestational age at which the antibody is first detected. Table 6.1 lists antibodies according to the frequency with which they are implicated in HDN. The approach to mothers with antibody against the Kell antigens is a special case and is described in detail below.

An antibody screen may detect antibody at the first prenatal visit or at 24–28 weeks. Following detection of an antibody, the immunoglobulin class and antigen specificity are determined. If the antibody is an IgG, and it is against an antigen of known clinical significance, then close prenatal monitoring is initiated. This laboratory evaluation complements the maternal history as a means of identifying mothers for monitoring. Once a mother has had a fetus with in utero compromise, the severity of HDN in subsequent antigen-positive fetuses is fairly consistent. Therefore, all subsequent pregnancies are considered at risk for in utero compromise until proven otherwise [127]. For women with this history, a systematic approach to determination of fetal risk should be pursued during subsequent pregnancies. This same approach should be considered for mothers with clinically significant antibodies found on routine testing, since even an antibody titer as high as 1 : 256 alone is not a specific indicator of fetal antigen status [35, 185].

The first step in a systematic approach is to determine the fetus's risk for expressing this antigen, which can start with paternal testing. Provided paternity is not in doubt, a homozygous negative antigen status for the father can be the end of the evaluation. If the father is heterozygous for the antigen, or if there is uncertainty regarding paternity, then a fetal sample should be tested directly for inheritance of the antigen. Detection of fetal antigen status can be accomplished for most antigens using fetal DNA obtained by chorionic villus sampling or amniocentesis [186]. The former can be done earlier in gestation but may have a small increased risk of fetal loss [187, 188]. An analysis of published reports of RhD typing using amniotic fluid cells found a positive predictive value of 100% and a negative predictive value of 96.9% for RhD [189]. Maternal blood is also being developed as a direct source of genetic material from fetal cells separated by fluorescent activated cell sorting [190–195] and as a direct source of cell-free fetal DNA [196]. DNA testing requires careful interpretation. It should include methods to confirm the fetal origin of the DNA and two independent tests to determine the identity of the gene studied, as well as parental testing that is consistent with the fetal findings [197]. In situations in which fetal DNA cannot be obtained or does not yield a definitive conclusion, fetal blood sampling for direct antigen determination is considered. If a woman has previously delivered a fetus with significant HDN, and the fetus under evaluation is known to be antigen positive, then some authors recommend planning an invasive study in mid second trimester

rather than using the antibody titer or biophysical profile as indicators of the need for invasive studies [127, 198].

For a woman who is identified to be at risk for HDN based on an antibody screen result, IAT continues to be the test that is used most widely to determine whether invasive testing should be done. Serial antibody titers are performed at regular intervals, usually every two to four weeks in the second trimester and every one to two weeks in the last trimester [199]. Each laboratory that performs serial measurement of maternal anti-blood-group antibodies determines a critical titer for the antibody titer as an indicator for invasive fetal studies. The critical titer is defined as the lowest titer that has been associated with fetal compromise defined as hydrops fetalis, severe intrauterine growth retardation, fetal distress precipitating delivery, or cardiorespiratory compromise at the time of delivery [200]. The critical titer is specific for a given antigen, since it varies with the antigen. A frequently cited critical anti-RhD titer done by the IAT is 1 : 16 or 1 : 32 for red cells suspended in saline. An additional criterion that may be employed is the sequential increase in titer greater than two dilutions, e.g. 1 : 4 increasing to 1 : 32.

Many attempts have been made to improve the predictive power of the maternal titer. The Auto Analyzer, an automated instrument for performance of anti-RhD titer only, uses enzyme-treated RBCs and reports the titer as international units or as micrograms of antibody based on a polyclonal standard. The Auto Analyzer gave a marginal improvement over manual titration of maternal samples in predicting amniocentesis results. This device is used widely in the UK and is used in Europe and Canada, but it has not become popular in the USA [201–203]. The Marsh score uses grading of the IAT red-cell agglutination reaction from 0 to 12 for each tube, as compared with the conventional grading of negative, weakly positive, and 1+ to 4+. The total agglutination score for all tubes is added to determine the Marsh score [204]. A study compared anti-RhD titration and the Marsh score with fetal hematocrit values obtained by cordocentesis. Both the conventional grading of titer and the Marsh score showed a strong correlation with fetal hematocrit, but the only statistical difference between the two tests was a greater specificity of a Marsh score of 57 compared with a titer of 1 : 16. The titer remained 100% sensitive to detection of fetal anemia to a value of 1 : 128, at which point the specificity was 64% [185]. Flow cytometry and enzyme-linked immunosorbent assay (ELISA) both can be used to determine the immunoglobulin class and the amount of antibody bound to standard test cells [205–209], but their role as tools to aid in the decision to perform invasive procedures awaits prospective trials using large numbers of patient samples [210].

Another method to improve the sensitivity and specificity of prenatal testing is assays that mimic the in vivo process of antibody adherence to the red cell followed by Fc binding by receptor and, in some cases, hemolysis. These tests include chemiluminescence [211, 212], the lymphocyte (K) [213] or monocyte (M) [139] antibody-dependent cell-mediated cytotoxicity assay (ADCC), and the monocyte monolayer adherence assay (MMA) [214]. Although some of these techniques appeared to represent an improvement over antibody quantitation techniques, in most cases the improvement is modest and their application has been limited by the technical demands of the test. A report of a forum of nine program directors of referral centers from seven countries in 1995 found only the Netherlands routinely incorporating a functional assay and only with anti-D [215]. These Dutch investigators reported on their experience over a five-year period using the monocyte ADCC assay as part of the testing in RhD-alloimmunized pregnancies. They found that the ADCC assay value of 50% was 100% sensitive and gave a false-positive rate of 39% for anti-RhD, whereas the comparative IAT value of 1 : 128 gave the same sensitivity but a higher false-positive rate of 47% [216]. Interestingly, this value of 1 : 128 for anti-RhD in the IAT was the same maximum titer that was 100% sensitive to prediction of fetal anemia by Moise and colleagues. The potential for a false-positive antibody titer measurement is demonstrated by these latter investigators, who described an IAT titer of 1 : 512 in the mother of an antigen-negative fetus [185].

The importance of ultrasound as a tool for fetal assessment and decision-making regarding invasive studies has increased in parallel with the improvement in technology and experience with this equipment. An ultrasound fetal biophysical profile is specific for severe HDN, in that the picture of edematous fetal skin, ascites, pleural effusion, and hepatosplenomegaly, in the context of maternal antibody, is unambiguously a picture of hydrops due to uncompensated severe anemia [217]. However, severe anemia will occur before the development of the ultrasound picture of hydrops. A fundamental finding, which was described early and remains valid in the time of frequently applied cordocentesis, is that the outcome of a fetus with HDN that develops hydrops is worse than that of a fetus that does not. Initially, this was due to the decreased absorption of intraperitoneally transfused RBCs in a hydropic fetus with poor fetal breathing movement [218, 219]. A subsequent study in which intravascular transfusions were used for all fetuses showed that hydropic fetuses still fared worse [220]. Thus, a goal of in utero management is prevention of fetal hydrops. Pursuit of this goal must be tempered by the knowledge that each cordocentesis carries a risk of fetal loss of 1–2% [167, 221–223]. Hence, an ideal of in utero transfusion is prevention of hydrops with the minimum number of transfusions.

In 2000, a consortium of physicians from eight institutions at sites in Chile, Saudi Arabia, Switzerland, and the USA reported that Doppler ultrasonography can identify fetuses with significant anemia. In this study, all identified at-risk fetuses had measurement of peak systolic velocity of the middle cerebral artery (MCA) immediately before cordocentesis. The investigators showed that there was a correlation between MCA velocity and fetal hemoglobin levels, such that significant fetal anemia could be detected by this noninvasive technique. Using the MCA velocity criteria chosen to identify significant anemia, the false-negative rate for this cohort was 0% and the false-positive rate was 13%. Importantly, the ability to identify fetuses with significant anemia was independent of ultrasound changes associated with hydrops. This was the first multi-institutional study of this approach [224]. The study had significant methodological limitations [225], but these are being addressed in another prospective trial [226]. The potential value of this technique is to eliminate the risk of cordocentesis for direct assessment of anemia and transfusion for many fetuses who are not at risk for the adverse effects of severe anemia. In the process of developing the correlation of the MCA velocity with hemoglobin values, the investigators established for the first time normal values with standard deviations for hemoglobin from the eighteenth week of gestation to term (Fig. 6.3) [226].

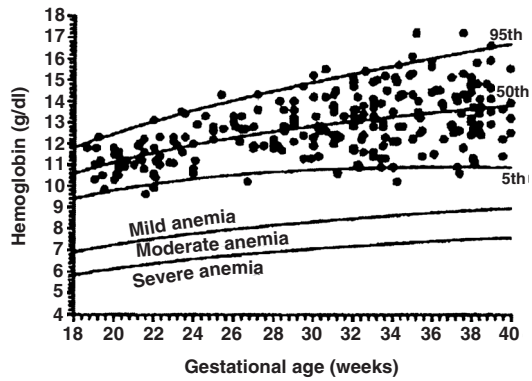

Fig. 6.3 Reference ranges for hemoglobin values based on samples from 265 normal fetuses, including curves representing the 95th, 50th, and 5th percentiles and curves describing values indicative of mild, moderate, and severe anemia for gestational ages 18–40 weeks [226]. Copyright © 2000 Massachusetts Medical Society. All rights reserved.

Invasive assessment of the fetus can be done by amniotic-fluid sampling or by obtaining fetal blood. Synonymous terms for fetal blood sampling include percutaneous umbilical-cord blood sampling (PUBS), cordocentesis, and funipuncture. Although cordocentesis has challenged the role of serial amniocentesis in the management of severe HDN, amniocentesis remains the invasive procedure of the intrauterine space with which there is the greatest experience, and it continues to be the most utilized [227, 228]. A contraindication to amniotic fluid sampling is an anterior placenta due to concerns of placental hemorrhage [127]. Potentially, amniocentesis can begin as early as 14 weeks in

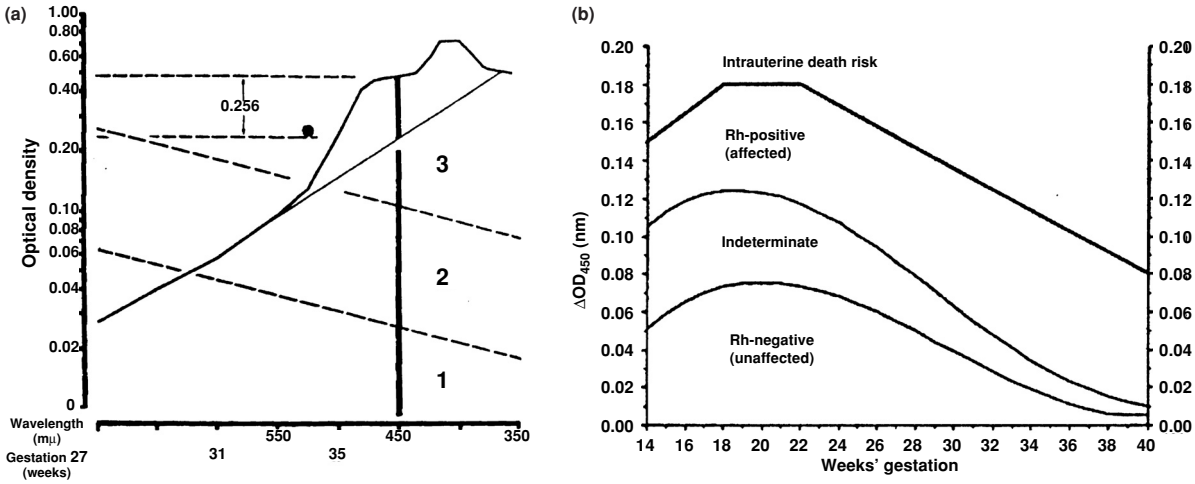

Fig. 6.4(a) This figure represents two superimposed graphs, but both use the *y*-axis value of optical density. The optical density (OD) of a sample of amniotic fluid is measured at wavelengths 700–350 nm (solid curves). The thick vertical line marks the OD at the wavelength characteristic for bilirubin absorption, 450 nm. The lower horizontal dashed line is drawn at the OD of bilirubin-free amniotic fluid, while the upper horizontal dashed line is drawn at the level of the measured OD of the patient sample. The difference between these two values, or the ΔOD_{450}, which is 0.206 in this case, is then plotted on the other superimposed curve using the *x*-axis values of gestational age (•). In this case, measured at 34.5 weeks' gestation, the value is in zone 3, or severe disease. Zone 1 represents values of unaffected fetuses and zone 2 represents that of fetuses with less severe disease. Reproduced with permission from *Pediatrics*. [233]. (b) Graph of Queenan's data of amniotic fluid ΔOD_{450} fetuses from 14 weeks of gestation, with curves describing boundaries between values representing fetuses that are unaffected, indeterminate, affected, or severely affected. Reprinted with permission from Elsevier. [365].

antigen-sensitized mothers. The fluid is evaluated for bilirubin pigment concentration by measuring the optical density of the amniotic fluid specimen at 450 nm, obtaining the spectral peak for bilirubin, and subtracting the value for the control fluid at 450 nm, producing the ΔOD_{450}. This value is then plotted on a graph, with ΔOD_{450} on the *y*-axis and gestational age on the *x*-axis; this is often referred to as a Liley curve, after the author who originated it. Liley's original graph used data from samples taken from 27 weeks' gestation to term and was divided into three regions by two curves (Fig. 6.4a) [229]. Later, Queenan and colleagues collected data for fetuses from 14 weeks to term and constructed a graph with four regions (Fig. 6.4b). Normally, amniotic fluid bilirubin concentration, as measured by ΔOD_{450}, increases from 14 weeks' gestation until a plateau at approximately 18–22 weeks. Then the concentration decreases until term, as the increasing volume of amniotic fluid reduces the concentration of bilirubin [230]. In a pregnancy with fetal hemolysis, however, the amniotic-fluid bilirubin concentration is sustained or increases after 22 weeks. Progressive increases in the amniotic-fluid bilirubin concentration above normal values are associated with a worsening state of hemolysis, a potential for worsening of the condition of the fetus in utero, and a higher likelihood of stillbirth, severe anemia, or high-output heart failure at the time of delivery [229–231].

Liley's original data were published in 1961, at which time the limit of neonatal viability was 32–34 weeks' gestation [229]. Before this report, the management of HDN was to use the maternal obstetrical history and antibody titer as criteria to determine whether a fetus would benefit from early delivery [232]. The use of the ΔOD_{450} and the Liley curve was

a major advancement in the predictive power [233]. However, with early delivery as the only management choice, the contemporary ability to support premature infants ex utero imposed considerable limitations on this approach. As methods of in utero support were developed, with first intraperitoneal [234] and then intravascular [167, 235] transfusions, there was a need for assays to guide the decision to initiate in utero transfusion before 27 weeks.

The initial effort to extend the amniotic fluid ΔOD_{450} to the second trimester was a direct extrapolation of Liley's curve [236, 237]. Simultaneous sampling of amniotic fluid and fetal blood showed the former to yield a false-normal result before 26 weeks at a significant frequency [238]. These data, and other data that showed some inaccuracy of the Liley method in the third trimester [239], persuaded many clinicians to use cordocentesis as the primary invasive diagnostic technique in the second trimester [240]. Subsequently, Queenan and colleagues obtained new ΔOD_{450} values from mothers with fetuses at 14–40 weeks' gestation [230]. A comparison of the Queenan and extended Liley graphs for interpretation of ΔOD_{450} values yielded a conclusion that although neither approach falsely identified fetuses as normal, the Queenan method was more likely to identify a fetus for in utero transfusions when it was not required [231].

Cordocentesis initially was performed as an endoscopically guided procedure, but with improved ultrasound equipment and increased experience, ultrasound-guided cordocentesis became the standard approach [167, 235]. Because it measures fetal hemoglobin directly, PUBS is considered the reference criterion against which other methods to assess fetal hemolysis and status are evaluated. Weiner and colleagues developed guidelines to interpret hemoglobin levels of the fetus and to assist in management decisions [240, 241]. Unfortunately, the process of obtaining this definitive information carries with it a significant increased risk of fetal loss and an increased risk of further elevation of maternal antibody titer when compared with amniocentesis. There are no definitive studies of critical titer, functional serological assays, the exact role and timing of amniocentesis, or cordocentesis, or of Doppler and fetal biophysical profiles [228]. Thus, authors on this subject continue to recommend using the expertise of the regional center to determine the approach for a particular pregnancy [120, 127]. The lower risks with amniocentesis compared with cordocentesis, of immediate fetal loss and of further increases in antibody titer secondary to procedure induced FMH, make amniocentesis a frequent choice as the first invasive procedure to evaluate fetal status. When amniotic-fluid pigment reaches the upper half of the Queenan's intermediate zone or Liley's zone 2 early in pregnancy, or more than four weeks before term if Queenan's upper zone or Liley's zone 3 is reached, most perinatologists will repeat the amniocentesis shortly after such unfavorable changes are noted. If the finding is confirmed, then direct sampling of fetal blood from a placental vessel under ultrasound guidance may be attempted at the same session.

Cordocentesis may be done to determine the fetal hemoglobin level based on concerns from an amniocentesis ΔOD_{450} or fetal ultrasound findings to confirm the antigen status of the fetus or due to a previous fetus being severely affected by HDN. Whenever PUBS is done, the requirements for intrauterine transfusion should be available. The decision to transfuse is based on the fetal hematocrit level, with 30% being the usual threshold [198, 240].

When successful, intrauterine transfusion will retard the rate of hemolysis by substituting antigen-negative donor cells for the fetal antigen-positive cells. Multiple transfusions may replace most of the patient's antigen-positive cells with antigen-negative donor blood. The reduction in antigen-positive cells will decrease the rate of hemolysis, will allow progressively increasing intervals between transfusions as the proportion of antigen negative cells increases, and will decrease bilirubin production. Subsequent transfusions may be done after a fixed interval from the first transfusion. An alternative approach is to use Doppler determination of MCA velocity as a technique to monitor

fetuses following an initial in utero transfusion to determine the timing of subsequent interventions [242]. The current approach of using a specific number of days to determine the next transfusion carries the risk that fetuses may be treated too late, with resulting increased risk of fetal demise, or too early, with excess risk due to the increased numbers of procedures. The intrauterine transfusion procedure can be repeated several times between 18 weeks and 36 weeks, as indicated. RBCs for transfusion will be negative for the antigen and irradiated due to the possibility of transfused lymphocytes causing graft-versus-host disease [243, 244]. Delivery before 36 weeks is also an option if transfusions are technically challenging or are not tolerated well by the fetus. Before planned premature delivery, the fetal assessment should include lung maturity by amniotic-fluid assays of lecithin-sphingomyelin ratio and the presence of phosphatidylglycerol [245, 246].

Plasmapheresis has long been used to manage antibody-mediated disorders [247]. More recently, intravenous immunoglobulin (IVIg) infusions have been employed in similar situations [248]. Plasmapheresis was first described as a means of controlling maternal antibody titer in 1977 [249]; other authors have also described its use [250]. The very short duration of reduction in antibody titer in maternal alloimmunization, combined with the demands of the plasmapheresis procedure itself, resulted in this procedure never being studied carefully or used widely. Similarly, IVIg had a short duration of effect and was associated with a high cost as well as some adverse effects [251, 252]. One use of these modalities is for fetuses too immature for in utero intervention [253]. Plasmapheresis is used to decrease the antibody titer, and then a regular IVIg infusion is given to maintain the decreased antibody titer. This approach is recommended only for mothers with a history of severely affected fetuses and only from the tenth week of gestation until the fetus is matured to an age at which in utero intravascular transfusion can be performed confidently; Fig. 6.5 is an algorithm that describes an approach to management of an Rh-sensitized pregnancy [254].

Postnatal interventions

Delivery of the antibody-sensitized mother and fetus

As an antibody-sensitized pregnancy passes the age of fetal viability (currently approximately 24–25 weeks) and nears term, the pediatric service in the hospital of delivery should be consulted [7]. If possible, the antibody-sensitized mother with progressive evidence of intrauterine fetal hemolysis should be delivered at a perinatal level III center with full neonatal intensive care capabilities.

The pediatric service should be notified of the estimated severity of fetal hemolysis and consulted about the possibilities and options for neonatal survival, resuscitation, and treatment [7]. We also recommend direct consultation between the neonatologist and the parents. Relative to the unaffected fetus matched for gestational age, the fetus with severe HDN appears to be at increased risk of neonatal respiratory distress syndrome. This is probably secondary to intrauterine fetal compromise and possibly is related to fetal hyperinsulinemia, which may downregulate fetal surfactant production. If the infant is delivered before term, then the combination of anemia, hyperbilirubinemia, and respiratory distress may require immediate attention to neonatal resuscitation and intensive care [7, 255].

The timing and mode of delivery can be planned according to fetal condition and obstetrical considerations. At 32–36 weeks' gestation, if an amniotic-fluid sample is taken for bilirubin pigment assay before planned induction or Cesarian section, then the same sample can be tested for neonatal surfactant production [256]. If delivery appears necessary before 34 weeks of gestation, then lung maturation can be accelerated by a 48-hour course of antenatally administered glucocorticoids [257]. For most purposes, betamethasone in appropriate doses appears preferable to other steroid preparations [258]. This approach appears to promote some favorable degree of lung maturation as early as 24–25 weeks' and as late as 32–33 weeks' gestation.

Unless antenatal evaluation shows evidence of severe fetal compromise already present, most

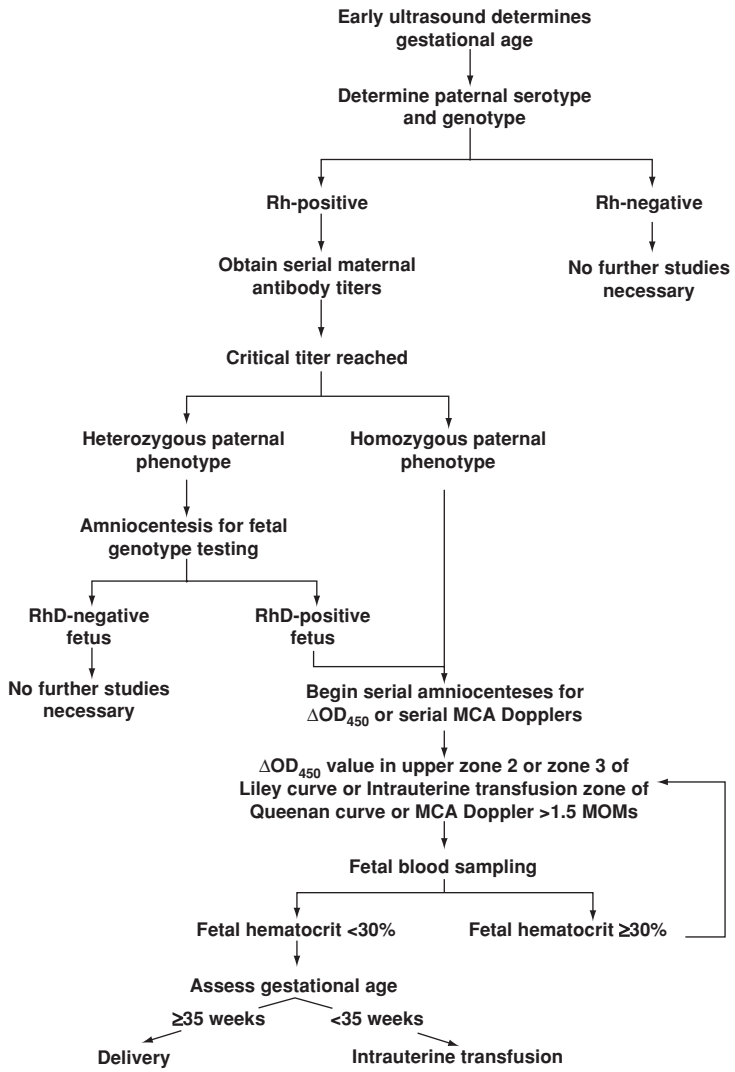

Fig. 6.5 Algorithm representing an approach to the management of a first affected fetus. For second affected fetuses, many authors recommend starting at the point of serial amniocenteses or, if available, serial (MCA) Doppler studies [254]. Reproduced with permission.

patients with HDN will tolerate delivery by induction of labor, with close electronic and bedside monitoring. Mild to moderately anemic fetuses generally tolerate labor well, although severely anemic fetuses and fetuses with incipient high-output cardiac failure may not [255]. Another consideration leading to early delivery, and considered by some authors to be an indication for delivery by Cesarean section, is severe maternal polyhydramnios. Cesarean section should be available immediately in case of fetal intolerance to labor or the development of untoward obstetrical complications, and it may be the preferred route of delivery for severe fetal compromise and other high-risk obstetrical indications.

Delivery-room management of the newborn

A pediatrician or pediatric team should attend the delivery, with the experience and equipment needed to begin neonatal stabilization and intensive care immediately [259]. In cases with antenatal evidence of severe sensitization and probable fetal anemia, group O, RhD-negative, and other appropriate antigen-negative packed RBCs and compatible plasma should be brought to the delivery room. Space and equipment should be prepared for immediate umbilical-vessel catheterization.

Cardiopulmonary resuscitation at delivery should proceed according to established guidelines. In addition, a specimen of cord blood should be sent immediately for determination of neonatal blood type, antibody titer, hemoglobin, and bilirubin concentration. Oxygen administration, endotracheal intubation, and assisted ventilation should be performed according to the infant's respiratory condition at birth. If the infant at delivery shows signs of severe anemia, high-output cardiac failure, peripheral edema, or shock, then an umbilical vein or artery should be cannulated as soon as possible; 50 ml/kg of neonatal blood should be withdrawn through the catheter in aliquots of 10 ml/kg and replaced promptly with 50 ml/kg of packed O, RhD-negative cells in 10 ml/kg aliquots over 20–30 minutes. Following emergency transfusion immediately after delivery, the infant should be admitted promptly to neonatal intensive care and evaluated further, as noted below. If the infant does not show immediate postnatal evidence of severe anemia or severe derangement of cardiac output, then the emergency transfusion as described above should be deferred until the infant is evaluated further in the nursery.

In the nursery, the Apgar scores, immediate postnatal events, and laboratory information should be reviewed in detail. Standard management for neonatal respiratory distress should be carried out, with surfactant administration and ventilatory assistance as indicated. In general, the most distressed infants will benefit most from early correction of their hematologic and cardiovascular complications, but only after immediate postnatal management has been initiated to stabilize ventilation, oxygenation, and acid–base balance. Infants with less severe respiratory distress and without evidence of profound anemia or cardiovascular compromise can be stabilized and observed without emergency transfusion until the postnatal course of their anemia and hyperbilirubinemia is evaluated further.

The most severely compromised hydropic infants may need inotropic support, paracentesis, and/or thoracentesis for aspiration of ascitic or pleural effusions [259, 260] and aggressive supplementation or virtual replacement of their intravascular red-cell supply early in the postnatal course. The mortality of hydrops fetalis remains substantial despite advances in obstetrical care and neonatal intensive care management [255, 260].

After initial neonatal stabilization and assessment, complete blood count and bilirubin concentrations should be determined again within four to six hours, as a guide to further management [261]. All other supportive measures for respiratory care, cardiovascular support, and prevention of infection should proceed according to standard neonatal intensive care practice.

Neonatal jaundice and hyperbilirubinemia

Pathophysiology

Many newborns have transient increases in their plasma indirect bilirubin concentrations during the first one to two weeks of postnatal life [262]. The hepatic conjugating system for bilirubin is relatively inactive before birth. In utero unconjugated bilirubin and structurally similar small molecules can cross the placenta for conjugation and excretion by the maternal liver and gastrointestinal tract. Postnatally, hepatic uptake of bilirubin increases the activity of the conjugating enzyme, uridinediphosphoglucuronate glucuronosyltransferase (UDPGT-1) [263]. As the conjugating enzyme is induced, canalicular transport, biliary excretion, and bacterial conversion of bilirubin to other porphyrin compounds mature during the first several postnatal days. While the newborn progresses from placental dependence to

self-sufficient excretion of bilirubin and bile products, plasma unconjugated bilirubin tends to increase for three to five days. It then stabilizes and gradually falls to normal adult levels. In newborns without hemolytic disorders the average plasma bilirubin level (predominantly unconjugated) increases to 6–8 mg/dl at day three to four, with a normal range of approximately 3–13 mg/dl [262]. The average peak bilirubin level is 1–2 mg/dl higher in breast-fed babies than in those fed formula milk. Breast feeding may also prolong neonatal jaundice by an additional one to four weeks, and occasionally longer [262, 264].

The term "neonatal hyperbilirubinemia" is generally reserved for cases in which jaundice appears in the first 24 hours of life, reaches levels greater than the normally expected physiologic upper limit of 15–20 mg/dl, or persists longer than a week. In addition to hemolytic disease resulting from blood-group incompatibilities, several other neonatal conditions increase the likelihood of hyperbilirubinemia [265]. The hemolysis associated with bruises or enclosed hemorrhages, and inherited hemolytic disorders such as glucose-6-phosphate dehydrogenase (G-6-PD) deficiency [34, 266] and hereditary spherocytosis [267, 268], may not produce anemia but will increase the production of bilirubin. Prematurity may predispose newborns to exaggerated or prolonged hyperbilirubinemia even with gestation as advanced as 36 weeks. The conjugating enzyme appears slower to activate, and full feeding and normal bacterial colonization may be delayed. Breast-feeding may inhibit activity of UDPGT-1 by a negative-feedback process, the exact mechanism of which is unclear [264]. In addition, breast milk in the proximal small bowel may promote enzymatic deconjugation and brush-border reabsorption of bilirubin in a process known as "enterohepatic circulation" [269, 270]. In some newborns, especially those under 37 weeks gestation, breast-milk intake may be inadequate for a week or longer. The combined effects of dehydration, increased enterohepatic circulation due to decreased peristalsis and defecation plus delayed bowel colonization may exaggerate the retention of bilirubin in these babies [271].

Genetic mutations in the gene coding for the conjugating enzyme UDPGT-1 may reduce its expression or impair its efficiency. To date, over 50 mutations of UDPGT-1 have been identified among people with Crigler–Najjar syndrome [272]. Crigler-Najjar syndrome type I is the more severe form and the original form described [273]. It is associated with absent or minimal UDPGT-1 activity, no increase in activity following administration of phenobarbital, severe, persistent elevation of bilirubin to 20–50 mg/dl in the absence of hemolysis or hepatocellular disease, and frequent hyperbilirubinemic encephalopathy. Frameshift, premature stop codon, splice site, and point mutations and multiple nucleotide deletions have all been identified as causative alterations of UDPGT-1 in Crigler–Najjar syndrome type I [272]. Infants with type II Crigler–Najjar syndrome have severely reduced, but detectable, UDPGT-1 activity. Unlike in type I patients, phenobarbital will produce a decrease of 30–80% in serum total bilirubin among infants with type II [272, 274]. Responsible mutations for Crigler–Najjar syndrome type II result in single amino-acid substitutions that are carried on both alleles and are associated with decreased conjugating activity. Outside the neonatal period, people with type II Crigler–Najjar syndrome experience recurrent or sustained unconjugated hyperbilirubinemia in the range of 7–20 mg/dl. Newborns with this disorder may have persistent severe hyperbilirubinemia, placing them at risk for kernicterus and often requiring exchange transfusion or intensive phototherapy, or both. Phenobarbital increases conjugating enzyme activity due to increased transcription from the UDPGT-1 locus via a phenobarbital-responsive enhancer element, with resultant increased UDPGT-1 protein [275].

Gilbert syndrome* is a disorder of bilirubin conjugation. In some populations of the Middle East, East Asia, and northern Canada, it occurs with a frequency of 10% or higher [272, 276–278]. Adults with Gilbert syndrome have recurrent, usually mild,

*Dr Gilbert first reported this entity in 1901 as "La cholémie simple familiale." He was French and his name is pronounced as the English words "Jill-bear."

unconjugated hyperbilirubinemia in the range of 2–6 mg/dl and often exaggerated at times of infectious illness such as influenza or gastroenteritis. Newborns with Gilbert syndrome have an increased risk of hyperbilirubinemia, but these newborns do not reach levels requiring intervention without accompanying increased bilirubin production, as can occur with G-6-PD deficiency [279]. Both UDPGT-1 and G-6-PD mutations are common in some populations in the Middle East and Asia. The Gilbert phenotype is associated with at least two types of genetic mutation. The most common mutation in European, Middle Eastern, and African ancestry peoples is in the noncoding promoter segment of the UDPGT-1 gene in a region that binds the transcription factor, TATAA protein. This DNA region is sometimes called the TATAA box for the sequence of the thymine and adenine nucleotides on the DNA molecule that binds the TATAA protein. The affinity of the TATAA binding protein for the TATAA box is related inversely to the number of TA repeats [277]. The addition of a TA repeat to the normal sequence $(TA)_6TAA$ within the promoter results in $(TA)_7TAA$ and decreases transcription from the UDPGT-1 gene. With the $(TA)_7TAA$ sequence, the UDPGT-1 protein produced is normal but is present in decreased quantity. It seems that by itself, the $(TA)_7TAA$ genotype, even in the homozygous state (7/7), does not result in an increased risk for hyperbilirubinemia. However, when hemolysis is increased, by whatever mechanism, the homozygous state (7/7) and the heterozygous state (6/7) are associated with an increased risk for hyperbilirubinemia [279]. In addition, there is some increased risk of hyperbilirubinemia attributed to breast feeding and pyloric stenosis in association with the (7/7) genotype [278, 280].

Among East Asian people with Gilbert syndrome, structural mutations in exon 1 of the gene for UDPGT-1 produce abnormal forms of the protein, some of which impair bilirubin binding by the enzyme. The most frequent associated polymorphism in a series of Japanese infants with prolonged jaundice was a glycine-to-arginine substitution at the 71 position of the peptide (G71R), but among a group of Taiwanese people with Gilbert syndrome, the TATAA promoter variant was most common [281, 282]. Unlike infants with the UDPGT-1 promoter mutations, the hyperbilirubinemia associated with the UDPGT-1 structural mutations can occur without other inciting factors for jaundice [281]. As expected, the combination of UDPGT-1 structural mutations with hemolysis or breast feeding can produce an exaggerated hyperbilirubinemia. Conjugation and excretion of bilirubin are increased by phenobarbital in patients with both the promoter and structural mutations for Gilbert syndrome.

Diagnosis and management

In addition to maternal-fetal and neonatal screening for blood-group incompatibilities and hemolytic disease, all clinicians caring for newborn infants should observe and screen newborns for jaundice during their nursery stay and at post-discharge follow-up visits [283]. Most newborns with jaundice have a mild, transient form, but a few who appear otherwise well will develop exaggerated or prolonged hyperbilirubinemia requiring treatment.

Evaluation of a newborn for jaundice, whether by inspection, semiquantitative estimation, or laboratory studies, should be related to the patient's age in hours [284]. An hour-specific approach to the baby's rate of increase in plasma bilirubin, traditionally used for assessment of hemolytic hyperbilirubinemia, is also useful to assess and roughly predict the rate of increase in nonhemolytic jaundice. Newborn nurseries should establish standard procedures for the timely recognition of early neonatal jaundice, and individual practitioners should incorporate a structured approach to its early recognition and anticipatory management including early post-discharge follow-up (Table 6.4) [261, 283, 284, 285].

Reports of kernicterus in previously "well" infants increased during the 1990s [286, 287]. Although the exact incidence of kernicterus is not known for the 1990s or the preceding decades, events associated with this perceived increase, and documented in some individual cases, include short hospital stay, discharge of preterm infants using the same criteria

Table 6.4 Observations to guide the formulation of a structured approach to hyperbilirubinemia

1. If the nursery stay is 2 days or less, the infant's peak bilirubin will probably occur after discharge.
2. During the first 3–5 postnatal days, the approximate upper limit for a "normal" increase in bilirubin is about 5 mg/dl/day, or about 0.2–0.25 mg/dl/h. In nonhemolytic jaundice, the rate of increase usually declines after the first 3–4 days.
3. With visible jaundice in the first 24 hours of life, serum total bilirubin usually exceeds 5 mg/dl and, therefore, is nearly always above the normal range.
4. Visible jaundice usually progresses cephalad to caudad. If "total-body" jaundice is seen during hours 24–48 of life, it is often associated with a serum total bilirubin of 10 mg/dl or greater, which may be above the normal range.
5. Infants of less than 37 weeks' gestation are more likely than full-term infants to have exaggerated or prolonged jaundice, especially if they are breast feeding.
6. Reassessment for jaundice should be part of a follow-up visit within a week or less after early discharge. If a newborn was visibly jaundiced on day 1 or had a serum or cutaneous bilirubin value above the normal range on day 2, reassess the baby for jaundice within 24–72 hours using hour-specific interpretation of the bilirubin level.
7. Suggested interventions for well term newborns at specific bilirubin levels are as follows [283, 285]:
   (a) At 15–20 mg/dl, follow the bilirubin closely until stable or declining.
   (b) At 20–24 mg/dl, or less if the rate of increase is rapid, start home or hospital phototherapy.
   (c) At 25 mg/dl or greater, hospitalize the infant, evaluate for underlying conditions, start intensive phototherapy, and prepare for a double-volume exchange transfusion.

These observations are based on multiple reviews of neonatal jaundice and on the authors' experience.

as for well term infants, lack of recognition or concern about early jaundice in the nursery, breast feeding, sometimes without monitoring or guidelines, and lack of follow-up within the first week of discharge. The message from this collective experience of adverse outcomes is that lack of attention to neonatal jaundice in the first week leaves some newborns at risk for severe hyperbilirubinemia and kernicterus.

Exchange transfusion

The distinction between early and late exchange transfusion in newborns with hemolytic disease is intended not to describe exclusive approaches to treatment but rather to identify infants most likely to benefit from transfusion within the first few hours after birth. A stepwise approach to neonatal transfusion in severe HDN is outlined in Table 6.5.

The traditional criteria for early exchange transfusion were based on clinical experience with large numbers of RhD HDN infants in several centers where the procedure was first described and documented to be beneficial [7, 261, 288]. The criteria, which developed with time and experience, were based on observation of the clinical course of neonatal RhD HDN with and without early exchange transfusion at a time of high morbidity and mortality from RhD HDN. In general, these guidelines were the result not of randomized controlled studies but of accumulated experience by practitioners who became skilled in both the art and the technology of managing severe RhD isoimmunization. These investigators found that severe anemia and hyperbilirubinemia already present at birth would progress almost inevitably to increasing severity and clinical deterioration within 24–48 hours unless the hematologic problem and the progression of hyperbilirubinemia were stabilized by exchange transfusion.

The criteria for early postnatal exchange transfusion (as soon as the infant was clinically stable) included a plasma indirect bilirubin above 5 mg/dl in cord blood, a cord-blood hemoglobin of less than 10 mg/dl, or evidence of disturbed oxygenation or impending high-output heart failure. If the initial criteria were not met, then a subsequent rapid fall in hemoglobin, especially combined with an increase in serum bilirubin greater than 1 mg/dl/h, predicted a subsequent course of severe

Table 6.5 Neonatal transfusion in hemolytic disease of the newborn

Emergency "partial exchange" transfusion
Indications: hydrops or severe neonatal cardiovascular compromise at the time of delivery
Goal: to stabilize intravascular volume and improve O_2-carrying capacity
Procedure: withdraw 10-ml/kg aliquots of patient blood and replace with 10-ml/kg aliquots of O-negative packed cells, to a total withdrawal/replacement volume of 50 ml/kg via umbilical artery or vein
"Early" whole-blood exchange transfusion
Indications: cord indirect bilirubin concentration >5–5.5 mg/dl, cord hemoglobin concentration <10–11 g/dl, or evidence of disturbed oxygenation or impending high-output heart failure
Goal: to correct early postnatal anemia and to slow the rate of postnatal increase in plasma bilirubin
Procedure: replace 150–180 ml/kg of patient's blood with reconstituted cross-matched O-negative whole blood, using either a continuous withdrawal/infusion technique or serial withdrawal and infusion of 5–10-ml/kg aliquots of whole blood
"Later" exchange transfusion
Indications: plasma indirect bilirubin concentration increasing by 0.5–1 mg/dl/h or exceeding 20 mg/dl, especially with a concomitant fall in hematocrit
Goal: to avoid potentially neurotoxic plasma bilirubin concentrations and to correct progressive postnatal anemia
Procedure: as for "early" whole-blood exchange transfusion
Supplemental RBC transfusion
Indications: progressive postnatal anemia without severe hyperbilirubinemia
Goal: to avoid profound anemia likely to result in decreased tissue oxygenation or cardiac compromise
Procedure: individualize patient assessment and treatment; transfuse with 10–15 ml/kg of compatible Rh-negative packed RBCs

RBC, red blood cell.

hyperbilirubinemia and progressive anemia with enough reliability to recommend exchange transfusion within the first 24 hours. After 24 hours, an increase in the serum indirect bilirubin to levels above 20 mg/dl was considered an indication for exchange transfusion to reduce the risk of bilirubin encephalopathy [288, 289]. After immediate postnatal stabilization, the development of progressive anemia without severe hyperbilirubinemia was an indication for supplemental red-cell transfusions, as indicated by the hemoglobin concentration and clinical assessment.

Prematurity, acidosis, asphyxia, and infection have often been considered to increase the risk of neonatal bilirubin encephalopathy (kernicterus) [290, 291]. Some authorities have recommended lower maximum bilirubin levels for exchange transfusion in such compromised babies and have also suggested algorithms for exchange transfusion based on birth weight, gestational age, and indicators of illness severity [292, 293]. Unfortunately, these guidelines have never been validated by adequate clinical trials [286, 294]. Although recently revised recommendations for intervention in hyperbilirubinemic term infants [285], are not intended for cases of neonatal hemolytic disease; the adjunctive use of phototherapy after initial exchange transfusion is still an appropriate way to attempt to maintain subsequent safe bilirubin levels.

Antenatal transfusion can modify the need for postnatal exchange transfusion by altering the rate of hemolysis and bilirubin formation. Many babies with antenatal transfusion(s) before delivery will show a change in the blood type to O-negative by replacement of fetal cells and possibly also by down-regulation of fetal erythropoiesis. In the immediate newborn period, jaundice may be mild and may not reach levels fulfilling the older criteria for exchange transfusion. Anemia at birth may be mild to severe, depending on the duration and aggressiveness of the

hemolytic process as well as the volumes and timing of antenatal transfusions. Although the infant's antigen-positive cells may have been replaced with antigen-negative donor cells, the antibody screen in the neonatal plasma usually will be positive and will remain so for several weeks. These antenatally treated patients may not need emergency transfusion or early exchange transfusion, but as many as half of the infants who receive in utero transfusion will require an RBC transfusion at an average age of five weeks [295]. Late anemia may be more likely among infants with HDN who did not receive a postnatal exchange transfusion [296]. Late anemia is attributed to persistently circulating antibody and to inadequate erythropoietin production for the degree of anemia [297]. A small randomized placebo-controlled trial showed a significant decrease in the number of transfusions among a group of neonates with RhD HDN who received erythropoietin compared with a control group [298]. However, supplementation with erythropoietin and iron to prevent late anemia of HDN remains experimental [299]. In addition, a few infants may develop slowly progressive hyperbilirubinemia to levels in the range of 20–25 mg/dl, which would warrant exchange transfusion in more severely affected babies. The early use of phototherapy in babies with slowly progressive hemolytic hyperbilirubinemia may not be adequate to avoid later exchange transfusion in all patients.

The role of intravenous immunoglobulin as an alternative therapy in the management of HDN was the subject of a Cochrane systematic review in 2002. Based on three studies that met the reviewers' criteria for inclusion [300–302], the review concluded that treatment with IVIg was associated with a decreased need for exchange transfusion for term and preterm infants with Rh- or ABO-incompatibility. However, it identified weaknesses of the studies, including the quality of the design of all studies and the small number of infants in each trial. Therefore, the reviewers recommended additional well-designed studies before concluding that IVIg could be recommended for infants with HDN to prevent the need for exchange transfusion [303].

Outcome

Studies of outcome following in utero transfusion (IUT) are sparse. With established hydrops, lung hypoplasia has been observed and attributed to volume effects of extravascular fluid and to immune-mediated injury [304]. An early study reported some abnormality among almost half of a group of infants who received in utero transfusion (IUT) [305]. More recently, global development and sensorineural function were evaluated at two years of age in 38 children who were survivors of an initial cohort of 52 consecutive fetuses receiving IUT. Thirty-five of 38 children had no identified disability, but three children had, respectively, severe, moderate, and possible disabilities [306]. A similar study, which tested 40 survivors of IUT between 12 and 62 months of age, found 38 children with normal results but identified one child with a severe hearing deficit and one child with unilateral spastic hemiplegia [307].

The largest study to date described the outcome of 69 children from a consecutive series of 92 fetuses that received IUT between 1987 and 1993. Seventy-two fetuses were born alive and survived the neonatal period. At the time of follow-up, at age six months to six years, 93% of the children had a normal neurologic examination and 3% had only mild dysfunction. Developmental progression was normal in 84% but slightly delayed in 9%. Among the infants in a control group comprised of very premature or small-for-gestational-age infants, and a separate control group that was representative of the general population, there was an 18% and 6% frequency, respectively, of developmental disability. In this cohort, an increased probability of neurologic abnormality was associated with a history of perinatal asphyxia or a lower cord-blood hemoglobin at birth (mean Hb = 4.0 for the neurologically abnormal group versus 6.0 for the neurologically normal group). The authors found no association between risk of developmental delay and presence of hydrops at first IUT or the number of IUTs [308].

Antigen-specific considerations in the management of hemolytic disease of the newborn

RhD

Anti-RhD no longer tops the list of the most frequently identified antibody of clinical significance among adult women in the USA. This represents a major accomplishment of the past 30 years in immunoprophylaxis and application of careful blood-banking procedures, as well as a decrease in the fertility rate [161, 309]. Anti-RhD does continue to comprise 15–20% of the total antibodies detected [109, 310], and it remains the most frequently reported cause of HDN. A study from Sweden of babies born between 1980 and 1991 found that anti-RhD accounted for 70% of infants requiring intervention for HDN. Bowman reported that antibody to RhD accounted for 96% of antibodies detected in patients referred from outside of Manitoba for severe HDN in the interval 1969–78, but this proportion had decreased to 81% in the period 1979–96 [127].

This high proportion of severe HDN due to anti-RhD is in spite of the stepwise decrease in Rh sensitization from the historical rate of 16% in first at-risk pregnancies to the 2% frequency following widespread use of postnatal immunoprophylaxis, and the decrease to 0.3% with combined prenatal and postnatal prophylactic anti-D [113, 114]. Caucasian ethnic groups have the greatest risk of HDN due to RhD incompatibility. They are reported to have a general frequency of 16% RhD-negativity, ranging from 11% for Scandinavians to 35% for Basques. In addition to the previously described risks for severe RhD HDN, gender appears to be a risk factor, with male fetuses developing worse anemia, being affected earlier in gestation, and having a more than ten times greater risk of developing hydrops than female fetuses [311]. HLA type may also affect disease severity. Although ABO-incompatibility decreases the likelihood that a mother will be immunized against RhD, there is no salutary effect on severity of RhD HDN once immunization has occurred. For 100 USA mothers with anti-RhD, 73 fetuses will be RhD-positive and 18 will meet criteria for invasive testing. Half of these 18 will require intrauterine transfusion and seven will survive, for an overall survival of 97% [198].

Rh CcEe

The non-D Rh antibodies are a significant cause of HDN. With the widespread application of effective prophylaxis against RhD HDN, the proportion of infants with HDN who are affected by these and other non-RhD antibodies is rising. Of the non-D Rh antigens, antibody to Rh E is the most commonly encountered among pregnant women, with a frequency of 1.1–1.8 per 1000 [312, 313]. Fortunately, less than 20% of at-risk infants show any signs of being affected [127, 314, 315]. In one case series, 13 of 62 direct antibody test (DAT)-positive infants received exchange transfusion for anti-E HDN [315].

Anti-Rh c, "little c," occurs less frequently than anti-Rh E, at an incidence of about 0.7 per 1000 pregnancies [312, 313], but 20–30% will require postnatal intervention [95, 127, 312, 314, 316]. Therefore, anti-Rh c is second only to RhD as a cause of HDN. Importantly, one report has described a frequency of 13% of anti-c-positive pregnancies resulting in severe anemia or hydrops [95]. Others have described this severe complication with anti-c, but at a lower rate [127, 317, 318].

Rh C, "big C," is reported to be the target of maternal hemolytic antibodies in 0.1–0.2 per 1000 pregnancies. Of those with anti-C antibodies, one-third show some sign of being affected, but less than 10% require any treatment. In utero intervention is required extremely rarely with anti-C, but the same monitoring approach, as used for anti-RhD, is recommended when maternal antibodies against these antigens are detected [319]. Antibody to the Rh C variant C^w is found in as many as 1% of pregnant women, but it is usually independent of antigen exposure, i.e. it is a so-called naturally occurring antibody. Naturally occurring antibodies are usually IgM, low-titer, and of no risk to the fetus. Anti-e antibody, as the least immunogenic of the Rh group antigens,

is uncommon and has been reported to cause only mild HDN [109, 320].

For Rh c, C, and E, there is a highly significant association of maternal antibodies with a history of transfusion [94–96]. This finding emphasizes the importance of eliciting the transfusion history from the patient and from all available medical records, since patient recall usually is imperfect. As described earlier, this association between HDN and a history of RBC transfusion has led to the recommendation of matching red cells for transfusion for these antigens as well as for Kell when blood is given to premenopausal females. Prenatal diagnosis using fetal-derived DNA is available for all of the Rh genes [321, 322].

Kell

Detection of maternal antibody to a Kell system antigen requires different considerations from those that apply to Rh antibodies. The Rh polypeptide is expressed at a late, relatively low-proliferative-potential stage of erythroid development. The Kell protein is the earliest erythroid-specific cell-surface antigen expressed (Fig. 6.2) [20]. Erythroid precursors begin to express the Kell protein at the stage of peak proliferative capacity, the primitive erythroid burst-forming unit (BFU-E), which is defined, using an in vitro assay, as a cell capable of producing 500 progeny at eight days and later in culture [323]. This finding explained the observations that (i) the amniotic fluid ΔOD_{450} was not a sensitive indicator of anemia for a fetus that was affected by anti-Kell HDN [55, 324, 325]; (ii) fetuses of mothers with anti-Kell had a lower reticulocyte and nucleated RBC count than fetuses with anti-RhD when matched for hematocrit and gestational age [59]; and (iii) in vitro assays showed a greater suppressive effect on the more primitive and proliferative BFU-E than on the erythroid colony-forming unit (CFU-E) when both monoclonal anti-K antibodies and serum from mothers with affected infants were added to in vitro assays [60]. Thus, investigators concluded that unlike the case of RhD, in which amniotic-fluid analyses of bilirubin could serve as an adequate surrogate for fetal status, for anti-Kell antibody this technique did not provide sufficient information to assess the degree of compromise of fetuses. Rather, direct methods to assess fetal hemoglobin level with PUBS were required [59, 61]. It appears that fetal MCA Doppler velocity measurements may be used for this evaluation up to 35 weeks' gestation in experienced hands [326].

Anti-Kell (K1) antibody is found in about 0.1% of pregnant women [327]. However, most of these women have developed antibodies due to transfusion exposure [97]. Since only 9% of European ancestry and 2% of African ancestry people are antigen-positive for K1, and almost all are heterozygous, the likelihood that a fetus will express K1 is small [328]. Antibody to Kell has been associated with a lower critical titer, 1 : 8, than for Rh antigens [56]. When an antibody to Kell is detected in initial prenatal screens, there is great importance in determining the paternal antigen status. With paternal heterozygosity and a titer of 1 : 8 or greater, fetal antigen determination is indicated to guide future monitoring. As expected, 5–15% of infants born to mothers with anti-K1 are affected [97, 127]. Of those infants born to mothers with anti-K1 and known to express the K1 antigen, 40% in one series were affected severely, 24% required phototherapy, and the remainder received no intervention [97]. In another series, 73% of antigen-positive infants were affected severely [61]. The antithetical Kel 2 (k) [329–332] also has been associated, rarely, with severe HDN. The other Kell-associated antigens, Kp^b (Kel4) [333] and Js^b (Kel7) [334, 335], have been associated with HDN, which, in the case of Js^b, was severe.

ABO

Anti-A or anti-B antibody is found in all immune-competent individuals who are not type AB. Only the 15% of newborns who are type A, B, or AB offspring of type O mothers are at risk for HDN, as ABO HDN is not reported among infants of type A or B mothers [336, 337]. However, only about 10% (0.3–4% of liveborn) of infants at risk are affected [338]. In

spite of the low proportion affected, in a retrospective report of 88 000 newborns in Australia, ABO HDN was the second in frequency, after prematurity, of the factors associated with jaundice and second, after RhD HDN, of the factors associated with jaundice requiring exchange transfusion [339]. Although ABO HDN is a common cause of HDN requiring postnatal management, hydrops has been attributed rarely to ABO HDN [141, 340–342]. Since the mere presence of these antibodies is not a reliable indicator of risk for significant ABO HDN, other useful indicators have been sought. A maternal anti-A or anti-B titer of 1 : 1024 or greater by IAT was found in mothers of all affected infants in one study [343] and, similar to RhD, the ADCC assay was found to be most predictive of HDN by Dutch investigators [139]. The maternal anti-A/B titer is typically four to eight dilutions greater than that found in cord blood. This is due to an inability of the IgM portion of the maternal anti-A/B activity to cross into the fetal circulation, and the multiple non-RBC fetal sites of A/B antigen expression [51].

The neonatal DAT is not a specific or sensitive indicator of the risk for ABO HDN, but a positive DAT is associated with a greater probability of requiring therapy compared with neonates with a negative DAT [338]. The lack of power of the DAT to predict disease is attributed to properties of the IgG subclasses. The false-positive results may be due to two phenomena. First, although IgG2 comprises approximately 25% of total fetal anti-A/B IgG [134, 343, 344], it does not produce hemolysis in vivo due to weak binding by the receptor Fcγ RII [128]. Second, IgG1 is capable of initiating hemolysis, but it appears that the amount of bound IgG required for hemolysis is greater than the threshold for detection in the DAT. The significant hemolysis with a negative DAT may be due to the binding of IgG3 antibodies, which has a bound IgG requirement for hemolysis that is below the detection threshold by DAT in some cases [343].

Classically, jaundice of ABO HDN occurs before 24 hours and is associated with icterus praecox, or jaundice before the usual time [183], but two case series have described jaundice after 48 hours of life in a significant proportion of affected infants [337, 338]. In historical series, phototherapy has been sufficient for 75–94% of infants with ABO HDN requiring therapy [339, 345, 346]. However, the bilirubin values that were used as criteria for exchange transfusion have changed since most of these infants were treated [283, 286]. The contemporary approach would likely result in fewer infants receiving an exchange transfusion. Significant anemia is rare among those with HDN due to ABO-incompatibility, and it is almost always associated with hyperbilirubinemia [338, 341, 343, 346]. Intravascular hemolysis, accompanied by hemoglobinuria with transient renal insufficiency [141–143, 340] and dermal erythropoiesis [347, 348] have been reported in fewer than ten individuals. As with other causes of neonatal hemolysis, late anemia is reported [349].

Duffy

Antibody to the Duffy antigens has been implicated as a rare cause of HDN requiring intervention. Although West African ancestry individuals have a high frequency of lacking the Fya and b antigens on RBCs, a US study of a defined racial population did not find an increased frequency of antibody to Fy^a among individuals self-classified as black compared with self-classified white individuals [350]. In this series, in which 45 patients had developed antibody to Fy^a, none had developed antibody to Fy^b. Fy^b is calculated to have an immunogenic potency one-eighth that of Fy^a [109], and it has not been implicated as a cause of HDN requiring treatment, except in a single case [16, 43, 73, 127]. In two case series that included pregnant women with anti-Fy^a, two-thirds of mothers had a history of blood transfusion [312, 351]. The anti-Fy^a titer has been a poor predictor of severity of neonatal disease, but investigators from Bristol, UK, reported that for a group of 68 mothers, titers of 1 : 32 or less by the low-ionic-strength solution (LISS) antiglobulin technique were predictive of mild or no disease [72]. Two case series described 40 Fy^a-positive infants born to mothers with anti-Fy^a. Of these 40 infants, two received intrauterine transfusions, three received exchange transfusions, and three were treated with

phototherapy alone [72, 314]. Fetal genotype determination of Fy^a has been described [72, 352]. Anti-Fy3 in association with HDN has been reported in one patient [74].

Kidd

Antibody to the Jk^a and Jk^b antigens has been associated with positive direct antiglobulin test and hyperbilirubinemia requiring phototherapy [79, 80, 127]. Although there are reports of infants receiving exchange transfusion for Jk HDN, there have been none since the higher thresholds for exchange transfusion became standard practice [283]. There are no reports of anti-Jk HDN requiring intrauterine transfusion [16, 43, 127].

MNSs

Detection of antibody to the M antigen is relatively frequent, but its association with HDN is very rare. This observation may be explained by the dominant production of IgM in response to exposure to the M antigen. Reports from Ohio, USA [353], and Manitoba, Canada [354], that described observations over two decades found no infants with worse than mild HDN among a combined group of 197 mothers with anti-M, but case reports have described severe HDN or in utero death in 12 infants [16]. Clinicians recommend using a woman's previous pregnancy history and antibody titer as guides. Based on their observation of 115 pregnancies with anti-M, De Young-Owens and colleagues recommended only a routine antibody titer at 28 weeks if the previous pregnancy history is negative and the initial titer is 1 : 4 or less [353]. Bowman recommended a titer of 1 : 64 as a threshold for further investigation when antibody that has been associated with significant HDN is detected [354]. Anti-N has been reported as a cause of HDN only once [88]. Anti-S and anti-s have been associated with significant HDN very infrequently [355–357]. The frequency of detected anti-S is about 5% that of anti-RhD in the post immunoprophylaxis era. A strong association with transfusion history is reported, but the antibody is too weak to titrate in two-thirds of the cases. Moderately severe HDN has occurred, and there is one case report of IUT for HDN due to anti-S [89, 127, 358]. The glycophorin B molecule carries the U antigen as well as S/s. Rarely, anti-U has been implicated in HDN, but among the 15 reported cases, all affected infants had an IAT titer of greater than 1 : 256 [90].

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7

Neonatal hemolysis

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This chapter focuses on the recognition and management of hemolysis in newborn infants (Table 7.1). Some of the common hemolytic anemias of childhood first appear in the newborn period, while others do not present until several months of age, and a few rare hemolytic disorders occur only in the neonatal period. These variations in the age that hemolytic anemia first presents reflect differences in neonatal erythropoiesis, hemoglobin synthesis, and the metabolism of newborn erythrocytes. When approaching an infant with a potential hemolytic disorder, the first issue to be addressed is whether there is evidence of increased red-cell destruction and accelerated production. If yes, then the next question is to consider whether the cause of neonatal hemolysis is due to extracellular (acquired) factors or an intrinsic (genetic) red-cell defect. Acquired disorders are those that are immune-mediated, associated with infection, or accompany some other underlying pathology. Inherited red-cell disorders are due to defects in the cell membrane, abnormalities in red-blood-cell (RBC) metabolism, or a consequence of a hemoglobin defect.

Evaluation of a neonate for hemolysis must be considered in the context of normal newborn physiology. The RBC lifespan in term neonates (80–100 days) and in premature infants (60–80 days) is shorter than in older children and adults (100–120 days) [1]. The reason for the reduced RBC survival observed in newborns is not known, although there are many biochemical differences between adult and neonatal RBCs [2–4]. Increased oxidant sensitivity of newborn red cells and relative instability of fetal hemoglobin have been considered as possible causes for this shortened lifespan [5]. Also, since hemoglobin F does not interact with 2,3-diphosphoglycerate (2,3-DPG), it is possible that unbound 2,3-DPG may interact with the membrane and cause injury, leading to early red-cell demise. To date, a definitive explanation of the "normal" shortened RBC lifespan of infant red cells remains elusive.

Table 7.1 Causes of hemolytic anemia during the newborn period

Acquired RBC disorders
Alloimmune hemolysis (Rh and ABO incompatibility)
Immune hemolytic anemia due to maternal disease (SLE) or drugs
Congenital infections (CMV, toxoplasmosis, syphilis)
Bacterial sepsis
Disseminated and localized intravascular coagulation
T-activation (necrotizing enterocolitis, sepsis)
Hereditary RBC disorders
Membrane defects (hereditary spherocytosis, hereditary elliptocytosis)
Enzyme deficiencies (glucose-6-phosphate dehydrogenase, pyruvate kinase)
Hemoglobinopathies (alpha-thalassemia syndromes, gamma-beta-thalassemia)
Unstable hemoglobins

CMV, cytomegalovirus; RBC, red blood cell; SLE, systemic lupus erythematosus.

Laboratory features of neonatal hemolysis

A common clinical presentation of hemolytic anemia in older children is anemia, reticulocytosis, and hyperbilirubinemia. In the newborn infant, however, because of the normal hematologic adaptations to extrauterine life, the degree of hyperbilirubinemia and reticulocytosis must be interpreted in terms of the values appropriate for gestational and postgestational age. Also, some tests used to assess RBC production and destruction in older children have limited usefulness in the newborn.

Evidence for anemia and increased red blood cell production

The normal hemoglobin (Hgb) concentration at birth is 14–20 g/dl (mean approximately 17 g/dl), decreasing to a level of 11 g/dl over three to four months [2, 6]. This hemoglobin decrement, referred to as the physiologic anemia of infancy, occurs as part of the normal adaptation to extrauterine life. It is a transition from an hypoxic milieu with an elevated RBC mass to a well-oxygenated environment with excess oxygen-carrying capacity. In the presence of mild hemolysis (commonly seen with ABO-incompatibility), the magnitude of anemia may be minimal and obscured by the physiologic anemia of infancy. In infants with more significant hemolysis, the extent of anemia also is a reflection of the infant's capacity for increasing RBC production. In many congenital hemolytic disorders, it is not uncommon to observe worse anemia in infancy compared with later in childhood, presumably a reflection of less available marrow capacity in neonates.

As fetal erythropoiesis is replaced, the mean corpuscular volume (MCV) decreases between birth (100–130 fl) and one year of age (70–85 fl). The elevated MCV at birth has implications on how we define neonatal microcytosis and thereby recognize certain disorders, such as alpha-thalassemia, in newborns [2, 7].

The normal reticulocyte count of children and older infants is 1–2%. The reticulocyte count in term infants ranges between 3% and 7% at birth, but this decreases to 1% to 3% by four days and to less than 1% by seven days of age [2]. In premature infants, reticulocyte values at birth are higher (6–10%) and may remain elevated for a longer period of time. With significant hemolysis, neonates usually demonstrate an increased reticulocytosis. However, as noted above, because the physiologic anemia of infancy is a response to excess oxygen-carrying capacity, mild hemolysis might not be sensed as an oxygen deficit and, hence, no increased erythropoiesis or reticulocytosis may be observed. Nucleated RBCs are seen in newborn infants, but they generally disappear by the third day of life in term infants and in seven to ten days in premature infants. The persistence of reticulocytosis or nucleated RBCs suggests the possibility of hemolysis. Hypoxia, in the absence of anemia, also can be associated with increased release of reticulocytes and nucleated RBCs. In the presence of hemolysis, the bone marrow manifests erythroid hyperplasia, but examination of the bone marrow is needed rarely to diagnose hemolysis in neonates.

The peripheral blood smear in many hemolytic disorders reveals abnormal red-cell morphology. In newborn infants, however, the peripheral blood smear can be misleading unless "normal" RBC morphology for this age group is appreciated. Morphological characteristics of RBCs are consistent with the normal state of relative hyposplenism in newborn infants [7]. Howell–Jolly bodies, target cells, siderocytes, and other bizarre forms are seen commonly [8], and this is even more marked in premature infants. Just as with many other characteristics of the neonatal RBC, the red-cell morphologic changes found in the newborn period disappear by three to four months of age. Despite these limitations, when considering the possibility of hemolysis, the peripheral blood smear should be examined because many conditions, such as hereditary spherocytosis, hereditary elliptocytosis disorders, alpha-thalassemia syndromes, and microangiopathic hemolytic anemia, can be identified by careful observations.

Evidence for increased red-blood-cell destruction

Elevated serum unconjugated (indirect) bilirubin concentration with normal liver function are a useful marker of accelerated red-cell destruction in children and adults. In neonates, however, normally there is a transient increase in serum bilirubin concentration, and this must be considered when evaluating for hemolysis. Bilirubin production in neonates is greater than in adults because of the increased circulating red-cell mass at birth, the decreased survival of neonatal red cells, and the increased enterohepatic circulation of bilirubin [9]. In addition, there is transiently reduced glucuronyl transferase activity, which impairs hepatic bilirubin conjugation [10]. As a consequence of these phenomena, physiological hyperbilirubinemia normally occurs in newborn infants, and bilirubin concentrations as high as 12 mg/dl (peak at four days) are seen in term neonates, and levels up to 15 mg/dl (peak at seven days) are observed in premature infants. However, in association with hemolysis, serum bilirubin concentrations exceed these physiological levels and clinical jaundice may appear before 36 hours of age. Moreover, since mild hemolysis can occur without anemia or significant reticulocytosis, excessive hyperbilirubinemia occasionally is the only manifestation of neonatal hemolytic disease. The decision to monitor closely neonatal serum bilirubin levels and to evaluate for underlying hemolysis is based on the Bhutani risk curves of total bilirubin concentration as a function of age [11]. Beyond the first few days of life, other metabolic problems and breast feeding can cause hyperbilirubinemia and further confound the recognition of increased red-cell destruction.

Carbon monoxide generation in humans is from the breakdown of heme. Carbon monoxide is transported in the blood as carboxyhemoglobin. Measurement of blood carboxyhemoglobin levels can be used as a marker of accelerated red-cell destruction [12]. Also, exhaled carbon monoxide from the lungs can be measured directly to document increased RBC destruction [13, 14]. A noninvasive instrument to measure exhaled carbon monoxide is commercially available and appears to be a reliable marker of increased breakdown of hemoglobin to bilirubin [15, 16]. A neonate with hyperbilirubinemia and increased carbon monoxide production needs close monitoring because the cause of the elevated bilirubin is due to ongoing destruction of RBCs. This infant needs further evaluation to determine the cause of hemolysis.

Elevated serum lactic dehydrogenase (LDH) due to the release of LDH2 isozyme from RBC also is a marker of accelerated erythrocyte destruction; however, it is usually hyperbilirubinemia that initiates a work-up for neonatal hemolysis.

Other tests are available to assess for hemolysis, but most have limited utility in newborns. Serum haptoglobin is an alpha-2 glycoprotein that reacts with free hemoglobin to form a complex that is removed by the reticuloendothelial system. Since synthesis of this protein is not stimulated by hemolysis or clearance of the hemoglobin–haptoglobin complexes, reduced levels of serum haptoglobin are a reasonable marker of intravascular hemolysis. In neonates, however, haptoglobin levels are low and normal child or adult values may not be achieved until several months of age [17]. Thus, the serum concentration of this protein is not a reliable parameter of hemolysis in newborns. A less commonly used assay is measurement of serum hemopexin, a serum protein that binds free heme and is decreased with hemolysis due to catabolism of the hemopexin–heme complex. Just as for haptoglobin, however, hemopexin synthesis is reduced in neonates, and this measurement has little clinical value. Free plasma hemoglobin can be detected during episodes of intravascular hemolysis when the haptoglobin molecule becomes saturated. Because the newborn does not synthesize appreciable amounts of haptoglobin, intravascular hemolysis can result more rapidly in elevated levels of plasma-free hemoglobin. However, the method used to detect plasma-free hemoglobin is very sensitive, and positive results can be seen due to hemolysis associated with sample collection, thus limiting the value of this diagnostic test.

Severe intravascular hemolysis often is associated with hemoglobinuria. More chronic intravascular hemolysis is identified by urinary hemosiderin, but this test is not used to any major extent in newborns.

Acquired hemolytic anemias in the newborn

Alloimmune hemolysis

Maternal sensitization to fetal RBC antigens inherited from the father and the ensuing placental transfer of maternal antibodies directed against fetal RBC antigens is the most common cause of neonatal hemolysis (see Chapter 6). The spectrum of clinical problems ranges from minimal anemia and hyperbilirubinemia to severe anemia with hydrops fetalis. At one time, before effective prevention of Rh sensitization was available, hemolytic disease of the newborn was responsible for more than 10 000 deaths annually in the USA [18], but now the overall incidence of alloimmune hemolysis has decreased dramatically. Nevertheless, hemolysis due to Rh D incompatibility does still exist worldwide in areas where immunoprophylaxis is not readily available. At our institution in California, we have observed hemolysis in several neonates whose mothers had resided in Mexico and did not receive immunoprophylaxis with previous pregnancies. Today, the majority of alloimmune hemolysis cases in neonates are due to ABO maternal-fetal incompatibility and, to a lesser extent, due to sensitization to Kell, Duffy, Kidd, and other Rh antigens. Since alloimmune hemolysis is the most common cause of neonatal hemolytic anemia, testing for antibody on neonatal RBCs is a necessary initial step in the evaluation of any newborn with a hemolytic anemia. This is accomplished with the direct antiglobulin test (DAT), also known as the direct Coombs' test, which detects the presence of antibody on RBC. Only after alloimmune hemolysis is ruled out should one consider the work-up for other causes of neonatal hemolytic anemia. The specific tests used to diagnose other causes of hemolysis are discussed later in this chapter.

Immune hemolytic anemia due to maternal disease

Maternal autoimmune hemolytic anemia or lupus erythematosus during pregnancy may be associated with passive transfer of immunoglobulin G (IgG) antibody to the fetus [19]. The diagnosis is suggested by the presence of neonatal hemolytic disease, a positive DAT, absence of Rh or ABO incompatibility, and antiglobulin-positive hemolysis in the mother.

Hemolytic anemia due to maternal drugs

Maternal drug intake can lead to hemolysis in the fetus and neonate. One such example is seen with dapsone (diaminodiphenylsulfone), which is used mainly in the treatment of leprosy and dermatitis herpetiformis. A dose-related hemolytic anemia is recognized as a complication of this drug therapy in normal adults and children. Pregnant women have received dapsone without ill effects [20, 21]; however, hemolysis also has occurred in mothers who were taking dapsone during pregnancy and their offspring [22]. In these cases, hemolysis ceased once dapsone exposure ended. One case report described an infant who developed hemolytic anemia from dapsone in breast milk [23]. Dapsone is structurally related to sulfonamides and may compete with bilirubin for binding to increase the possibility of kernicterus, and it has been recommended that dapsone therapy be discontinued one month before the expected date of birth [24].

Infection

Congenital infections due to cytomegalovirus (CMV), toxoplasmosis, or syphilis, as well as bacterial sepsis, can be associated with hemolytic anemia; frequently, some degree of thrombocytopenia also exists. In association with congenital infections, often there is hepatosplenomegaly and other stigmata such as cataracts and microcephaly. In cases of

bacterial sepsis, both the direct and indirect bilirubin may be elevated. The mechanism of hemolysis is not defined clearly, but it is thought to be related in part to RBC sequestration and macrophage activation associated with infection. In addition, certain bacteria may produce enzymes (phospholipases, neuraminidase) that affect the membrane lipids or alter the normal RBC glycoprotein surface structure, thereby leading to shortened survival (see T-activation, below)

Microangiopathic (schistocytic) anemias

The presence of many fragmented RBC or schistocytes in the peripheral blood suggests that hemolysis is due to altered erythrocyte–blood-vessel wall interactions. Abnormalities of the placental microcirculation or macrovascular anomalies such as an umbilical-vein varix are rare causes of congenital schistocytic anemia [25]. Also, schistocytic hemolytic anemia occurs as a component of disseminated intravascular coagulation (DIC) and this is secondary to the deposition of fibrin within the vascular walls. When erythrocytes interact with fibrin, fragments of RBCs are broken off, producing fragile schistocytes that are removed by macrophages in the reticuloendothelial tissues. The hemolytic–uremic syndrome (HUS) represents a localized form of intravascular coagulation that is characterized by varying combinations of thrombocytopenia, renal disease, and hemolytic anemia. Hemolysis is characterized by RBC fragmentation, presumably for the aforementioned reasons. Of interest, some of the pathology of the hemolytic uremic syndrome may be related to T-activation (see below)

T-activation

T-activation is a form of polyagglutination due to alterations in RBC membrane glycoprotein structure, thereby leading to agglutination in the presence of most ABO compatible adult serum [26]. The altered membrane structure is due to enzyme (neuraminidase) cleavage of sialic acid residues from membrane sialoglycoproteins, thereby exposing a hidden galactosyl residue, the T antigen. The unmasked T antigen binds with anti-T immunoglobulin M (IgM) antibodies that are present in most human adult plasma, resulting in agglutination in vitro and possible hemolysis in vivo. In clinical practice, T-antigen exposure is associated with infections with neuraminidase-producing organisms, particularly with *Clostridium perfringens* and *Streptococcus pneumoniae*. However, bacteriodes, *Escherichea coli*, and other Gram-negative organisms also have been implicated. T-transformed red cells may be destroyed quickly by immune-mediated intravascular hemolysis and/or removed more rapidly from the circulation because of a reduced membrane surface charge caused by the decreased membrane sialic acid content. Anti-T IgM antibodies are absent at birth and in early infancy but are present later in most adults. It is presumed that the formation of these antibodies is due to antigenic stimulation by intestinal flora that possess T-like antigens, analogous to the development of ABO antibodies secondary to environmental antigenic stimuli.

Neonates with necrotizing enterocolitis (NEC) have been considered to be particularly susceptible to T activation and its resulting complications. It has been postulated that neonatal RBCs exposed to locally released bacterial neuraminidase from devitalized bowel results in T-activation [27–29]. Of interest, invasive *Streptococcus pneumoniae* infection is being recognized increasingly as an important cause of atypical hemolytic uremic syndrome in children [30]. Circulating neuraminidase produced by pneumococci has been postulated to cause exposure of the T cryptantigen in renal endothelium, red cells, and platelets with subsequent binding of anti-T IgM, resulting in renal failure, microangiopathic hemolytic anemia, and thrombocytopenia.

T-activation should be suspected in the clinical setting of sick or septic infants with intravascular hemolysis and with hemoglobinuria and hemoglobinemia following transfusion of blood products or when there is unexplained failure to achieve an expected post-transfusion hemoglobin increment. It can be recognized in the laboratory by compatibility testing where agglutination

is observed when donor serum and patient RBCs are mixed. Unfortunately, however, current blood-banking practices do not include a routine minor cross-match (donor serum and patient RBC) when infants under four months of age are transfused; this is because the formation of alloantibodies at this age is extremely rare. As a consequence, T-activation may not be recognized before transfusions are given. If there is any clinical suspicion of T-activation, then the compatibility of neonatal RBCs and donor plasma needs to be assessed.

It has been reported that transfusion of adult blood components to infants with T-activation can result in severe and sometimes fatal intravascular hemolysis, and routine screening of infants with NEC for T activation has been recommended by some to avoid transfusion-related morbidity and mortality [31, 32]. The necessity for routine screening and special transfusion protocols for infants with NEC, however, is highly controversial. Current approaches vary from no routine screening, to routine screening of infants with NEC or sepsis, to screening high-risk infants after they manifest hemolysis. Once identified, neonates with T-activated RBCs can be transfused with washed or plasma-reduced RBCs or platelets. If plasma products are needed, low-titer anti-T plasma should be used.

Vitamin E deficiency

Premature infants are endowed at birth with significantly less vitamin E than term infants; unless supplemental vitamin E is provided, this deficiency state persists for two to three months. Vitamin E is an antioxidant compound vital to the integrity of erythrocytes; in its absence, these cells are susceptible to lipid peroxidation and membrane injury. One clinical consequence of vitamin E deficiency is that hemolytic anemia can occur in small premature infants (weighing less than 1500 g) at six to ten weeks of age [33, 34]. This hemolytic anemia, which is characterized by reduced vitamin E levels and increased RBC peroxide hemolysis, disappears rapidly following vitamin E administration. A logical conclusion is that vitamin E deficiency might contribute to the anemia of prematurity in a more general sense. In fact, in one study, premature infants given daily vitamin E (15 IU per day) had higher hemoglobin levels and lower reticulocyte levels than a control group not given the vitamin [33]. However, subsequent studies found no hematologic benefit to the administration of 25 IU of vitamin E daily to premature infants [35]. Although it has become standard practice to administer vitamin E to all premature infants, the hemoglobin nadir in these babies is still lower than that in term newborns, indicating that anemia is caused largely by other factors, such as erythropoietin deficiency.

Infantile pyknocytosis

Pyknocytes are abnormal erythrocytes with a distorted, irregularly contracted appearance with few, irregular, short projections. Pyknocytes account for up to 2% of the erythrocytes in a term infant's peripheral blood smear and up to 6% of the erythrocytes in a preterm infant's peripheral blood smear [36]. Infantile pyknocytosis is an infrequently observed hemolytic anemia of variable severity. It was initially described by Tuffy and colleagues [37] and has been noted by several investigators since that time [38–41]. For unclear reasons, it is not commonly recognized today, with only rare case reports [41]. Clinical manifestations include hemolysis, pallor, jaundice, and, occasionally, hepatosplenomegaly. Laboratory features include anemia, reticulocytosis, and an elevation in aspartate aminotransferase (AST). Increased numbers of pyknocytes and polychromasia are apparent on the peripheral blood smear. Of interest, transfused erythrocytes also have a shortened survival [39], suggesting an extraerythrocyte factor is responsible for the disorder. Treatment is transfusion for symptomatic disease. Pyknocytes may be seen with other hemolytic disorders, such as Heinz body hemolytic anemia [42], G-6-PD deficiency [38], pyruvate kinase deficiency, and vitamin E deficiency [33]. Hereditary elliptocytosis and microangiopathic hemolytic disorders may produce similar-appearing RBCs. These causes of hemolytic

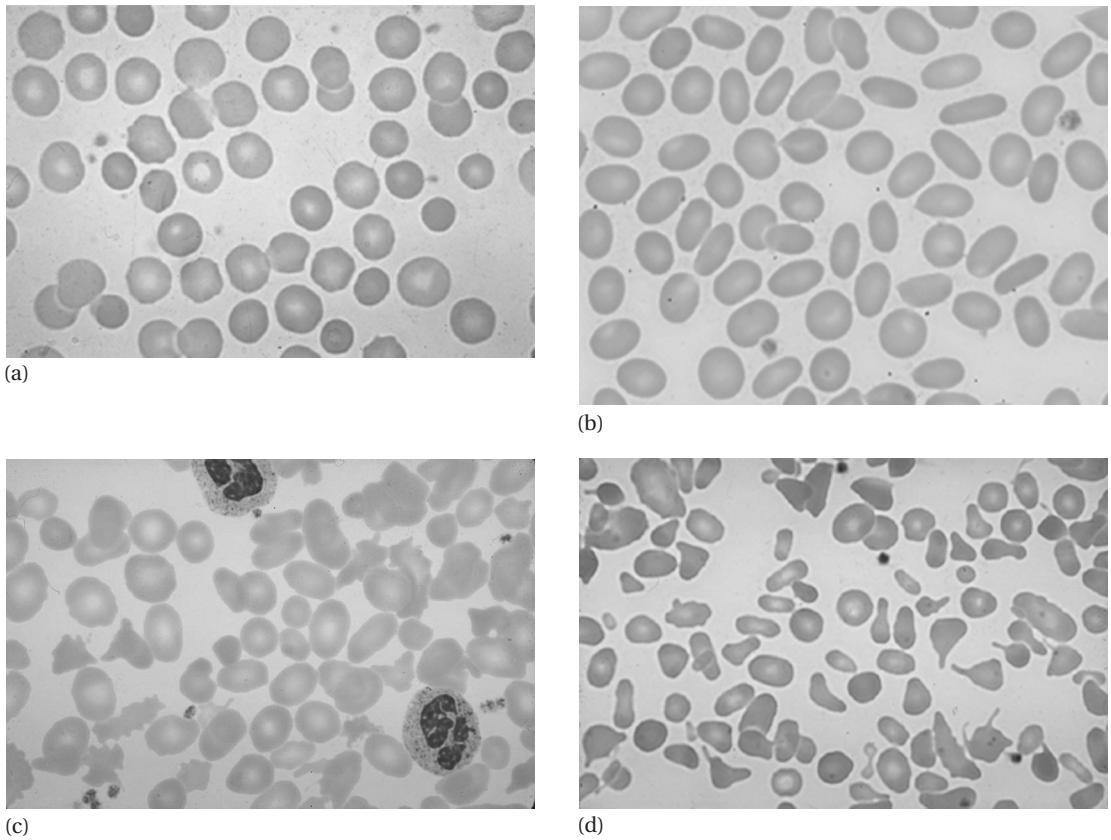

Fig. 7.1 Photomicrographs of blood smears from patients with different hereditary red-blood-cell (RBC) membrane disorders. (a) Hereditary spherocytosis; (b) mild, asymptomatic common hereditary elliptocytosis characterized by numerous elliptocytic cells; (c) hereditary elliptocytosis with chronic hemolysis with the presence of elliptocytes and rod-shaped cells and poikilocytes; (d) hereditary pyropoikilocytosis with a large number of microspherocytes and micropoikilocytes and relative paucity of elliptocytes.

anemias should be considered in the evaluation of an infant with proposed infantile pyknocytosis.

Hemolysis due to hereditary red-blood-cell membrane disorders

The RBC membrane structure has three major components: (i) a bilayer of phospholipids intercalated with molecules of unesterified cholesterol and glycolipids; (ii) integral membrane proteins (antigens, receptors) that penetrate or span the lipid bilayer, interact with the hydrophobic lipid core, and are bound tightly to the membrane; and (iii) a separate protein network that forms the membrane cytoskeleton (spectrin, ankyrin, and several other proteins) interacting with both the integral membrane proteins and the lipid bilayer. Abnormalities in the quantity and/or quality of these proteins and their interaction with each other are the cause of the two most common inherited RBC membrane disorders, hereditary spherocytosis and hereditary elliptocytosis. Numerous DNA mutations have been identified, and several excellent reviews of the advances in these

diseases are available [43, 44]. Both hereditary spherocytosis and hereditary elliptocytosis are associated with neonatal hemolysis.

Hereditary spherocytosis

Hereditary spherocytosis is the most common of the hereditary hemolytic anemias among people of Northern European background. In the USA, the incidence of the disorder is approximately 1 in 5000 [45]. In most affected families, hereditary spherocytosis is transmitted as an autosomal dominant trait, and identification of the disorder in multiple generations of affected families is the rule. Nearly one-quarter of all newly diagnosed patients do not demonstrate a dominant inheritance pattern, and the parents of these patients are clinically and hematologically normal. New mutations have been implicated to explain some of these sporadic cases. Also, an autosomal recessive mode of inheritance accounts for 20–25% of all hereditary spherocytosis cases, and it is manifested only in homozygous or compound heterozygous individuals and often is associated with severe hemolytic anemia [46, 47].

The hallmark of the disorder is the presence in the circulation of spherocytes, RBCs that have become spheroid because of a loss of membrane surface (Fig. 7.1a). This occurs because of inherited mutations in genes for components of the membrane cytoskeleton (spectrin, ankyrin, band 3). These protein abnormalities weaken the stability of the interactions between the cytoskeleton and membrane lipid bilayer, resulting in a loss of vertical integrity of the cell membrane. As a consequence, there is vesiculation, with loss of bits of the bilayer and, thereby, progressive loss of membrane surface area. As RBCs become more spherical, there is a loss of flexibility and increased vulnerability to entrapment in the spleen, and this is where metabolic depletion and macrophage interactions lead to hemolysis (Fig. 7.2). Removal of the spleen allows hereditary spherocytes to have a near-normal lifespan, although the cytoskeletal defects and abnormal RBC shape persist.

The clinical features of hereditary spherocytosis encountered most commonly are anemia, jaundice, and splenomegaly. However, signs and symptoms are highly variable, with respect to both age of onset and severity. At one extreme, some mild cases may escape recognition for many years into adulthood, while, at the other extreme, hydrops fetalis with fetal death has been reported [48]. About 30–50% of adults with the disorder have a history of jaundice during the first week of life [49, 50]. The magnitude of hyperbilirubinemia occasionally requires exchange transfusion [51]. In contrast to the frequency of hyperbilirubinemia, most newborns with hereditary spherocytosis are not anemic at birth [49]. However, in many infants hemolytic anemia develops rapidly in the first month of life and can require one or more RBC transfusions. It is thought that this change reflects maturation of the splenic filtering function and development of the splenic circulation. Within a few months, erythropoiesis increases, anemia improves, and the need for red-cell transfusions disappears in all but the most severely affected infants. Beyond the neonatal period, jaundice is rarely intense. Even when patients have no detectable jaundice, there usually is laboratory evidence of ongoing hemolysis. Splenomegaly is the rule, and, in large family studies, palpable spleens have been detected in over 75% of affected members. No apparent correlation exists between spleen size and disease severity.

The diagnosis of the typical case of hereditary spherocytosis in older children is suggested by the complete blood count (CBC) (decreased to normal hemoglobin, increased reticulocytes, increased mean corpuscular hemoglobin concentration (MCHC), some spherocytes on the blood smear) and laboratory evidence of increased RBC destruction (increased bilirubin). In the newborn, morphologic assessment of the RBCs sometimes is difficult because even normal neonates may possess a minor population of spherocytes, and, conversely, infants with hereditary spherocytosis may have fewer spherocytes than will be the case later in life. The diagnosis is confirmed by the RBC osmotic fragility test; however, there once was concern whether the disorder could be diagnosed in the newborn period because fresh neonatal red cells normally are relatively resistant to osmotic lysis, while incubated infant RBCs are

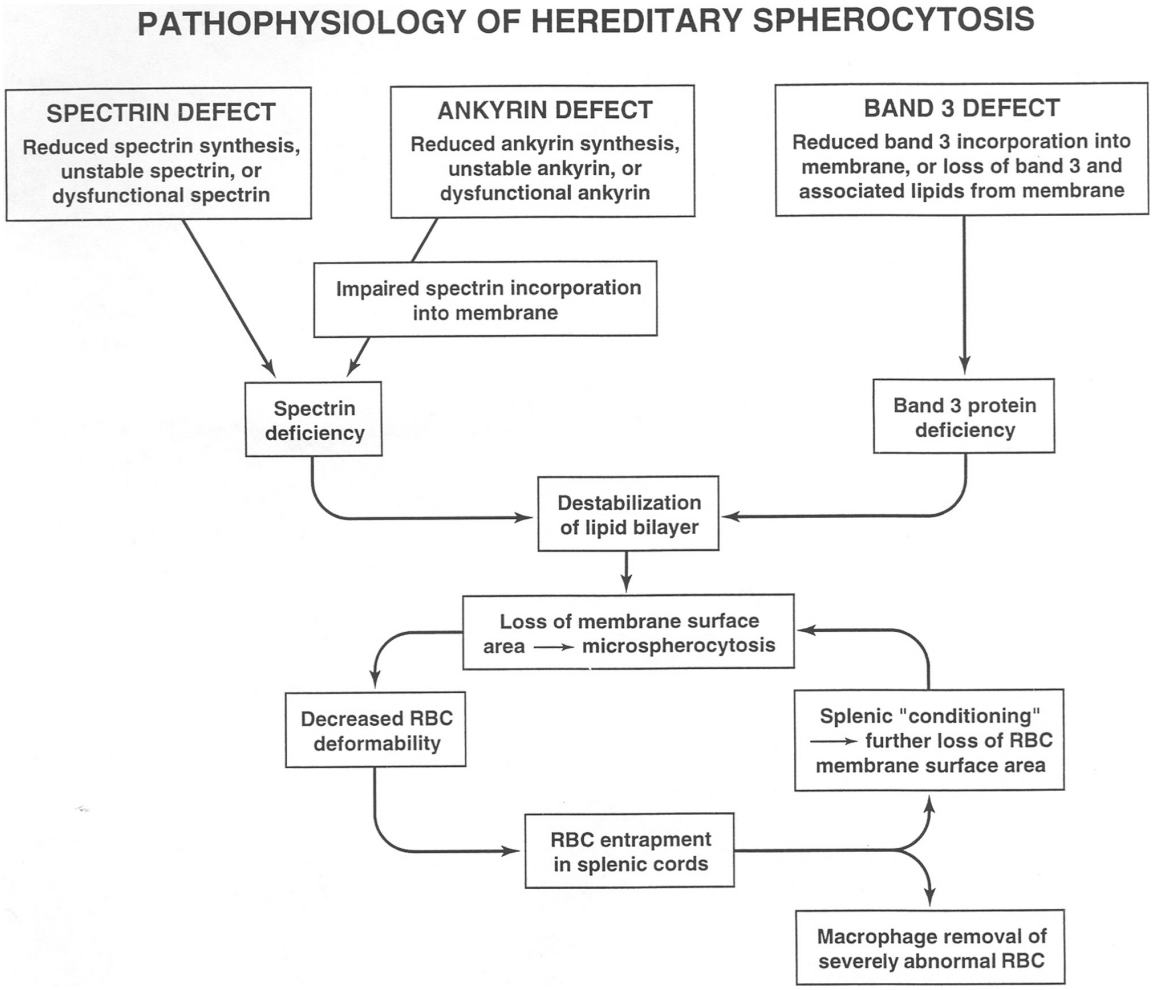

Fig. 7.2 The pathophysiology of hereditary spherocytosis is a consequence of spectrin, ankyrin, or band 3 abnormalities, which lead to membrane instability, loss of lipid microvesicles, decreased red cell surface area, reduced cellular deformability, and stagnation of red blood cells in the splenic cords. Schema modified from Palek and Jarolim [58].

osmotically more fragile. However, it has since been shown that the osmotic fragility test to detect hereditary spherocytosis in the newborn period can be used if there are appropriate normal neonatal RBC controls [52]. Alloimmune hemolysis, which also generates spherocytes, must always be ruled out in the work-up of a suspected case of hereditary spherocytosis. Spherocytes are commonly observed in ABO incompatibility but not in Rh disease. Blood typing and a direct antiglobulin test (DAT) usually confirm a diagnosis of ABO incompatibility, although the DAT occasionally is negative and thus misleading. In this situation, evaluation of the family for other individuals affected with spherocytosis may point to a hereditary rather than an acquired cause. Sometimes it is necessary to wait until the infant is three to four months of age to obtain a definitive laboratory diagnosis of hereditary spherocytosis; at this time, the confounding effects of maternal antibody and fetal RBCs are no longer present. Studies of

membrane proteins have been extremely important in enhancing our understanding of the biology of hereditary spherocytosis; however, these analyses, which are not available widely, have little clinical utility above that of the incubated osmotic fragility.

Treatment during the newborn period is directed toward management of hyperbilirubinemia and may require phototherapy and/or exchange transfusion in severe cases. In affected infants who also are homozygous for the mutation responsible for Gilbert syndrome, hyperbilirubinemia almost always requires phototherapy [53]. Occasionally, RBC transfusions may be required at birth for management of symptomatic anemia [48]. A common occurrence is the appearance of a transient but severe anemia during the first 20 days of life due to underproduction of erythropoietin in the face of continuing hemolysis [54]. These infants should be monitored closely following discharge from the nursery. Folic acid is required to sustain erythropoiesis: 0.5–1.0 mg per day is used for older children; for young infants, our pharmacy prepares an oral solution (1 ml of a 50-μg/ml solution). Splenectomy is the definitive treatment for hereditary spherocytosis; however, it is best deferred until the child is at least five years old because of the increased risk of overwhelming sepsis with encapsulated organisms such as *Haemophilus influenzae* or *Streptococcus pneumoniae* [55] that occurs following splenectomy in infants and young children. It has been suggested that partial splenectomy may reduce the rate of hemolysis without increasing the risk of overwhelming infection, and this is a possible option in very young children with severe hemolysis [56].

Hereditary elliptocytosis

Hereditary elliptocytosis is an autosomal dominant clinically heterogeneous group of disorders caused by mutations of RBC membrane cytoskeletal proteins (usually spectrin or protein 4.1). These protein abnormalities weaken skeletal protein interactions, disrupt the horizontal integrity of the membrane, and thereby increase RBC mechanical fragility [43, 57, 58]. The hereditary elliptocytosis variants occur with an estimated frequency of 1 in 5000, occurring in all racial and ethnic groups, although they are somewhat more common among blacks. Most individuals with common hereditary elliptocytosis have no clinical abnormalities or anemia, although the peripheral blood smear can be striking, with 15–100% elliptocytes (Fig. 7.1b). In a small fraction (5–20%) of common cases, there is some degree of hemolysis, usually compensated but occasionally leading to anemia (Hgb 9–12 g/dl, reticulocytes 20–25%). The peripheral blood smear in these hemolytic cases reveals poikilocytes in addition to elliptocytes. It is of interest that in families with common hereditary elliptocytosis, some individuals have a chronic hemolytic disorder while others do not, thus indicating that other genetic factors modify disease expression. Both homozygous and compound heterozygous forms of the disease also occur; these cases are associated with clinically severe hemolytic anemia. Morphological characteristics include marked poikilocytosis, microelliptocytosis, and red-cell fragmentation (Fig. 7.1c). In many cases, both parents have nonhemolytic common hereditary elliptocytosis. Hereditary pyropoikilocytosis (HPP) is a recessively inherited severe hemolytic anemia characterized by RBC membrane budding, red-cell fragments, microspherocytes, and poikilocytes seen on the peripheral smear (Fig. 7.1d). The MCV may be extremely low (30–50 fl), while the MCHC is normal. The osmotic fragility is increased. From a clinical perspective, it is difficult to distinguish HPP from homozygous or doubly heterozygous common hereditary elliptocytosis. Once regarded as a separate condition, HPP is biochemically related to common hereditary elliptocytosis, occurs in families where other members have common hereditary elliptocytosis, and now is considered to be a variant of this disorder. In childhood, affected individuals manifest moderately severe hemolytic anemia (hemoglobin concentration 5–9 g/dl), reticulocyte count 13–35%). Most but not all patients are black. The osmotic fragility is normal in most nonhemolytic forms of common hereditary elliptocytosis but is increased in those hemolytic variants with poikilocytosis and fragmentation. No treatment is needed for most patients, although splenectomy later in childhood is beneficial for those with chronic hemolysis.

Infantile poikilocytosis is a particularly interesting variant of hereditary elliptocytosis that occurs in the newborn period. Affected young infants have moderately severe hemolytic anemia and hyperbilirubinemia in the newborn period, the latter often necessitating exchange transfusion [59, 60]. The blood smear is characterized by marked red-cell fragmentation and poikilocytosis in addition to elliptocytosis. These morphologic changes are indistinguishable from those noted in patients with HPP. However, in contrast to HPP, hemolysis lessens gradually, and the clinical and hematologic features convert to those of mild hereditary elliptocytosis by 6–12 months of age [59]. In these infants, RBC membrane mechanical fragility is strikingly abnormal, probably a consequence of the destabilizing influence of large amounts of free intraerythrocytic 2,3-diphosphoglycerate (2,3-DPG) a byproduct of the presence of fetal hemoglobin [61]. As fetal hemoglobin levels decline postnatally in affected infants, membrane mechanical fragility improves, hemolysis disappears, and RBC morphology undergoes a transition from poikilocytosis to elliptocytosis. At birth, it is difficult to predict which patients will have transient poikilocytosis with ultimate recovery into common hereditary elliptocytosis and which are destined to have lifelong HPP with hemolysis. Close observation is necessary.

Hemolysis due to hereditary enzyme abnormalities

Glucose is the main metabolic substrate for RBCs. It is metabolized by the glycolytic or "energy-producing" pathway and, to a much lesser extent, by the hexosemonophosphate (HMP) shunt or "protective" pathway (Fig. 7.3). The initial and most important reaction of the HMP shunt is catalyzed by glucose-6-phosphate dehydrogenase (G6PD), a critical enzyme for protecting RBCs from oxidant injury. The major products of glycolysis are adenosine triphosphate (ATP), a source of energy for numerous RBC membrane and metabolic reactions, and 2,3-DPG, an important intermediate that modulates hemoglobin-oxygen affinity. A critical enzyme for the generation of ATP is pyruvate kinase. Hemolytic anemia due to G6PD deficiency is very common, while glycolytic enzymopathies are rare. Abnormalities in several glycolytic enzymes have been described, although over 90% of cases associated with hemolysis are due to pyruvate kinase deficiency. Hyperbilirubinemia, anemia, and even hydrops fetalis can be seen with inherited RBC enzymopathies. Overviews of this group of disorders are available elsewhere [44, 62].

Glucose-6-phosphate dehydrogenase deficiency

G6PD deficiency is a sex-linked disorder that affects millions of people throughout the world, particularly in Mediterranean countries, Africa, and China. In almost all G6PD-deficient individuals, hemolysis and anemia are present only during infections or after exposure to medications that are potent oxidants. In the steady state, there are no symptoms or laboratory signs of anemia. The clinical heterogeneity of G6PD deficiency is due to the very large number of different mutations, usually single amino-acid substitutions, that lead to altered enzyme function [63, 64]. Normal RBCs contain abundant amounts of reduced glutathione (GSH), a sulfhydryl-containing tripeptide that serves as an intracellular antioxidant, neutralizing peroxides that form during metabolism or are introduced directly from the extracellular environment. Because of their enzyme deficiency, G6PD-deficient RBCs have a limited capacity to regenerate GSH from oxidized glutathione (Fig. 7.3). In the absence of GSH, RBCs are vulnerable to oxidant injury. The effects of oxidants on RBCs are multifocal. Denatured globin precipitates, termed Heinz bodies, bind to the cell membrane, unfavorably altering its structure and function. Membrane lipid peroxidation may contribute to altered function. The activity of intracellular enzymes may decline. The ultimate result of these insults is hemolysis.

The normal or wild-type enzyme is referred to as G6PD B, and over 400 other variant enzymes have been identified and characterized on the basis of biochemical properties. The World Health Organization (WHO) has further classified these different G6PD variants on the magnitude of the enzyme deficiency

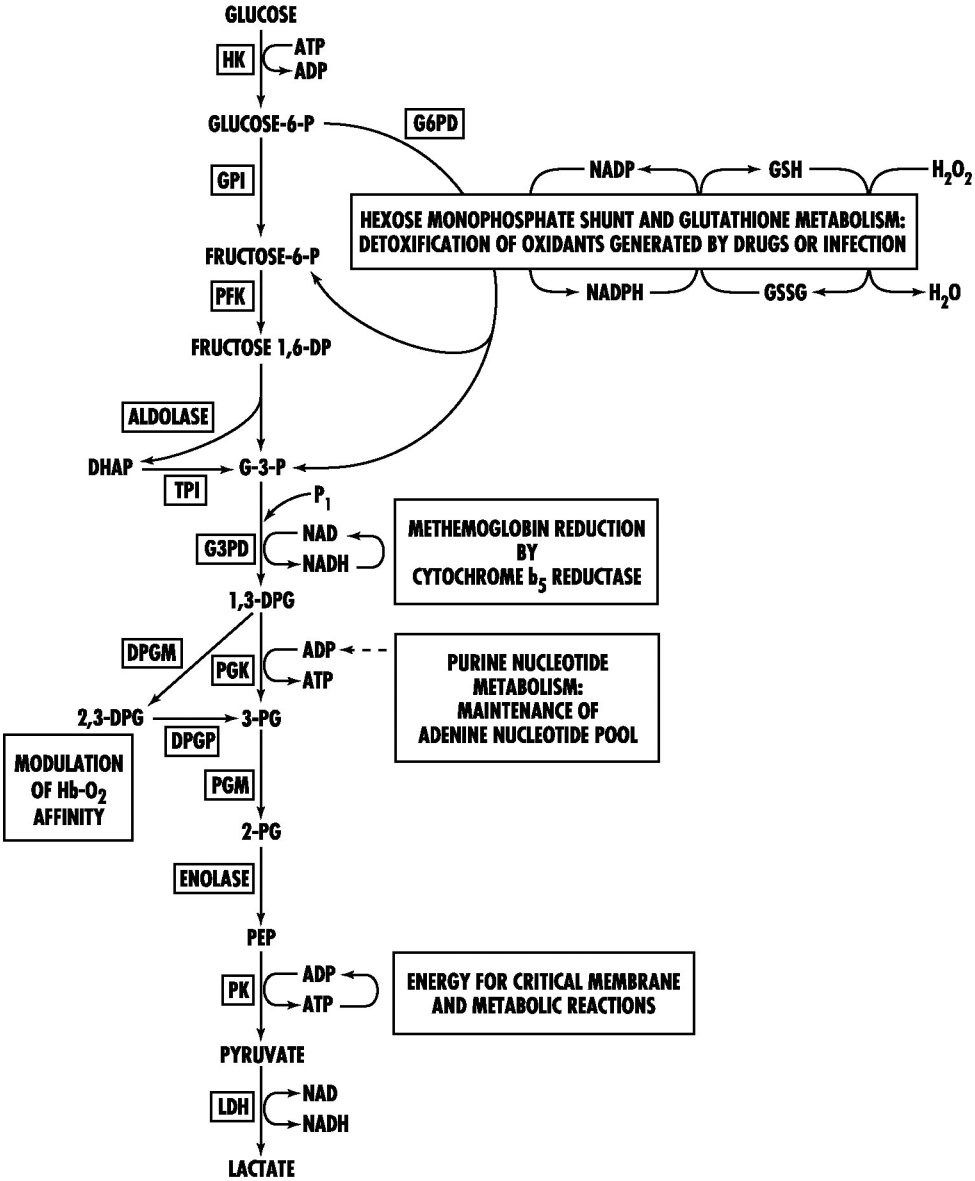

Fig. 7.3 Overall red cell metabolism and function.

Substrate abbreviations: (GSH) reduced glutathione; (GSSG) oxidized glutathione; (G-3-P) glyceraldehyde-3-phosphate; (DHAP) dihydroxyacetone phosphate; (1,3-DPG) 1,3-diphosphoglycerate; (2,3-DPG) 2, 3-diphosphoglycerate; (3-PG) 3-phosphoglycerate; (2-PG) 2-phosphoglycerate; (PEP) phosphoenolpyruvate.

Cofactors: (ADP) adenosine diphosphate; (ATP), adenosine triphosphate; (NAD) nicotinamide adenine dinucleotide; (NADH) reduced nicotinamide adenine dinucleotide; (NADP) nicotinamide adenine dinucleotide phosphate; (NADPH) reduced nicotinamide adenine dinucleotide phosphate.

Enzymes are shown in boxes: (HK) hexokinase; (G6PD) glucose 6 phosphate dehydrogenase; (GPI) glucosephosphate isomerase; (PFK) phosphofructokinase; (TPI) triosephosphate isomerase; (G3PD) glyceraldehyde-3-phosphate dehydrogenase; (PGK) phosphoglycerate kinase; (DPGM) diphosphoglycerate mutase; (DPGP) diphosphoglycerate phosphatase; (PGM) phosphoglycerate mutase; (PK) pyruvate kinase; (LDH) lactate dehydrogenase.

From: Glader B. [62]. Reproduced with permission.

Table 7.2 Compounds associated with hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficiency

Drugs and chemicals that are unsafe for class I, II, and III G6PD variants
Furazolidone (furoxone)
Methylene blue
Nalidixic acid (neggram)
Naphthalene (mothballs)
Nitrofurantoin (furadantin)
Phenazopyridine (pyridium)
Phenylhydrazine
Primaquine
Sulfacetamide
Sulfamethoxazole (gantanol)
Sulfanilamide
Sulfapyridine
Thiazolesulphone
Toluidine blue
Trinitrotoluene (TNT)
*Drugs and chemicals that are safe for class II and III G6PD variants**
Acetaminophen
Ascorbic acid
Aspirin
Chloramphenicol
Chloroquine
Colchicine
Diphenhydramine
Isoniazid
Menadione sodium bisulfite
Phenacetin
Phenylbutazone
Phenytoin
Probenecid
Procainamide
Pyrimethamine
Quinidine
Quinine
Streptomycin
Sulfamethoxypyridazine
Sulfisoxazole
Trimethoprim
Tripelennamine
Vitamin K

*In most cases, safety for class I G6PD variants is not known.

From Glader [62].

and also the severity of hemolysis [65]. Class I variant patients have very severe enzyme deficiency (less than 10% of normal) and have chronic hemolytic anemia; these patients are very rare. Class II variant patients have severe enzyme deficiency, but there is usually only intermittent hemolysis. A common variant in this class is $G6PD^{Mediterranean}$, found in Caucasians whose origins are in the Mediterranean area and the Near East. Class III variant people have moderate enzyme deficiency (10–60% of normal) with intermittent hemolysis, usually associated with infection or drugs. A very common variant in this class is $G6PD^{A-}$, found in 10–15% of African-Americans and with similar frequencies in western and central Africa. Hemolysis in children with class III variants usually is mild and self-limited, but in class II variants, hemolysis may be quite severe and the hemoglobin may fall to life-threatening levels. Moreover, severe hemolysis can occur in children with class II G6PD variants following exposure to fava (broad) beans. A list of drugs implicated as causes of hemolysis in G6PD deficiency is given in Table 7.2.

The diagnosis of G6PD deficiency in older children is suggested by the sudden appearance of a nonimmune hemolytic anemia in association with infection or the administration of drugs. Cells that appear as if a "bite" had been taken from them (due to splenic removal of Heinz bodies) are seen occasionally on the peripheral blood smear. Supravital stains of the peripheral blood with crystal violet may reveal Heinz bodies during hemolytic episodes. Although screening tests are available, definitive diagnosis requires assay of RBC G6PD activity. Measurement of enzyme activity may not reveal the deficiency in African-Americans immediately after a hemolytic episode, because the population of deficient cells has been eliminated or, in transfused patients, because of the presence of normal, enzyme-replete RBC. Repeating the assay after at least three months ensures that any transfused cells are gone and that the population of deficient cells has been regenerated so that a more accurate determination of the presence of G6PD deficiency can be made. In some cases, specific G6PD mutations can be identified by

DNA analysis, but this is used rarely in normal clinical situations [64, 66].

Hemolysis and/or hyperbilirubinemia resulting from G6PD deficiency is well documented in the newborn period [67], and may occur in utero [68]. Close monitoring of serum bilirubin levels in infants known to be G6PD-deficient is warranted [68–70]. From a practical perspective, neonatal hyperbilirubinemia occurs frequently with G6PD^{Mediterranean} (class II), while African-American infants with G6PD A$^-$ (class III) people appear to be at much less risk. Of interest, African-American infants with G6PD A$^-$ appear to be at minimal risk, although black African infants have an increased incidence of neonatal hyperbilirubinemia [71, 72]. Moreover, in the latter group, untreated hyperbilirubinemia frequently leads to kernicterus with severe neurologic injury or death [72, 73]. Since black Africans and African-Americans have the same G6PD A$^-$ genotypes, the adverse outcomes in Africa are thought to relate to local customs and differences in oxidant exposure. Another example suggesting that local environmental variables are probably important is the observation that the incidence of hyperbilirubinemia in G6PD-deficient infants born in Australia to Greek immigrants is lower than that noted in deficient infants in Greece [74]. Herbs used in traditional Chinese medicine and clothing impregnated with naphthalene also are examples of covert oxidants to which susceptible infants may be exposed. Moreover, drugs (e.g. sulfonamides), chemicals (e.g. ascorbate), and fava-bean ingestion by mothers in late gestation have been implicated as the inciting stimulus of hemolysis in newborns [75, 76].

In some cases, the hyperbilirubinemia seen in G6PD-deficient infants reflects accelerated red-cell breakdown, but often there is no obvious RBC destruction or oxidant exposure and it has been suggested that hyperbilirubinemia may have another etiology, possibly related to impaired liver clearance of bilirubin. In support of this hypothesis are the observations that production of carboxyhemoglobin a marker of hemolysis or RBC breakdown, is the same in G6PD^{Mediterranean}-deficient neonates with and without hyperbilirubinemia [77]. It is now thought that the variable degree of hyperbilirubinemia in G6PD-deficient neonates reflects the presence or absence of the variant form of uridine-diphosphoglucoronylsyl transferase responsible for Gilbert's syndrome [68]. The relative importance of the latter is underscored by the observation that most jaundiced G6PD-deficient neonates are not anemic and that evidence for increased bilirubin production secondary to hemolysis often is lacking [78].

Therapy for neonatal hemolysis and hyperbilirubinemia resulting from G6PD deficiency includes phototherapy or exchange transfusion to prevent kernicterus, RBC transfusion for symptomatic anemia, removal of potential oxidants that may be contributing to hemolysis, and treatment of associated infections. In infants known to be G6PD-deficient, prevention of severe hyperbilirubinemia by administration of a single intramuscular dose of Sn-mesoporphyrin, an inhibitor of heme oxygenase, is highly effective and safe [79].

A study from Nigeria has reported a much poorer outcome for G6PD-deficient infants born at home, presumably a reflection of delayed identification and treatment of hyperbilirubinemia in these neonates [72]. In the USA, there is concern that changes in healthcare delivery with early discharge of newborn infants may have similar consequences. In support of this is the report of four newborn infants with G6PD deficiency (three African-American, one mixed Peruvian/Chinese) who developed kernicterus following early hospital discharge [80]. This report is disturbing because kernicterus in the USA has been rare in recent years and the adverse outcomes in these four cases occurred despite adherence to the early neonatal discharge guidelines of the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists.

Neonatal screening for G6PD deficiency has been very effective in reducing the incidence of favism later in life in Sardinia [81] and in other regions where this potentially fatal complication is common [67]. In the USA, where the most common G6PD mutation is the A$^-$ variant found in people of African descent (who are not susceptible to favism and in whom life-threatening hemolytic

episodes are rare), neonatal screening has not been thought to be cost-effective. Similarly, prenatal diagnosis utilizing molecular techniques potentially is available but the benign course of most G6PD variants has precluded its development [82]. It could be argued that certain Mediterranean populations, especially Kurdish Jews with a 60–70% incidence of G6PD deficiency, might benefit from knowing their G6PD status and thereby avoiding obvious oxidant exposures. The class I G6PD variants associated with chronic hemolysis are so rare as to render screening impractical.

Routine blood-bank screening likewise appears to be unwarranted, and G6PD deficiency is not considered a problem in transfusion medicine. Even in areas where G6PD deficiency is endemic, screening of blood donors is not required. One careful evaluation of the recipients of G6PD-deficient blood uncovered no deleterious consequences [83]. However, patients receiving G6PD Mediterranean blood may have an increased serum bilirubin and lactate dehydrogenase (LDH) concentration following transfusion, and this can be confused with a transfusion reaction [84]. In premature infants, simple transfusions with G6PD-deficient red cells have been associated with hemolysis and severe hyperbilirubinemia requiring exchange transfusion [85]. Also, massive intravascular hemolysis has occurred in an Indian neonate following an exchange transfusion with G6PD-deficient blood [86]. In view of these occurrences, it has been recommended that in areas where G6PD deficiency (presumably class II variants) is common, donor blood should be screened for the enzyme before transfusing premature infants [85] or using the blood for a neonatal exchange transfusion [86]. Currently, this recommendation is not standard blood-banking practice.

Pyruvate kinase deficiency

Deficiency of erythrocyte pyruvate kinase is an autosomal recessive disorder that occurs in all ethnic groups [44]. Although it is the most common of the Embden–Meyerhof glycolytic pathway defects, it is rare in comparison to G6PD deficiency. Over 400 cases, mostly in northern Europeans, have been described [87]. A particularly high frequency exists among the Pennsylvanian Amish people, in whom the disorder can be traced to a single immigrant couple [88]. Pyruvate kinase is one of the two key enzymatic steps that generate ATP in RBCs. Because nonerythroid tissues have alternative means of generating ATP, clinical abnormalities in pyruvate kinase deficiency are limited to RBCs. More than 130 pyruvate kinase mutations have been defined at the nucleic-acid level and many more have been defined in terms of abnormalities of the pyruvate kinase protein [87]. Reflecting this genetic diversity, the hemolytic anemia that characterizes pyruvate kinase deficiency varies considerably in severity from family to family. Approximately one-third of pyruvate kinase-deficient individuals have a history of neonatal hyperbilirubinemia. Jaundice tends to appear early (on the first day of life) and may require exchange transfusion [89]. Death or kernicterus may occur. Severe intrauterine anemia and hydrops fetalis have been reported [87].

The diagnosis of pyruvate kinase deficiency should be considered in a jaundiced newborn with evidence of nonimmune hemolysis and in the absence of infections or exposure to hemolytic agents. Hemoglobinopathies and membrane disorders should be ruled out by examination of the blood smear and other appropriate diagnostic tests before proceding to assay of red-cell pyruvate kinase activity. RBC morphology in pyruvate kinase deficiency usually is normal, although a few dense cells with irregular margins (echinocytes) are seen occasionally. Pyruvate kinase heterozygotes are clinically and hematologically normal, although they have roughly half the normal amount of RBC pyruvate kinase activity.

Failure to demonstrate pyruvate kinase deficiency in the face of chronic hemolysis necessitates considering some of the other less common enzymopathies that occur in children. Glucose phosphate isomerase (GPI) deficiency is the second most common glycolytic enzymopathy associated with hemolysis. The

clinical manifestations of this disorder are identical to those of pyruvate kinase deficiency. The severity of hemolysis varies considerably. Anemia and hyperbilirubinemia complicate the postnatal course in many patients [90, 91]. Hydrops fetalis with death in neonates has been reported [92–94].

Phosphoglycerate kinase (PGK) deficiency is unique amongst glycolytic enzymopathies in that it is X-linked and most severely PGK deficient individuals with hemolysis also have neurologic abnormalities (seizures, mental retardation, aphasia, movement disorders).

Phosphofructokinase (PFK) deficiency is a unique cause of hemolytic anemia; red cells show partial PFK deficiency, with about 50% normal enzyme activity, although there is a profound enzyme deficiency in muscle associated with a severe myopathy [95, 96].

Triose phosphate isomerase (TPI) deficiency associated with hemolytic anemia has been reported in at least 25 individuals. A unique feature of this enzymopathy is an early onset of a severe neurologic disorder characterized by spasticity, motor retardation, and hypotonia. Most affected patients die before they are five years old [97–99].

Hexokinase deficiency is a rare cause of hemolysis that has been identified in over 20 individuals [100, 101]. Splenectomy ameliorates but does not cure the hemolytic process.

Pyrimidine 5′nucleotidase (P5′N) deficiency is the fourth most common enzyme abnormality associated with hereditary hemolytic anemia [102, 103]. Approximately 30 kindred representing wide geographic distribution have been reported, with a predisposition for people of Mediterranean, Jewish, and African ancestry. In all families studied, the disorder follows an autosomal recessive pattern. P5′N catalyzes the degradation of pyrimidine nucleotides to inorganic phosphate and the corresponding pyrimidine nucleoside [104, 105]. The most reasonable explanation for hemolysis in P5′N deficiency is that retained aggregates of ribosomes produce direct membrane injury, akin to that observed with Heinz bodies. The disorder is characterized by mild to moderate anemia, reticulocytosis, and hyperbilirubinemia. RBC morphology is unique in that marked basophilic stippling is present. Basophilic stippling of RBCs is the morphologic equivalent of partially degraded ribosomes. Diagnosis requires a specific spectrophotometric enzyme assay.

Hemolysis due to hemoglobin abnormalities

General considerations

The earliest embryonic hemoglobins are composed of zeta globin chains (forerunners of alpha globin chains) and epsilon chains (forerunners of gamma globin and beta globin chains) (Fig. 7.4). The transition from zeta to alpha globin chains is complete by the end of the first trimester. Epsilon chains disappear more slowly and are replaced first by gamma globin chains and then by the beta globin chains of adult hemoglobin. Fetal hemoglobin ($\alpha 2,\gamma 2$) is the major hemoglobin found in fetuses after the first trimester, while hemoglobin A ($\alpha 2,\beta 2$) is the major hemoglobin in children and adults. At the time of birth, 60–90% of the hemoglobin found in the normal term infant is fetal hemoglobin. After birth, gamma globin synthesis declines rapidly as beta globin production increases; by six months of age, the proportion of hemoglobin F is approximately 5%. Only trace amounts of hemoglobin A_2 ($\alpha 2,\delta 2$) and gamma globin chain tetramers ($\gamma 4$), or hemoglobin Barts, are present in cord blood. With postnatal maturation, the hemoglobin A_2 level increases gradually to the adult level of 2–3%, while hemoglobin Barts quickly disappears. In contrast to the major changes in globin during development, the heme moiety is unchanged in structure in embryonic, fetal, and postnatal hemoglobin molecules.

Hemolysis occurs in hereditary abnormalities due to decreased production of normal hemoglobins (thalassemia syndromes) or the production of qualitatively abnormal hemoglobins (sickle cell disorders, unstable hemoglobins). Those due to alpha globin chain abnormalities are manifested at birth, while those due to beta globin abnormalities may not be

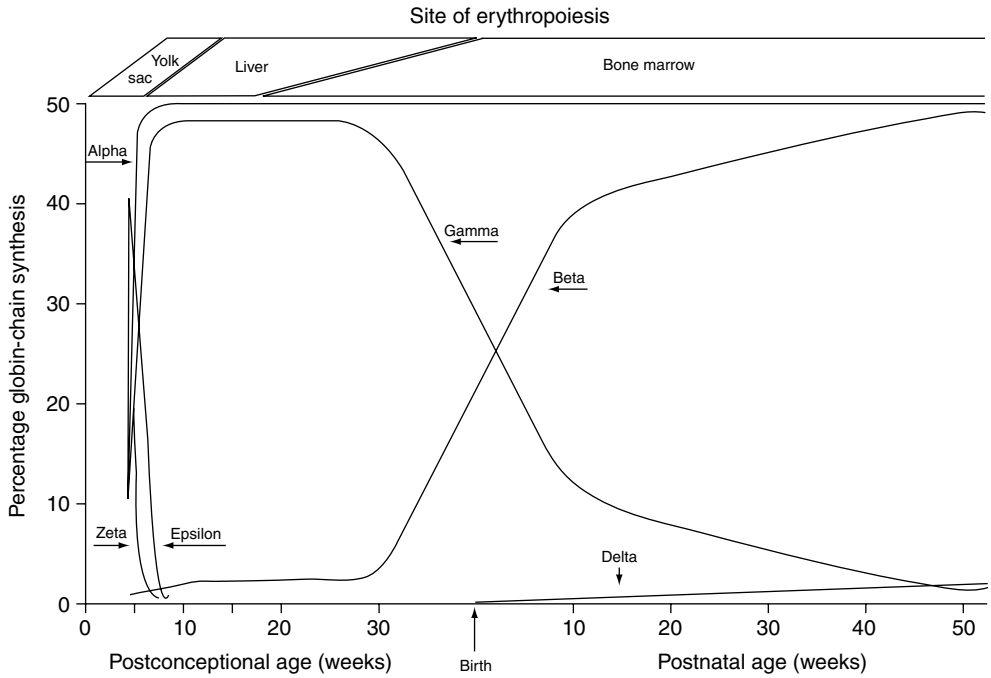

Fig. 7.4 Summary of fetal and infant hemoglobin synthesis. Reproduced with permission from Stockman and Pochedly [172].

apparent until four to six months of age, when the switch from hemoglobin F to hemoglobin A synthesis reveals the defect. In contrast, rare gamma globin mutations are evident in fetal and neonatal life but disappear by three months of age, when gamma globin synthesis is replaced by beta globin synthesis.

Thalassemia syndromes

Thalassemia syndromes are characterized by diminished or absent production of normal alpha globin polypeptides (alpha thalassemias) or beta globin chains (beta thalassemias). The decreased globin synthesis causes a microcytic anemia, the severity of which depends upon the remaining number of functional alpha and beta genes. However, since non-thalassemic globin chains are produced at a normal rate, the resulting imbalance of globin chain production also can contribute to the pathophysiology. For example, in alpha thalassemia, there is diminished synthesis of alpha globin chains, leading to a relative excess of gamma or beta chains. In the fetus, the excess gamma chains form tetramers ($\gamma 4$), also known as hemoglobin Barts, while beyond the neonatal period, the excess beta chains form tetramers ($\beta 4$), also known as hemoglobin H. The opposite is true of beta thalassemia, in which excess alpha globin chains accumulate. Aggregates of free alpha chains lead to RBC membrane damage and rapid hemolysis. Moreover, hemoglobin Constant Spring (Hgb CS) is an alpha globin mutant that modifies alpha thalassemia syndromes, while Hgb E, a beta globin variant, influences beta thalassemia.

Alpha thalassemia

Alpha thalassemia is of particular importance to neonatologists because its clinical manifestations are present in utero and at birth. These disorders

Table 7.3 Alpha thalassemia syndromes

Alpha thalassemia syndromes	Genotype	Clinical features
Normal	(αα/αα)	
Silent carrier	(–α/αα)	
Alpha thalassemia trait	(– –/αα) or (–α/–α)	Mild microcytic anemia
Hemoglobin H disease	(– –/–α)	Mild moderate hemolytic anemia
Homozygous alpha thalassemia	(– –/– –)	Severe anemia hydrops fetalis
Hemoglobin Constant Spring (Hgb CS) syndromes		
Heterozygous Hgb CS	(α^{cs}α/αα)	Similar to alpha thalassemia trait
Hgb H disease with Hgb CS	(α^{cs}α/– –)	Similar to Hgb H disease
Homozygous Hgb CS	(α^{cs}α/α^{cs}α)	Similar to Hgb H disease

occur worldwide, with an increased frequency in Africa, the Mediterranean, and throughout Asia, where the more severe forms of alpha thalassemia are found. The genes directing alpha globin synthesis are duplicated on the short arm of chromosome 16. Thus, with two genes per chromosome, there is a total of four genes (αα/αα) that control normal alpha globin polypeptide production. The alpha thalassemia syndromes are due to deletions of alpha globin genes, although nondeletional forms of alpha thalassemia occur much less commonly. Since the nonthalassemic globin chains are produced at a normal rate, gamma globin tetramers (γ4 or hemoglobin Barts) and beta globin tetramers (β4 or Hgb H) appear in substantial quantity as the number of functional alpha globin genes decreases. In families with alpha thalassemia, an infant can inherit one, two, three, or four alpha thalassemia gene deletions, giving rise to the following four clinical alpha thalassemia syndromes: silent carrier state, alpha thalassemia trait, hemoglobin H disease, and homozygous alpha thalassemia (Table 7.3).

The silent carrier state (alpha thalassemia 2 in older literature) occurs in individuals lacking one functional alpha globin gene (–α/αα). These individuals have no clinical manifestations and are hematologically normal. At birth, the one distinguishing feature is a slightly increased concentration of hemoglobin Barts (1–3%) compared with that seen in normal neonates (less than 1%). Beyond the newborn period, there is no anemia or microcytosis, and both Hgb F and Hgb A_2 percentages are normal.

Alpha thalassemia trait (alpha thalassemia 1 in older literature) is due to a deficiency of two alpha globin genes. Deletion or nonfunction of two alpha globin genes, in *cis* or *trans*, is associated with mild microcytic anemia, without hemolysis or reticulocytosis. These individuals may have a mild anemia with microcytic RBCs. Even neonates are microcytic (i.e. MCV less than 100 fl). The cord blood of infants with alpha thalassemia trait usually contains 3–10% Hgb Barts, and this can be useful in identifying new patients at birth. There are no significant clinical abnormalities associated with alpha thalassemia trait, and beyond the newborn period this mild microcytic anemia can be mistaken for iron deficiency. In most cases beyond the newborn period, the presumptive diagnosis of alpha thalassemia trait is made after iron deficiency, beta thalassemia trait, and hemoglobin E disorders have been ruled out by specific tests. Of interest, the alpha globin gene deletions occurring in Southeast Asians with alpha thalassemia trait are in *cis*, or located on the same chromosome (– –/αα), whereas people of African descent with alpha thalassemia trait have their alpha globin gene deletions in *trans*, on different chromosomes (–α/–α). As a consequence, the more serious alpha thalassemia disorders (Hgb H disease and homozygous alpha thalassemia, see below) occur almost exclusively in Southeast Asians and rarely, if ever, in people of African ancestry.

Hemoglobin H disease occurs in individuals with one functional alpha globin gene. In Southeast Asia, it has been estimated that 13 000–16 000 infants with

Hgb H are born annually, translating into nearly 700 000 affected individuals living with Hgb H disease. This disorder occurs most commonly when there is a deletion of three genes (––/–α), in which case one parent usually has alpha thalassemia trait (––/αα) while the other parent is a silent carrier for alpha thalassemia (αα/α–). Similarly, individuals who inherit the alpha thalassemia trait genotype on one chromosome and have a Constant Spring gene on the other chromosome (––/$\alpha^{cs}\alpha$) also have hemoglobin H disease (see below). During the newborn period, Hgb H disease is characterized by marked hypochromia and microcytosis and cord blood with high concentrations of Hgb Barts (20–30%). Beyond infancy, the imbalance in globin chain synthesis is associated with beta globin excess and the presence of Hgb H. The latter is detected as a rapidly migrating hemoglobin (5–40% total Hgb) on electrophoresis of a fresh blood sample on cellulose acetate at alkaline pH. When erythrocytes from a patient with an intact spleen are incubated with the dye brilliant cresyl blue, small inclusions of precipitated Hgb H are seen.

Beyond infancy, this disorder is characterized by a mild to moderate hemolytic anemia, fatigue, and splenomegaly. Parents of infants who have hemoglobin H disease should be instructed to avoid oxidant agents that can cause hemolysis (the same list that is given to patients with G6PD deficiency, see Table 7.2). Although these infants are usually only mildly anemic, they may experience significant episodes of hemolysis during infections or exposure to oxidant agents. Older patients with severe anemia and hypersplenism occasionally benefit from splenectomy, but this is rarely necessary in childhood.

Homozygous alpha thalassemia (hydrops) is due to a deletion of all four alpha globin structural genes (––/––). Family studies of children with homozygous alpha thalassemia reveal that both parents have alpha thalassemia trait (––/αα). The absence of functioning alpha globin genes is incompatible with extrauterine life. The reason that fetuses survive in utero is thought to be due to the persistent function of hemoglobin Portland, an embryonic hemoglobin ($\xi_2\gamma_2$). Hemoglobin Barts, which has an extremely high oxygen affinity, impairs oxygen delivery and contributes to the pathophysiology of this disorder. The beneficial effect of Hgb Portland is thought to be related to its normal oxygen affinity. Hemoglobins present in cord blood represent tetramers of non-alpha globin chains, with approximately 70–80% Hgb Barts, small amounts of Hgb H, and small amounts of Hgb Portland. The anemia is severe (Hgb concentration 3–10 g/dl) and erythroblastosis is present [106].

Physical findings in affected infants include marked pallor, edema, hepatosplenomegaly, and a variety of other congenital abnormalities [107]. Most fetuses with homozygous alpha thalassemia and who are not aborted are usually stillborn, but several have been born alive, resuscitated, and placed on chronic RBC transfusion programs [108–110]. If a suitable donor is available, these infants can be considered for curative bone-marrow transplantation [111]. Experimental treatment of homozygous alpha thalassemia in utero by means of hematopoietic stem-cell transplantation is currently under evaluation.

The natural history of the Hgb Barts hydrops fetalis syndrome has been described in 65 infants in Thailand: 25% died in utero, 18% died during delivery, and 54% died within one hour of delivery. Abnormalities noted at autopsy included gross enlargement of the placenta, heart, liver, spleen, and adrenal glands. Moreover, there was significant retardation of brain growth, and many were less than 60% of the expected weight for the gestational age. Also of interest was the observation that some infants had relatively few abnormalities at autopsy.

In addition to the fetal problems that occur during gestation, there also are significant maternal complications. A variable amount of fetal edema develops early in gestation (18–28 weeks), and this can be detected by fetal ultrasound [112]. The vast majority of mothers carrying fetuses with homozygous alpha thalassemia develop toxemia of pregnancy [113]; the reason for this is unknown. Also, vaginal delivery is complicated by the retention of large bulky placentas, and occasionally there is postpartum hemorrhage [112, 113]. It has been estimated that in the absence of obstetrical care, up to 50% of mothers carrying hydropic fetuses suffer lethal complications.

For all of the above reasons, it is important to identify those families at risk for bearing children with homozygous alpha thalassemia. Specifically, if one partner in a relationship is noted to have alpha thalassemia trait (microcytosis not related to iron deficiency, beta thalassemia trait, or Hgb E), then it is important to assess the other partner [114]. Moreover, if alpha thalassemia trait is first noted in a child, then it is important to study both parents, since families may anticipate having more children [115]. Genetic counseling for these high-risk families should include a discussion of the risks to the fetus as well as to the mother. In families at risk where pregnancy has occurred already, prenatal diagnosis is available [116, 117]. During the first trimester of pregnancy, both chorionic villus sampling (at 8–12 weeks) and amniocentesis (at 16–18 weeks) can provide fetal cells for DNA analysis [106, 116]. This approach also has been used to detect hemoglobin H disease during pregnancy [118]. During the second or third trimester of pregnancy, ultrasound-guided fetal blood sampling, which detects 78–99% of Hgb Barts, has been used to diagnose the cause of fetal hydrops [118]. This information may be useful in deciding whether to terminate a pregnancy early before the mother is at risk for developing toxemia.

Hemoglobin Constant Spring is due to a mutation that alters the normal translation codon for terminating alpha globin chain synthesis, thereby leading to the production of an elongated alpha globin variant (alpha CS globin). However, this structural abnormality has an alpha thalassemic phenotype because the output of alpha^{cs} globin is less than 1% of normal [119, 120]. For unknown reasons, the α^{cs} allele is found only where the other alpha globin allele on the same chromosome is normal ($\alpha^{cs}\alpha$). Heterozygous Hgb CS ($\alpha^{cs}\alpha/\alpha\alpha$) appears like the alpha thalassemia silent carrier state (or, occasionally, like the alpha thalassemia trait), with normal hematologic findings except for a small amount of Hgb CS (1–2%), seen as a slow-migrating hemoglobin on electrophoresis (cellulose acetate, alkaline pH). The simultaneous inheritance of an α^{cs} allele and alpha thalassemia trait ($\alpha^{cs}\alpha/--$) results in a phenotype that is similar to that seen in Hgb H disease, characterized by a hemolytic anemia with 10–15% Hgb H and 2–3% Hgb CS. It is estimated that this variant of Hgb H disease in Southeast Asians may be as common as that due to deletions of the alpha globin gene. Homozygous Hgb CS ($\alpha^{cs}\,\alpha/\alpha^{cs}\,\alpha$) at one time was thought to be similar to alpha thalassemia trait, since both were associated with only two functioning alpha globin genes. However, it is now recognized that individuals homozygous for the Hgb CS mutation have a clinical syndrome more like that seen with Hgb H disease. There is splenomegaly and a mild hemolytic anemia, the RBCs are normocytic (not microcytic), and there is marked basophilic stippling. Moreover, beyond infancy, there is an accumulation of Hgb Barts instead of Hgb H. One possible explanation for this hemolytic anemia is that α^{cs} mRNA is unstable, thus precipitating and causing cell-membrane injury and basophilic stippling.

Beta thalassemia

Like alpha thalassemia, beta thalassemia is found in regions of the world where malaria was formerly endemic: Southeast Asia, India, Africa, and the Mediterranean basin. The beta thalassemias are a consequence of mutations that impair the normal process of beta globin chain production. There are two beta globin genes, one located on each chromosome 11. Heterozygous beta thalassemia (beta thalassemia trait) is due to inheritance of one beta thalassemia gene, resulting in a lifelong mild hypochromic microcytic anemia (Hgb 8–11 g/dl) appearing at several months of age. The clinically important disorders seen in this population include homozygous beta thalassemia and Hgb E/beta thalassemia.

Homozygous beta thalassemia is due to the inheritance of two beta thalassemic genes, leading to a marked reduction or absence of beta globin chain production. Although deletion of the beta globin locus is an occasional cause of beta thalassemia, most cases are caused by point mutations that affect transcription, mRNA processing, or translation [121–123]. There are two general types of homozygous beta thalassemia, β^0 and β^+. In β^0 thalassemia, no beta globin is produced by the thalassemic locus, whereas in β^+ thalassemia, there is

reduced but measurable output of beta globin. The severity of homozygous beta thalassemia (or beta thalassemia major) is greatest when two β^0 thalassemia genes are inherited and is usually much milder when two β^+ thalassemia genes are inherited. Severe beta thalassemia is associated with lifelong hemolytic anemia, dependence on regular RBC transfusions for survival, and the gradual development of transfusion-associated hemosiderosis [123]. The clinical abnormalities of beta thalassemia are not evident at birth but first present after three months of age, when beta globin normally becomes the dominant form of non-alpha globin that is synthesized. Although affected newborns appear normal, the diagnosis of β^0 thalassemia can be made at birth when hemoglobin electrophoresis or other hemoglobin analysis fails to detect any hemoglobin A. However, diagnosis of β^+ thalassemia by these techniques in the newborn period is not possible because the reduced amount of hemoglobin A produced overlaps the range for normal babies. Direct identification of beta thalassemia mutations by DNA diagnostic techniques is increasingly available and does allow the identification at birth of all infants with beta thalassemia major. Today, however, these techniques are used most commonly for prenatal diagnosis of beta thalassemia syndromes. DNA can be obtained during mid trimester from fetal amniocytes (at 15–17 weeks) or during the first trimester from chorionic villi (at 9–11 weeks); the assay can be completed within a few days, allowing families to make informed decisions regarding termination of pregnancy [124]. The implementation of a strategy of carrier detection, genetic counseling, and prenatal diagnosis in countries where beta thalassemia is common has led to a striking reduction in the number of births of infants with beta thalassemia major [125].

Hemoglobin E/beta thalassemia

Hemoglobin E is the second most common hemoglobin variant in the world (Hgb S being the most common) and is seen predominantly in people from Southeast Asia. At the border of Thailand, Laos, and Cambodia, there is a 20–40% prevalence of Hgb E. The structure of Hgb E differs from Hgb A by the replacement of lysine for glutamic acid at position 26 of the normal beta globin chain due to a DNA substitution of adenine for guanine at codon 26 of the beta globin gene. This mutation also alters RNA processing, thus resulting in decreased production of functional beta globin mRNA, reduced beta globin chains, and a mild thalassemic phenotype. The thalassemic component of the condition is mild (a β^+ thalassemia), so that hemoglobin E carriers are microcytic but not anemic. Even hemoglobin E homozygotes have little or no anemia. However, coinheritance of hemoglobin E trait and β^0 thalassemia trait can give rise to a transfusion-dependent form of beta thalassemia major [126]. As with other types of beta thalassemia major, clinical abnormalities are not seen until the infant is three to six months of age. However, the presence of hemoglobin E is detected easily at birth by hemoglobin electrophoresis or related techniques. Infants found to have an FE hemoglobin screening pattern need careful follow-up to exclude the possibility of hemoglobin E beta thalassemia. DNA-based detection of the hemoglobin E mutation is feasible [127] and has been applied to both prenatal and neonatal diagnosis.

Hgb E/beta thalassemia is much more common than homozygous beta thalassemia in Southeast Asia, and the same is true for refugees to the USA. During 1990–95, the Californian newborn hemoglobinopathy screening program identified 29 neonates with beta thalassemia major, 9 of whom were Southeast Asian, and 26 neonates with hemoglobin E/beta thalassemia, 25 of whom were Southeast Asian [128]. These data reflect in part the large influx of Southeast Asians into California. However, there also has been a marked decrease in the incidence of beta thalassemia major in other ethnic groups (e.g. Italians, Greeks) at high risk for having children with beta thalassemia major. The clinical course of Hgb E/beta thalassemia is quite variable. In some cases, the clinical features are identical to those seen in homozygous beta thalassemia and the anemia is sufficiently severe to require regular RBC

transfusions, iron chelation therapy, and, in some patients, stem-cell transplantation. In other cases, the anemia is milder, with Hgb levels of 7–8 g/dl and no need for RBC transfusion therapy. One explanation for this difference, just as in the case for homozygous beta thalassemia, is the concomitant inheritance of alpha thalassemia genes, which decreases the globin chain imbalance and, hence, the degree of RBC membrane damage and magnitude of hemolysis.

Gamma thalassemia

Large deletions within the beta globin gene cluster may remove both gamma globin genes ($^{A}\gamma$ and $^{G}\gamma$) as well as the delta and beta globin genes. The resulting gamma delta beta thalassemia is lethal in the homozygous state, but in the heterozygote it produces a transient but moderately severe microcytic anemia in the newborn. Over the first few months of life, the anemia improves without specific therapy and eventually the hematologic picture is that of beta thalassemia trait. At least eight different gamma delta beta deletions have been reported, all but one in families of European origin [122].

Sickle cell disease

The sickle hemoglobinopathies are beta globin mutations that, like beta thalassemia, do not become clinically evident until the infant is several months of age. Sickle hemoglobin (Hgb S) is found throughout the world, with an increased incidence in central Africa, the Near East, the Mediterranean, and parts of India. Inheritance of the sickle gene follows an autosomal dominant pattern; from a practical perspective, four distinct sickle syndromes are recognized: sickle cell trait (Hgb AS), homozygous sickle cell disease (Hgb SS), hemoglobin SC disease, and hemoglobin S beta thalassemia. Beyond the neonatal period, hemoglobin electrophoresis on cellulose acetate at pH 8.6 is the most practical definitive test to differentiate the various sickling disorders. Under these conditions, hemoglobin S has a very distinct mobility, which distinguishes it clearly from Hgb A, F, and C. At birth, as part of newborn screening programs, acid citrate agar electrophoresis allows for a better separation of Hgbs A and F and, thus, a clearer definition of sickle trait versus sickle cell anemia. Alternatively, high-performance liquid chromatography (HPLC) allows for very discrete separation of hemoglobins, and this has been adopted by some states as the newborn screening test of choice [128]. Results of such screens are expressed as a pattern describing the hemoglobins present in order of descending concentration (Table 7.4). Newborn screening for hemoglobinopathies is further discussed in Chapter 8.

Table 7.4 Hemoglobin patterns observed in newborn screening

Hemoglobin screening pattern	Diagnosis
FA	Normal
FAS	Sickle trait
FS	Sickle cell anemia
FSA	Sickle beta thalassemia
FSC	Hgb SC disease
F	Homozygous beta thalassemia
FAE	Hgb E trait
FE	Hgb EE
FEA	Hgb E/β-thalassemia

Patterns are reported by the hemoglobins that are present in order of decreasing concentration.

Sickle cell anemia, the most severe of the disorders, is the result of inheritance of two β^S mutations (substitution of valine for glutamic acid at the sixth amino acid on the beta globin chain), one from each parent. Sickle β^0 thalassemia, phenotypically identical to sickle cell anemia, is caused by inheritance of one β^S and one β thalassemia mutation. The third common form of sickle cell disease, hemoglobin SC disease, is somewhat milder than sickle cell anemia or sickle β^0 thalassemia. It is the consequence of inheritance of one β^S mutation and one β^c mutation (the substitution of lysine for glutamic acid at the sixth amino acid on the beta globin chain). Although no clinical abnormalities associated with sickle cell disease are present at birth, early diagnosis is important because two potentially fatal but largely

preventable complications may occur during the first year of life [129]. The first is the splenic sequestration crisis, an unpredictable pooling of large numbers of RBCs in the spleen, which leads to a rapid decrease in hematocrit and, in the most severe cases, cardiovascular collapse and death. The second is overwhelming septicemia, usually caused by *S. pneumoniae* or *H. influenzae*. The unusually high susceptibility to infection with encapsulated organisms such as *S. pneumoniae* is the consequence of functional asplenia, which commonly appears by one year of age in sickle cell anemia or sickle β^0 thalassemia infants (but not until later in hemoglobin SC disease). Prompt treatment of splenic sequestration with RBC transfusions is lifesaving, so that parents are taught to recognize early manifestations such as splenic enlargement, lethargy, and pallor. Overwhelming sepsis can be prevented in most instances by early immunization with *H. influenzae* and conjugated pnuemococcal vaccines, beginning at two months of age, and by institution of prophylactic penicillin at a dose of 125 mg twice daily [130]. It is the need to institute these prophylactic measures within the first one to two months of life that provides a compelling rationale for the neonatal diagnosis of the sickling disorders. In many states, all newborns are screened for these disorders, whereas in others only high-risk ethnic groups are targeted. Usually, a dried sample of blood on filter paper, collected at the same time as other screening tests for inherited metabolic disorders, is used, but cord blood is also satisfactory. Tests that quantitate the amount of hemoglobin S, such as HPLC, thin-layer isoelectric focusing, or electrophoresis on both cellulose acetate (in an alkaline buffer) and citrate agar (in an acid buffer), are adequate, but sickle solubility tests or the sodium metabisulfate "sickle prep" are not, because sickle cell disease cannot be distinguished from sickle cell trait and the tests are not sensitive enough to detect reliably the small percentage of hemoglobin S present in RBCs of the newborn. Prior transfusion with normal adult red cells may cause a false-negative newborn screening result and delay the diagnosis [131]. Extensive experience with mandatory statewide screening for all infants has been accumulated in New York [132], California [133], and elsewhere [134].

Infants who are born to mothers with sickle cell disease present more of a clinical problem during gestation and the neonatal period than infants who actually have sickle cell disease. Spontaneous abortion, intrauterine growth retardation (approximately 15%), stillbirth (6%), preterm labor and delivery, and perinatal mortality (approximately 15%) are all more frequent in infants of mothers with sickle cell anemia [135]. These problems are thought to be due to abnormalities of the placenta, such as small size, infarction, and an increased incidence of placenta previa and abruptio placentae. These alterations appear to be the consequence of sickle vaso-occlusive events within the maternal side of the placental circulation; they are not caused by the presence of the sickle trait, beta thalassemia trait, or hemoglobin C trait in the infant, because no hematologic disease is associated with the carrier state for these mutations, even in adult life when they are expressed fully, except under conditions of extreme hypoxia.

One caveat regarding sickle trait blood is that blood from an adult donor who has sickle trait should not be used for exchange transfusions in the newborn, particularly if hypoxemia is present, because sickle trait RBCs in this setting may contribute to a fatal outcome [136].

Unstable hemoglobinopathies

These disorders are due to amino-acid substitutions that decrease heme binding to globin and/or alter the normal tertiary structure of hemoglobin. As a consequence of these changes, hemoglobin is unstable as manifested by intracellular denaturation and Heinz body formation. Several unstable hemoglobins have been described [137, 138]; generally, these disorders are characterized by mild to moderate hemolysis associated with Heinz bodies. The overall clinical course may mimic G6PD deficiency. Specific diagnosis of unstable hemoglobinopathies is made by detecting hemoglobin instability at 50°C or in the presence of isopropanol [139]. Neonatal

problems due to unstable hemoglobins are unusual, since most described variants are due to beta globin chain abnormalities. Two unique hemoglobinopathies have been described in association with neonatal hemolysis: hemoglobin F-Poole and hemoglobin Hasharon. In all likelihood. however, some of the presently undiagnosed transient hemolytic anemias of infancy probably are due to unstable fetal hemoglobin variants.

Hemoglobin F-Poole [140] is a mutant fetal hemoglobin in which the one hundred and thirtieth residue of the gamma globin chain contains glycine instead of tryptophane. As a consequence of this mutation, hemoglobin F-Poole is unstable (heat and isopropanol instability) and associated with Heinz body hemolytic anemia during the first weeks of life. In the one reported case of this disorder, all signs of hemoglobin instability and hemolysis disappeared after six weeks of age.

Hemoglobin Hasharon differs from hemoglobin A by an amino-acid substitution at the forty-seventh residue of the alpha globin chain (aspartic acid is replaced by histidine). Adults heterozygous for hemoglobin Hasharon generally are asymptomatic although rarely there may be evidence of mild hemolysis [141]. A premature infant with this alpha chain mutation was reported to have moderate but persistent hemolytic disease throughout the first weeks of life [142]. Since hemolysis disappeared at the time beta globin chain synthesis became maximal, it was suggested that hemolysis may have been due to instability of the fetal form of this abnormal hemoglobin.

Methemoglobinemia

Although methemoglobinemia is not a hemolytic disorder, we have included a brief discussion of this problem here because maintaining low levels of methemoglobin nicotinic adenine is dependent on glycolysis for reduced nicotinamide adenine dinucleotide (NADH) production, and some of the congenital causes of this disorder are related closely to defects described elsewhere in this chapter. Methemoglobin is an oxidized derivative of hemoglobin in which heme iron is in the ferric (Fe^{3+}) or oxidized state rather than the ferrous (Fe^{2+}) or reduced state. Because methemoglobin is unable to bind oxygen, the presence of significant amounts of this respiratory pigment affects blood oxygen transport adversely. Normally, small amounts of methemoglobin are formed daily in vivo by the action of endogenous agents, which may include oxygen itself (auto-oxidation). However, as methemoglobin forms, it is reduced rapidly through the action of red-cell NADH and cytochrome b_5 reductase (also known as NADH-methemoglobin reductase), so that in normal individuals, levels of methemoglobin seldom exceed 1%. A second reduced nicotinamide adenine dinucleotide phosphate (NADPH)-methemoglobin reductase is also present in red cells but is not functional under normal physiologic conditions. The latter enzyme is activated only by certain redox compounds, and this is the basis for treatment of methemoglobinemia with methylene blue. Methemoglobinemia usually is a combined result of acquired environmental abnormalities, deficiency of cytochrome-b_5 reductase, and/or the presence of one of the M hemoglobins.

Acquired methemoglobinemia can occur in normal individuals following exposure to chemicals, such as aniline dyes, that readily oxidize hemoglobin iron. Newborns are particularly susceptible, because fetal hemoglobin is oxidized more readily to the ferric state than is hemoglobin A [143] and also because RBC cytochrome-b_5 reductase activity is low during the first few months of life [144]. Merely marking the diapers of newborns with aniline dyes has caused clinically significant methemoglobinemia.

The best known of the chemicals that cause methemoglobinemia are nitrites, either contained within ingested material or converted from nitrates by the action of intestinal bacteria. Fertilizers are rich in nitrites and the washout can contaminate well water and cause methemoglobinemia in infants [145, 146]. Also, foods with a high nitrate content (e.g. cabbage, spinach, beets, carrots) can produce methemoglobinemia in infants [147]. Diarrheal disorders

during infancy have been associated with transient methemoglobinemia, possibly related to production of nitrites in the bowel [148–152]. Another nitrate association is the observation of methemoglobinemia following the administration of nitrous oxide to babies for treatment of persistent pulmonary hypertension [153–157].

Acidosis due to renal tubular disease also may exacerbate methemoglobinemia [149, 158]. Prilocaine administered before birth to provide local anesthesia can produce methemoglobinemia in both the mother and the infant [159]. Moreover, other local analgesics, including eutectic mixture of lidocaine and prilocaine (EMLA), can cause slight methemoglobinemia in the newborn, but usually this is not clinically significant and should not proscribe its use [160–163]. Other drugs, including over-the-counter medications and the dye henna also can cause methemoglobinemia [149, 161, 164]. During an eight-month period, Hjelt and coworkers studied 415 neonates in the neonatal intensive care unit (NICU) and 8% had methemoglobinemia. Prematurity, length of hospitalization, diarrheal illness, parenteral nutrition, and the use of antibiotics were associated strongly with the presence of methemoglobin [165].

Congenital methemoglobinemia is due to inherited disorders of hemoglobin structure or to a severe deficiency of NADH methemoglobin reductase activity. The inherited abnormalities of hemoglobin structure that give rise to methemoglobinemia, known collectively as the hemoglobin M disorders, are rare autosomal dominant defects caused by point mutations that alter a single amino acid in the structure of normal globin. The altered conformation that ensues favors the persistence of the ferric rather than the ferrous form of heme iron. The normal methemoglobin reductive capacity of the RBC cannot compensate for such instability of ferrous heme. Of the seven known hemoglobin M mutations, two affect the alpha globin chain, three alter the beta globin chain, and two involve the gamma chain. Most of these mutations are histidine-to-tyrosine mutations in the critical contact points of the heme moiety and the globin molecule. Only the alpha and gamma globin chain mutations are associated with neonatal methemoglobinemia, because these are the globin chains in hemoglobin F, the predominant hemoglobin found in neonatal RBCs. Neonatal methemoglobinemia is transient when produced by one of the two gamma chain mutations, hemoglobin FM-Osaka [166, 167] or hemoglobin FM-Fort Ripley [168], because the normal developmental switch from fetal to adult hemoglobin eliminates all but a trace of the mutant hemoglobin. Hemoglobin M heterozygotes inheriting alpha or beta globin mutations appear cyanotic their entire life because of the increased methemoglobin levels present in their RBCs, but they are otherwise asymptomatic. No therapy is needed (and none is possible). The homozygous state is incompatible with life. Diagnosis of hemoglobin M disorders is made by hemoglobin spectroscopy.

NADH-methemoglobin reductase (cytochrome-b_5 reductase) deficiency is an uncommon autosomal recessive disorder. Heterozygotes are asymptomatic and do not have methemoglobinemia under normal circumstances. If challenged by drugs or chemicals that cause methemoglobinemia, however, patients become cyanotic and symptomatic at doses that have no effect in normal individuals. Homozygotes have lifelong methemoglobinemia at a level of 15–40% and are cyanotic but otherwise asymptomatic, unless exposed to toxic agents. Three different types of hereditary NADH-cytochrome b_5 reductase deficiency are recognized. Type I deficiency is limited to RBCs and is manifested by methemoglobinemia only. Type II deficiency is due to a widespread enzyme deficiency and is characterized by mental retardation in addition to methemoglobinemia. Type III deficiency occurs in RBCs, leukocytes, and platelets, but the only clinical manifestation is methemoglobinemia. The gene for NADH-cytochrome b_5 reductase is located on chromosome 22. At least 18 different NADH-cytochrome b_5 reductase mutations, mostly missense, have been described in the three recognized types of enzyme deficiency [169, 170]. Diagnosis of NADH-methemoglobin reductase deficiency is by assay of the RBC enzyme activity, a

procedure available only in specialized hematology laboratories.

The main clinical manifestation of methemoglobinemia is cyanosis not resulting from cardiac or respiratory disease. Cyanosis present at birth suggests hereditary methemoglobinemia, whereas that appearing suddenly in an otherwise asymptomatic infant is more consistent with acquired methemoglobinemia. The blood is dark and, unlike deoxygenated venous blood, does not turn red when exposed to air. Rapid screening for methemoglobinemia can be done by placing a drop of blood on filter paper and then waving the filter paper in the air to allow the blood to dry. Deoxygenated normal hemoglobin turns red, whereas methemoglobin remains brown. Using this technique, methemoglobin levels of 10% or more can be detected [171]. More accurate determination of methemoglobin levels are accomplished in the blood-gas laboratory by co-oximetry or in the clinical laboratory using a spectrophotometer. Cyanosis is first clinically evident when methemoglobin levels reach approximately 10% (1.5 g/dl), but symptoms attributable to hypoxemia and diminished oxygen transport do not appear until levels increase to 30–40% of total hemoglobin. Death occurs at levels of 70% or greater. Methemoglobinemia is not associated with anemia, hemolysis, or other hematologic abnormalities.

In newborns, treatment with intravenous methylene blue (1 mg/kg as a 1% solution in normal saline) is indicated when methemoglobin levels are greater than 15–20%. The response to methylene blue is both therapeutic and diagnostic. Methemoglobin levels decrease rapidly, within one to two hours, if methemoglobinemia is caused by a toxic agent or by a deficiency of NADH-methemoglobin reductase. In contrast, the hemoglobin M disorders do not respond to methylene blue. Reappearance of methemoglobinemia after an initial response to methylene blue suggests a deficiency of NADH-methemoglobin reductase or the persistence of an occult oxidant. A poor response to methylene blue also is seen in G6PD-deficient individuals; this occurs because G6PD is responsible for generation of NADPH, the required cofactor for the reduction of methemoglobin by methylene blue. In general, most infants with hereditary methemoglobinemia are asymptomatic and require no therapy. Older children are sometimes given daily administration of oral ascorbic acid or methylene blue to decrease cyanosis for cosmetic reasons. Methylene blue produces blue urine, but this is harmless.

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8

Neonatal screening for hemoglobinopathies

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Introduction

The history of neonatal screening for sickle cell disease (SCD) and other hemoglobinopathies began 30 years ago with the demonstration that hemoglobin electrophoresis could be applied successfully to samples of umbilical-cord blood or capillary blood collected on filter paper [1–3]. This approach provided adequate differentiation of important adult hemoglobin (Hb) variants, such as sickle hemoglobin (Hb S) and Hb C, from normal adult hemoglobin (Hb A) in the presence of large amounts of fetal hemoglobin (Hb F). Statewide programs initiated in New York in 1975 and Colorado in 1979 demonstrated the feasibility of universal screening by public health laboratories that utilized filter paper samples already being collected for phenylketonuria screening [4, 5]. In 1986, the demonstration that prophylactic penicillin markedly reduces the incidence of pneumococcal sepsis, the leading cause of death in children with SCD, provided a strong incentive for neonatal screening [6]. One year later, a consensus development conference of the National Institutes of Health reviewed evidence that neonatal screening, when linked to timely diagnostic testing, education, and comprehensive medical care, markedly reduces morbidity and mortality from SCD in infancy and early childhood [6–8]. The panel recommended that all neonates in the USA be screened for SCD [9]. Since then, most states in the USA, and a number of other countries, have implemented neonatal screening for hemoglobinopathies.

Approximately 2000 infants with SCD are identified annually by US neonatal screening programs [10, 11]. Data from a number of programs indicate that mortality from SCD during the first three to four years of life has been reduced markedly by universal screening and appropriate follow-up and treatment [5, 12–18]. Screening also can identify infants with other hemoglobinopathies, including some with severe forms of β-thalassemia and most with α-thalassemia, as well as hemoglobinopathy carriers (i.e. those with hemoglobin traits). The benefit of presymptomatic diagnosis for these other disorders is less clear than for SCD. Many of these results may have important genetic implications for families [19].

The purpose of this chapter is to provide an overview of neonatal screening for SCD and other hemoglobinopathies, with specific information about the implications and appropriate follow-up for infants with common abnormal results. Hemoglobinopathies are complex, and appropriate follow-up and diagnostic testing require expertise beyond that of most providers of neonatal care. Most neonatal screening programs provide practitioners with guidance about neonatal screening results that is tailored to local resources and utilizes the expertise of local or regional consultants. In many areas, follow-up and diagnostic testing are coordinated or provided directly by the screening program without additional costs to the family.

Screening programs and methods

Forty-seven of the 50 states in the USA, the District of Columbia, USA, Puerto Rico, and the Virgin Islands currently provide universal screening for SCD. The other three states offer screening by request. Screening is also performed in areas of other countries, including the UK, France, Brazil, and Ghana [20–23]. Most screening programs in the USA use isoelectric focusing (IEF) of an eluate from the dried blood spots that also are used to screen for hypothyroidism, phenylketonuria, and other disorders [10, 24, 25]. A few programs use high-performance liquid chromatography (HPLC) or cellulose acetate electrophoresis as the initial screening method. Many programs retest specimens with positive results using a second, complementary electrophoretic technique, HPLC, immunologic tests, or DNA-based assays [10, 24, 25].

The sensitivity and specificity of current screening methodologies are excellent, but a few infants with SCD, even in areas with universal screening, may be missed because they are never screened. Infants with SCD who are screened may go undiscovered because of extreme prematurity, mislabeled specimens, clerical errors in the laboratory, or the inability to locate affected infants after discharge from the nursery [5, 24, 26–29]. Blood transfusions administered before collection of a screening sample alter the results and have caused infants with SCD to go undetected [30]. Thus, it is imperative that all infants, including those born at home, are screened and that the initial screening test is always obtained before any blood transfusion, regardless of gestational or postnatal age. Information requested on screening forms should be provided accurately and completely to facilitate the follow-up of positive results. In regions that have not yet implemented universal screening, neonatal screening for SCD should be requested for all high-risk infants (i.e. those of African, Mediterranean, Middle Eastern, Indian, Caribbean, and South and Central American ancestry). Any high-risk infant not screened at birth, or for whom neonatal screening results cannot be documented, should be screened for hemoglobinopathies before two months of age.

Hemoglobins identified by neonatal screening are reported in order of quantity. Because more Hb F than Hb A is present at birth, most normal infants show FA (Hb F and Hb A) results. Like normal infants, those with hemoglobinopathies show a predominance of Hb F at birth. In addition to Hb F, infants with SCD have Hb S in the absence of Hb A (FS), Hb S with another hemoglobin variant such as Hb C (FSC), or a quantity of Hb S greater then Hb A (FSA). Hundreds of other Hb variants also may be identified. Most of these variants are associated with few or no clinical consequences, but some are associated with anemia or other problems. Many screening programs also detect and report Hb Bart's, which is indicative of α-thalassemia.

Sickle cell disease

SCD is an autosomal recessive disorder caused by a number of different genotypes (Table 8.1). Approximately 65% of patients have homozygous disease (Hb SS). Other forms of SCD are caused by coinheritance of Hb S from one parent and Hb C (Hb SC disease) or β-thalassemia (Hb S β^+-thalassemia or Hb S β^0-thalassemia) from the other parent. All SCD genotypes are characterized by hemolysis and unpredictable episodes of vaso-occlusion, which cause tissue ischemia and variable degrees of chronic organ damage. Acute complications that rapidly can become life-threatening include pneumococcal sepsis, splenic sequestration, acute chest syndrome, and stroke. Presymptomatic diagnosis through neonatal screening affords the opportunity to prevent some of these complications and, through family and provider education, to ensure prompt recognition and treatment of those that occur.

As shown in Table 8.1, a number of different neonatal screening results may be indicative of SCD [24, 31]. Infants with FS screening results may have a variety of genotypes with a wide range of clinical severity. Most infants with FS have Hb SS, but other less common conditions include other forms of SCD, such as sickle β^0-thalassemia and sickle $\delta\beta$-thalassemia, and sickle hereditary persistence of fetal hemoglobin (HPFH), which is a benign condition. Most infants

Table 8.1 Sickle hemoglobinopathies: neonatal screening and diagnostic test results

					Hematologic studies by 2 years				
Disorder	Approximate % of US patients	Neonatal screening results[a]	Hb separation by 2 months of age[a]	Serial CBC, reticulocytes	MCV[b]	Hb A_2[c] (%)	Hb F (%)	Hb F distribution	DNA dot blot
SS	65	FS	FS	Hemolysis and anemia by age 6–12 months	N or ↑[d]	<3.6[d]	<25	Heterocellular	β^S
SC	25	FSC	FSC	Mild or no anemia by age 2 years	N or ↓	NA[e]	<15	NA[f]	β^S β^C
S β^+-thalassemia	8	FSA or FS[g]	FSA	Mild or no anemia by age 2 years	N or ↓	>3.6	<25	NA[f]	β^A β^S
S β^0-thalassemia	2	FS	FS	Hemolysis and anemia by age 6–12 months	↓[d]	>3.6[d]	<25	Heterocellular	β^A β^S
S δβ-thalassemia	<1	FS	FS	Mild anemia by age 2 years	↓	<2.5	<25	Heterocellular	β^S
S-HPFH	<1	FS	FS	Benign condition. No hemolysis or anemia	N or ↓	<2.5	25–35	Pancellular	β^S

CBC, complete blood count; Hb, hemoglobin; N, normal; ↑, increased; ↓, decreased.

Table shows typical results, but exceptions occur. Some rare sickle cell disease (SCD) genotypes (e.g. SD_{Punjab}, SO_{Arab}, SC_{Harlem}, S Lepore, SE) are not included.

[a]Hemoglobins reported in order of quantity (e.g. FSA = F>S>A).

[b]Normal MCV: ≥70 at 6–12 months, ≥72 at 1–2 years.

[c]Hb A_2 results vary somewhat depending on laboratory methodology.

[d]Hb SS with coexistent α-thalassemia may show ↓MCV and Hb A_2 >3.6%; neonatal screening results from such infants usually show FS Bart's.

[e]NA, not applicable: quantity of Hb A_2 cannot be measured by hemoglobin electrophoresis or column chromatography in presence of Hb C.

[f]NA, not applicable: test not indicated.

[g]Quantity of Hb A at birth sometimes insufficient for detection.

with sickle β^+-thalassemia show FSA, but some have FS screening results when the quantity of Hb A at birth is insufficient for detection [32]. The coinheritance of α-thalassemia, usually indicated by the presence of Hb Bart's (e.g. FS Bart's), may complicate differentiation of SCD genotypes in some infants [33]. For infants with screening tests indicative of possible SCD, confirmatory testing of a second blood sample should be accomplished by two months of age so that parental education, prophylactic penicillin, and comprehensive care can be implemented promptly [18, 24]. Confirmatory testing can be performed using hemoglobin electrophoresis (cellulose acetate and citrate agar), IEF, HPLC, and/or DNA-based methods. In some areas, such testing is provided by the screening program without additional cost to the family. Solubility tests to detect Hb S are inappropriate screening and confirmatory tests, in part because high levels of Hb F and relatively low concentrations of Hb S during infancy cause false-negative results in SCD.

Hemolytic anemia and clinical signs and symptoms of SCD are rare before two months of age and develop variably thereafter (Table 8.1). Thus, for infants with FS screening results, serial complete blood counts (CBCs) and reticulocyte counts may not clarify the specific diagnosis during early infancy. In selected cases, testing of parents or DNA analysis

Table 8.2 Non-sickle hemoglobinopathies identified by neonatal screening

Screening results	Possible condition	Clinical manifestations
F	Homozygous β^0-thalassemia	Severe thalassemia
	Premature infant	Repeat screening necessary
FE	EE	Microcytosis with mild or no anemia
	E β^0-thalassemia	Mild to severe anemia
FC	CC	Mild microcytic hemolytic anemia
	C β^0-thalassemia	Mild microcytic hemolytic anemia
FCA	C β^+-thalassemia	Mild microcytic anemia

may be helpful [24]. Unless SCD has been excluded definitely, all infants with FS screening results should be started on prophylactic penicillin by two months of age, and parents should be educated about the importance of urgent medical evaluation and treatment for febrile illness and for signs and symptoms indicative of splenic sequestration [18, 24]. All children with SCD should receive the pneumococcal conjugate vaccine beginning at two months of age. An overview of comprehensive medical care for SCD is beyond the scope of this chapter but is provided elsewhere [24, 34, 35]. Many neonatal screening programs provide resources and links to sickle cell consultants to facilitate referral of infants with SCD to providers and clinics with appropriate expertise. It is important that such referrals occur early in infancy, before symptoms develop.

Non-sickle hemoglobinopathies

Neonatal screening identifies some infants with non-sickle hemoglobinopathies, a heterogeneous group of disorders characterized by variable degrees of hemolysis and/or ineffective erythropoiesis (Table 8.2) [19, 24, 36–40]. Infants with results that show only Hb F may be normal infants who do not yet have Hb A because of prematurity or they may have β-thalassemia major, a cause of severe transfusion-dependent anemia that develops later during infancy. Infants with an F screening result need repeat testing to differentiate those with β-thalassemia from normal infants and also to identify any premature infants with SCD and other hemoglobinopathies. Hb E is a common β-globin variant in people of Southeast Asian ancestry. Infants with FE (Hb F + Hb E) may have homozygous Hb E, which is asymptomatic, or Hb E β^0-thalassemia, which is variably severe [36–39]. Family studies, DNA analysis, or repeated hematologic evaluation during the first one to two years of life are required to differentiate these two possibilities. It is important to note that most infants with β-thalassemia syndromes (i.e. β-thalassemia minor and β-thalassemia intermediate) have normal screening results (FA) and, thus, are not identified by neonatal screening [19]. As is the case for families with SCD, education about clinical manifestations, symptoms, treatment, and options such as family testing and genetic counseling should be offered to affected families [19, 24]. Because the diagnostic evaluation of many of these hemoglobinopathies is complex, providers are encouraged to utilize the expertise of local or regional consultants identified by neonatal screening programs.

Alpha-thalassemia syndromes

The α-thalassemias occur commonly in people of African, Mediterranean, and South and Southeast Asian ancestry. The red cells of newborns with α-thalassemia contain Hb Bart's, a tetramer of γ-globin. Many, but not all, neonatal screening programs detect and report Hb Bart's [19, 24, 36, 41–43]. As shown in Table 8.3, infants with Hb Bart's at birth

Table 8.3 Alpha-thalassemia syndromes identified by neonatal screening

Screening result	Possible condition	Clinical manifestation
FA + Bart's	α-Thalassemia silent carrier	Normal CBC
	α-Thalassemia minor	Microcytosis with mild or no anemia
	Hb H disease	Mild to moderately severe microcytic hemolytic anemia
	Hb H Constant Spring	Moderately severe hemolytic anemia
FAS + Bart's	α-Thalassemia with structural	Clinical manifestations, if any, depend on
FAC + Bart's	Hb variant	characteristics of structural variant (e.g. Hb E) and
FAE + Bart's		severity of α thalassemia
FE + Bart's		

CBC, complete blood count; Hb, hemoglobin.

Table 8.4 Hemoglobinopathy carriers identified by neonatal screening

Screening result	Possible condition	Clinical manifestation
FAS	Hb S carrier (Sickle cell trait)	Normal CBC; Generally asymptomatic
FAC	Hb C carrier	No anemia; Asymptomatic
FAE	Hb E carrier	Asymptomatic; Normal or slightly decreased MCV without anemia
FA Other	Other Hb variant carrier	Depends on variant; Most have no clinical or hematologic manifestations

CBC, complete blood count; Hb, hemoglobin; MCV, mean cell volume.

may be silent carriers or may have α-thalassemia minor, Hb H disease, or Hb H Constant Spring disease. Silent carriers, the largest group with Hb Bart's at birth, have a normal CBC. People with α-thalassemia minor generally show a decreased mean cell volume (MCV) with mild or no anemia. Many, but not all, infants with α-thalassemia minor have a decreased MCV at birth (<96 fl). Newborns with relatively large amounts of Hb Bart's (more than 10% by IEF or more than 25–30% by HPLC) or those who develop more severe anemia need diagnostic testing and consultation with a pediatric hematologist to diagnose and treat more serious forms of α-thalassemia, such as Hb H disease or Hb H Constant Spring disease [24, 43, 44]. The identification of Hb Bart's in infants of Asian, Greek, Cypriot, or Turkish ancestry may have important genetic implications because subsequent parental testing may identify couples at risk for pregnancies complicated by hydrops fetalis [19, 24, 45]. Such risk is rare in other groups, such as people of African ancestry and most Mediterraneans. (See also Chapter 7.)

Carriers of hemoglobin variants

Approximately 50 infants who are carriers of hemoglobin variants (i.e. infants with hemoglobin traits) are identified for every one with SCD [24]. Many screening laboratories confirm the carrier state by testing the original screening specimen by using complementary methodology (see Screening programs and methods, above). Some programs recommend testing a second blood specimen for confirmation of carriers. Carriers generally are asymptomatic (Table 8.4); thus, identification of a carrier state is of no immediate benefit to the infant. However, parents are entitled to the information and can benefit from knowing the child's carrier status, in part because parental testing may indicate risk for SCD or other hemoglobinopathies with subsequent pregnancies; such information may influence reproductive decision-making. Therefore, parents of infants who are detected to be carriers should be offered education and testing for themselves and their extended family [9, 18, 24]. Such testing may

raise concerns about mistaken paternity and, thus, the availability and implications of parental testing are often discussed initially with the mother. Testing of parents and other potential carriers includes CBC and hemoglobin separation by hemoglobin electrophoresis, IEF, or HPLC. If the MCV is borderline or decreased, then accurate quantitation of Hb A_2 by column chromatography or HPLC and of Hb F by alkali denaturation, radial immune diffusion, or HPLC is also needed to identify those with β-thalassemia.

Unidentified hemoglobin variants

Hundreds of less common hemoglobin variants are detected by neonatal screening, many of which are not identifiable by the procedures available in neonatal screening or clinical laboratories. Each year, thousands of infants with unidentified hemoglobin variants are detected by neonatal screening programs [10, 46]. The definitive identification of these variants may require extensive laboratory evaluation, such as amino-acid sequencing or DNA analysis, and is rarely accomplished, in part because of limited reference laboratory capacity. Most infants with unidentified Hb variants are heterozygotes, and most will have no clinical or hematologic manifestations. However, some variants, such as unstable hemoglobins or those with altered oxygen affinity, may be associated with clinical manifestations, even in heterozygotes (see Chapter 7). Other variants have no clinical consequences in heterozygous or homozygous individuals but may cause SCD when coinherited with Hb S and, thus, have potential clinical and genetic implications [31].

Follow-up of infants with unidentified Hb variants is problematic, in part because uncertainty may cause frustration and anxiety for families. In most cases, the quantity of Hb A is equal to or greater than the quantity of the unidentified hemoglobin; if so, and if there is no anemia or neonatal jaundice and the family history is negative for anemia or hemolysis, then no further hematologic evaluation may be necessary. Alternatively, some recommend repeat hemoglobin analysis by electrophoresis, IEF, or HPLC, and/or obtaining a CBC, reticulocyte count, and peripheral smear for red-cell morphology between 6 and 12 months of age. Fetal hemoglobin (γ-globin) variants disappear by one year of age, and the absence of anemia or hemolysis may be reassuring for parents of infants with hemoglobin variants that persist (α- or β-globin variants). For some families, it may be appropriate to offer testing of parents by hemoglobin electrophoresis, IEF, or HPLC and/or CBC, blood smear, and reticulocyte counts. The absence of hematologic abnormalities in a parent with the same Hb variant as the child may be reassuring to both the family and the pediatric provider. Definitive identification of unidentified variants may then be reserved for the less common circumstances listed in Table 8.5 [31, 47, 48]. In such cases, the evaluation may require protein sequencing, DNA analysis, or HPLC combined with electrospray mass spectrometry in a specialized reference laboratory [49].

Table 8.5 Indications for definitive identification of unidentified hemoglobin (Hb) variants detected by neonatal screening

Infant with unexplained hemolysis
Parent or other family member with unidentified variant and clinical evidence of hemolysis or abnormal oxygen affinity
Infant without Hb A (i.e. presumptive homozygote or compound heterozygote), especially if Hb S also present
Unidentified variant present in one parent and a second Hb variant, especially Hb S, in the other parent

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9

Polycythemia and hyperviscosity in the newborn

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Introduction

Polycythemia of the newborn is first mentioned in the Bible as Esau and Jacob are described at the time of their birth. Esau appears to be the recipient of a twin-to-twin transfusion (Genesis 25 : 25: "The first one emerged red . . ."). There is little in the modern medical literature concerning polycythemia in the newborn until the early 1970s [1–5]. During this time, there were a number of case reports and small series of infants with various symptoms that were thought to be secondary to an elevated hematocrit and blood viscosity. It was not until the 1980s that several investigators systematically examined the association between polycythemia, hyperviscosity of the blood, and organ-system dysfunction. These studies have done much to enlighten our understanding of the relationships between abnormalities of the hematocrit, blood viscosity, organ blood flow, and organ function. There has been little new information over the past ten years. However, the dissemination of this knowledge has provided a clinical approach that is based on well-defined data and has clarified the role of polycythemia as an etiologic factor for organ dysfunction in the neonate.

Definitions

Definitions of polycythemia and hyperviscosity have varied by study and methodology. Common variables have been the source of the blood sample and the age of the infant at the time of measurement [6–11]. In many studies, a hematocrit value of 65% or above has been diagnostic for polycythemia. Using cord-blood from appropriate-for-gestational-age (AGA) infants, Gross and colleagues defined hyperviscosity as a value that was two standard deviations greater than the mean (Fig. 9.1) [5]. Using blood samples from three different sites (peripheral vein, umbilical vein, and capillary), Ramamurthy and Brans defined hyperviscosity as a value that was three standard deviations from the mean [6]. This coincided with an umbilical venous hematocrit value of 63% or above. This study also found that capillary samples were higher than those from the peripheral vein, which in turn were greater than those from the umbilical vein. This is consistent with findings published previously by Oh and Lind [7]. In their study, the capillary hematocrit was consistently 10% higher than simultaneously obtained peripheral venous samples. Data from this study have been replotted and shown in Fig. 9.2. Based on population data from many sources, it is now accepted that polycythemia should be defined as a hematocrit value of 65% or above from a large, freely flowing peripheral vein. In a parallel manner, hyperviscosity should be defined as a value of more than two standard deviations from the mean.

Incidence

The incidence of polycythemia is 2–5% of all infants born at term [6, 8–11]. Factors known to influence

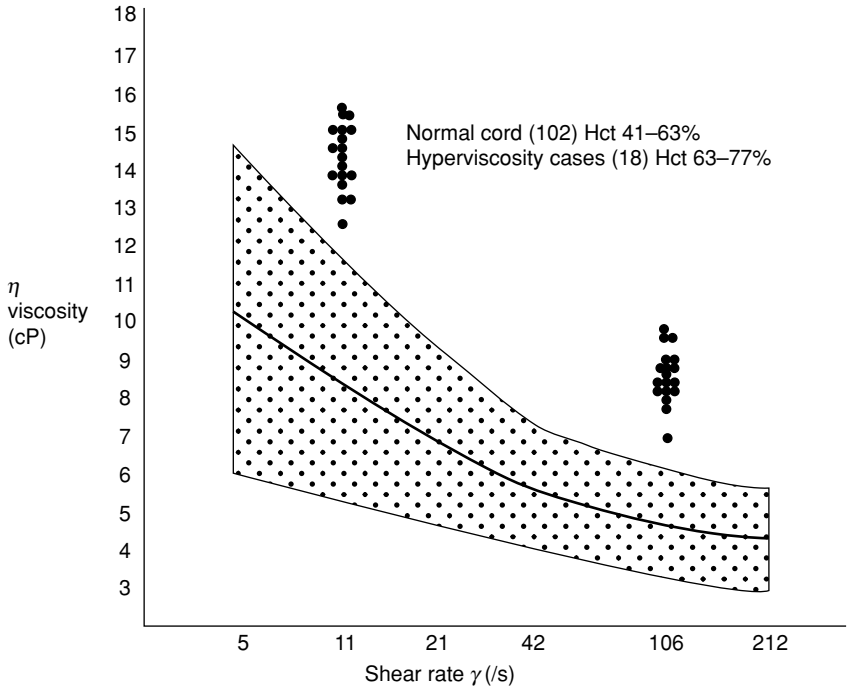

Fig. 9.1 The shaded area represents the viscosity of cord blood at shear rates from 2 to 212/s for 102 healthy full-term appropriate-for-gestational-age infants (mean ± 2 SD). Viscosity for 18 "symptomatic" infants is plotted at shear rates of 11/s and 106/s. Hematocrit (Hct) values for each group are indicated. From Gross *et al.* [5] with permission.

Table 9.1 Factors that influence hematocrit in the perinatal period

Timing of cord clamping
Relative height of infant to placenta before cord clamping
Altitude
Sampling site
Postnatal age
Intrauterine growth
Fetal hypoxia

the hematocrit at birth are listed in Table 9.1. Delay in clamping of the umbilical cord will result in a significant increase in hematocrit and blood volume [12–15]. From birth to 6–12 hours of age, the hematocrit will increase due to shifts in body water [11, 16]. By 24 hours of age, the hematocrit will become similar to the value at birth and will remain relatively stable. Infants who experience fetal distress, with abnormalities in fetal growth, and of mothers with poorly controlled diabetes have an increased incidence of polycythemia. Acute fetal distress results in a shift of blood volume from the placenta to the fetus, leading to an increased blood volume and red-cell mass. Those infants who experience intrauterine growth retardation or fetal hyperglycemia exist in a relatively hypoxic intrauterine environment. This results in increased erythropoietin production, increased red-cell mass, and a greater likelihood of polycythemia. Birth at centers located at higher elevations also is associated with a greater incidence of polycythemia. This was documented in two studies by Wirth and colleagues [8, 9], who noted that the incidence of polycythemia in Denver, Colorado (1610 m above sea level), was twice that in Norfolk, Virginia (sea level).

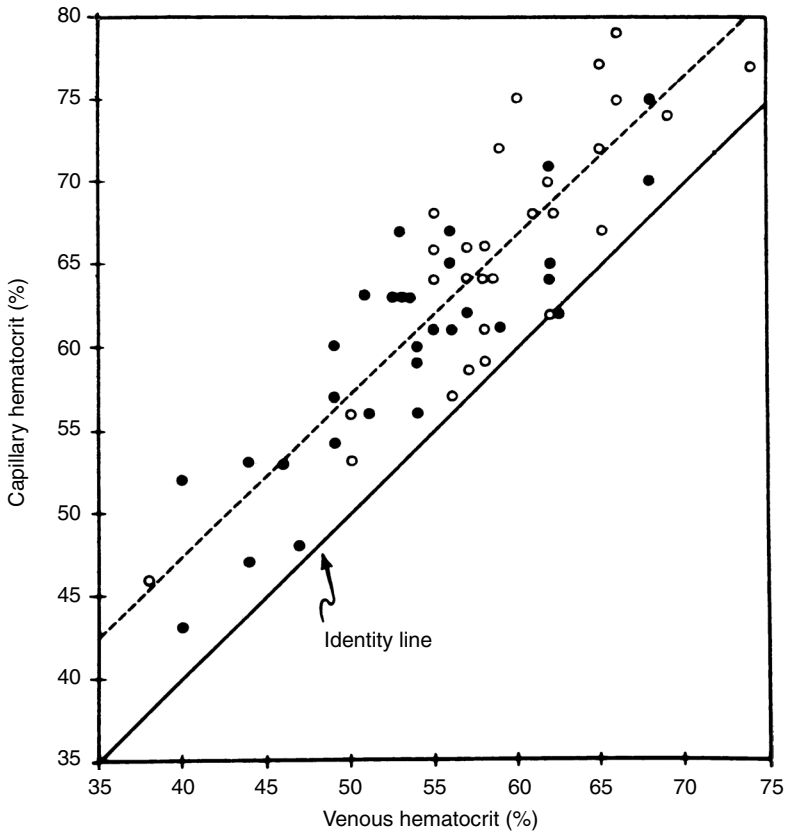

Fig. 9.2 Correlation between capillary and venous blood hematocrit in newborn infants. (o) 1–6 h; (•) 12–24 h. From Rosenkrantz and Oh [66] with permission.

Viscosity

It is important to have an understanding of the physics of the flow of fluids to understand how blood viscosity affects blood flow in the newborn infant. It will also allow for the understanding of the clinical symptoms observed in infants with an elevated hematocrit.

Definition

Viscosity, as defined by Poiseuille, is the ratio of shear stress to shear rate, as demonstrated in the formula below [17]:

$$\eta = \frac{(p - p')r^4\pi}{8lQ} = \frac{\text{shear stress}}{\text{shear rate}}$$

in which η is blood viscosity (dynes/s/cm^{-2}, or poise), $p-p'$ is the pressure gradient along the blood vessel, r is the radius and l the length of the blood vessel, and Q is blood flow. The shear stress that represents the pressure gradient along the blood vessel is expressed in dynes [18]. The shear rate, which represents the velocity between two fluid planes, divided by the distance between them, is expressed as s^{-1}.

As demonstrated in Poiseuille's original work, the ratio of shear stress to shear rate, or viscosity, of a

fluid is constant. However, this is true only for homogeneous or Newtonian fluids. Blood is a suspension of particles in a solution and does not behave as a Newtonian fluid. The viscosity of blood does not remain constant with variations in shear stress and shear rate. This can be demonstrated in vitro using a cone/plate viscometer, such as that described by Wells and colleagues [19]. In this device, shear rate can be varied and the resultant shear stress measured.

Shear rates in large vessels such as the aorta are greater (100–300/s) than those observed in small vessels such as arterioles (11–25/s) [18]. Using the above formula, one would then predict that the viscosity of the blood would be lower in large vessels and higher in small blood vessels (see later in this chapter for exceptions to this rule). While knowing the shear rate in a particular vessel will allow an estimate of the blood viscosity and a microviscometer will allow in vitro measurement of blood viscosity, there are multiple factors that can vary the in vivo viscosity of the blood. These other factors are reviewed below.

Factors that affect blood viscosity

It is pertinent to understand all of the factors that contribute to whole-blood viscosity, although the primary determinant of blood viscosity in the newborn is the red-blood-cell (RBC) concentration.

Hematocrit

The hematocrit, a reflection of the RBC concentration, has a logarithmic relationship with blood viscosity at shear rates (Fig. 9.3). The greatest changes occur at the lowest shear rates and at hematocrits that exceed 65% [16, 20].

Plasma proteins

The plasma has a viscosity of 1.0–1.5 centipoise (cP), similar to the viscosity of water [21–23]. Water, with a viscosity of 1 cP, behaves as a Newtonian fluid, as

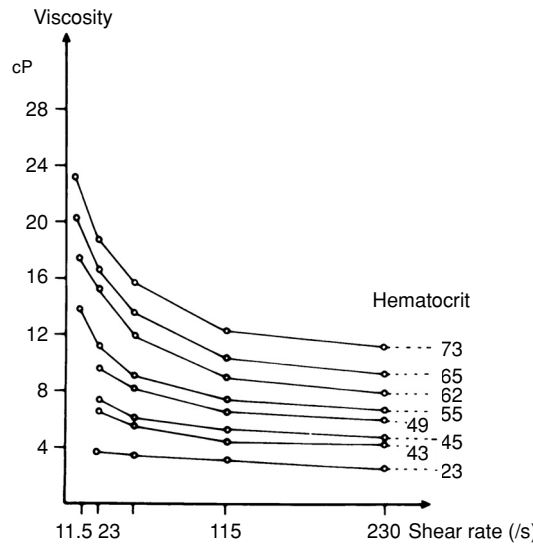

Fig. 9.3 Variation of whole-blood viscosity in eight newborn infants at different hematocrit values and shear rates. From Bergqvist [24] with permission.

the viscosity is constant at all shear rates. As such, the plasma protein contributes little to blood viscosity under normal conditions. Adult syndromes of hyperviscosity can be attributed to hyperproteinemia in conditions such as diabetes and Waldenstrom's macroglobulinemia. These are not conditions observed in the newborn [24–26].

Red-blood-cell deformity

The RBC is the major contributor to whole-blood viscosity, because it is the most prominent particle suspended in the blood and because of its intrinsic properties. The RBC consists of a membrane that moves around a body of internal fluid, making it a dynamic particle [23, 27]. The surrounding membrane is quite deformable, with the RBC of the newborn having a greater degree of deformability than that of the adult [26, 28]. The viscosity of the internal fluid of the RBC will increase with cell age [29]. The internal viscosity also appears to increase with decreasing blood flow and external shear rate.

White blood cells

The white blood cell of the newborn is larger and less deformable than the RBC. It has been shown that extremely high concentrations of white blood cells, as observed in congenital leukemia, can increase the whole-blood viscosity [30–32].

Fibrinogen

Due to its low concentration in the blood of the newborn, fibrinogen contributes little to whole-blood viscosity [22].

Platelets

Although they are relatively inflexible particles, platelets do not appear to affect blood viscosity in the normal state. In adults with vaso-occlusive disease, platelet aggregates may affect the viscosity of the blood in narrowed vascular areas [21]. Platelet aggregates do not appear to be a factor in the blood viscosity of newborn infants with normal hematocrits or polycythemia.

Blood pH

Whole-blood viscosity increases with pH below 7.00 [21, 33]. This is due to a shift of the fluid into the RBCs with acidosis. This may be one of the factors responsible for an increase in blood viscosity in asphyxia, along with the associated placental transfusion that also increases the blood volume of the infant.

Vessel size

In large blood vessels, such as the aorta, blood flow and shear rate (100–300/s) are high. Therefore, the apparent viscosity of the blood is low. The opposite is true in small blood vessels. Blood flow and shear rate are low (11–25/s) and viscosity is high. As shown in Fig. 9.3, changes in hematocrit cause the greatest changes in viscosity in the small blood vessels.

While blood behaves as a non-Newtonian fluid in large and small blood vessels, the opposite is true in the capillaries of most organs. The diameter of capillaries is in the range of 3–5 μm while the RBC has a diameter of 8.5 μm. As shown by Fahraeus and Lindqvist, viscosity actually decreases with diminishing size of the capillary (see Fig. 9.4) [34]. This phenomenon has been confirmed in vivo in capillaries as narrow as 3 μm [23]. The term "bolus flow" has been given to this phenomenon. It reflects high hemodynamic efficiency. Measurements of the viscosity in the capillary are in the range of 1.3 cP, similar to those of plasma and water. As observed in Fig. 9.4, the hematocrit of the blood does not affect the viscosity in the capillary. Thus, it is important to keep in mind that in vitro measurements of blood viscosity may not reflect the viscosity of the blood in the capillary. Dintenfass has suggested that viscosity may increase again as the capillary size decreases below 4 μm [21]. The variation in capillary size in different organs may therefore explain why changes in hematocrit and viscosity seem to have an effect on flow in some organs but no effect in others.

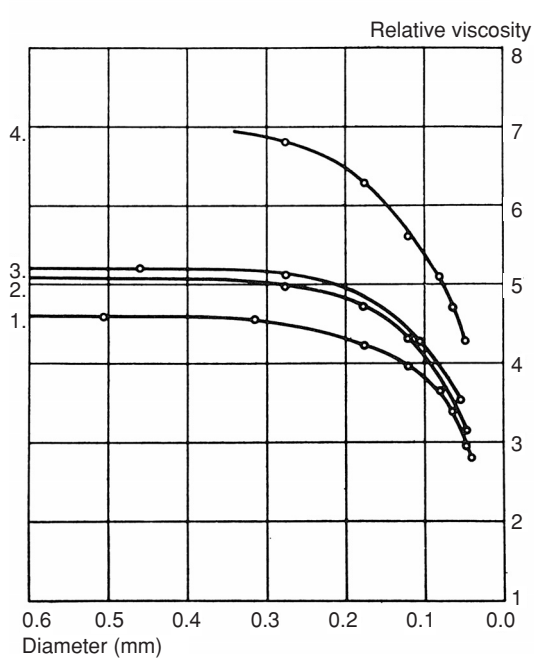

Fig. 9.4 Blood viscosity in small capillaries. From Fahraeus and Lindqvist [34] with permission.

Hemodynamics

Many of the clinical problems observed in infants with polycythemia and hyperviscosity have been ascribed to disturbances in organ blood flow. Therefore, it is essential to understand how changes in hematocrit and blood viscosity affect the blood flow of the different organs of the newborn infant.

Cardiopulmonary blood flow and function

The primary change in cardiac function is decreased output, which is associated with an increase in arterial oxygen content, no change in oxygen transport/delivery or consumption in the myocardium or whole body, and increased pulmonary and, to a lesser extent, systemic resistance [35–43]. These findings have been observed in both appropriate animal models and human newborn infants.

The decrease in cardiac output appears to be the result of a reduction in stroke volume and heart rate, or both [43]. In our own studies of polycythemic infants, we found that heart rate increased following a partial exchange transfusion (PET) with Plasmanate® to decrease the hematocrit and maintain blood volume [39]. Swetnam and colleagues found that following PET, there was an increase in heart rate, stroke volume, and cardiac output as well as systemic oxygen transport [43]. Studies of myocardial oxygenation have shown that polycythemia and changes in blood viscosity do affect blood flow but do not adversely affect oxygen transport and consumption [37].

The changes in systemic and pulmonary circulation with changes in hematocrit have been demonstrated in both newborn animal and human studies [38, 40]. Fouron and Hebert have shown in the newborn lamb model that pulmonary resistance increases to a greater extent than systemic resistance with increases in hematocrit [40]. At hematocrit values of 70%, pulmonary vascular resistance was equal to systemic resistance. The change in pulmonary resistance will also change the direction in blood flow through the ductus arteriosus if it is still patent. These findings are similar to those found in a study of human infants [44]. The change in pulmonary resistance with elevation of the hematocrit is thought to explain, in part, the cardiopulmonary symptoms that have been reported by Gatti and colleagues as well as the plethoric or cyanotic appearance observed by many clinicians [41].

Gastrointestinal blood flow and oxygenation

Gastrointestinal blood flow has been studied in animal models [35, 42]. In the newborn piglet, polycythemia is associated with a decrease in blood flow but normal oxygen transport. However, oxygen extraction and uptake are reduced, suggesting abnormalities in local regulation of oxygen uptake that are not related to oxygen availability. The underlying etiology is unclear.

Liver and pancreatic function have been reported in the term newborn with polycythemia [45]. Compared with infants with normal hematocrit, the study found an elevated bile concentration in the serum and a low lipase and trypsin activity in duodenal juice on the first day of life. PET tended to normalize the bile acid and lipase concentrations. These findings suggest that polycythemia affects the enterohepatic circulation of bile acids and the exocrine pancreatic function during the first days of life. However, it should be noted that there were no clinical symptoms associated with these findings. Therefore, the implications for short- and long-term management as well as nutritional consequences are not clear.

Renal blood flow and function

Kotagal and Kleinman studied renal function in a puppy model of normovolemic polycythemia [46]. Renal blood flow was unaffected by the elevation in hematocrit. However, the decrease in plasma volume resulted in a lower plasma flow and glomerular filtration rate (GFR). The urine output was also lower, as was the Na^+ and K^+ excretion. There was no change in systemic blood pressure or renal blood flow. Therefore, the calculated renal vascular resistance was not increased by the change in hematocrit. This suggests that the Fahraeus–Lindqvist effect (decreased

viscosity as vessel size decreases) is present in the kidney.

In a study of renal function in normovolemic and hypervolemic newborn infants, Oh and colleagues found that renal function was affected by blood volume [12]. One group of infants had late cord clamping and a mean hematocrit of 62%, while those with immediate cord clamping had hematocrit of 50%. The group with late cord clamping had higher blood and RBC volume, mean arterial blood pressure, and renal blood flow as well as a greater GFR and urine output compared with the group with immediate cord clamping and normal blood volumes.

The apparent discrepancy between these two studies can be explained by the methodology. In the puppy model, blood volume was held constant so that plasma volume and flow were reduced in subjects with polycythemia. In the human study, the expanded blood volume was accompanied by a normal plasma volume and flow. Therefore, renal function in infants is a function of not only hematocrit but also blood volume. Infants with normovolemic polycythemia would be expected to have a reduction in renal function, while those with an increased blood volume should have normal renal function.

Carcass blood flow

Studies of isolated muscle in calf and dog models have shown that despite changes in blood flow associated with polycythemia, oxygen transport, and uptake are normal in resting and working muscle [47–50]. Studies of peripheral blood flow in newborn infants show an increase in blood flow following PET [43]. Transcutaneous oxygen measurements are normal in polycythemia [50]. These studies suggest that polycythemia and hyperviscosity do not adversely affect oxygenation of the carcass.

Brain blood flow and oxygenation

In 1982, we published the first study to document changes in the cerebral circulation of polycythemic newborn infants using Doppler techniques [39]. Polycythemic infants were found to have a significant reduction in cerebral blood-flow (CBF) velocity compared with similar term infants with normal hematocrit and blood viscosity values. Following PET to lower the hematocrit, the CBF velocity measurements increased and were similar to those of the control infants.

Jones and colleagues demonstrated in newborn lambs that CBF was correlated inversely with the arterial oxygen content of the blood in studies in which hematocrit and oxygen levels were varied independently [51]. Viscosity, which was not measured, presumably varied with the changes in hematocrit. Since the blood viscosity was not measured, Jones and colleagues could not state conclusively that it played a role in the observed changes in blood flow. The group concluded that arterial oxygen content was the primary determinate of CBF when other variables, such as cerebral metabolic rate, are constant.

To answer the question about the role that blood viscosity might play in the cerebral circulation, we designed a study in newborn lambs in which arterial oxygen content and blood viscosity were varied independently [52]. Using isovolemic exchange transfusion of newborn lamb RBCs, we increased the hematocrit, arterial oxygen content, and blood viscosity. This was associated with a decrease in the CBF. Sodium nitrite was then infused to cause methemoglobin formation and to decrease the arterial oxygen content while maintaining the increased blood viscosity. The CBF values increased to control levels. Cerebral oxygen delivery was constant throughout the study. From this, we concluded that the decreased CBF observed with polycythemia and hyperviscosity is due to the associated increase in arterial oxygen content.

Following these studies, Goldstein and colleagues varied blood viscosity by increasing the concentration of fibrinogen in the blood to see whether this would affect CBF in a newborn-lamb model [53]. Again, it was found that CBF varied with the arterial oxygen content, and not with the blood viscosity. Lastly, we found in a study of newborn lambs that polycythemia does not affect the uptake of oxygen

or the metabolic rate of the brain, as long as usual brain substrates are available [54].

In conclusion, brain blood flow is decreased in infants with polycythemia. Blood viscosity is not responsible for this reduction in blood flow. Therefore, it would appear that the Fahraeus–Lindqvist effect is functional in the vasculature of the brain. That is, classic cerebral autoregulation is intact in infants with polycythemia and who have not suffered from some type of brain injury such as brain hypoxia or trauma.

Fetal blood flow and oxygenation

There is little information on the in utero effects of polycythemia and hyperviscosity on the fetus. Tenenbaum and colleagues performed isovolemic packed-red-cell transfusion on fetal lambs using adult sheep RBCs [55]. This resulted in an increase in hematocrit and viscosity, no change in venous oxygen content, and a decrease in umbilical blood flow and oxygen delivery. However, there was an increase in oxygen extraction, so that fetal oxygen uptake was not affected by the polycythemia. It should be noted that it is not clear what the effects of hypervolemic polycythemia might have on the fetus. This is an important point, as shifts in placental–fetal blood volume distribution are not uncommon (e.g. fetal hypoxia). In addition, there are no data on polycythemia and hyperviscosity and regional blood flow and oxygenation in the fetus.

Etiologies

There are three major categories of etiologies or clinical scenarios in which polycythemia and hyperviscosity may be observed. As outlined in Table 9.2, these include chronic fetal hypoxia, acute fetal hypoxia/asphyxia, and delayed clamping of the umbilical cord at delivery. Other less common causes and associations include maternal–fetal hemorrhage, fetofetal transfusion, and chromosomal abnormalities, including trisomy 21 and Beckwith–Weidemann syndrome [2, 9, 13–15, 56–58].

Table 9.2 Etiologies and syndromes associated with polycythemia

Acute hypoxia
Chronic hypoxia
Infant of diabetic mother
IUGR, SGA
Pre-eclampsia
Placental insufficiency
Neonatal thyrotoxicosis
Maternal smoking
High altitude
Intrauterine transfusion
Fetofetal transfusion
Maternal–fetal transfusion
Genetic syndromes
Trisomy 21, 18, 13
Beckwith–Weidemann syndrome

IUGR, intrauterine growth retardation; SGA, small for gestational age.

In the past, the most common cause of polycythemia was placental–fetal transfusion via delayed clamping of the umbilical cord. To avoid this problem, the cord should be clamped within 10–15 seconds of delivery of the body [15].

As discussed earlier in this chapter, altitude of the external maternal environment and intrinsic oxygen concentration are important but uncontrollable causes of polycythemia [8, 9].

Perinatal asphyxia and fetal hypoxia remain significant causes of polycythemia. Philip and colleagues examined placental residual volumes and neonatal outcomes [59]. Small placental residual blood volume was associated with fetal distress and low Apgar scores. Similar observations were made by Flod and Ackerman and Yao and Lind [60, 61]. Oh and colleagues demonstrated that intrauterine hypoxia resulted in a shift of blood from the placental compartment to the fetus [62]. There was a correlation between the length of the hypoxic event and the shift in blood volume. The data suggested that fetal vasodilatation associated with fetal hypoxia was part of the mechanism.

Fetuses with abnormal growth (small (SGA) or large (LGA) for gestational age) are at high risk

Table 9.3 Frequency of clinical symptoms observed in association with polycythemia

Clinical symptoms	Gross *et al.* [5] ($n = 18$) (%)	Ramamurthy and Brans [6] ($n = 54$) (%)	Black *et al.* [94] ($n = 111$) (%)	Goldberg *et al.* [93] ($n = 20$) (%)
Cyanosis	89	17	7	nr
Plethora	83	63	nr	nr
Tremulous/jittery	67	13	nr	nr
Abnormal EEG	33	nr	nr	nr
Seizures	28	0	0	nr
Respiratory distress	44	4	10	15
Cardiomegaly	17	nr	nr	85
Lethargy/poor feeding	nr	50	+	55
Hyperbilirubinemia	50	6	nr	5
Abnormal blood smear	50	nr	nr	nr
Thrombocytopenia	39	nr	nr	25
Hypoglycemia	33	nr	27	40
Hypocalcemia	6	nr	nr	0

EEG, electroencephalogram; nr, not reported or examined; +, greater incidence compared with control group.

for polycythemia [63]. This would appear to be secondary to chronic fetal hypoxia, which leads to increased erythropoietin levels, which in turn cause an increase in RBC production [64]. Over time, the red-cell mass increases to increase the oxygen-carrying capacity and maintain a normal oxygen content in the face of a low PaO_2. Clinical examples include pregnancies complicated by placental insufficiency and increased fetal metabolism. Placental insufficiency frequently exists in pregnancy-induced hypertension, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, post-term pregnancy, and maternal cigarette smoking. Poorly controlled diabetes during pregnancy is associated with an increased fetal metabolic rate, as is fetal hyperthyroidism [65].

Symptoms

This section will attempt to sort the clinical symptoms associated with polycythemia by organ system and to provide a physiologic explanation for the symptomptology. Table 9.3 lists symptoms that have been observed in populations of newborn infants with polycythemia and their frequency, as reported in several different series [66]. In addition, Gatti and colleagues reported prospectively on a population of 629 infants; twenty-five were polycythemic but none had any symptoms [41].

Investigators have noted an increased incidence of polycythemia in the SGA population of infants. While some have attributed observed abnormalities to polycythemia, Hakanson and Oh found the incidence of abnormalities in SGA infants with a normal hematocrit to be similar to that in those with polycythemia [67]. This observation serves as an important point. Many of the symptoms observed in infants with polycythemia are attributable to other perinatal problems associated with polycythemia, such as asphyxia and chronic hypoxia, and are not related directly to the polycythemia and hyperviscosity.

Abnormalities of blood volume

When the population of all polycythemic infants is studied, blood volume is increased for body weight at all gestational ages [12, 68, 69]. However, there is a fair amount of variability from infant to infant

due to the multiple etiologies for the polycythemia. Saigal and Usher showed a significant relationship between infants with increased blood volume and infants with cardiorespiratory problems [56]. Brans and colleagues and Thorton and colleagues demonstrated that polycythemic infants do not differ from infants with normal hematocrits when total body water, mean extracellular water, mean intracellular water, and mean interstitial water are compared [69, 70]. Plasma volumes also appear to be normal.

Cardiopulmonary symptoms

Cyanosis, tachypnea, cardiomegaly, and "plethora" of the lung fields on X-ray film of the chest are frequent findings reported in infants with polycythemia [4, 5, 41, 56, 71]. Although the PaO_2 may be normal, many of these infants have a red–blue color. The respiratory distress may be due, in part, to an elevated pulmonary vascular resistance and increased intrapulmonary shunting secondary to the increase in blood viscosity. Most investigators have reported complete resolution of the respiratory symptoms with PET.

In 1995, Scott and Evans reported on a set of monochorionic twins in which the recipient twin developed ischemia and gangrene of a lower leg [72]. We have observed a similar situation in a set of preterm monoamniotic twins. The recipient had an elevated hematocrit. Within an hour of birth, there was decreased perfusion of one leg. The systemic blood pressure was normal to elevated and cardiac performance was poor. An ultrasound examination revealed a clot in the distal aorta that extended into the iliac artery. The clot, causing aortic obstruction and increased afterload, was thought to be responsible for normal to increased systemic blood pressure and poor cardiac function. Various thrombolytic agents were not helpful, and the infant was too small to introduce a catheter into the aorta for perfusion of local thrombolytic therapy. Recent literature warns of the risk of intraventricular hemorrhage (IVH) in such preterm twins. The clinical picture was consistent with prenatal development of the clot.

Gastrointestinal symptoms

Multiple investigators have reported on infants with polycythemia and poor feeding or vomiting [64, 73, 74]. In addition some studies have suggested an association between polycythemia and the development of necrotizing enterocolitis (NEC) [75, 76]. Many of these infants have other risks for the development of NEC, including intrauterine growth retardation, asphyxia, or both. Thus, it is unclear whether polycythemia is responsible for the development of the NEC or the other associated perinatal complications.

LeBlanc and colleagues created a model of polycythemia in the newborn puppy [77]. The incidence of intestinal histology that was consistent with NEC was 58%. The intestinal blood flow and oxygen uptake data published by Nowicki and colleagues suggest that the bowel may experience some hypoxia in the unfed state [35].

In a randomized study of infants with polycythemia, Black and colleagues found that 6% of the observed or untreated infants had serious gastrointestinal symptoms whereas 51% who received a PET exhibited serious gastrointestinal symptoms [78]. One-third of the treated infants had radiographic evidence of pneumotosis intestinalis. This study suggests that the most important risk factor for the development of NEC is an exchange transfusion to reduce the hematocrit, and not polycythemia. Of interest is that an isovolemic exchange transfusion with packed RBCs was used to increase the hematocrit in the experiments of both LeBlanc and Nowicki.

Renal symptoms

Acute renal failure has been reported in a term infant with polycythemia [79]. The mechanism for alterations in renal function has been clarified by the studies of Kotagal and Oh [12, 46]. In infants with a normal blood volume, plasma volume is decreased, as is renal plasma flow and GFR. In infants who are hypervolemic, the plasma volume, renal plasma flow, and GFR will be normal. The cause of renal

dysfunction in any particular infant may be complicated further by acute tubular necrosis (ATN) secondary to perinatal asphyxia. Therefore, the abnormalities in renal function in any individual infant with polycythemia may be multifactorial.

Hypoglycemia

Hypoglycemia is frequently a problem in infants with polycythemia, even after correcting for factors such as intrauterine growth retardation. Leake and colleagues and Creswell and colleagues have examined this problem utilizing the newborn lamb [80, 81]. Both groups speculated about decreased glucose production and increased glucose uptake, but a definitive conclusion could not be derived from their work; thus, there was no final explanation. Data from our own experiments in polycythemic lambs who were made hypoglycemic support the hypothesis that the hypoglycemia is secondary to a reduced plasma volume [82]. Glucose exists almost exclusively in the plasma. Therefore, the glucose-carrying capacity is reduced. Combined with decreased blood flow in many organs, extraction of glucose must be increased to meet the metabolic requirements of the body. Therefore, the plasma glucose concentration, especially the venous concentration, will be lower than normal.

Hematologic symptoms

Both thrombocytopenia and low antithrombin III (AT-III) levels have been reported in infants with polycythemia [83–92]. Disseminated intravascular coagulation (DIC) has been reported by one investigator but not confirmed by others. Explanations for the low platelet count include impaired production secondary to tissue hypoxia, predominance in the marrow of erythropoietic cells, slow spleen blood flow, and decreased plasma fraction with normal concentrations. The platelet count consistently returns to normal in all of these infants.

Low AT-III is observed in asphyxiated infants. Therefore, the low concentrations may be secondary to asphyxia, which is also responsible for polycythemia.

Neurologic symptoms

Newborn period

Numerous papers dating back to the 1950s have reported neurologic abnormalities in newborn infants with polycythemia and hyperviscosity. Symptoms have included lethargy, irritability, tremulousness, and seizures. Several papers have also reported cerebral infarction.

In a controlled study, Goldberg and colleagues demonstrated abnormalities in the Prechtl and Brazelton examinations in infants with polycythemia [93]. All of these infants improved spontaneously over time. Those infants that had hemodilution with PET improved at the same rate as those who did not receive any therapy. Van der Elst and colleagues also performed Brazelton examinations in a group of infants with polycythemia and in a control group [73]. The polycythemic infants were randomized further either to be observed or to receive a PET. At ten days of age, the two polycythemic groups had similar Brazelton examinations. The control group had significantly better examinations than either of the polycythemic groups.

It would appear that polycythemic infants are different in their state behavior and neurologic function during the first week of life. Normalization of hematocrit and blood viscosity does not seem to affect the short-term outcome.

Long term

There have been six series of patients that have been followed long term (eight months to six years) in order to obtain an understanding of the role that polycythemia in the newborn period might play in the long-term neurologic development. The first of the studies was by van der Elst and colleagues, who followed the group of infants described above to eight months of age [73]. Both polycythemic groups and the control group had normal neurologic

examinations and developmental scores (modified Griffith developmental score).

Goldberg and colleagues also re-examined their subjects at eight months of age [93]. The Bayley scales of infant development, Milani–Comparetti postural reflex examination, medical history, and neurologic and physical examinations were completed on all three groups of infants. There were no differences in the Bayley scales among the three groups. Neurologic abnormalities were found in all three groups, polycythemic-treated (67%), polycythemic-observed (50%), and controls (17%). While no statistical differences were found, the two polycythemic groups appeared to be very similar to each other but had more problems when compared with the control group. There was a high incidence of spastic diplegia in the two polycythemic groups.

Black and colleagues have followed two populations of infants with polycythemia. In the initial study, there were 111 polycythemic infants and 110 control infants [94]. Forty-two of the polycythemic infants received a PET to lower their hematocrit after birth. The decision to lower the hematocrit was not done by randomization; rather, the physician in the nursery made the decision. Sicker infants tended to be those who received the partial exchange transfusion. Follow-up examinations were done at one and three years and included the Bayley scales, the Denver developmental screening test, physical and neurologic examinations, and a medical history. There were no differences in the two polycythemic groups or control groups in mental performance, but a significant number of the polycythemic infants, independent of therapy, had motor delay compared with the control group. Twenty-five percent of all polycythemic infants had neurologic abnormalities, especially spastic diplegia. Forty-three percent of the treated infants and 35% of the observed polycythemic group had some handicap compared with 11% of the control group ($P < 0.005$).

In a second study of polycythemic infants by Black and colleagues, in which the efficacy of PET was studied, 93 infants were randomized to treatment or observation [95]. There was no differentiation between infants who had symptoms in the newborn period and those who were asymptomatic. Eighty percent of infants were examined at one and/or two years of age (59% at one year, 61% at two years). No differences were detected between the two groups at the one-year follow-up. At two years of age, the treated group had fewer neurologic abnormalities. Forty-nine of the original 93 infants were evaluated again at school age (seven years of age) [96]. There were no differences between the treated and non-treated polycythemic children.

Host and Ulrich reported on a group of polycythemic infants who were part of a community health study [74]. At 2.5 years of age, a Denver developmental screen and a health questionnaire were administered. A second questionnaire was administered at six years of age. Eighty percent of the infants with a venous hematocrit of 65% or above completed the study. All were normal, except for one child who had hypocalcemic seizures.

Bada and colleagues studied a population of polycythemic infants who were randomized to received a PET or observation along with a control group [97]. Follow-up at 30 months of age revealed no differences in the three groups. A multivariant analysis of the various perinatal risk factors was performed. The analysis revealed that outcome was related highly to perinatal risk factors other than polycythemia.

There are several points that become clear from a review of the data. Polycythemia appears to be part of the fetal adaptive process for acute and chronic hypoxia. Hypoxia is known to cause irreversible brain injury. Exchange transfusion to lower the hematocrit in the newborn period does not change long-term neurologic function in this population, although polycythemia is a marker for an increased risk of long-term neurologic dysfunction. The demographic data of Black and Bada indicate that infants who are symptomatic in the newborn period and have late sequelae are the same infants who experienced an adverse intrauterine environment or perinatal hypoxia or asphyxia [95–97]. Therefore, it would appear that it is the hypoxic–ischemic events that trigger the adaptive fetal response that increases the hematocrit and is responsible for the cerebral dysfunction. This would be consistent with the

observation that PET to reduce the hematocrit in the newborn does not prevent long-term neurologic dysfunction.

Asymptomatic newborn infants

Most studies suggest that infants with an elevated hematocrit but who are asymptomatic are at a minimal and nonquantifiable risk for adverse outcome. In light of our current understanding of the pathophysiology of the etiology of the neurologic dysfunction in polycythemia, there does not appear to be sufficient evidence to recommend exchange transfusion in this population.

Management

Partial exchange transfusion to reduce the hematocrit should be reserved for newborns with symptoms that can be attributable directly to the elevation in hematocrit and in which there is evidence that the reduction of the hematocrit will correct the observed problems. Such clinical conditions include respiratory distress with cyanosis, renal failure, and hypoglycemia. Before the exchange transfusion is performed, other explanations for the observed symptoms should be explored. Once it is clear that polycythemia appears to be the etiologic explanation for the infant's problems, then a PET should be undertaken. Colloidal fluids and crystalline fluids appear to have equal efficacy in PET [98, 99]. To determine the volume of the exchange transfusion, the following formula may be used [100]:

$$\frac{[\text{hematocrit (observed)} - \text{hematocrit (desired)}] \times \text{blood volume}}{\text{hematocrit (observed)}}$$

Summary

The incidence of neonatal polycythemia and hyperviscosity is between 1% and 5%, making it a common occurrence. However, it has become a less common problem in the practice of newborn medicine, as timely cord clamping is now standard obstetric practice and the American Academy of Pediatrics no longer recommends screening the hematocrit in otherwise normal infants [101]. PET should be employed to correct problems that are clearly attributable to polycythemia and hyperviscosity. It should be emphasized that there is no clear evidence that PET will affect the long-term developmental and neurologic outcome of infants born with polycythemia and hyperviscosity.

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10

Newborn platelet disorders

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Newborn platelet disorders

Bizzozero is credited with the first description of platelets, the last blood cell to be described [1]. Similarly, the understanding of platelet formation has lagged behind the study and understanding of erythropoiesis and myelopoiesis. The isolation of thrombopoietin in 1994 [2–6], the development of better techniques to isolate megakaryocytes [7–17], and the advent of molecular biology have brought about a much better understanding of megakaryocytopoiesis/thrombopoiesis, as well as the final steps of platelet production and release. Yet, our understanding of newborn platelets is incomplete. In this chapter, we will discuss briefly the ontogeny of hematopoiesis, megakaryocytopoiesis, thrombopoiesis, and platelet production, concentrating on the differences between adult and fetal processes. The majority of the chapter is devoted to platelet disorders of the newborn.

Megakaryocytopoiesis and thrombopoiesis in the newborn

Hematopoiesis

Our current understanding of hematopoiesis is based on the hypothesis that there is a hematopoietic stem cell capable of differentiating into all hematopoietic cell lines, including megakaryocyte/platelet lineage [18–22]. Hematopoietic stem-cell-transplantation animal models and cell-culture techniques, developed in the 1960s and 1970s, documented the ability of bone-marrow cells to reconstitute all hematopoietic lineages, which proved the existence of this hematopoietic progenitor cell [23]. For instance, Till and McCulloch in 1961 [23] documented that hematopoietic stem cells could reconstitute the blood in animals given otherwise lethal doses of radiation. Lineage-specific in vitro assays for myeloid, erythroid, megakaryocytic, and multilineage progenitor cells were first developed in mice [23–26] and now are available for human progenitors [27–30]. Recently, assays for high-proliferative-potential colony-forming cells have become available for both rodent models and humans [31–34].

The better understanding of these in vitro colony assays made clear that progenitor cells require specific cytokines in culture to undergo proliferation and final differentiation. Several lineage specific and nonspecific factors were isolated and tested in these culture systems [18, 21]. The results of these experiments showed that stem cells and primitive progenitor cells require a combination of growth factors for optimal growth and differentiation, while lineage-committed progenitor cells are dependent on lineage-specific factors to undergo final differentiation. It is still controversial whether this process is stochastic (a random process) in nature or deterministic, or perhaps a combination of both [21, 35–44].

Megakaryocytopoiesis and platelet formation

Megakaryocytes arise from the pluripotential stem cell in the bone marrow (Fig. 10.1). The

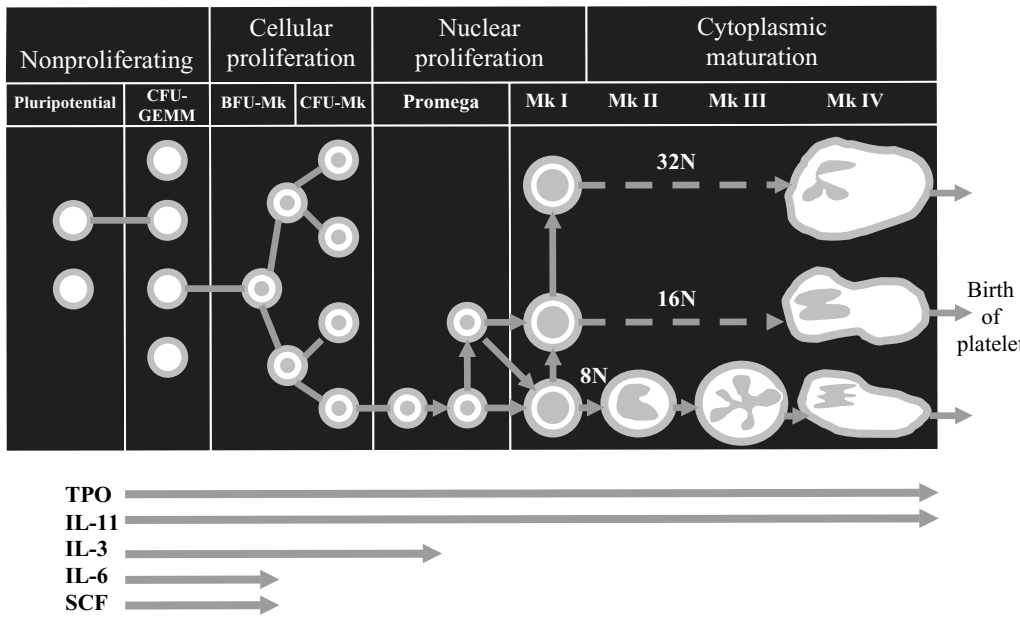

Fig. 10.1 Megakaryocytopoiesis/thrombocytopoiesis begins with a pluripotent, nonproliferating stem cell that transitions to a proliferating multilineage progenitor cell compartment capable of cell division but limited differentiation. Further proliferation occurs at the level of the committed progenitor cells, BFU-Mk (primitive) and CFU-Mk (more mature), which are capable of differentiation but not self-renewal. Further differentiation occurs with transition into a precursor compartment of morphologically recognizable megakaryocytic cells. These cells have limited cell-division capabilities but are capable of nuclear DNA amplification, doubling DNA content with each endoreduplication without nuclear or cell division. These cells also increase in size (horizontal axis of graph), concomitant with the increase in DNA content of the cell or ploidy (vertical axis of the graph). As the ploidy increases, the nucleus of the cell becomes more lobulated, ultimately producing the large megakaryocyte with a multilobulated nucleus. The bottom of the graph illustrates the major cytokines that influence megakaryocytopoiesis, thrombopoiesis, and platelet release and their range of action.

characteristics of this stem cell are still unclear. The transplantation experiments by Till and McCulloch documented that spleen cells can reconstitute the entire hematopoietic system and therefore contained a hematopoietic stem cell [23]. The stem cell differentiates into progenitors that have potential for differentiation to all cell lines, but as differentiation occurs, these progenitors lose pluripotentiality and become lineage-committed [18]. The earliest lineage-committed megakaryocyte progenitor is the burst forming unit-megakaryocyte (BFU-Mk) [45]. This cell can be grown in vitro, where it is characterized by large colonies. This cell differentiates into the more mature progenitor, the colony forming unit-megakaryocyte (CFU-Mk) [30, 45–48], a cell also capable of making in vitro colonies that contain fewer cells (3–50) per colony. The bone marrow contains both CFU-Mk and BFU-Mk; peripheral blood has a relative increase in BFU-Mk due mostly to a relative loss of circulating CFU-Mk [49, 50]. Fig 10.1 illustrates how the stem cell differentiates into multipotential progenitor cells. A bipotential progenitor cell capable of erythroid and megakaryocytic development may also represent a step in this continuum of differentiation [51]. The early progenitors have a high proliferative capacity, require multiple cytokines for proliferation [52, 53], but have a lower differentiation potential. As the cells commit to the megakaryocyte line, they become more dependent on thrombopoietin

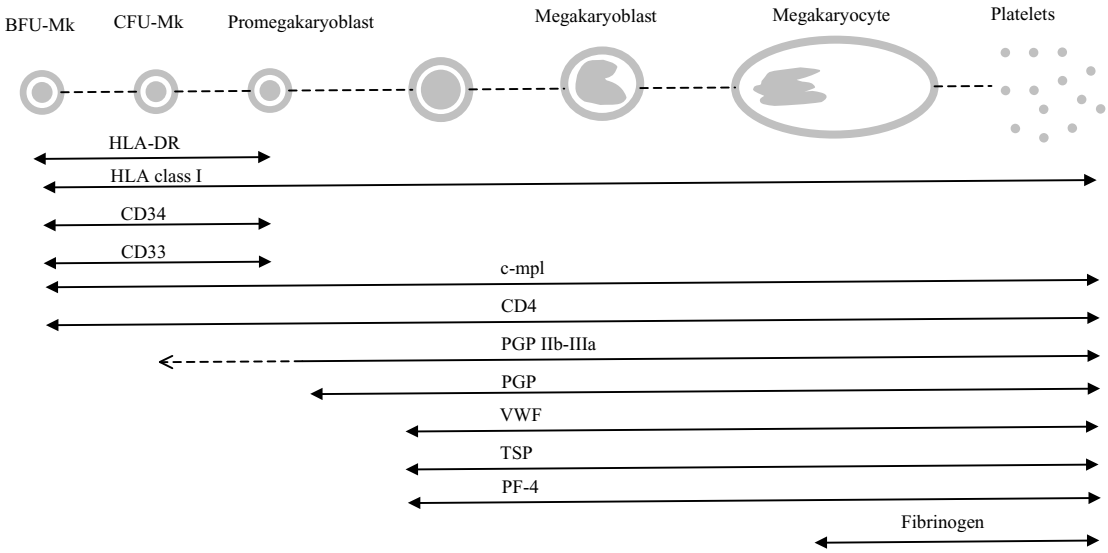

Fig. 10.2 The expression of various antigens as they appear and disappear on the surface of megakaryocyte progenitors, precursors, megakaryocytes, and platelets. PGP, platelet growth factor; TSP, thrombospondin; PF-4, platelet factor 4.

stimulation and lose proliferative capacity, but they become more able to differentiate and mature [18, 54]. The megakaryocyte is a peculiar cell that, as it matures, undergoes nuclear duplication without undergoing cell division. This process is called endoreduplication. The cell becomes larger and increases the amount of DNA per cell, or ploidy [12, 15, 55–60].

Fetuses and newborns are difficult to study, but cord blood is rich in progenitor cells, with premature infants having the greatest number of circulating and marrow progenitors per 10^5 cells compared with older children and adults [49, 50, 61–64]. The difference is not only in numbers. BFU-Mk tends to have fewer foci and far more cells in newborn infants' blood, either full-term or premature, than in older children's or adults' blood [50]. The cell size of megakaryocyte colonies from newborn blood is smaller than the cell size of colonies formed by progenitors of older children or adults [50, 65, 66].

In rodent bone marrow, a small acetylcholinesterase-positive cell already committed to the megakaryocytic lineage, but not easily identified morphologically, may represent a transition cell from committed progenitor to identifiable megakaryocyte [67]. Using monoclonal antibodies against platelet glycoprotein (PGP) IIb/IIIa or Ib, a human equivalent cell can be identified [48]. Recognizable megakaryocytes are large multilobulated cells. As megakaryocytes mature, they increase in size and lobulation. The content of DNA, or ploidy, increases in parallel. Megakaryocytes have been classified morphologically as type I to type IV, from small and immature to large and mature, dependent on their size, degree of nuclear maturation, and degree of cytoplasmic maturation [55]. Fetal and newborn megakaryocytes are smaller than those of older children and adults [68, 69]. The size may remain the same or increase slightly during gestation [68, 70, 71]. Megakaryocyte ploidy also is significantly lower in fetal megakaryocytes than in those of older children and adults [72]. This finding has also been documented in cultured megakaryocytes [47, 65, 66, 73].

PGPs IIa, V, and Ib are almost platelet-specific. During development, these proteins appear on the surface of megakaryocytes. Vainchenker and Kieffer reviewed the differentiation markers during megakaryocytopoiesis (Fig. 10.2) [46]. The PGP IIb–IIIa can be detected with the use of monoclonal antibodies in a small round cell called the

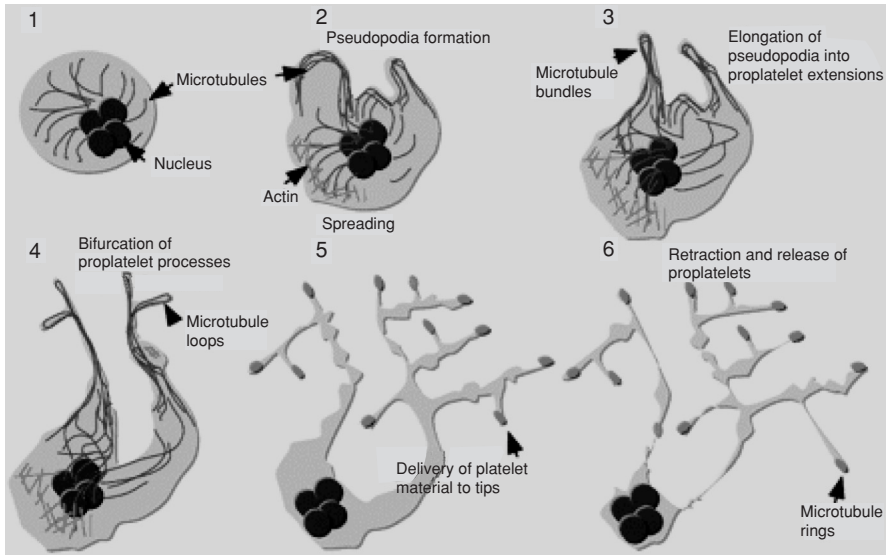

Fig. 10.3 Stage 1: megakaryocyte undergoes polyploidization and cytoplasmic maturation. Stage 2: during the initial stages of proplatelets formation, megakaryocytes remodel their cytoplasm into thick pseudopodia. Stage 3: pseudopodia contain bundles of microtubules situated just beneath the protruding membrane. Blunt pseudopodia elongate into proplatelet processes. Stage 4: proplatelets frequently bend and branch. Stage 5: these proplatelet processes form constrictions along their length, giving the beaded appearance to proplatelets. Stage 6: platelets are released from the tips of the proplatelets. Adapted with permission from Italiano *et al.* [85].

promegakaryoblast [48, 74]. Vinci and colleagues demonstrated that PGP Ib appeared later in differentiation [74]. Other proteins, such as PGP IV, von Willebrand factor (VWF), thrombospondin receptor, and fibrinogen, appear even later [74, 75]. Several investigators in this group have reported the presence of CD34 in early megakaryocyte progenitors [76] and the presence of c-mpl and CD4 in progenitors, differentiated megakaryocytes, and platelets [77, 78]. Although there is overlap of antigen expression, Vainchenker and collaborators have defined the phenotypes of cells committed to megakaryocyte differentiation, including the burst-forming unit-erythroid megakaryocyte (BFU-E/Mk), BFU-Mk, CFU-Mk, the promegakaryoblast, and the differentiated megakaryocyte [51, 53, 76–81].

Fully mature megakaryocytes develop elongated pseudopods known as proplatelets. These pseudopods have a microtubular arrangement and distribution of organelles that resemble the structure of platelets [82–85]. New technologies allowing for video recording of megakaryocytes in culture suggest that platelets bud off these pseudopods [85]. The younger the megakaryocyte, the thicker is the pseudopod and the larger is the platelet. Thus, early platelet release from a megakaryocyte, as occurs during response to thrombocytopenia, leads to large platelet formation. Fig. 10.3 illustrates the proplatelet model of platelet production proposed by Italiano and colleagues [85].

Platelet structure, function, and numbers

Platelet structure

The platelet is a small discoid particle, a fragment of megakaryocytes, which is released into circulation. Because of their small size, platelets are propelled to the periphery of the vascular blood flow and placed in a strategic position to react to tissue injury and the presence of collagen and von Willebrand factor (VWF) on the surface of the endothelium.

Platelet anatomy

The surface of the platelet is smooth, except for the opening of the canalicular system. The latter is a complex system of communicating channels within the platelet that allows easy access for the platelet contents to the extracellular space. The shape of the platelet is maintained by a cytoskeleton. Its structure includes a lipid bilayer membrane supported, like the red cell, by a spectrin-based cytoskeleton. A microtubular coil is wrapped under the surface, and an actin-based network of filaments forms a net that fills the cytoplasm. Inside the platelet there are several organelles. The α-granules are storage granules that contain matrix adhesive proteins, P-selectin (which is not present on the surface of the resting platelet), PGP Ib–IX–V complex, the major platelet integrin PGP IIb–IIIa ($\alpha_{II}\beta_{III}$ integrin), fibrinogen, fibronectin, thrombospondin, vitronectin, and coagulation factors, including VWF. The second set of platelet granules is the dense granules. These are less abundant and smaller and contain platelet agonists such as adenosine diphosphate (ADP) and serotonin. In addition, platelets contain normal organelles, such as lysosomes and mitochondria [86–88, 91, 92].

Platelet surface

The platelet surface contains multiple receptors that determine the reactivity of the platelet and its relationship to the exterior of the platelet. Receptors interact with agonists and adhesive molecules as well as cytokines, producing the necessary intracellular signaling for platelet function. There are several types of receptors on the surface of the platelet. The integrins represent the major class of adhesive and signaling molecules in many cell types. The platelet has integrins of three different families (β_1, β_2, and β_3). The major platelet integrin, $\alpha_{IIb}\beta_3$ (also called glycoprotein IIb/IIIa), is expressed uniquely in platelets, is the fibrinogen receptor, and is essential for platelet aggregation. This integrin has low affinity for ligand in the unstimulated platelet. Platelet agonists cause an inside-out signaling process that activates $\alpha_{IIb}\beta_3$ integrin and causes clustering of its molecules. The second most important integrin on the platelet surface is the collagen receptor, $\alpha_2\beta_1$ integrin (glycoprotein IIa/IIIa). There are several other integrins present on the surface of platelets, but their roles are not defined as clearly as the previously mentioned integrins. The von Willebrand factor receptor (VWFR) on the surface of the platelet is a member of the leucine-rich repeat family of receptors, the PGP Ib–IX–V. This receptor is essential in the initiation of platelet adhesion. However, its role in platelet physiology is far more extensive. It activates $\alpha_{IIb}\beta_3$ integrin by initiating the so-called inside-out signaling mechanism. It interacts with P-selectin in unactivated platelets and with Mac-1 in leukocytes. It also interacts with the contact factors of coagulation high-molecular-weight kininogen, thrombin, and factors XI and XII. The thrombin receptors are transmembrane receptors. The first of these receptors to be identified was the protease activation receptor 1. Several other receptors of this family have now been identified on the platelet. There are three ADP receptors on the platelet: $P2Y_1$, $P2Y_{12}$, and P2X. In addition, the platelet has receptors for thromboxane, prostaglandins, platelet-activating factor, adenosine, vasopressin, chemokines, and serotonin, and the β2-adrenergic receptor. The immunoglobulin superfamily is also represented on the surface of the platelet, including intracellular adhesion molecule 2 (ICAM-2) and platelet-endothelial cell-adhesion molecule 1 (PCAM-1), and tyrosine kinase receptors such as c-mpl, the thrombopoietin receptor. It is clear from the abundance of these receptors that the platelet is involved in more than hemostasis: it also plays a critical role in atherosclerosis, tumor metastasis, antimicrobial host defense, and inflammation [93].

Platelet size

Platelets vary in size and density. The advent of automated cell counters allows for the direct measure of platelet size. The mean platelet volume (MPV) of normal platelets varies from 6 to 12 fl. It has a normal distribution [94]. The measured MPV can increase with time from blood drawing

when collected in ethylenediamine tetra-acetic acid (EDTA) [95–98]. The MPV is more stable in citrated blood [96, 98]. The initial response to acute thrombocytopenia is an increase in platelet size [99]. In animal models, this response precedes the increase in megakaryocyte numbers and platelet numbers [99]. Most disorders of platelet destruction are associated with increased platelet size [94]. It is often stated that large platelets are young platelets. However, this is controversial and current evidence does not support this concept. Platelet size appears to be determined at the megakaryocyte stage, and released large platelets remain large until removed from circulation [100–102].

Newborn platelet structure

Platelet anatomy

Very little is known about the structure of newborn (full-term or premature) or fetal platelets. Most studies have concentrated on analyzing the numbers or the function of platelets, paying little attention to the structure. Saving and colleagues [103] studied 32 term infants, 39 sick preterm infants, and 15 normal adults. Adult platelets formed greater numbers of pseudopods, contained larger glycogen storage, more visible microtubular structures, and markedly fewer α-granules, and had a smaller area and perimeter than newborn or premature infant platelets.

Platelet surface

In particular, the epinephrine receptor density of neonatal platelets is decreased compared with adult platelets [104]. This renders platelets hypoaggregable with epinephrine. The density of other receptors, such as fibrinogen receptor and the PGP IIb/IIIa ($\alpha_{IIb}\beta_3$), is normally distributed in neonatal platelets. The VWFR, PGP Ib/IX/V, and the collagen receptor, PGP IIa/IIIa ($\alpha_2\beta_1$), appear to have a normal distribution and density in neonatal platelets [105–109]. Some investigators have found lower expression of some of the adhesion molecules in neonatal platelets [110, 111].

Platelet size

There are a limited number of studies of platelet size in the newborn. Kipper and coworkers reported the MPV in 27 full-term and 10 premature infants [112]. The full-term infants had an MPV of 8.47 fl, while the premature infants had an MPV of 8.08 fl. There was no difference between the two groups. Thrombocytopenic infants had a higher MPV. Arad and coworkers studied 52 infants with a birth weight greater than 2 kg and 15 infants with a birth weight of less than 2 kg [113]. The MPV was 8.45 + 1.10 fl in the first group and 8.67 + 0.82 fl in the second group. MPV increased in both groups during the first 20 days of life. Patrick and coworkers studied 78 full-term infants and 65 premature infants [114]. The full-term infants had an MPV of 9.06 + 0.85 fl, while the premature infants had an MPV of 8.51 + 0.97 fl. The analysis was performed four hours after collection of the sample. The swelling of platelets that occurs in EDTA would be expected to have reached a maximum by this time. The difference between the two groups was significant. There was a large variation around the mean, however. Using flow cytometry to measure platelet volume, Meher-Homji and colleagues studied fetal blood from the fourth to the fortieth week of gestation; they found that the MPV decreased during gestation [115].

Platelet function

Shape changes

Platelet activation by contact with collagen or VWF or by agonist induces predictable shape changes in the platelet. These changes are dependent on a complex process of the platelet cytoskeleton and the dynamics of actin polymer. Upon activation, the platelet loses its discoid shape and becomes spheroid. Finger-like projections develop from the periphery of the platelet; then the platelet flattens and broad lamellae are extended. Next, the flattened platelet squeezes the organelles into the center of the platelet, creating a fried-egg-like appearance. Finally, the flattened platelet generates new filopods [116].

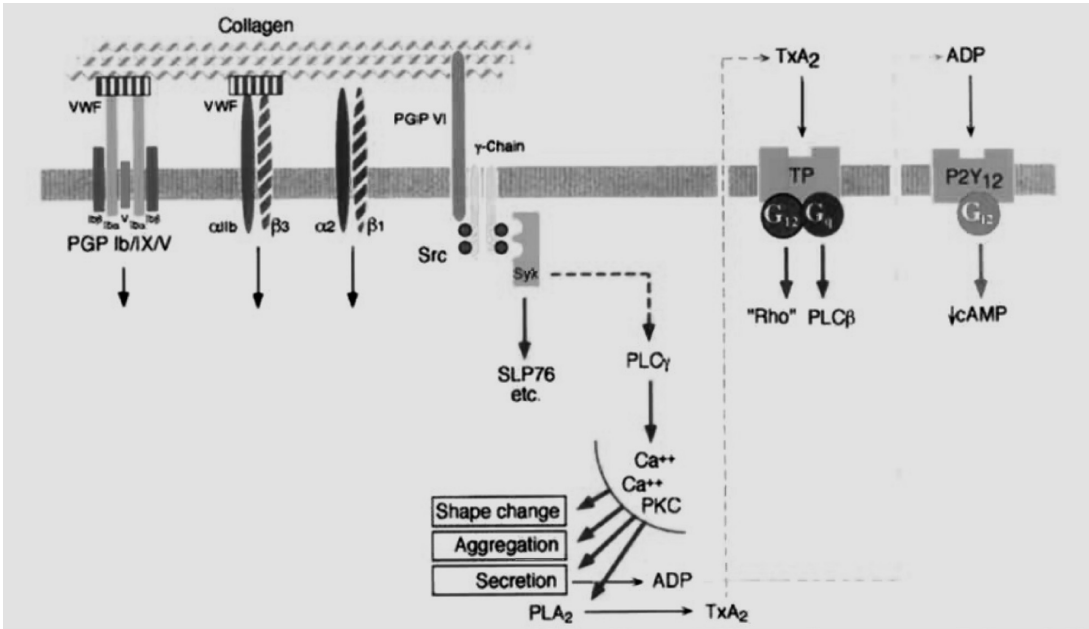

Fig. 10.4 Platelet activation by collagen. Platelets appear to use several different molecular complexes to support platelet activation by collagen. These include the von Willebrand factor (VWF)-mediated binding of collagen to the platelet glycoprotein (PGP) Ib/IX/V complex and the $\alpha_{IIb}\beta_3$integrin; a direct interaction between collagen and the $\alpha_2\beta_1$ integrin; and the PGP VI/γ–chain complex. Clustering of PGP VI results in the phosphorylation of tyrosine residues in the γ-chain followed by the binding and activation of the tyrosine kinase Syk. One consequence of Syk activation is the phosphorylation and activation of phospholipase C_γ (PLC_γ), leading to phosphoinositide hydrolysis, secretion of adenosine diphosphate (ADP), and the production and release of thromboxane A2 (TxA_2). ADP and TxA_2 bind to their own receptors in platelets, generating signals that support the more direct effects of collagen. cAMP, cyclic adenosine monophosphate; PLA_2, phospholipase A2. Reproduced with permission from Woulfe *et al.* [117].

Adhesion

VWF on the surface of injured endothelium binds transiently at a high sheer rate to its platelet receptor PGP Ib-IX-V on the platelet surface, causing platelet rolling on the surface of the injured tissue. Signaling from the bound PGP Ib-IX-V leads to cytoskeleton rearrangement and activation of $\alpha_{II}\beta_{III}$ integrin. The rolling platelet then binds to collagen and VWF. Binding of collagen to its receptors $\alpha_2\beta_1$ integrin and PGP VI, and of VWF to its receptors $\alpha_{II}\beta_{III}$ integrin and PGP Ib-IX-V, then generates a more permanent adhesion. Collagen binding to PGP VI generates clustering of the receptor and phosphorylation of its γ-chain by the Src family of tyrosine kinases. The phosphorylation creates an SH_2 motif recognized by Syk, which leads to phosphorylation of the G2 isoform of phospholipase C (PLC). PLC hydrolyzes phosphoinositol diphosphate to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 opens the Ca^{++} channel and DAG activates protein kinase C (PKC). This signaling then leads to shape changes, secretion, and aggregation (Fig. 10.4) [117].

Aggregation

Further extension of the platelet plug occurs through platelet aggregation mediated by the agonist released from adherent platelets. The contact

between platelets is maintained by the binding of fibrinogen and VWF to their activated platelet receptor $\alpha_{II}\beta_{III}$ integrin. ADP and thromboxane A_2 (TxA_2), released by collagen binding, activate platelets through G-protein-mediated signaling, leading to activation of "Rho" and PKC and inhibition of adenyl cyclase, which in turn leads to platelet shape changes, secretion, and aggregation. Activation of thrombin on the surface of the activated platelet furthers the role of thrombin in the activation of platelets and formation of the platelet plug.

Newborn platelet function

Platelet aggregation

Uniform platelet aggregation measured by an optical instrument was first described in 1962 [118, 119]. Most of the information regarding platelet function in the newborn comes from studies performing this test. However, the difficulties in obtaining an adequate sample from cord blood or from venopuncture in the newborn or premature infant have hampered these studies and made the interpretation of the results difficult. Yet, the information gathered from these studies has demonstrated significant aggregation defects in newborn platelets.

The bleeding time

The bleeding time is a test of primary whole hemostasis. A clean cut in the skin is made and the time to clotting is measured. There are several techniques available for measuring the bleeding time, but the most commonly used is the Ivy technique. Unfortunately, this technique is not easily applicable to the newborn. Several investigators have adapted the bleeding time for use in the newborn and have achieved consistent results [120–122]. However, as a general screening tool, the bleeding time has not been found useful in the adult population. Given the adaptations needed to perform the test in the newborn, it is better used by centers that are proficient with this test and not as a general screen. The bleeding time in newborns is similar to or shorter than that of adults [120–122]. The shortening of the bleeding time may be due to increased hematocrit and/or the increased VWF levels found in the newborn [123, 124].

The PFA-100

This is an instrument that measures in vitro assessment of primary hemostasis. This instrument measures the time that it takes for a blood sample to occlude an aperture of 150 μm as it forms a platelet plug. Several studies have now documented that newborns have a shortened closure time compared with adults [125–127]. Even when adjusted for the hematocrit effect, newborns still have a shortened closure time due to the higher concentration of VWF [126, 127].

Flow cytometry

Platelet function can be studied with flow cytometry. Upon agonist stimulation, platelets express different proteins on the surface (e.g. P-selectin) and rearrange others (e.g. PGP IIb–IIIa). This technique solves the major obstacle in assessing platelet function in the newborn – the volume of the sample required to perform the test. This technique requires a very small sample of blood. As described below, several investigators have demonstrated platelet aggregation defects in the newborn using this technique [106, 108–110, 128, 129].

Newborn platelet function assessment

Upon receptor ligand binding, signaling pathways trigger platelet shape change, granular release, platelet adhesion, and platelet aggregation (Fig. 10.4). Newborn platelets are relatively hypoaggregable compared with those of older children and adults. As discussed in Chapter 13, in spite of the hypofunction of platelets and deficiencies in coagulation factors, the newborn is in a hypercoagulable state.

Earlier studies using platelet-aggregation studies documented hypoaggregable platelets in the newborn [130–134]. However, some of the abnormalities found were attributed to degranulation of platelets during the birthing process [135]. Significant differences are found between different agonists. Neonatal platelets have a poor response to epinephrine. This is due to the decreased expression (about 50%) of α2-adrenergic receptors in neonatal platelets [104]. Thrombin-induced aggregation is decreased only slightly in newborn platelets, while arachidonic acid, collagen, and ADP-induced aggregation show a more significant decrease [106, 107, 128, 130, 131, 133, 136, 137]. The receptors for these agonists, however, are present in normal amounts on the surface of neonatal platelets. High concentrations of thrombin induce a greater amount of arachidonic acid production in neonatal platelets than in adult platelets. However, stimulation with collagen, epinephrine, or ADP produces less arachidonic acid. Downstream activation of signaling molecules is diminished in the newborn [138]. Activation of PLC, PKC, and intracellular Ca^{++} mobilization are all impaired. This is most likely due to decreased GTPase activity of the G proteins. Phosphatidylserine and other negatively charged lipids form part of the inner membrane of the platelet. Upon activation, phosphatidylserine is relocated to the surface of the platelet, where it can bind coagulation factor zymogens and initiate coagulation on the surface of the platelet. Newborn platelets produce greater numbers of microparticles containing phosphatidylserine than do adult platelets. However, the procoagulant activity is decreased. This activity can be corrected by the addition of factor V that is deficient in newborn plasma [139].

Platelet function defects in the newborn

Most defects of platelet function have mild to moderate clinical symptoms. It is unusual for these defects to be detected at birth. Two disorders with moderate to severe symptoms can present at birth: Bernard–Soulier and Glanzmann thrombasthenia.

Bernard–Soulier

Bernard–Soulier is a moderate to severe platelet function defect characterized by mild thrombocytopenia, giant platelets, and mucosal-type bleeding. It is a very rare disorder, with an incidence of 1 in 1 000 000. It is a defect in the VWFR PGP Ib-IX-V. The receptor includes four distinct transmembrane peptides, PGP Ibα, PGP Ibβ, PGP IX, and PGP V. The genes correspond to the superfamily of genes with the leucine-rich repeats motif. Although they are located in different chromosomes, their structure is similar, suggesting an ancestral gene. The gene for PGP Ibα maps to chromosome 17, PGP Ibβ maps to chromosome 22, and PGP IX and PGP V map to chromosome 3, 3q21 and 3q29, respectively. The majority of defects have been associated with mutations in PGP Ibα, but defects in PGP Ibβ and PGP IX have been described. To date, no mutation of PGP V has been described [140, 141]. In spite of its severity, newborns with this disorder do not bleed. However, severe bleeding in an infant with DiGeorge syndrome and cardiac disease may be due to coexisting Bernard–Soulier [142]. Lopez and colleagues reviewed the Bernard–Soulier syndrome. These authors provide an excellent review of the molecular defects, as well as the clinical features, of this rare disorder [141].

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare inherited disorder of platelet function. It is caused by a deficiency or abnormality of the surface-membrane PGP IIb–IIIa complex. Thrombasthenia is a severe platelet function disorder. It is an autosomal recessive disorder [143, 144]. It has been classified according to the expression of the PGP IIb–IIIa complex on the surface of the platelets. Type I Glanzmann thrombasthenia expresses less than 10% of the receptor, type II expresses 15–25% of the receptor, and the variant type expresses 50% to normal levels of the receptor and represents a dysfunctional receptor defect. Both genes for the PGP IIb and PGP IIIa coexpress to chromosome 17q21–23. Thrombasthenia may be due to defects in either gene. George and colleagues

Table 10.1 Bleeding in patients with Glanzmann thrombasthenia

	No. of affected patients	Frequency (%)
Symptoms		
Menorrhagia	54/55	98
Easy bruising, purpura	152/177	86
Epistaxis	128/177	73
Gingival bleeding	97/177	55
Gastrointestinal hemorrhage	22/177	12
Hematuria	10/177	6
Hemarthrosis	5/177	3
Intracranial hemorrhage	3/177	2
Visceral hematoma	1/177	1
Severity		
Requirement for red-cell transfusions	86/112	77

Adapted with permission from George *et al.* [143].

described the clinical picture of thrombasthenia in 177 patients, 64 from a closely followed cohort in Paris and 113 from case reports and case series in the literature [143]. In the neonate, generalized purpura or petechiae and bleeding from circumcision are the most common manifestations. Seven of the 64 patients from Paris presented with generalized purpura in the neonatal period. Isolated reports of neonates have described more serious hemorrhages [145]. In the older child, nosebleeds, easy bruising, and menorrhagia (particularly with the first period) are the most common manifestations. Gastrointestinal bleeding, intracranial bleeding, joint bleeding, and bleeding from surgery or trauma can also occur (Table 10.1) [143]. Many patients will require blood-product transfusions during their lifetime. However, the majority of bleeding episodes are not severe and patients lead reasonably normal lives. The diagnosis of thrombasthenia is made by the clinical picture, normal platelet count, normal coagulation screen, failure of platelets to aggregate with most agonists, normal ristocetin-induced platelet aggregation, and decreased or absent PGP IIb–IIIa complex on the platelets, as measured by flow cytometry. In cases of variant Glanzmann thrombocythemia, gene analysis may be performed to identify the abnormal receptor. The disorder is more common in the Middle East [144]. Treatment consists of supportive measures and platelet transfusions when hemorrhage cannot be stopped. Platelet transfusions are also effective to cover surgery and delivery of a pregnant woman. There is a concern that a patient with thrombasthenia may become sensitized to platelets because the PGP IIb–IIIa is very antigenic and present in normal platelets. However, only one patient in the Paris series developed antibodies, and these were not clinically significant [143]. In a few isolated reports, human recombinant activated factor VII was effective in controlling hemorrhage in patients with thrombasthenia [146].

Other platelet dysfunction disorders

Other inherited platelet function defects are mild and therefore rarely present in the newborn period. These disorders cause nosebleeds, easy bruising, and menorrhagia as their most common presenting symptoms and, therefore, they are not seen in the newborn. However, these patients may bleed with surgery. Although these disorders are rare, they need to be considered at any time when there is unexplained bleeding in the neonate, particularly with bleeding during surgery.

Pseudo-von Willebrand disease

This is a very rare autosomal dominant inherited disorder of hyperfunction of the VWFR PGP Ib-V-IX. This disorder is similar to von Willebrand disease type 2b, in that there is increased plasma clearance of the high-molecular-weight multimers of VWF due to the increased binding to platelets. However, in pseudo-von Willebrand disease, the defect resides in the platelets, and not in the VWF. The diagnosis is made by performing platelet aggregations with low concentrations of ristocetin as the agonist. Normal platelets will not aggregate in this test, but platelets from a patient with type 2b von Willebrand disease or pseudo-von Willebrand disease will aggregate. If normal plasma is substituted for the patient's

plasma in the test, then the platelets from the von Willebrand disease type 2b patient will behave normally, while the platelets from pseudo-von Willebrand disease will aggregate in the presence of normal plasma alone [147, 148].

Collagen receptor defects

Collagen receptor is involved in platelet adhesion. Congenital defects associated with three collagen receptors have been described. A case of mild bleeding disorder with deficiency of PGP Ia–IIa has been described. Deficiency of PGP IV (CD36) occurs in 3% of Japanese and African-American people and in 0.3% of Caucasians, but it is not associated with a bleeding disorder. A subject with deficiency of PGP VI and bleeding disorder has been reported; the parents had no bleeding symptoms and about 50% of the normal levels of PGP VI [149–153].

Secretory platelet disorders

Secretory platelet disorders have defective platelet granules. The Gray platelet syndrome is a mild bleeding disorder in which platelets and megakaryocytes lack α-granules. The storage-pool defect (δ-storage-pool defect) is an autosomal dominant disorder associated with absent dense granules. There are other disorders with associated congenital abnormalities that are also δ-storage-pool defects. The Hermansky–Pudlak syndrome consists of tyrosinase-positive oculocutaneous albinism, reticuloendothelial deposits of ceroid in macrophages, gastrointestinal lesions, and pulmonary fibrosis. It is a defect of lysosomal assembly and trafficking. At least four gene defects have been implicated in this disorder. In the animal model of Hermansky–Pudlak syndrome, 14 different genes are involved. In humans, HSP-1 localizes to chromosome segment 10q23 [154, 155], HSP-2 is a defect in the β3A subunit of AP-3 adaptor protein, HPS-3 localizes to 3q24 [156], and HPS-4 localizes to 22q11.2–12.2 [157]. The Chediak–Higashi syndrome is a syndrome of oculocutaneous albinism with abnormal granules in hematopoietic cells and other cells with recurrent pyogenic infections. The gene has been identified and localizes to 1q42–43 [158, 159]. Dense-granule storage-pool defects have also been described in the thrombocytopenia and absent radii syndrome (TAR) and in Wiskott–Aldrich syndrome (WAS) [160, 161]. The αδ-storage-pool defects lack both alpha and dense granules.

Congenital platelet-activation defects

Defects in agonist-induced activation of platelets have been described. Defects in arachidonic acid activation have abnormal platelet aggregation with ADP, epinephrine, and collagen stimulation, but normal aggregation with arachidonic acid. Cyclo-oxygenase deficiency, thromboxane synthetase deficiency, and epinephrine stimulation deficiency defects have been described. All cause mild bleeding disorders [162].

Therapy

In the newborn, especially for severe bleeding, the therapy of choice is platelet transfusions. Several reports suggest that therapy with activated factor VII may also be effective in stopping hemorrhage in these disorders [146]. While these disorders may respond symptomatically to the administration of desmopressin at a dose of 0.3 mg/kg, this drug can induce hyponatremic seizures in neonates, infants, and young children [163]. If used in such children, the patient's fluid balance needs to be monitored closely, with avoidance of free water.

Drug-induced platelet function defects

Many drugs can cause platelet function abnormalities. Most of these cause in vitro defects with little clinical significance. However, there are some drugs that deserve mention because of their use in perinatal medicine.

Cyclo-oxygenase inhibitors

The ingestion of aspirin or indomethacin by pregnant women has been associated with increased risk

of hemorrhage in the newborn. Aspirin ingestion within two weeks of parturition increased mucosal bleeding in the newborn. Ingestion of indomethacin by mothers increased the risk of intracranial hemorrhage (ICH) in newborn infants. Low-dose aspirin used in the management of maternal hypertension may not have as high a risk of increased bleeding in the newborn as indomethacin [164, 165].

Nitric oxide

Nitric oxide (NO) inhibits in vitro activation of the fibrinogen/VWF receptor, PGP IIb–IIIa [166]. Several studies have documented a direct effect of inhaled nitric oxide (iNO) on neonatal platelet aggregation, as measured by aggregometry, flow cytometry, or the bleeding time [167–169]. Most newborn infants requiring iNO are premature or have essential pulmonary hypertension, both conditions having increased risk of bleeding and particularly ICH. To date, there has not been a report of increased risk of bleeding in infants treated with iNO, but given their increased risk of bleeding and the effect of NO on platelets, these infants should be watched carefully for increased risk of bleeding.

Ethanol

Ethanol inhibits platelet function and crosses the placental barrier. In vitro neonatal platelets are affected as well as adult platelets. This suggests that maternal ingestion of ethanol in the peripartum period may increase the risk of neonatal platelet dysfunction [170]. This phenomenon has not been studied in vivo.

Other conditions affecting platelet function

Renal failure and hepatic failure affect platelet function. However, these have not been implicated in increased risk of bleeding in the neonate. Bilirubin [171] and phototherapy [172] also inhibit platelet function; however, in spite of wide use of phototherapy in jaundiced babies, there is no report of increased incidence of hemorrhage. Extracorporeal membrane oxygenation (ECMO) can cause both thrombocytopenia and platelet dysfunction [173, 174]. These patients have multiple factors that can induce bleeding, so it is difficult to tease out the direct cause of bleeding.

Table 10.2 Platelet counts in full-term newborns

Ref.	Source	*n*	Platelet count (/mm^3)
Ablin *et al.* [176]	Heel puncture	105	190 000 ± 94 000
Sell and Corrigan *et al.* [177]	Umbilical cord	45	325 000 ± 50 000
Aballi *et al.* [175]	Capillary blood	88	250 000 ± 125 000

Therapy

Regardless of the cause of acquired platelet dysfunction, the therapy for severe hemorrhage is the same, i.e. platelet transfusions. Minor bleeding may respond to desmopressin therapy, but there is a risk of hyponatremia-induced seizures [163]. As stated before, some reports suggest that therapy with activated factor VII may be effective in the treatment of severe hemorrhage due to platelet dysfunction, but this needs further study.

Newborn thrombocytopenia

Newborn platelet count

The platelet count in the newborn is similar to that in older children and adults, ranging from 150 000 to 450 000/mm^3 (Table 10.2) [175–178]. Aballi and colleagues [175] reported a mean platelet count in full-term newborns of 250 000/mm^3. However, the range was from 117 000/mm^3 to 450 000/mm^3. Ablin and colleagues [176] reported a mean platelet count of 190 000/mm^3 with a range from 84 000/mm^3 to 478 000/mm^3. Only 4% of full-term babies had a platelet count below 150 000/mm^3. Sell and Corrigan [177] reported a mean platelet count in full-term newborns of 325 000/mm^3, with a standard error of 50 000/mm^3. Premature infants have a wider range

Table 10.3 Platelet counts in normal preterm newborns

Ref.	*n*	Age		Platelet count × 10^3/mm^3		
		Gestation	Postnatal	Mean	SD	Range
Fogel *et al.* [179]	73		0–28 days	212		156–302
Aballi *et al.* [175]	273		0–2 days	220	68	55–518
	194		2 weeks	260	100	33–574
	112		4 weeks	309	125	85–682
Appleyard and Brinton [180]	60		Birth	203		80–356
	40		10 days	399		172–680
	40		28 days	384		212–625
Sell and Corrigan[a] [177]	8	27–30 weeks	<36 h	280	110	
	29	31–33 weeks	<36 h	295	115	
	24	34–36 weeks	<36 h	305	90	
	45	37–41 weeks	<36 h	295	114	
Hathaway[b]		27–31 weeks	Birth	275	60	
		32–36 weeks	Birth	290	70	

[a] Published reference and personal communication.

[b] Data from personal series combined with reported series of others.

SD, standard deviation.

Adapted with permission from Naiman [250].

for normal platelet count, but the mean platelet count does not differ from that of older children or adults (Table 10.3, Fig. 10.5) [175, 177–181]. In the series by Aballi and colleagues, 14% of premature infants had a platelet count below 150 000/mm^3 [175]. In the series by Sell and Corrigan [177], the mean platelet count of infants of 27–30 weeks' gestation was 280 000 ± 110 000/mm^3, for those of gestational age 31–33 weeks it was 295 000 ± 115 000/mm^3, and for those of 34–36 weeks' gestational age it was 305 000 ± 90 000/mm^3. Fig. 10.5, derived from the data of Aballi and colleagues, illustrates the wider range of platelet counts seen in the premature compared with the full-term newborn. The wide use of percutaneous umbilical-vein blood sampling (PUBS) has allowed for the development of standard blood counts in fetuses from 18 weeks to 40 weeks. The mean platelet count is slightly lower at earlier gestation, rising slightly to the normal range seen at full term, but it never falls below 150 000/mm^3 [182] (Table 10.4). Neonatal thrombocytopenia can be classified by its mechanism,

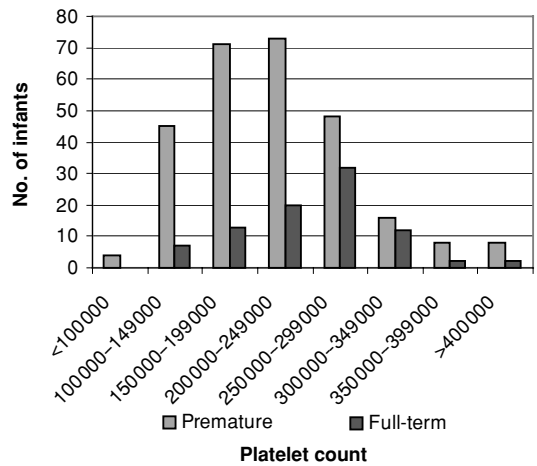

Fig. 10.5 Platelet counts in the first two days of life. Graph adapted with permission from Aballi *et al.* [175].

i.e. increased platelet destruction, decreased platelet production, or a mix of the two. Because of limited information, the precise mechanism of many of the neonatal thrombocytopenias is speculative.

Table 10.4 Evolution of platelet counts in 163 normal fetuses during pregnancy

		Platelet count/mm^3	
Weeks of gestation	*n*	Mean	SD
18–20	25	242 100	34 480
21–22	55	258 200	53 650
23–25	61	259 430	42 450
26–30	22	253 540	36 600

Adapted with permission from Forestier *et al.* [182].
SD, standard deviation.

Nevertheless, increased platelet destruction is a prominent feature of the most common neonatal thrombocytopenias. However, they often have a component of failure of platelet production. The diagnostic approach to neonatal thrombocytopenia can also be classified as dependent on maternal or infant factors. In particular, thrombocytopenia in the healthy newborn infant has different clinical implications and a different differential diagnosis compared with thrombocytopenia in the ill newborn. We shall consider the infant and maternal factors, as well as the mechanism of thrombocytopenia, as we describe the different conditions that affect the newborn.

Maternal factors

Maternal factors affecting an infant's platelet count include drugs, antibodies, and procoagulant factors. Immune thrombocytopenia can be acquired passively, as in immune thrombocytic purpura (ITP) and lupus erythematosus, or it may be drug-induced. It also may be part of an active process in which alloimmune thrombocytopenia is the most common cause. Immune thrombocytopenias are secondary to platelet destruction. However, they also may have a component of suppression of platelet production. Placental infarctions and vascular abnormalities can lead to thrombocytopenia of the infant. The precise mechanism leading to platelet consumption is not clear. Platelets may be consumed within the vascular abnormality or as a result of disseminated intravascular coagulation (DIC). The trigger for consumption of platelets may be either the release of thromboplastic substances or platelet damage.

Maternal thrombocytopenia

The assessment of hemoglobin concentration in pregnancy is routine. Since this measure is automated, the platelet count also is measured. As a result, asymptomatic thrombocytopenia is seen frequently in pregnancy. Boehlen and co-workers [184] suggest that a platelet count of <75 000 in a mother needs to be worked up. It is unclear what to do for those mothers whose platelet count falls between 75 000 and 116 000. Mothers with platelet counts >116 000 should be followed like any other pregnancy (Table 10.5). Several reviews suggest a rational approach to maternal thrombocytopenia [184, 189–193]. The causes of thrombocytopenia include gestational thrombocytopenia, spurious thrombocytopenia, pre-eclampsia, hypertension, hemolysis elevated liver enzymes low platelet count (HELLP) syndrome, drug induced, infection, autoimmune, malignancy, splenomegaly, thrombotic thrombocytopenic purpura (TTP), and hemolytic uremic syndrome (HUS) [187–189, 194]. Discussion of this topic is beyond the scope of this book and its relevance is only in that these maternal thrombocytopenias may affect the infant's platelet count. The risk of developing thrombocytopenia for the infant of a mother with thrombocytopenia is small, but it increases with the severity of maternal thrombocytopenia [184, 185].

Several series have been published looking at platelet counts in mothers and their newborn infants. Burrows and Kelton studied 2284 consecutive deliveries over a one-year period. The rate of maternal thrombocytopenia was 9.1%. Neonatal platelet counts were available in 1550 newborns; thrombocytopenia was present in 55 of these, a rate of 4.1%. Only two of these infants had a platelet count below 50 000/mm^3 [183]. Of the entire cohort, 1357 women were normal as defined prospectively in the study. One hundred and twelve of these women had platelet counts of 150 000/mm^3 or less,

Table 10.5 Diagnosis and incidence of maternal and neonatal thrombocytopenia according to maternal platelet count

	Maternal platelet count				
Characteristic	>150 000/mm^3	116 000–149 000/mm^3	75 000–115 000/mm^3	50 000–74 000/mm^3	<50 000/mm^3
No. of women ($n = 6770$)	5984	621	144	15	6
Thrombocytopenia of unknown origin ($n = 738$)		602 (97%)	129 (90%)	7 (47%)	0
Thrombocytopenia of known origin ($n = 48$)		19 (3%)	15 (10%)	8 (53%)	6 (100%)
HELLP syndrome/ pre-eclampsia/ hypertension		17	10	5	4
ITP		1	2	1	0
Other		2	3	2	2
No. of analyzed newborns ($n = 6103$)	5375	577	132	15	4
Thrombocytopenic newborns ($n = 33$)	23 (0.4%)	6 (1.0%)	2 (1.5%)	2 (13.3%)	0
Severe neonatal thrombocytopenia (<20 000/mm^3)	3 (0.1%)	0	1 (0.8%)	0	0

Adapted with permission from Boehlen *et al.* [184].

HELLP, hemolysis elevated liver enzymes low platelet count.

ITP, immune thrombocytopenic purpura.

the lowest count being 97 000/mm^3. The incidence of newborn thrombocytopenia in all normal mothers was 1.9%, in nonthrombocytopenic mothers 1.5%, and in thrombocytopenic mothers 4.3%. None of these rates was significantly different. No infant born to a "normal" thrombocytopenic mother had a platelet count below 100 000/mm^3. Only two infants in the entire series had evidence of hemostatic defect and a platelet count below 50 000/mm^3. They were both born to mothers with normal platelet counts and were found to have neonatal alloimmune thrombocytopenia (NAIT). Boehlen and coworkers defined a range of normal platelet count for pregnant women [184]. They studied 6770 pregnant women and 287 nonpregnant controls. The platelet count range for pregnant women was 116 000–346 000/mm^3 (2.5–97.5 percentile). From 5984 women with platelet counts above 150 000/mm^3, 23 (0.1%) infants had thrombocytopenia. Of the 621 mothers with a platelet count of 116 000–149 000/mm^3, six (1.0%) infants had thrombocytopenia, none severe. In a study of a seven-year period of 15 663 deliveries, platelet counts were obtained in 15 607. A total of 1027 mothers had platelet counts below 150 000/mm^3 for an incidence of 6.6% [185]. Severe maternal thrombocytopenia with a platelet count below 100 000/mm^3 occurred in only 181 (1.2%) women. Cord-blood platelet counts were determined in 15 932 infants out of the 16 068 infants born to the 15 607 mothers. Nineteen infants had a cord-blood platelet count below 50 000/mm^3, a prevalence of 0.12%. Six infants had a platelet count below 20 000/mm^3. Ten of 19 infants with thrombocytopenia were born to mothers with thrombocytopenia, but only three were born to mothers with severe thrombocytopenia. A total of 756 women had gestational thrombocytopenia. Only one infant with thrombocytopenia was born

Table 10.6 Incidence of fetal or neonatal thrombocytopenia according to mother's health status and platelet count

Mother's health status	No. of infants with thrombocytopenia	Mother's platelet count		
		$<150\,000/mm^3$	$>150\,000/mm^3$	Total
		No. affected/total no.	No. affected/total no.	No. affected/total no.
Normal	1	1/756 (0.13%)	0/13 169 (0%)	1/13 925 (0.01)
Hypertensive disorders of pregnancy	5	4/216 (1.8%)	1/1198 (0.08%)	5/1 414 (0.35%)
ITP	4	2/31 (6.4%)	2/15 (13.3%)	4/46 (8.7%)
SLE	0	0/8 (0%)	0/47 (0%)	0/55 (0%)
NAIT	9	3/3 (100%)	6/15 (40%)	9/18 (50%)
Other	0	0/13 (0%)	0/0 (0%)	0/13 (0%)
Total	19	10/1027 (0.97%)	9/14 444 (0.06%)	19/15 471 (0.12%)

Adapted with permission from Burrows and Kelton [185].

ITP, immune thrombocytopenic purpura; NAIT, neonatal alloimmune thrombocytopenia; SLE, systemic lupus erythematosus.

to these mothers. This infant had trisomy 21 and transient myeloproliferative syndrome of the newborn. The incidence of neonatal thrombocytopenia in the infants of mothers with platelet counts below $150\,000/mm^3$ was 0.97%; in infants of mothers with platelet counts above $150\,000/mm^3$ it was 0.06%. The incidence of neonatal thrombocytopenia in the infants of mothers that were classified as normal by the investigators was 0.01%, with only one infant having thrombocytopenia. In mothers where NAIT was suspected, 50% of the infants had severe thrombocytopenia.

The incidence of neonatal thrombocytopenia in infants of mothers with ITP was not affected by maternal platelet count. The incidence of neonatal thrombocytopenia in these infants is higher than in infants of mothers without ITP (Table 10.6). Human immunodeficiency virus (HIV) can cause thrombocytopenia. In a series of 29 thrombocytopenic HIV-infected mothers, only one infant developed thrombocytopenia [186]. TTP and HUS can be severe complications of pregnancy. Untreated TTP in pregnancy has a high risk of mortality and morbidity in both the infant and the mother. With aggressive plasmapheresis therapy, pregnancy can be successful and infants do not develop thrombocytopenia [187, 188].

The results of the above studies suggest that a platelet count below $75\,000/mm^3$ in a mother needs to be worked up. It is unclear what to do for those mothers whose platelet count falls between $75\,000/mm^3$ and $116\,000/mm^3$. Mothers with platelet counts above $116\,000/mm^3$ should be followed like any other pregnancy (Table 10.5).

Maternal immune thrombocytopenic purpura

The increased recognition of maternal thrombocytopenia has led to the increased recognition of ITP during pregnancy. ITP is more common in women, and the majority of women with ITP are of childbearing age [195]. Two questions are critical in the management of ITP in pregnancy: how to manage the thrombocytopenia in the mother and how to treat and/or prevent thrombocytopenia in the newborn. Burrows and Kelton carried out a comprehensive review of the literature on this subject, encompassing the period between January 1980 and December 1990 [196]. They looked at the reported number of pregnancies with ITP and at the number of infants born to these mothers with a platelet count of $50\,000/mm^3$ or lower. They also recorded the time and site of collection of the infants' blood samples. They analyzed 885 pregnancies and 893 living

infants. In 288 infants with blood samples obtained at birth from a cord-blood sample, a platelet count below 50 000/mm^3 was reported in 10.1% of infants and a platelet count below 20 000/mm^3 was reported in 4.2%. No infant suffered an ICH. The mode of delivery (Cesarian section or vaginal) did not make a difference. In 56 studies describing 552 pregnancies and 557 liveborn infants, the neonate's blood sample was obtained at an undetermined time. Seven infants had major events, including five ICHs; two of these five infants died. Overall, the authors concluded that the risk of thrombocytopenia for the infant is low. Christiaens and colleagues [197] reviewed retrospectively all pregnancies with a diagnosis of ITP admitted to the University of Utrecht ($n = 95$) or New York–Cornell Medical College ($n = 33$) between 1984 and 1995. Thirty-four women had delivered at least two children while they had ITP. The investigators found a strong correlation between the first-born infant's platelet count and the platelet count of the next sibling. During the first two weeks of life, 24 of the 68 infants reached a platelet count nadir of less than 100 000/mm^3 and 12 infants reached a count of less than 50 000/mm^3. Two infants had ICHs and their platelet counts were 5000/mm^3. Seven of the 22 mothers with a history of splenectomy and a platelet count below 50 000/mm^3 had infants with severe thrombocytopenia. When splenectomy was excluded, the maternal platelet count lost its significance. Samuels and colleagues studied prospectively 162 pregnant women with ITP. Eighty-eight had been diagnosed previously with ITP; 74 were diagnosed during pregnancy [198]. Thirty-five of the mothers diagnosed previously with ITP and three of those diagnosed during pregnancy delivered infants with platelet counts below 100 000/mm^3. All 18 infants with platelet counts below 50 000/mm^3 were born to mothers diagnosed previously with ITP. Two infants had ICH. Previous maternal diagnosis of ITP conferred a 20% chance of delivering a thrombocytopenic infant. In an attempt to accurately predict neonatal thrombocytopenia, Berry and colleagues performed cordocentesis in a series of expectant mothers with ITP. They did not find this technique reliable in predicting thrombocytopenia in the newborn [199, 200]. Several investigators have reviewed their experience with ITP in pregnancy. The severity of ITP, splenectomy, presence of antiplatelet antibodies, the site of obtaining the newborn's platelet count, and other parameters have been studied without yielding any reliable predictors of the newborn's platelet count [201–204]. The severity of maternal thrombocytopenia, a history of splenectomy, and having a previous child with severe thrombocytopenia have been associated with lower platelet counts in the newborn, but the data are conflicting [205, 206]. Therapy of maternal ITP also does not affect or predict the neonatal outcome [207]. The current recommendation, based on the very low risk of severe thrombocytopenia, is to manage the pregnant woman with ITP like any other patient – treating the ITP appropriately, but using only obstetric indications for Cesarian delivery [205, 208, 209]. This is not to say that severe complications cannot occur. ICH, when it occurs, usually is a postnatal event, but there is at least one reported case of intrauterine ICH [210]. The overall risk of severe thrombocytopenia (platelet count below 50 000/mm^3) in the newborn of a mother with ITP is about 5%, and the risk of thrombocytopenia with a platelet count below 20 000/mm^3 is only 1% [206].

Neonatal lupus erythematosus

The neonatal lupus erythematosus syndrome is due to the transfer of maternal antibodies to the infant. Burrows and Kelton reviewed the incidence of neonatal thrombocytopenia in their institution over a seven-year period [196, 211, 212]. The few mothers with lupus did not deliver thrombocytopenic infants. Most infants exposed to maternal anti-SSA/Ro antibodies do not develop neonatal lupus [213]. The skin manifestations often develop after birth [213–215]. The most common manifestation of this disorder is skin lesions and cardiac disease, including congenital heart block and cardiomyopathy [211, 214]. Hematological abnormalities are not uncommon. Weston and colleagues reported 18 infants with neonatal lupus seen by the authors. In three infants, the skin lesions were present at birth. Cholestatic hepatitis was seen in three infants, and four infants had

thrombocytopenia [214]. Thrombocytopenia can be the initial manifestation of neonatal lupus [216, 217]. Hariharan and colleagues also reported an infant with neonatal lupus and thrombocytopenia as part of a syndrome of microvascular hemolysis [218]. Mothers of infants with neonatal lupus may be asymptomatic. If symptomatic, the most common symptom in these mothers is the Sjögren syndrome [213]. Although neonatal lupus is a rare cause of newborn thrombocytopenia, it needs to be part of the differential diagnosis of neonatal thrombocytopenia. Infants with severe thrombocytopenia (platelets $< 50\,000\,\mu l$) born to mothers with ITP have been treated with IVI g and/or corticosteroids with good responses [219].

Maternal hypertension

Maternal pre-eclampsia and maternal hypertension can also induce thrombocytopenia in the newborn [220–225]. Brazy and colleagues [220] studied 28 premature infants of mothers with severe gestational hypertension. Fifty-seven percent of these mothers were thrombocytopenic and 36% of the infants were thrombocytopenic. Three infants had evidence of DIC and their mothers had platelet counts below $50\,000/mm^3$. There was a close correlation between maternal platelet count and newborn platelet count. The authors postulate that a common etiology causes the thrombocytopenia in both mother and child, particularly since in the most severe cases both infant and mother had evidence of DIC. Burrows and Andrew studied 831 newborns (632 full-term, 199 premature) of normotensive mothers and 520 newborns born to 607 mothers with hypertension [221]. Eighteen of 831 (2.2%) control infants were thrombocytopenic compared with 48 of 520 (9.2%) in the cohort of infants born to hypertensive mothers. The rate of newborn thrombocytopenia in full-term infants was not different in those born to normotensive mothers compared with those born to hypertensive mothers. Premature babies born to hypertensive mothers had a rate of thrombocytopenia of 20.5%, significantly different from the rate of 4% in infants of normotensive mothers. Sibai and colleagues studied 43 infants of 48 mothers with severe pre-eclampsia/eclampsia compared with 43 age-matched controls [222]. Eleven (26%) infants were thrombocytopenic, but only four had platelet counts below $100\,000/mm^3$. There were ten infants with ICH born to hypertensive mothers. Nine of these infants were thrombocytopenic. This did not differ from the control normotensive group, in which there were nine infants with ICH and eight with thrombocytopenia. Thrombocytopenia was highly associated with perinatal asphyxia. Koenig and Christensen found that 11 of 72 (15%) infants born to hypertensive mothers had thrombocytopenia [224]. In infants born to hypertensive mothers, the platelet count usually falls to $50\,000–100\,000/mm^3$, but bleeding manifestations are unusual. Platelet recovery usually occurs within five to seven days.

Maternal drugs

Drugs that cause thrombocytopenia in the adult may also cause thrombocytopenia in the newborn infant. Most of these drug-induced thrombocytopenias are due to immune-mediated platelet destruction [226, 227]. Thrombocytopenia is reported as an adverse drug reaction with increasing frequency [228]. A large number of drugs have been reported as causing thrombocytopenia. Among the noncytotoxic drugs reported, sulfonamides, cinchona alkaloids, diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), gold salts, penicillamine, anticonvulsants, and heparins are the most frequently associated with thrombocytopenia (Table 10.7). Some drugs, such as sulfonamides and trimethoprim–sulfamethoxazole (co-trimoxazole) have early onset of thrombocytopenia, while a long interval to development of thrombocytopenia is seen more commonly with gold salts, penicillamine, and valproic acid [229]. Maternal therapy with thiazides can cause thrombocytopenia in the infant without necessarily affecting the mother's platelet count [230, 231]; hydralazine also has been implicated in this form of thrombocytopenia [232]. However, the frequency of thrombocytopenia in infants of hypertensive mothers and the rarity of thiazide-induced

Table 10.7 Drugs associated frequently with drug-induced thrombocytopenia

NSAIDs
Propionic acids
Indoles
Gold salts
Penicillamine
Sulfonamides
Trimethoprim–sulfamethoxazole (co-trimoxazole)
Quinine
Quinidine
Thiazides
Valproic acid
Carbamazepine
Vaccines

NSAID, nonsteroidal anti-inflammatory.
Adapted with permission from Pedersen-Bjergaard *et al.* [229].

infant thrombocytopenia suggest that these drugs may not be involved directly [231, 233]. These observations emphasize further the need for studies to determine hematologic abnormalities in infants that may be associated with mothers taking various medications. Tolbutamide is another drug that has been implicated as a cause of newborn thrombocytopenia [234]. Anticonvulsants have been reported to cause thrombocytopenia in the newborn [235–237], as has glucagon infusion [238]. Of particular interest is the development of heparin-induced thrombocytopenia (HIT) in the newborn. HIT is a frequent complication of heparin therapy in adults, with an incidence reported to vary widely from 1% to 30%. It usually develops after a few days of therapy with heparin. Transient mild thrombocytopenia can occur in the first two days of therapy with heparin. This is not immune-mediated and patients recover in spite of continuation of heparin therapy. There are no thrombotic complications of this transient thrombocytopenia. Immune-mediated HIT is associated with a high risk of thromboembolic complications. It is due to an antibody to heparin–platelet factor 4 complex [239]. The thrombocytopenia resolves within 48 hours of stopping heparin. HIT is not common in children, but serious thrombotic complications have been reported in children and newborns [240–247]. Ranze and colleagues reviewed HIT in children and newborns [248]. Spadone and colleagues studied 1329 infants admitted to their nursery [249]; they suspected HIT in 34 infants. They were able to demonstrate antiplatelet antibodies consistent with HIT in 14 infants. Aortic thrombi were documented in 11 of 13 infants with antibodies. This study had an incidence of 1.5% of HIT in the newborn nursery. It is important to remember that even small amounts of heparin can induce the syndrome. Just the heparin in a central venous nutrition solution or the heparin needed to keep a butterfly needle patent can be enough to cause HIT. The therapy for this disorder is stopping heparin. The platelet count recovers within five to seven days [249]. If anticoagulation is needed, then low-molecular-weight heparin has been used, but this also can cause HIT and cross-react with the antibodies made against full-length heparin. An alternative treatment is the use of antithrombin agents [240, 242, 243].

Placental abnormalities

Neonatal thrombocytopenia has also been reported with placental abnormalities. The majority of the cases are associated with placental chorioangiomas, but cord aneurysms, placenta previae, and abruptio placenta have also been reported to cause neonatal thrombocytopenia [250–258].

Infant factors

Thrombocytopenia with platelet counts of less than 100 000/mm^3 occurs in up to 80% of sick infants [259]. Thrombocytopenia occurs in as many as 20% of all infants admitted to the neonatal intensive care unit [260, 261]. Nevertheless, in 60% of these infants, no cause for the thrombocytopenia is found. Many factors in the newborn have been found to cause thrombocytopenia. Castle and colleagues [262] were able to identify a mechanism for thrombocytopenia in 80% of thrombocytopenic infants (Fig. 10.6). They also found an incidence of thrombocytopenia of 22% among infants admitted to neonatal intensive care

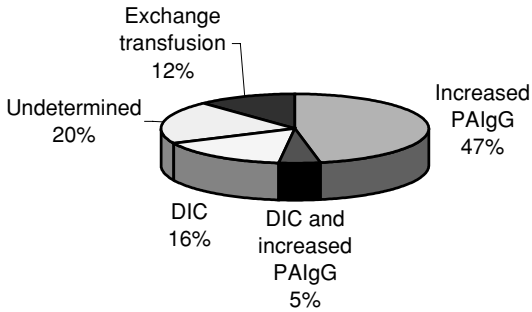

Fig. 10.6 Mechanism of neonatal thrombocytopenia. Adapted with permission from Castle *et al.* [262].
DIC, disseminated intravascular coagulation; PAI_gG, platelet-associated immunoglobulin G.

units. Asphyxia, as reflected by an Apgar score of less than 7, was present in 70% of the thrombocytopenic infants. Evidence of DIC was present in 21% of the thrombocytopenic infants. Exchange transfusion could have caused thrombocytopenia in 12% of these infants. There was an elevated platelet-associated immunoglobulin G (PAIgG) in 47% of the thrombocytopenic infants, and 5% had both elevated PAIgG and evidence of DIC [262]. Although 52% of the thrombocytopenic infants had increased PAIgG suggestive of immune destruction of platelets, only 9% of their mothers had elevated PAIgG and 5% had serum platelet-binding IgG. There is no clear explanation for the increased PAIgG seen in these babies, except that control nonthrombocytopenic babies did not have increased levels of PAIgG. Whether this increased PAIgG represents true immune-mediated thrombocytopenia is still unclear. Other investigators have found increased PAIgG in newborn infants [263]. Castle and colleagues studied 11 thrombocytopenic newborns with platelet-survival studies. Ten showed decreased survival. The one exception was a newborn whose platelet count had returned to a normal level when studied [264]. In six of these infants, bone-marrow megakaryocytes were examined; the number of megakaryocytes was normal in all. Hence, it appears that the thrombocytopenia in most newborn infants is due to increased destruction.

Infection (viral or bacterial), necrotizing enterocolitis (NEC), hyperbilirubinemia, exposure to phototherapy, occurrence of respiratory distress syndrome, the use of assisted ventilation, the presence of an indwelling umbilical line, being small for gestational age, occurrence of asphyxia, meconium aspiration, cold injury, polycythemia, and pulmonary hypertension have all been associated with thrombocytopenia [265, 266]. Bleeding complications occurred in 22% of the thrombocytopenic babies in one study [259]. The overall mortality among the thrombocytopenic babies was 34%, which is significantly higher than the 5% mortality among babies without thrombocytopenia. Even in this group of sick infants, maternal factors were important: 8% had antiplatelet antibodies either in the maternal serum or in the baby's serum, and for 4% of the sick infants the mothers gave a history of having ingested, before delivery, drugs known to cause thrombocytopenia. The incidence of intraventricular hemorrhage (IVH) is higher in thrombocytopenic infants and serious neurological consequences of the hemorrhage also are greater in thrombocytopenic infants than in nonthrombocytopenic infants [260]. Thrombocytopenia in the healthy newborn has different clinical implications and a much lower incidence than thrombocytopenia in the ill newborn. For instance, in two of the series discussed earlier in this section [183, 185], only 4.1% of healthy newborns had thrombocytopenia and only two (0.14%) newborns had a platelet count below 50 000/mm^3 [183]. The incidence of neonatal thrombocytopenia in infants of insulin-dependent diabetic pregnant women was 21.4% significantly different from the rate in normal pregnancies. Gestational diabetes did not increase the risk of neonatal thrombocytopenia. In the seven-year extension of their study, Burrows and Kelton analyzed 15 471 pregnancies, with cord-blood platelet counts available in 93.8% of the infants [185]. Nine infants had platelet counts below 50 000/mm^3. This gave an overall prevalence of neonatal thrombocytopenia of 0.12%. Table 10.8 shows the number of infants and the incidence of neonatal thrombocytopenia in the most commonly seen disorders in pregnancy and in normal pregnancies. Sainio and

Table 10.8 Incidence of neonatal thrombocytopenia

Mother's health status	No. of infants with thrombocytopenia	Incidence (%)
Normal	1	0.01
Hypertensive disorders of pregnancy	5	0.35
Immune disorders of pregnancy	4	2.8
Immune thrombocytic purpura	4	8.7
Systemic lupus erythematosus	0	0
Neonatal alloimmune thrombocytopenia	9	50
Other	0	0
Total	*19*	*0.12*

Adapted with permission from Burrows and Kelton [196].

colleagues [267] found an incidence of thrombocytopenia (platelet count below 150 000/mm^3) of 2% in 4489 full-term infants and of severe thrombocytopenia (platelet count below 50 000/mm^3) of 0.24%. All cases of severe neonatal thrombocytopenia were due to NAIT. Several other investigators have confirmed the prevalence of neonatal thrombocytopenia (platelet count below 150 000/mm^3) of approximately 0.5% and of severe thrombocytopenia (platelet count below 50 000/mm^3) of 0.1–0.2% [267–270].

Immune-mediated thrombocytopenia

Immune-mediated neonatal thrombocytopenia can be either an active process or a passive acquisition of antibodies against platelets. The principal disorder that involves an active immune response is NAIT. Passive antibodies can be a result of maternal ITP, maternal systemic lupus erythematosus, or hemolytic disease of the newborn, or secondary to transfusions. Drug-related immune thrombocytopenia and infection-related immune thrombocytopenia can be both passive and active processes.

Neonatal alloimmune thrombocytopenia

NAIT is a rare disorder, but it is the most important thrombocytopenia seen in full-term healthy newborn infants. Harrington and colleagues first described the disorder in 1953 as an unusual variety of idiopathic thrombocytopenia of the newborn [271]. It is the platelet-equivalent of the hemolytic disease of the newborn. Mothers exposed to a platelet-surface antigen that they do not possess develop an IgG antibody against the antigen. The antibody crosses the placenta and causes thrombocytopenia in the infant [272]. This is a disorder of increased platelet destruction and the infant has normal levels of thrombopoietin [273]. There may also be a component of marrow suppression. Sera from HPA-1b mothers suppressed in vitro growth of CFU-Mk [274]. Infants with immune amegakaryocytic thrombocytopenia have been reported. In these three cases, the thrombocytopenia was due to maternal anti-HLA-A2 antibodies, which also suppressed megakaryocytes [275]. Another infant had anti HPA-2a antibodies [276].

Clinical presentation

The typical presentation of the first affected infant of a pregnant mother with NAIT is that of a healthy baby who develops diffuse petechiae, bruising, hemorrhage, and jaundice. Pearson and colleagues [272] in 1964 described nine infants from six families that illustrate the natural history of this disorder. They documented the presence of antibodies in the children and in the mothers directed against antigens present on the platelets of the fathers and the infants but not the mothers. Some of the infants were the firstborns in their families, a feature of NAIT that is distinctly different from the picture seen in Rh hemolytic disease of the newborn. Some of the infants were normal at birth but rapidly developed petechiae, bruising, and hemorrhage. Jaundice within the first 48 hours of birth was a common finding. ICH was documented in two of the nine cases, and a third infant had hydrocephalus probably due to ICH. In the review of the literature of that time, Pearson and colleagues documented a fatality rate

of 12%. The thrombocytopenia tended to get worse in the first two days of life and then began to recover. Platelets became normal by the second to third week of life. Five infants were treated with steroids with more rapid resolution of the thrombocytopenia. Three mothers received steroids before delivery in their second affected pregnancies. The investigators felt that this therapy may have improved the course of the illness of the second infant. The second infant is often affected more severely, and intrauterine ICH is not infrequent.

The diagnosis, history of the previous pregnancy, and genetic understanding of the disease guide the approach to the infant at risk. The understanding that antibodies are formed early in gestation and that the risk begins in utero has prompted some investigators to change the name of this disorder to feto-maternal alloimmune thrombocytopenia (FMAIT) [277]. Deaver and colleagues [278] reviewed 58 cases and found that 81% of the patients had petechiae or ecchymoses, jaundice and anemia were present in 16%, and ICH occurred in 28%, with 9% occurring in utero. Mueller-Eckhardt and colleagues [279] reviewed 88 patients prospectively and found a similar incidence of symptoms, except that 10% were asymptomatic and thrombocytopenia was discovered incidentally (Table 10.9).

Table 10.9 Clinical spectrum of 58 cases from a literature review by Deaver *et al.* [278] and in 88 children with neonatal alloimmune thrombocytopenia (NAIT) reported by Mueller-Eckhardt *et al.* [279]

Finding	No. (%) (Deaver *et al.*)	No. (%) (Mueller-Eckhardt *et al.*)
None		9 (10)
Petechiae/ecchymoses	47 (81)	79 (90)
Jaundice	16 (28)	29 (39)
Anemia	14 (24)	15 (19)
Gastrointestinal bleeding	11 (19)	26 (30)
Melena		24
Hematemesis		2
Hemoptysis		7 (8)
Soft-tissue hematoma	6 (10)	58 (66)
Bleeding circumcision	4 (7)	
Hematuria	4 (7)	3 (3)
Conjunctival/retinal hematoma	3 (5)	6 (7)
Vaginal bleeding	1 (2)	
Suspected intracranial hemorrhage		
Total	16 (28)	12 (14)
In utero	5 (9)	5 (6)
Death	5 (9)	1 (1)

Adapted with permission from Deaver *et al.* [278] and Mueller-Eckhardt *et al.* [279].

Antigens associated with neonatal alloimmune thrombocytopenia

The presence of unique platelet antigens was suspected in the early 1950s, as documented in a description of NAIT in 1953 [271]. In 1959, van Loghem and colleagues [280], and in 1961, Shulman and colleagues [281], described the first platelet antigen. This antigen is the most common cause of NAIT in the Caucasian population. It was initially named Zw^a or PlA1. The current terminology for this antigen is HPA-1a. The initial nomenclature for these antigens came from the name of the patient. The major impetus for antigen definition was NAIT. Independently described cases led to a multitude of names for one antigen and multiple and varied names for antigens. In 1990, dem Borne and Decary [282] proposed a simplified system. Each antigen, defined by antibody specificity, was given a human platelet antigen (HPA) number. The Zw^a/PlA1 antigen received the designation of HPA-1, with its two serological forms designated as HPA-1a for the common form and HPA-1b for the less common form, the latter corresponding to PLA2 or Zw^b. This nomenclature has simplified the system vastly. However, with the definition of these platelet antigens at the molecular level, the nomenclature does not reflect accurately the scientific knowledge of the antigens, and a molecular designation of the antigens has been proposed [283]. In spite of its scientific deficiencies, for clinicians the current classification works well and helps us to understand the antigens involved in sensitization. There are two types of clinically relevant platelet alloantigens. The first type, or type I,

includes antigens that the platelet shares with other cells. This includes the blood group ABH antigens and the HLA class I antigens. The second group is the platelet-specific antigens defined by the HPA classification. Thirteen HPA antigens have been defined [284], some of them representing private antigens defined by single cases of NAIT and some that have yet to be assigned numbers. For the purposes of NAIT, the first five are the most commonly encountered antigens. We now know that these antigens are genetic polymorphisms in the major PGPs. The majority of these antigens reside in the PGP IIIa, but there are polymorphisms in PGP IIb, PGP Ia, PGP Ibα, and PGP Ibβ (Table 10.10) [285]. The frequency and immunogenicity of these antigens determine the incidence of NAIT. The frequency of each antigen varies within ethnic groups [286, 287]. The majority of cases of severe NAIT in Caucasians are due to anti-HPA-1 antibodies, while in Japan the most frequently involved antigen is HPA-4 [286]. About 2.5% of the Caucasian population is homozygous for the HPA-1b antigen. Mueller-Eckhardt and coworkers [279] examined 348 women with normal platelet counts and who gave birth to thrombocytopenic infants. Of 36 infants with specific antiplatelet antibodies, 78% had antibodies against HPA-1a, 19% had antibodies against HPA-5b, and 3% had antibodies to other antigens.

Table 10.10 Specific platelet alloantigens implicated in neonatal alloimmune thrombocytopenia (NAIT)

System	Antigen	Glycoprotein	Amino-acid substitution
HPA-1 (=Zw=PlA)	HPA-1a	IIIa	Leu33
	HPA-1b		Pro33
HPA-2 (=Ko)	HPA-2a	Iba	Thr145
	HPA-2b		Met145
HPA-3 (=Bak=Lek)	HLA-3a	IIb	Ile843
	HPA-3b		Ser843
HPA-4 (=Yuk-Pen)	HPA-4a	IIIa	Arg143
	HPA-4b		Gln143
HPA-5 (=Br=Hc=Zav)	HPA-5a	Ia	Glu505
	HPA-5b		Lys505
HPA-6w (=Ca=Tu)		IIIa	Gln489
	HPA-6wb*		Atg489
HPA-7w (=Mo)		IIIa	Ala407
	HPA-7wb*		Pro407
HPA-8w (=Sr)		IIIa	Cys636
	HPA-8wb*		Arg636
HPA-9w (=Max)		IIb	Met837
	HPA-9wb*		Val837
HPA-10w (=La)		IIIa	Gln62
	HPA-10wb*		Arg62
HPA-11w (=Gro)		IIIa	His633
	HPA-11wb*		Arg633
HPA-12w (=Iy)		Ibb	Glu15
	HPA-12wb*		Gly15
HPA-13w (=Sit)		Ia	Met799
	HPA-13wb*		Thr799

*Rare or private antigens.

Reproduced with permission from Kaplan [285].

Incidence

The initial estimates of the incidence of NAIT were of 1 infant per 5000–10 000 births [272]. More recent estimates from prospective evaluation of pregnant mothers are of an incidence of 1 infant per 1000–2000 pregnancies [183, 185, 279, 288–291], but it can be as high as 1 in 800 pregnancies [268]. A high proportion of babies with NAIT are born to primigravidas. Forty-two percent of infants with NAIT in Mueller-Eckhardt and coworkers' series were born to first-time mothers [279]. Not all mothers with a set-up for incompatibility and who deliver incompatible babies develop antibodies. Durand-Zaleski and coworkers [327] prospectively studied 2066 primiparas and 6081 newborn infants. Fifty-two of these women were HPA-1b homozygous, but two fathers were also HPA-1b homozygous. The authors were able to enroll in the study protocol 45 of these women; four developed antibodies. Two of these women had thrombocytopenic infants. Hence, for the entire cohort of mothers, the prevalence of sensitization was 1 in 500 but the prevalence of NAIT was 1 in 1000. Of the 5632 infants tested for platelet counts, 48 (0.9%) were thrombocytopenic and 5 were HPA-1a born to HPA-1b mothers, for an incidence of NAIT of 0.09%. In this study, 10% of mothers at risk of sensitization became sensitized and 50% of those sensitized had an infant with NAIT. It is not

clear yet what makes the mother more likely to be alloimmunized or what makes the infant more likely to be thrombocytopenic. There are both maternal and infant factors that need to be considered, but HLA class I and class II genotypes may have been implicated as risk factors for sensitization [289, 292–296]. Incompatibility set-up for HPA-5b has a higher prevalence than for HPA-1a. However, the frequency of NAIT is lower. HPA-5b is, however, the second most common antigen to cause NAIT in the Caucasian population [268, 291, 297, 298]. HPA-5b also may cause a less severe disease [298]. Significant NAIT can also occur in the presence of HPA-3a incompatibility [299, 300]. Antibodies to HLA antigens are found frequently in pregnant women. Whether anti-HLA antibodies can cause NAIT is controversial [301, 302], but clear cases of NAIT in the presence of anti-HLA antibodies are well documented in the literature [301, 303, 304]. Several private or rare antigens can also cause NAIT, including anti HPA-1b and anti HPA-5a incompatibility [305–316].

Immune response

As noted above, only 5–10% of HPA-1b homozygous mothers become sensitized to their HPA-1a positive infants [292, 294]. Reznikoff-Etievant and coworkers described the association of maternal sensitization with the presence of the HLA-A1 and HLA-B8 MHC class I antigens [317] and an even stronger association with the presence of the MHC class II antigen DR3 [318]. Other investigators confirmed these observations [292]. De Waal and coworkers found an even stronger association with the superantigen DRw52. This antigen includes DR3, DR5, DRw6, and Drw8. In the group of mothers with NAIT, only DR3 and DRw6 were present, but all mothers sensitized to infant platelets were DRw52-positive [319]. Valentin and coworkers reported that all immunized mothers were DRw52-positive and that the majority of these women were DRw52a-positive [295]. Using molecular technology to define the DR antigen, 91% of the responder mothers are DRB3*0101 and 94% are DQB1*0201. However, not all responders are positive for these antigens, and not all HPA-1b homozygous mothers with an HPA-1a-positive infant who also have these DR antigens become sensitized [320]. The mother who is negative for these antigens is rarely sensitized. The negative predictive value is greater than 99% [321], but the positive predictive value of the DRB3*0101 antigen is only 35%. Sensitization to HPA-5b does not seem to occur in the first pregnancy as often as that for HPA-1a [298]. Mothers who develop antibodies are more likely to be DRw6-positive [322]. Semana and coworkers [323] have further dissected the association of HLA type and alloimmunization in HPA-5b. They found a negative association with DRB1*0301. When they analyzed the DR6 generic group, they found an increased frequency but not significant of the antigen in sensitized mothers. When they analyzed the alleles that were more frequent in the sensitized mothers, they found increased presence of an amino-acid sequence in the β chain GAA-GAC nucleotide sequence. Mawas and coworkers found that sensitized mothers with anti-HPA-1a antibodies who produced IgG3 subclass antibodies were more likely to have infants with severe thrombocytopenia [324]. The role of T-cells has not been studied well, but Maslanka and coworkers demonstrated a restricted T-cell response [325].

Maternal anti-HPA-1a antibodies are not detected in all cases of HPA-1a NAIT [291, 326]. A better understanding of the immune response may help us to further characterize and predict which mothers will react to which antigens. Ability to predict maternal response would make screening for this disorder a reality. Several investigators have analyzed the possibility of screening for NAIT. Durand-Zaleski and coworkers selected for screening mothers homozygous for HPA-1b and typed the fathers to define the infants at risk. They screened 2066 mothers. They also screened newborns by obtaining a platelet count at birth. They found that screening newborns is more cost-effective than screening mothers, but it misses the in utero risk for the infant without a previous sibling with NAIT [327]. Doughty and coworkers screened 3473 women for their HPA-1 status as well as HLA-DR52a type. The investigators felt that screening would be a worthwhile process [328].

Williamson has reviewed the data for screening and concluded that there are still issues to resolve before screening can be implemented [321].

Diagnosis

One in ten thrombocytopenic infants will have NAIT [291]. In particular, in an otherwise healthy full-term infant who develops bruising and petechiae, NAIT should be high on the differential diagnosis. If NAIT is suspected, blood should be obtained from both the mother and the father, if available. The mother's serum should be tested for the presence of specific antiplatelet antibodies. This testing should be done in an experienced laboratory. Antiplatelet antibodies can be tested by an enzyme-linked immunosorbent assay (ELISA)-based, antigen-capture assay that tests maternal serum against immobilized platelet glycoprotein targets [329, 330]. If maternal antibodies are negative but the clinical picture is consistent, then infant serum and platelets may be tested. It is important to remember that maternal serum is not positive for antibodies in all cases of NAIT [193, 291, 326, 331]. Determining the maternal and paternal genotype also is important in the diagnostic work-up. Most laboratories that deal with NAIT have now moved to DNA-based testing to determine the maternal and paternal genotypes. This testing is important to predict the risk of the next infant, since 75–90% of second at-risk infants will be affected [193, 332, 333].

Therapy

Severe thrombocytopenia is a clear indication for platelet transfusions in the newborn [261, 334]. Random donor platelet transfusions will not give an adequate response. A poor response to random donor platelets in this clinical setting is also solid evidence for the diagnosis of NAIT [335, 336]. Traditionally, the therapy of choice in the infant with a platelet count below 30 000/mm^3 or with clinical bleeding is the transfusion of maternal platelets. Random donor platelets will raise the platelet count by 7000–30 000/mm^3 for 24–48 hours [335, 337–339] while one unit of washed (to decrease the amount of antibody transferred with the plasma in the platelet transfusion) maternal platelets will increase the platelet count to the normal range and will last for at least five to ten days [335, 336, 339, 340]. In most infants, one platelet transfusion is all that is needed, since recovery should occur within two weeks. If maternal platelets are not available, then HPA-1a-negative platelets are as effective in infants in whom there is an anti-HPA-1a antibody. Where the technology to provide HPA-1b, HPA-5a homozygous platelets is available, then this is a faster and more efficient therapy [341, 342]. Obtaining maternal platelets can be difficult and, if the infant has been transferred to a tertiary care center away from the mother, nearly impossible. This product containing platelets positive for both HPA-1b and HPA-5a should be able to treat more than 95% of affected infants in a Caucasian population [341]. The product could be fresh from an available pool of donors or from frozen product [343]. In the case of Japanese or Korean ethnicity, the likely antigen is HPA-4b (Yuka) and the blood services should provide the appropriate product. Intravenous immunoglobulin (IVIG) can be of use in the therapy of NAIT, as first reported by Sidiropoulos [344]. The platelet count rises within 36–48 hours from the start of IVIG [279, 345, 346]. Steroids have also been used in the therapy of the newborn with NAIT [272, 279, 335]. Neither of these therapies alone (steroids or IVIG) is appropriate in the infant with NAIT. When maternal or type-specific platelets are not available, then a combination of random donor platelets with IVIG (1 g/kg/dose once or twice, or 400–500 mg/kg/day for five days) may be indicated while awaiting the availability of appropriate platelet transfusions [193, 279].

The second-born infant of a mother of a NAIT-affected infant has a 75–90% chance of being also affected [193, 332]. ICH complicates the course of NAIT in as many as 20% of infants, half of which may occur prenatally [279]. The hemorrhage may occur in the first trimester of pregnancy [347]. The mortality of NAIT is due mostly to ICH [272, 279]. A head sonogram should be done to document the presence or absence of ICH. If maternal or antigen-negative platelets are not available, then the affected infant whose platelet count falls below 20 000–30 000/mm^3 should receive IVIG, 1 g/kg/dose given for one or two

days together with random donor platelets. Maternal or antigen-compatible platelets should be administered as soon as they are available.

Because ICH occurs in utero in 50% of cases, the therapy for these infants needs to be prenatal. To design a logical antenatal therapeutic approach, it is important to have (i) an accurate diagnosis of the infant at risk, (ii) a definition of the risk to the infant, and (iii) an effective therapy.

Accurate diagnosis of the infant at risk To define the diagnosis accurately, of NAIT, it is important to know the platelet genotype of the mother and the father. In the case of an HPA-1b homozygous mother, determining the genotype of the father defines the infant's risk. A father who is homozygous for HPA-1a defines a risk of 100% for the infant, while a heterozygous father implies a 50% risk for the infant. Most laboratories familiar with the assessment of families at risk for NAIT use molecular testing instead of serologic testing to define the parents' genotypes. The next test in the diagnosis is ascertaining the genotype of the fetus. Using molecular technology, there is no need for fetal blood sampling, and amniocentesis or chorionic villi biopsy at 13–18 weeks of gestation can provide the DNA needed to make the diagnosis of an affected infant. The only way to find out whether the infant is thrombocytopenic is to perform fetal blood sampling. This technique carries a risk of premature labor and hemorrhage in the normal population and an even higher risk in a thrombocytopenic infant, even when performed at centers with great expertise. It is thus controversial as to whether a blood sample is required. If done, HPA-1b/1b platelets must be available to treat the thrombocytopenic fetus.

Assessment of risk to the infant Contrary to Rh disease, the titer of antiplatelet antibody has not been predictive of the presence or the severity of disease. The antibody titer tends to decrease through gestation. In general, the second infant will have equal or more severe disease than the first affected infant, and the severity of the disease in the first infant can predict the risk for the second infant. Infants whose previously affected sibling had ICH have a higher risk for developing severe NAIT. In particular, those infants whose sibling had an intrauterine ICH have an even higher risk for severe disease. So far, no other predictive parameter exists to help in the therapeutic decision process.

Prenatal therapy In 1988, Kaplan and colleagues reported on the treatment of nine women and their infants, with assessment of the infants' platelet count by fetal blood sampling. Six infants were treated successfully with transfusion of maternal platelets in utero. All infants did well, and only one required postnatal platelet transfusions [331]. One mother received five days of IVIG, 400 mg/kg/day, with no apparent effect on the infant's platelet count. In 1988, Bussel and coworkers reported that giving weekly IVIG to the mother prevented ICH and improved platelet counts of fetuses and infants affected by NAIT [326]. Several case reports have administered IVIG to the fetus in utero, with mixed results [348, 349]. Controversy still exists regarding the use of fetal blood sampling, the use of IVIG administered weekly to the mother, and the use of weekly intrauterine fetal platelet transfusions. Bussel and coworkers have reported three series of patients using IVIG administered to the mother weekly, with and without additional corticosteroids [350–352]. The initial report included 18 mothers treated with weekly IVIG and dexamethasone. The steroid had to be stopped after five subjects because of oligohydramnios and growth retardation in the fetus. None of the infants suffered ICH [350]. A second report included 51 mothers and 55 infants. Beginning at the twentieth to twenty-fourth week of gestation, mothers were randomized to receive weekly IVIG, with or without a lower dose of dexamethasone. No infant had ICH, and dexamethasone did not make a difference. Twelve of 47 infants tested after the start of therapy failed initial therapy, and nine mothers were then treated with prednisone 60 mg per day. Five of these nine responded to the rescue therapy [351]. In the most recent series of 107 pregnancies, the investigators found that in utero ICH was the only predictive

parameter for the next sibling [352]. Although 47% of the infants had lower platelet counts than their previously affected siblings, the severity of thrombocytopenia was not predictive of severe disease in the second pregnancy. Only one infant of the first pregnancy had an ICH, while 20 of the 98 siblings had had an ICH. Infants with HPA-1 incompatibility had more severe disease than those with other HPA incompatibilities. The majority of but not all infants respond with an increased platelet count when their mothers are treated with weekly IVIG, with or without steroids. Other investigators have reported failure of weekly IVIG [353, 354], including an infant with in utero ICH while the mother was on weekly IVIG [355]. These treatments appear to decrease the incidence of ICH as compared with the historical incidence of ICH in siblings. Murphy and coworkers [359] have reported the use of weekly intrauterine fetal platelet transfusions with great success. Intrauterine platelet transfusions for NAIT have been used as a single transfusion [331, 356, 357] and as series of multiple transfusions [358–362]. In 1988, Kaplan and coworkers reported their experience with seven infants with NAIT. Diagnostic cordocentesis was done at 20–24 weeks' gestation when possible. However, three patients were referred late in pregnancy, so that first testing was done at 32 or 38 weeks. Six fetuses had intrauterine platelet transfusions and all had a rise in the platelet count. One infant had repeated cordocentesis and three transfusions, documenting that these transfusions last only a short time [331]. Overton and coworkers reported their experience with weekly platelet transfusions [358]. Twelve pregnancies in eight women were treated. First sampling began at 22–24 weeks of gestation, with a planned elective Cesarian delivery at 32 weeks. All fetal counts increased after transfusion, but the rate of fall of the platelet count was 40 000/mm^3 per day. One infant died from exanguination and another died with evidence of placental insufficiency but not of hemorrhage. The authors also combine the reported experience of weekly transfusions in the care of NAIT [360–362] of a total of 223 transfusions in 55 pregnancies, with a procedure-related fetal loss of three. This gives a risk of fetal loss of 1.3% per transfusion and 5.5% per pregnancy. However, Sainio and coworkers had no fetal losses in 13 pregnancies but had 3 near-misses with fetal hypotension and bradycardia [362]. In general, the treatment of NAIT in North America has been with weekly maternal IVIG, with or without steroids, and platelet transfusions at the time of fetal blood sampling. In Europe, the approach has been to use weekly platelet transfusions as the main form of therapy. Spencer and Burr [363] performed a Medline search of 376 articles covering the years 1966–98. Treatment of HPA-1a-incompatible pregnancies with weekly maternal IVIG increased the likelihood of a neurologically normal outcome (relative risk (RR) 1.68, range 1.3–2.2). Treatment with only platelet transfusions had an RR of increasing the likelihood of a neurologically normal outcome of 1.63 (range 1.1–1.21). However, cordocentesis carries a risk of fetal loss. Platelet transfusion also may cause problems with thrombosis in addition to bleeding. A meta-analysis of the literature determined that the best results were achieved by the use of IVIG, but intrauterine transfusions had almost as good a risk ratio as weekly maternal IVIG. The recommendations and guidelines for antenatal therapy reflect the uncertainty regarding the best approach to the prenatal care of an infant at risk of NAIT [193, 364].

Based on the risk of severity of disease derived from the incidence of ICH in the previous sibling, Bussel and Kaplan have recommended tailored therapy according to a set of risk categories (Table 10.11) [333]. This schema classifies the patient's risk depending on the previous sibling's history. If the previous infant had an ICH before 28 weeks of gestation, then the second infant is assigned to extremely high risk. If the sibling had an ICH at 28–36 weeks, then the second infant is assigned to very high risk. If the sibling had a perinatal ICH or an initial platelet count below 20 000/mm^3, then the second infant is assigned to high risk. If the sibling had an initial platelet count above 20 000/mm^3 and no ICH, then the second infant is assigned to standard risk. Treatment can then be given using this schema, with more

Table 10.11 Risk classification for prenatal therapy of alloimmune thrombocytopenia

Risk category	Characteristics
Extremely high	Previous sibling had an antenatal ICH that occurred before 28 weeks of gestation
Very high	Previous sibling had an antenatal ICH that occurred at 28–36 weeks of gestation
High	Previous sibling had a perinatal ICH or an initial platelet count below 20 000/mm^3
Standard	Previous sibling had an initial platelet count above 20 000/mm^3 and no ICH

Adapted with permission from Bussel and Kaplan [333].

aggressive therapy for the highest-risk group and lesser therapy for the standard-risk group. The prenatal therapy for the extremely high-risk pregnancy, suggested by Bussel and Kaplan is a combination of weekly maternal IVIG at 1 g/kg/dose and oral prednisone at 1 mg/kg/day beginning at 13 weeks of gestation. Umbilical-cord sampling should be done at the earliest possible time, i.e. 20 weeks' gestation. Compatible platelets must be available to give to fetuses that do not have a safe platelet count (above 35 000–50 000/mm^3). In fetuses that do not respond to medical therapy, weekly compatible platelet transfusions should be used. If there is a response to therapy with an increase in the fetal platelet count, then umbilical-cord-blood sampling should be done four to six weeks later. An elective delivery at 32 weeks should be planned for infants cared for by platelet transfusions. Responders may be delivered at 36–38 weeks' gestation. Prenatal treatment for very high-risk infants could start with administration of weekly IVIG to the mother beginning at 13 weeks' gestation, but steroid therapy should be reserved for non-responders when fetal cord-blood sampling is carried out at 20 weeks' gestation. High-risk infants and standard-risk infants could be treated with weekly maternal IVIG. Fetal blood samples in the high-risk group should be obtained at 20–24 weeks' gestation to assess response. It is not clear whether early sampling is needed for standard-risk infants or whether sampling should be done only before elective delivery. Elective delivery could be vaginal if the platelet count of the fetus is above 50 000/mm^3, otherwise Cesarian delivery is advised. Because of the aggressive nature and the risks to the fetus of fetal blood sampling and intrauterine transfusion [365, 366], several investigators have proposed similar combined approaches to prenatal therapy of NAIT [277, 361, 362, 367].

Other complications

The most serious complication of NAIT is ICH. The incidence of ICH in NAIT has been reported to be between 10% and 30%. Half of these occur in utero. Unfortunately, hemorrhage can occur very early in pregnancy [279]. The prognosis of infants that suffer an ICH is thought to be very poor [368, 369]. More recent experience suggests that infants that have been aggressively treated prenatally may not have as bad a prognosis. Bussel and colleagues reported seven infants with ICH and good outcome [370]. Sharif and Kuban reported their experience with eight infants treated with aggressive prenatal therapy. All did well [371]. Other serious complications of NAIT are iatrogenic. Complications are both from blood sampling and from therapy itself. A case of autoimmune neutropenia secondary to fetal therapy with IVIG has been reported [372]. Hypoglycemia and hyperinsulinism can occur in NAIT, as happens in Rh alloimmunization [373]. An infant with alloimmune thrombocytopenia and immunodeficiency has been reported. A sibling also had NAIT and lymphopenia but recovered [374].

Hemolytic disease of the newborn

Immune thrombocytopenia may occur in association with erythroblastosis fetalis [375–378]. In this condition, the decrease in platelet count may be due to the associated hyperbilirubinemia, to phototherapy, to DIC, or to the isoimmune reaction per se. This form of thrombocytopenia, which occurs in the more severely involved infants [375, 378, 379], improves following exchange transfusion [380]. Some of these infants may have hemorrhagic manifestations and DIC [375]. Thrombocytopenia often occurs following

exchange transfusion [381, 382]. Platelet counts may drop to 50 000–100 000/mm^3, but bleeding manifestations are uncommon. The onset of thrombocytopenia may be delayed by 24 hours [383]. Platelet counts recover within two to three days. However, the use of blood products containing platelets with decreased capacity for survival, such as old or frozen blood [382], or performance of repeated exchange transfusions may lead to more severe post-exchange transfusion thrombocytopenia [384].

Other causes of neonatal thrombocytopenia

Ill premature newborns with thrombocytopenia frequently have elevated platelet-associated immunoglobulin [262]. This may represent either an immune mechanism for the thrombocytopenia or just passive acquisition of immunoglobulins by young platelets, as the platelets of healthy neonates also have increased levels of immunoglobulins [385]. In one series, an elevated PAIgG was found in 47% of the thrombocytopenic infants, and 5% had both elevated PAIgG and evidence for DIC (Fig. 10.2) [262]. Although 52% of the thrombocytopenic infants had increased PAIgG, suggestive of immune destruction of platelets, only 9% of their mothers had elevated PAIgG, and 5% of the mothers had serum platelet-bindable IgG. There is no clear explanation for the increased PAIgG seen in these babies, except that control nonthrombocytopenic babies did not have increased levels of PAIgG. Whether this increased PAIgG represents true immune-mediated thrombocytopenia is still unclear. It appears that in most newborns with thrombocytopenia, the thrombocytopenia is due to increased platelet destruction [386].

Infection

Septic infants frequently are thrombocytopenic; 52–77% of these infants have decreased platelet counts [383, 387–390]. Thrombocytopenia may be the earliest hematologic manifestation of infection, and it may be a useful parameter to follow [383, 387]. Most likely, increased platelet destruction is the major cause of thrombocytopenia. Thrombocytopenia develops quickly after the onset of symptoms of infection [383, 387, 390]. Platelets transfused to infected infants have a shortened survival [390]. Many infants have increased PAIgG [386, 389], and the mean platelet volume (MPV) measured with an automated counter is increased [386]. Although thrombocytopenia is severe during acute infectious diseases, serious bleeding complications are not common. If the infection resolves, then thrombocytopenia resolves promptly. The mean duration of thrombocytopenia is five to six days (range one to ten days) [383, 387, 388, 390]. Septic bacteremic infants have lower platelet counts than infants with suspected infection but with negative blood cultures [391]. There also is some evidence that infection-related thrombocytopenia may have a component of marrow suppression as the mechanism of thrombocytopenia. Murray and Roberts reported marked reduction of circulating megakaryocyte progenitors (BFU-Mk and CFU-Mk) in thrombocytopenic versus nonthrombocytopenic infants [392]. Two of these infants had bacteremia. Sola and coworkers have described higher levels of thrombopoietin (TPO) in newborn cord blood than in adult peripheral blood [393]. In thrombocytopenic septic infants, they found an elevated TPO level with a broad range (<41–1112 pg/ml). In infants with decreased bone-marrow megakaryocytes, they found very high levels of TPO, but not as high as the levels found in adults with similar decreases in bone-marrow megakaryocytes [394]. Other investigators have also found elevated levels of TPO in the newborn [391, 395, 396], with a wide range in septic newborns [391, 395]. As suggested by Sola and colleagues, the very high levels found in some of these infants suggest that there is a component of marrow failure in the thrombocytopenia of infection [394].

Both Gram-negative and Gram-positive bacteria can cause thrombocytopenia in the newborn. Organisms that have been associated with thrombocytopenia include beta-hemolytic *Streptococcus*, group B, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Citrobacter diversus*, *Escherichia coli*,

Pseudomonas aeruginosa, *Haemophilus influenzae*, *Flavobacterium meningosepticum*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, and anaerobes such as *Viellonella* [383, 397, 398]. This long list suggests that any organism capable of causing severe disease in the newborn may also cause thrombocytopenia.

Viral and parasitic infections also can cause thrombocytopenia, particularly the organisms associated with the "toxoplasma, rubella, cytomegalovirus, herpes (TORCH) syndrome" group of infections [399–406]. The cause of this form of thrombocytopenia is unclear, but a mixture of platelet destruction and suppression probably contributes to the decreased platelet count. Chesney and coworkers [407] described intranuclear inclusions in megakaryocytes in congenital cytomegalovirus (CMV) infection. In the mouse model, these inclusions are viral particles causing dysmorphic megakaryocytes [408]. Herpes virus can cause suppression of CFU-Mk in vitro [409]. Parvovirus B19 usually causes hydrops fetalis, but it also can cause thrombocytopenia. Forestier and coworkers found thrombocytopenia in 11 of 13 fetuses with parvovirus B19 infection [410]. Postnatal infection is also associated with thrombocytopenia [411]. A combination of anemia and thrombocytopenia based on marrow suppression from parvovirus B19 has been reported in transient erythroblastopenia of childhood [412, 413]. In vitro parvovirus B19 suppresses megakaryocyte colony formation in both humans and the mouse animal model [414, 415]. Neonatal enterovirus infection can cause thrombocytopenia, especially in the presence of hepatitis and DIC [416–418]. In a series of 57 infants with enterovirus infection, five had severe thrombocytopenia [418]. Coxsackie virus [419, 420], adenovirus [421], Epstein–Barr virus [422], and dengue virus [423] can cause neonatal thrombocytopenia. Although HIV is associated rarely with thrombocytopenia in infants and children [424], it can present with thrombocytopenia in the newborn. In a study from South Africa, neonatal thrombocytopenia was associated with HIV so often that the authors suggested that a platelet count be used in the newborn as a potential screen for HIV in an area of high prevalence [425]. Like CMV and parvovirus, HIV can directly infect megakaryocytes [426] and cause suppression of in vitro colony growth.

The very-low-weight premature infant is at risk of fungal infection. *Candida* species are the most frequently isolated fungus in these infants [427–429]. Thrombocytopenia is a frequent manifestation of this disorder, with an incidence of 79–100% of these infants being thrombocytopenic [428–431]. *Candida glabrata* sepsis may be associated with less severe thrombocytopenia [432]. The magnitude of this problem in the neonatal intensive care unit is so great that prophylaxis with fluconazole has been suggested [433, 434]; two prophylactic trials have documented some success. One trial documented decreased colonization [435] and the other a decrease both in colonization and in invasive disease [436]. Malassezia fungemia also can cause thrombocytopenia [437]. As more fungi and yeast species are isolated in this neonatal population, it is more likely that more species will be associated with thrombocytopenia.

Necrotizing enterocolitis

NEC is frequently associated with thrombocytopenia [438, 439]. As many as 90% of the infants with NEC studied by Hutter and colleagues [439] had platelet counts below 150 000/mm^3, and 55% had platelet counts below 50 000/mm^3. Of the latter, 55% had bleeding complications, one-third of these being serious enough to be considered contributory to the infant's death. Six of 14 infants studied had evidence of DIC. The mean duration of thrombocytopenia was seven days (range 1–31 days). Increased platelet destruction is the most probable mechanism for the associated thrombocytopenia in NEC. Kosloske and colleagues did not find severe thrombocytopenia to be an indicator for surgery in NEC [440]. Ververidis and coworkers reviewed 58 cases of NEC, Bell's stage II or III, treated in their institution between 1995 and 1998. They found that thrombocytopenia and/or a rapid fall in the platelet count was a poor prognostic factor. The nadir platelet

count was lower in stage III patients than in stage II patients. The greater the extent of the disease, the lower the platelet count was. Patients who died had lower platelet counts than those who survived. None of the patients with a platelet count above 100 000/mm^3 died. Severe thrombocytopenia had a positive predictive value of 89% in predicting intestinal gangrene. A rapid fall in platelet-count had a positive predictive value of 92%, although severe thrombocytopenia was more sensitive [441]. The shortened survival of platelet transfusions in these infants suggests a platelet-consumption process. However, the cytokine levels in these patients give a mixed picture, suggesting a combined process with suppression as well as decreased survival [442, 443]. Proinflammatory cytokines are elevated in NEC, particularly platelet-activating factor (PAF), interleukin 1 (IL-1), and tumor necrosis factor (TNF) [444–446]. In an animal model, inhibition of PAF prevents the development of NEC [447]. Caplan and colleagues propose that these cytokines have an etiologic role in the development of NEC [442, 445, 448]. These factors have been associated with reactive thrombocytosis [449–451], but it is unclear what role they may play in the development of thrombocytopenia in NEC.

Chromosomal abnormalities

Newborns with Turner syndrome, trisomy 13, trisomy 18, or trisomy 21 can present with thrombocytopenia [452–458]. In a retrospective review of 5194 fetal blood samplings, Hohlfeld and coworkers reported that 3 of 44 (6%) fetuses with Down syndrome, 26 of 30 (86%) fetuses with trisomy 18, 5 of 16 (31%) fetuses with Turner syndrome, 6 of 11 (54%) fetuses with trisomy 13, and 3 of 4 (75%) fetuses with triploidy were thrombocytopenic [458]. The hematologic picture of fetuses with triploidy, in addition to thrombocytopenia, includes platelet anisocytosis and anemia, with marked macrocytosis and anisopoikilocytosis. These findings in a fetal blood sample should alert the clinician to the potential diagnosis of triploidy [459]. Hord and colleagues reviewed 31 newborns with Down syndrome. After excluding six infants because of the presence of diseases that may cause thrombocytopenia, they found that 7 of 25 were thrombocytopenic (28%), with platelet counts from 40 000 to 100 000/mm^3 [457]. Thuring and Tonz studied 70 newborns with Down syndrome, 10 with trisomy 18, and 6 with trisomy 13. Sixty percent of the infants with a trisomy had thrombocytopenia. The average platelet count in Down syndrome was 104 600/mm^3, with a median of 90 500/mm^3 [456]. Infants with Down syndrome have multiple other hematological abnormalities, including transient myeloproliferative syndrome, leukemia, polycythemia, and macrocytosis [460–464]. These abnormalities suggest that there is a gene, or genes, important in the control of hematopoiesis in chromosome 21 [465]. Jacobsen syndrome, a deletion at 11q23, also is associated with thrombocytopenia [466].

Malformations

Thrombocytopenia has been associated with the giant hemangioma syndrome [467], or Kasabach–Merritt syndrome (KMS). Hemangiomas are present at birth; they may be large or small, single or multiple. These vascular tumors tend to disappear by the fourth year, but they may enlarge during the first six months of life [468]. Hemangiomas associated with the KMS tend to be large. Bleeding manifestations frequently are preceded by enlargement and hardening of the hemangioma. During the initial period of enlargement, vital structures may be compromised, and bleeding manifestations can become so serious as to require transfusion therapy. Few fatalities have been reported in this condition. It is clear now that the vascular tumors associated with the KMS are not true childhood hemangiomas but are Kaposiform hemangioendotheliomas [469–472]. In this form of thrombocytopenia, there is local consumption of platelets and clot formation with fibrin consumption [473, 474]. Also, there is destruction in the spleen of platelets that have been damaged within the vascular abnormality. KMS also can be associated with cardiac failure and hydrops fetalis [475, 476].

Several therapeutic modalities have been used for treating the giant hemangioma syndrome. Surgical removal of the vascular tumor may be complicated by the mutilating effects of surgery, infections, and hemorrhagic complications [477], but it can be an effective form of therapy with careful screening of candidates for surgery [478]. Radiotherapy also has been used, but the secondary effects to the underlying bony structures should be considered before giving radiation [479]. Corticosteroids at conventional doses or at very high doses may help to resolve the thrombocytopenia and also induce shrinkage of the tumor [480–484]; however, the improvement is not complete. With the theoretical consideration that thrombosis may lead to necrosis and shrinking of the tumor, the antifibrinolytic agents ε-aminocaproic acid (EACA) and tranaxemic acid have been used in this condition, with some success [481, 485, 486]. Antiplatelet and anticoagulant drugs also have been used. Ezekowitz and colleagues reported interferon alfa-2a therapy of 20 infants with life-threatening hemangiomas, four of which had KMS. Eighteen of the 20 infants responded with shrinkage of the tumor from 50% to 100%. One of the four infants with KMS did not respond and died [487]. Lack of response to interferon alpha2a also has been reported [488]. Interferon is not a benign form of therapy [489]. In 53 children with hemangiomas treated with interferon grade 3 and 4 (severe) toxicities occurred in 58% and 17% of patients, respectively, and all patients had at least one form of toxicity. Other approaches may be available, including cytotoxic chemotherapy [490], intralesional steroids [491], embolization [492], and laser therapy [493]. In an animal experimental model, a form of thrombopoietin was able to increase the animals' platelet counts [494].

A reasonable approach to therapy is to begin with the least harmful approach [495–499] but to consider all available options. Blood-product support must be administered in the form of platelets, red cells, and plasma, as needed. A course of corticosteroid therapy should be started at a dose of prednisone 2–4 mg/kg/day. This therapy requires about one week to produce a beneficial effect. Transfusions of platelets and red cells may be required in the interim. If steroids do not work, a trial of EACA, at an initial dose of 200 mg/kg orally, followed by 100 mg/kg every six hours, can be started. EACA may be more effective if prednisone is given at the same time. For patients who require a rapid reduction of tumor size because of compromise of vital structures, surgery or radiotherapy must be considered. Interferon therapy should be considered if steroids fail. A rational decision regarding therapy can be made only by evaluating the risks and benefits of these forms of therapy for the individual patient.

In patients who do not respond to medical therapy and for whom radiation therapy and surgery are being considered because of persistent severe thrombocytopenia, one also should consider defining the vascular supply of the hemangioma with angiography. Once the vascular supply is known, sclerosing agents may be injected into the vascular bed of the lesion. Hemangiomas of the eyelid have improved following injection of steroids, such as triamcinolone and dexamethasone sodium phosphate, as sclerosing agents [491]. We have observed that injection of sclerosing agents into the vascular bed of a hemangioma may give only a temporary response, requiring a second or third injection of the sclerosing agent.

Inherited disorders

Thrombocytopenia in the newborn can be a manifestation of congenital pancytopenia. Thrombocytopenia and pancytopenia also can be part of inherited metabolic disorders [106]. The mechanism for this form of thrombocytopenia is not known.

Fanconi anemia

Fanconi described in 1927 a lethal familial anemia [500]. Fanconi anemia can present in the newborn with thrombocytopenia [501]. It is an autosomal recessive disorder. The infant may present with thrombocytopenia alone, with pancytopenia, or with dysmorphic features only. The associated congenital abnormalities of hypopigmented

and hyperpigmented skin lesions, microcephaly, small size, urinary-tract abnormalities, and upper-extremity radial-side abnormalities involving the thumb should alert the physician to the possibility of Fanconi anemia. Of 388 patients reported by Butturini and colleagues, 332 developed hematologic abnormalities at a median age of seven years (range birth to 31 years), with an actuarial risk of developing these abnormalities of 98% [501]. Much has been learned about the molecular nature of this disorder. However, the diagnosis is still based on the susceptibility of these patients' cells to DNA damage. Fanconi anemia cells are hypersensitive to both cytotoxic and clastogenic effects of DNA cross-linking agents [502]. The cross-linking agent diepoxybutane (DEB) has been used effectively to diagnose Fanconi anemia and is the standard diagnostic test to date [503, 504]. Peripheral blood chromosomal studies, looking for unstable chromosomes and increased chromosomal fragility and rearrangements on exposure to DEB, define the diagnosis. The varied presentation of this disorder makes it difficult to rely on any one aspect of the disease [501]. Most patients present later than the newborn period, with cytopenias or dysmorphic features. Based on somatic fusion cell studies, Fanconi anemia can be classified into eight complementation groups: A–C, D1, D2, and E–G. Except for complementation groups B and D1, six of these genes have been cloned. A review of the mechanism by which these orphan proteins protect cells from damage and maintain hematopoiesis is beyond the scope of this chapter. Fanconi anemia is discussed in Chapter 5, and excellent reviews of the proposed mechanism have been published [505–508]. Patients with Fanconi anemia are also at risk of cancer. Approximately 17% of 1301 patients reviewed by Alter developed a cancer [509]. Leukemia, myelodysplastic syndrome, solid tumors of the head and neck, and liver tumors constitute the majority of tumors affecting these patients.

Dyskeratosis congenita

Dyskeratosis congenita is an inherited disorder of connective tissue and bone-marrow failure. The major clinical manifestations are dermatologic. The classic dermatological triad of reticulated pigmentation of the skin with associated atrophy and erythema, dystrophy of the nails, and leukoplakia of the oral and genital mucosa is the most consistent clinical manifestation. Most patients will develop marrow failure by 20 years of age. It is now clear that immunologic abnormalities occur in a subgroup of these patients [510]. Another group of patients develop pulmonary problems, and 8% develop malignancy [510]. The gene responsible for this disorder has been cloned and localizes to Xq28. The protein produced is dyskerin. The disease is inherited as an X-linked recessive disorder in most patients. In 16 of 92 families, there were females affected. Some but not all of these had a history of consanguinity, suggesting that there are autosomal recessive and autosomal dominant forms of the disease [510]. The disorder can present as isolated thrombocytopenia, but it has not been reported in newborns [511]. Further discussion of this and other marrow-failure disorders is presented in Chapter 5. An excellent review of the clinical and biological aspects of this disorder has been published [510].

Thrombocytopenia and absent radii syndrome

Shaw and Oliver first described thrombocytopenia and absent radii (TAR) syndrome in 1959 [512]. Radial dysplasia is a common genetic abnormality. Carroll and Louis [513] found that 4 of 53 patients with radial dysplasia had hematologic abnormalities, two having Fanconi anemia and two with TAR syndrome. Hall and coworkers reviewed 40 cases of TAR in 1969 [514]. Until recently, this was the only series of patients reported. In their series, the inheritance pattern of the TAR syndrome is consistent with an autosomal recessive pattern of inheritance. However, the incidence among siblings of 1:7 is lower than the expected rate [514]. Both males and females are affected, but there is a predominance of females. The onset of symptoms usually occurs very early in life. Half of the patients have onset of hemorrhagic manifestations in the first week of life, and

most develop thrombocytopenia by four months of age [514]. However, one patient was 47 years old at presentation [515]. Most patients had leukemoid reactions, usually at the time of thrombocytopenic exacerbations. Leukocytosis and eosinophilia are frequent hematologic findings. Megakaryocytes are either absent or markedly decreased in the bone marrow. When megakaryocytes are present, they are small and immature in appearance. In the series by Hedberg and Lipton, which added 60 more cases to the Hall *et al*. series, bone-marrow examinations reported that megakaryocytes were absent in 28 of 52 and decreased in 22 of 58 patients; in two patients, there were normal numbers of megakaryocytes [516]. These investigators reported that 21 of 77 cases died, 14 dying in the first four months of life. All deaths occurred in patients with platelet counts below 30 000/mm^3. In Hall and colleagues' series, 8 of 16 patients with platelet counts below 10 000/mm^3 died; after improvement of supportive care from 1969 to 1988, only three of ten such infants died [514]. Storage pool and collagen aggregation platelet-function defects have been described in this syndrome [160, 517, 518]. Platelet survival may be decreased [517]. Other investigators have not found a defect in platelet function or survival [519]. Anemia has been attributed to hemorrhage and secondary iron deficiency. The radial abnormalities do not include the thumbs. However, the radial abnormalities can range from hypoplasia to total absence of the radius [514]. Ulnar and hand abnormalities are common associated abnormalities, occurring in 75–78% of patients [514, 516]. Abnormalities of the humerus also occur in 40% of the patients; these include phocomelia, resulting in an abnormality similar to thalidomide-induced limb abnormalities. Cardiac defects are also seen in one-third of these patients, the most common being tetralogy of Fallot, atrial septal defect, and ventricular septal defects [514, 516]. The differential diagnosis of TAR syndrome must include consideration of Fanconi anemia. In Fanconi anemia, the absence of thumbs, onset of thrombocytopenia later in childhood, and the characteristic increased chromosomal fragility after challenge with DEB should help distinguish it from the TAR syndrome. Thalidomide therapy can produce similar osseous abnormalities. However, thrombocytopenia is not a common finding with thalidomide use, and maternal intake of this drug is now unheard of. The Holt–Oram syndrome (hereditary heart disease plus skeletal malformations) is in the differential, but this syndrome is associated rarely with thrombocytopenia and, if it is present, is not severe. Roberts syndrome (tetraphocomelia, cleft lip and palate, intrauterine growth retardation, failure to thrive) is usually not associated with hematologic abnormalities. Thrombocytopenia and radial abnormalities can be seen in DiGeorge syndrome and in other syndromes belonging to the deletion of chromosome 22q11 spectrum [520, 521], including Bernard–Soulier syndrome [142]. Chromosomal analysis looking specifically for this deletion should be carried out to exclude this syndrome. Table 10.12 shows the clinical characteristics of TAR syndrome, Fanconi anemia, Holt–Oram syndrome, Roberts syndrome, and 22q11 deletion syndrome.

Prognosis

The prognosis in the TAR syndrome is dependent on the severity of the hemorrhagic manifestations. If the patient survives the first year of life, then the hemorrhagic manifestations resolve and thrombocytopenia disappears in most cases [514], with only one fatality reported with thrombocytopenia beyond 14 months of age [516]. However, infection, surgery, and other stresses can cause recurrent thrombocytopenia. In some cases, the platelet count may remain slightly low. Milk allergy has been suggested as an exacerbating factor in this syndrome [522]. Greenhalgh and colleagues reported a clinical study of 34 patients with TAR syndrome recruited through their genetic services [523]. The platelet count was documented in 17 patients at birth; 14 (82%) had a platelet count below 50 000/mm^3. Platelet counts fluctuated during childhood and adulthood. Of the 28 cases where details regarding the lower-limbs were available, 13 (47%) had lower-limb abnormalities. Fourteen (47%) cases had cows' milk intolerance. Renal abnormalities occurred in 23%. Cardiac

Table 10.12 Comparison of thrombocytopenia and absent radii (TAR) syndrome, Fanconi anemia, Holt–Oram syndrome, Roberts syndrome and "CATCH 22"

Syndrome	Hematological findings	Skeletal abnormalities	Other	Genetic abnormalities	Prognosis
TAR	Thrombocytopenia in all Other cell lines normal	Bilateral absence of radii Thumbs always present	Cardiac anomalies (15–30%)	Normal autosomal recessive	Excellent if survives past first year of life
Fanconi anemia	Thrombocytopenia may be first symptom Aplastic anemia follows, usually at age 5–10 years	Thumb abnormalities, including absent thumbs	Renal abnormalities, skin abnormalities Microcephaly	Hypersensitivity to DEB with increased chromosomal breaks Autosomal recessive	Poor because of aplastic anemia and secondary malignancies
Holt–Oram	Normal	Characteristic thumb defect Terminal upper-extremity defects	Cardiac anomalies common	Normal chromosomes Autosomal dominant	Dependent on cardiac defect
Roberts	Normal	Tetraphocomelia, cleft lip and palate Thumbs may be present	Dysmorphic features	Subtle centromeric puffing Autosomal recessive	Failure to thrive
DiGeorge	Thrombocytopenia extremely rare and mild (2/558)	Skeletal abnormalities are minor in 17% Radial aplasia extremely rare (one report)	Cardiac abnormalities, abnormal face, thymic hypoplasia, cleft palate, hypocalcemia (CATCH 22)	Deletion 22q11 Sporadic or autosomal dominant	Good, but dependent on severity of cardiac and immune abnormalities

DEB, diepoxybutane.

Table 10.13 Features of thrombocytopenia and absent radii (TAR) syndrome in 34 patients compared with previous studies

Feature	This study	Previous studies	Reference
Bilateral radial aplasia	100%	100%	[514]
Thrombocytopenia	100%	100%	[514]
Cows' milk intolerance	47%	62%	[516, 522, 672]
Lower-leg involvement	47%	40%	[516]
Cardiac anomaly	15%	22–33%	[514, 516]
Renal anomaly	23%	3%	[514]
Cleft palate	3%	1 case	[673]
Uterine +/− genital anomaly	6%	3%	[514]
Facial capillary hemangioma	24%	–	–
Intracranial vascular malformation	3%	–	–
Sensorineural hearing loss	3%	–	–
Epilepsy	6%	–	–
Scoliosis	3%	–	–
Neural tube defect	3%	1 case	[514]

Reproduced with permission from Greenhalgh *et al.* [523].

abnormalities occurred in 15%. Of the 34 cases, two died, one of complex cardiac disease and the second of ICH at three months of age. Table 10.13 presents the clinical features of the series of Greenhalgh *et al.* compared with previous reports. The presence of renal abnormalities, facial hemangiomas, and seizures are much greater in this series than reported previously.

Pathogenesis

Because the platelet count recovers after the first year of life, Freedman [524] has suggested that megakaryocyte progenitors are present in this disorder. Although the inheritance pattern of TAR syndrome suggests an autosomal recessive inherited disorder, the pattern is not perfect and suggests that there may be several forms of this disorder. Before the isolation of TPO and its receptor c-mpl, Homans and colleagues reported a child with TAR syndrome whose bone marrow failed to grow megakaryocytic colonies in culture (CFU-Mk). The patient had increased megakaryocyte colony-stimulating activity (MK-CSA), which probably represented increased TPO levels, suggesting a stem-cell-failure syndrome [525]. We described a case with normal levels of functional TPO and abnormal in vitro CFU-Mk. This subject had large colonies of very small megakaryocytes, suggesting abnormal megakaryocyte development [526]. Bone-marrow megakaryocytes were also small and had low ploidy [526]. Sekine and coworkers also reported a child with normal TPO levels and abundant but abnormal CFU-MK [527]. Ballmaier and coworkers studied five subjects with TAR syndrome. Thrombopoietin levels were high in all subjects. In vitro bone-marrow megakaryocyte colony formation was tested in one subject. This subject's colonies failed to grow under TPO stimulation [528]. The TPO receptor was present on the platelets of the patients, but upon TPO stimulation, platelets failed to increase tyrosine phosphorylation of proteins in all but one patient. This latter finding suggests a defective signaling through the TPO receptor. A second publication involving one more patient showed failure of Jak-2 phosphorylation in TAR platelets [529]. Jak-2 is an important signaling molecule downstream from c-mpl, the TPO receptor. Letestu and coworkers studied nine patients with TAR syndrome. They found elevated TPO levels in the six patients tested. The CFU-Mk were markedly decreased. However, in liquid cultures, the TAR syndrome patients demonstrated an increase in growth of an unusual population of cells that were CD41a-positive but CD42a-negative. The finding that a combination of stem cell factor (SCF), interleukin 2 (IL-2), and interleukin 6 (IL-6) are better stimulators of in vitro growth than TPO suggests that there is a maturational defect in megakaryopoiesis in TAR syndrome patients [530]. In addition, these investigators found decreased c-mpl expression on TAR platelets and a relative increase in an isoform that has no cytoplasmic tail and may not transduce signal, consistent with the findings of Ballmaier and colleagues. The c-mpl and TPO genes are normal at the genomic DNA level in TAR syndrome [530]. The Hox gene complex is important in bone development and hematopoiesis. This dual role made these genes, particularly Hox 10 and 11, attractive as potential candidates to explain the TAR syndrome [531–535].

Fleischman and coworkers could not demonstrate mutations in HoxA10, HoxA11, or HoxD11 genes in TAR syndrome [536]. A closely related syndrome with thrombocytopenia and radioulnar synostosis [537–539], however, is due to mutations in the HoxA11 gene [540]. This syndrome is inherited as an autosomal dominant disorder [537], and aplastic anemia develops in adulthood [530]. These studies, as Letestu and coworkers hypothesized, suggest that the thrombocytopenia in TAR syndrome is characterized by dysmegakaryocytopoiesis, with blockade at an early stage of megakaryocyte differentiation. Although TPO and c-mpl genes are normal, the regulation of c-mpl is abnormal. Improvement of the platelet count as the patient gets older suggests a defect in an age-dependent c-mpl regulatory gene or genes. As the patient gets older, the gene or genes is/are no longer necessary, and the platelet count improves.

Treatment

The therapy for the TAR syndrome is supportive, with platelet transfusions for hemorrhage, red-cell transfusions for anemia, and avoidance of those stresses that worsen the thrombocytopenia, including cows' milk. The critical period for these children is the first months of life. Given that mortality is reduced markedly with platelet counts above 30 000/mm^3, and is highest with platelet counts below 10 000/mm^3, maintaining a platelet count greater than 20 000–30 000/mm^3 in these infants is a reasonable therapeutic approach. Using apheresis single-donor units of platelets can decrease platelet sensitization. Corrective therapy for the congenital bone abnormality should be delayed until the hemorrhagic manifestations have improved after the first year of life. Corticosteroids, IVIG, and splenectomy have been tried, but the beneficial effects have been inconsistent. Successful therapy with IL-6 has been reported in one child [541]. There is also a single case report of erythropoietin therapy in TAR syndrome. An adult with TAR syndrome had an increase in platelet count during therapy with human recombinant erythropoietin [542]. Given the in vitro resistance to TPO and the poor signaling through TPO, the role of high-dose TPO in this disorder is not clear. Mothers who have infants with TAR syndrome should be advised of the possibility of having another affected child. Prenatal diagnosis by ultrasound examination should identify an affected child by 16 weeks of gestation. The prenatal diagnosis of TAR syndrome can be made by ultrasound or by percutaneous umbilical cord blood sampling (PUBS) [543–549]. Once a child survives the first year of life and the hemorrhagic manifestations virtually stop, the life expectancy becomes normal. Although many hematopoietic failure disorders have an increased risk of malignant transformation, this has not been documented for TAR syndrome; however, two cases of leukemia in TAR syndrome have been reported [550, 551].

Amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (cAMT) is a rare bone-marrow-failure syndrome that presents initially with thrombocytopenia but can progress either to aplastic anemia or to leukemia. Alter and Young, in an excellent review of cAMT, describe the clinical presentation and progression of 21 subjects with this disorder presenting with no physical abnormalities [552]. Fifteen of these patients presented with thrombocytopenia in the first week of life, and five presented after age two years. In addition, they also review the reports of another 17 children who presented with thrombocytopenia, but with physical abnormalities that did not fit any other syndrome. In the infants without physical abnormalities, the male-to-female ratio is 1.1. The inheritance pattern from these families could be autosomal recessive or perhaps X-linked. Although at presentation the patients have normal hemoglobin, the red cells are macrocytic and the patients have elevated hemoglobin F. Aplastic anemia developed at a mean of 3.7 years in 11 of the 26 patients without physical abnormalities. Transformation to leukemia has been reported; therefore, this is another bone-marrow-failure syndrome with increased propensity to malignant transformation [553]. We now understand better

the pathophysiology of this disorder. Guinan and coworkers found decreased CFU-Mk in children with cAMT [554]. Stimulation with either GM-CSF or IL-3 failed to increase the number of CFU-Mk in four of five patients. Muraoka and coworkers studied a patient with cAMT and showed that the CFU-Mk were not responsive to TPO. When they looked at RNA expression of the c-mpl gene, it was markedly decreased [555]. Ihara described a point mutation in the c-mpl gene of a Japanese patient with cAMT [556]. Since then, several investigators have described mutations in this gene as the cause of cAMT [557–559]. Van den Oudenrijn and coworkers describe mutations in the c-mpl gene in four of five patients with cAMT [557]. Ballmaier and coworkers found c-mpl mutation in eight of eight patients with cAMT tested [559]. Both of these groups of investigators found decreased CFU-Mk, failure to respond to TPO in vitro, and elevated TPO levels in the patients' plasma. However, van den Oudenrijn and coworkers found that functional polymorphisms exist in the c-mpl gene. These investigators stress that mutations found in the gene need to be studied in vitro to document that they do lead to a dysfunctional c-mpl [560]. Mutations in the TPO receptor, c-mpl, are the cause of hematopoietic failure in some, if not most, patients with cAMT. Hallett and colleagues reported a case of cAMT with duplication of chromosome 3q21–26 close to the location of the TPO gene, suggesting that in this case disturbance of TPO itself may be the cause of the cAMT [561]. TPO not only stimulates platelets but also is antiapoptotic to stem cells [562]. This latter effect would explain the marrow failure that develops in these patients. This disorder has a high mortality, but improved supportive care with platelet transfusions has decreased the risk of early death and, perhaps, increased the risk of malignant transformation [552]. Cytokine therapy has not been very successful and, given the nature of the defect, it is unclear whether TPO therapy would be effective [554]. These patients have been treated successfully with hematopoietic stem-cell reconstitution [563].

Macrothrombocytopenias

Newborn thrombocytopenia can occur even if megakaryocytes are present in the bone marrow. Some of these syndromes are part of functional platelet disorders such as Bernard–Soulier syndrome [141], gray-platelet syndrome [564–566], May–Hegglin anomaly [242], Fechtner syndrome, Sebastian syndrome, Alport/Epstein syndromes [567], Paris–Trousseau syndrome [568], Mediterranean macrothrombocytopenia [569], and Montreal platelet syndrome [570]. Some of these disorders are inherited as autosomal dominant, while others as recessive. May–Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, Alport syndrome, and Epstein syndrome are macrothrombocytopenias with leukocyte inclusions. They have different, although related, clinical presentations and distinct morphological abnormalities. In spite of their clinical and morphological differences, they may be due to mutations in the same gene, the human non-muscle myosin IIA gene [571]. In these syndromes, there is decreased platelet survival and perhaps decreased production of platelets. The thrombocytopenia tends to be milder than the amegakaryocytic thrombocytopenias, and the bleeding manifestations, such as easy bruising and petechiae, are identified later in childhood. However, a careful history will often reveal easy bruising in the first year of life. These patients do not require specific therapy for the thrombocytopenia, although occasionally they may require platelet transfusions for severe bleeding.

Wiskott–Aldrich syndrome

The Wiskott–Aldrich syndrome (WAS) is an X-linked inherited disorder associated with immunodeficiency, eczema, and thrombocytopenia [572]. The thrombocytopenia not only consists of decreased number of platelets but also is associated with the presence of small dust-like platelets. Bone-marrow megakaryocytes are normal to increased [572]. Thrombocytopenia at birth may be the first

manifestation of the disease [573]. Sullivan and coworkers obtained information in 154 patients with WAS [574]. Persistent thrombocytopenia was a requirement for entry in the study. The triad of thrombocytopenia, eczema, and immunodeficiency was present in only 30% of the patients. Eighty-four percent of the patients had symptomatic thrombocytopenia before being diagnosed with WAS. The most common symptoms were petechiae and purpura in 78% of patients. Only 20% of the patients had hematologic manifestations alone, without infectious manifestations. Thrombocytopenia was treated with steroids, with 10 of 31 treated patients responding. No patient responded to IVIG alone. Fifty-nine patients had splenectomy and 54 (92%) had a sustained rise in platelet count. There were nine (15%) deaths due to bacteremia in the splenectomized group.

The gene responsible for WAS has been isolated to Xp11.23 [575]. Mutations in this gene are responsible for WAS as well as for a group of patients with X-linked thrombocytopenia [576]. The exact function of this gene is not known, but it participates in cytoskeletal organization [577]. Haddad and colleagues did not find a defect in platelet production and hypothesized that the platelet defect may be in cytoskeletal assembly of the circulating platelets [578].

These patients are at risk of hemorrhage, infection, malignancy, and development of autoimmune diseases [574]. Because of these complications, supportive therapy alone is not enough, and hematopoietic stem-cell reconstitution is the recommended therapy. Results from this form of therapy depend on the donor type, with matched-sibling transplantation having the best results [579].

X-linked thrombocytopenias

X-linked thrombocytopenia can be part of the spectrum of WAS [580]. These patients, like those with WAS, will have small platelets [574, 578]. X-linked thrombocytopenia also can be associated with macrothrombocytopenia. Several case reports have described patients with or without associated anemia. Thompson and colleagues described a case of X-linked thrombocytopenia and thalassemia [581]. This disorder was linked to Xp11–12 [582] and, more recently, to a mutation in the GATA-1 gene [583]. Nichols and colleagues first reported a patient with anemia and thrombocytopenia with a mutation in the GATA-1 gene [584]. Since then, several other investigators have added to the list of reported patients with X-linked thrombocytopenia, with and without anemia [585, 586]. These patients present with variable levels of thrombocytopenia associated with bleeding symptoms. These patients do not have the immunologic disorders seen in WAS.

Other inherited and congenital thrombocytopenias

In addition to the syndromes discussed already, amegakaryocytic or hypomegakaryocytic thrombocytopenia in the newborn is part of several other syndromes. Many of these syndromes include multiple congenital abnormalities.

Thrombocytopenia also has been associated with hearing impairment, ophthalmoplegia, and radial hypoplasia, including hypoplasia of the thumbs [587]. The thrombocytopenia is mild, and the disorder is inherited as a dominant character. Many other hypomegakaryocytic/thrombocytopenic syndromes have been reported, associated with various congenital abnormalities, including deafness and renal abnormalities [588], cerebral malformations [589], absence of the corpus callosum [590], and neurological dysfunction [591].

Metabolic disorders

Various metabolic disorders have been associated with neonatal thrombocytopenia. This includes infants of diabetic mothers [592]. These infants have an increased incidence of polycythemia and are at higher risk of developing thrombi [592, 593]; both conditions are associated with thrombocytopenia in the newborn. It is unclear whether the metabolic condition or polycythemia or thrombi or a host of

other complications that may be present in these infants [594] are the cause of thrombocytopenia in infants of diabetic mothers. Other metabolic conditions that have been associated with neonatal thrombocytopenia include propionic acidemia [595, 596], hyperthyroidism [597], cobalamin C deficiency [598], hypercalcemia with subcutaneous fat necrosis [599], and mevalonic acidemia [600].

Thrombosis

The triad of gross hematuria, palpable kidney, and thrombocytopenia in the newborn nursery is considered evidence of renal-vein thrombosis [601–603]. The thrombosis is due to a multitude of precipitating causes. In a review, this classic triad was present in only 13% of cases [604]. The investigators suggest that the presence of any of the three signs should be considered as potential renal-vein thrombosis. Other thrombi can cause thrombocytopenia. Thrombocytopenia also is seen in the nonbacterial endocardial thrombosis syndrome [605]. Most of these infants have severe respiratory disease, and there is an increased incidence of persistent fetal circulation [605]. The mechanism for this form of thrombocytopenia is unknown. Other reported sites of thrombosis causing thrombocytopenia include the umbilical vein or artery, aortic thrombus, and chorionic vessels [606–609]. The thrombocytopenia is most likely due to consumption of platelets, and any large thrombus may cause it.

Neonatal thrombotic thrombocytopenic purpura, sporadic or familial, has been reported [610]. Repeated episodes suggest the Upshaw–Schulman syndrome, which may be due to deficiency of the von Willebrand metaloprotease [611].

Miscellaneous disorders

Newborns exposed to low ambient temperatures can develop hypothermia. Among infants whose temperature drops below 32°C in the absence of other illnesses, thrombocytopenia is a common finding. Six of seven infants reported by Cohen and colleagues [612] developed thrombocytopenia. Platelet counts decreased further on rewarming in these infants. Recovery then takes about seven days. The mechanism for this form of thrombocytopenia is not clear.

Polycythemia in newborns also has been associated with thrombocytopenia; 19% of the infants studied by Katz and colleagues [613] had platelet counts below 140 000/mm^3. Plasma exchange rapidly corrected the thrombocytopenia. The mechanism of thrombocytopenia in this condition is unclear. Thrombocytopenia also can occur in infants who are small for gestational age [614–617]. The mechanism for the thrombocytopenia is not known, although in one series, associated neonatal complications that could explain the thrombocytopenia were present in almost half of the infants [617]. Meconium aspiration syndrome also is associated with thrombocytopenia. In a series of 356 infants with lung disease, 16 had thrombocytopenia. Twelve of the 16 were among the 90 infants with a diagnosis of perinatal aspiration syndrome. Pulmonary hypertension was present in all the patients who had thrombocytopenia and aspiration pneumonia [618]. Pulmonary hypertension was not associated with thrombocytopenia when it was a primary finding or when it was associated with respiratory distress syndrome. The mechanism for the thrombocytopenia is unclear.

Other conditions associated with thrombocytopenia include asphyxia [619, 620] hyperbilirubinemia, phototherapy [259], mechanical ventilation [266], extracorporeal membrane oxygenation (ECMO) [621, 622], cyanotic congenital heart disease [623], and the presence of an indwelling umbilical line [259].

Thrombocytopenia also is part of the spectrum of diseases associated with bone-marrow infiltration. These disorders are mentioned because they have to be considered in the differential diagnosis of thrombocytopenia of the newborn. Congenital leukemia, the myeloproliferative syndrome associated with Down's syndrome, osteopetrosis, and the histiocytoses are diseases to consider as possible causes of thrombocytopenia. These disorders are discussed extensively in Chapter 16.

Therapy

Newborns with secondary thrombocytopenia, regardless of cause, are at risk of hemorrhage. In general, when these newborns have platelet counts above 50 000/mm^3, the risk of bleeding is small [261, 334, 624]. If the platelet count is below 20 000/mm^3, then the risk of bleeding increases [261, 516, 625]. There may be some increased risk for bleeding with platelet counts between these values, but there is little information regarding the role of transfusion. Twenty percent of thrombocytopenic infants have severe thrombocytopenia [262]. Newborns with hemorrhage are more likely to have a coagulopathy in addition to thrombocytopenia [626]. The best therapy is correction of the underlying disorder. In an infant with clinical bleeding and secondary thrombocytopenia, platelet transfusions may be required until the underlying cause of thrombocytopenia is resolved. If, in addition, there is evidence of DIC, then replacement of coagulation factors with fresh frozen plasma may be necessary. A dose of 1×10^{10} platelets per kilogram of body weight is sufficient to increase the platelet count of a newborn to 100 000/mm^3. There are no clear guidelines as to when an infant needs platelet transfusions. Andrew and colleagues randomized infants with platelet counts between 50 000/mm^3 and 100 000/mm^3 to receive or not receive platelet transfusions. Platelet transfusions did not make a difference in this study [334]. There are no prospective data documenting the level of platelet count at which an infant needs platelet transfusion. Because of this lack of information, the current guidelines for a platelet-count threshold to transfuse platelets vary from a platelet count of 20 000/mm^3 to a platelet count of 50 000/mm^3 [627–630]. Murray and colleagues reviewed their experience with platelet transfusions in neonates [261]. During the three-year review period, there were 901 admissions, with 53 (6%) neonates with severe thrombocytopenia. Severe thrombocytopenia was defined as a platelet count below 50 000/mm^3. Forty-four (8%) of 543 premature infants and 9 (1.6%) of 543 term newborns had severe thrombocytopenia. Premature infants received platelet transfusions if their platelet count was below 30 000/mm^3 or if they were deemed to be at high risk of hemorrhage. Nineteen premature infants with severe thrombocytopenia were considered stable and were not transfused. None of them had a hemorrhage. Only two of the nine full-term infants with severe thrombocytopenia were transfused; both had a platelet count below 30 000/mm^3. Of the seven not transfused, three had platelet counts below 30 000/mm^3 but were clinically stable and were not transfused. None of the full-term neonates with severe thrombocytopenia had significant hemorrhage and none died [261]. A safe guideline is to transfuse neonates with platelet counts below 30 000/mm^3, particularly if they are ill or premature. A careful prospective study of neonatal platelet transfusion would be helpful to establish better platelet-transfusion criteria.

Diagnostic approach

Thrombocytopenia is a common finding in the intensive-care nursery. The approach to the diagnosis of thrombocytopenia must then be tailored to the individual infant. Thus, our first consideration should be the clinical findings.

The differential diagnosis in a sick newborn differs from the differential diagnosis in a healthy newborn. As shown in Table 10.14, the sick newborn may become thrombocytopenic from a variety of neonatal complications. Infection, asphyxia, meconium aspiration, respiratory distress syndrome, polycythemia, NEC, and the presence of an indwelling umbilical catheter all can cause thrombocytopenia.

In a sick newborn with thrombocytopenia and who also is very small, it may be justifiable to provide specific treatment for the underlying condition and treat the thrombocytopenia symptomatically. When the newborn's underlying condition improves, the thrombocytopenia should also improve, usually within five to seven days [262]. Thrombocytopenia develops late (median postnatal age of eight days) in late-onset sepsis and NEC. NEC is the major

Table 10.14 Possible causes of thrombocytopenia in the sick newborn[a]

Infection (sepsis)
Bacterial
Viral
Mycotic
Protozoal
Asphyxia
Meconium aspiration
Pulmonary hypertension
Respiratory distress syndrome
Small for gestational age
Necrotizing enterocolitis
Polycythemia
Cold injury
Hemolytic disease of the newborn
Hyperbilirubinemia
Phototherapy
Thrombosis
Diffuse intravascular coagulation
Extracorporeal circulation

[a]If thrombocytopenia lasts for more than a few days, then consider further diagnostic work-up.

precipitant of thrombocytopenia. Murray and colleagues found that this type of thrombocytopenia can last longer (median 8 days, range 1–37 days) [261]. Persistent thrombocytopenia should alert the physician to look for other causes of thrombocytopenia. For a healthy newborn, findings in the maternal history, the platelet count, and the infant's physical examination are the keys to the differential diagnosis (Fig. 10.7). A maternal history of drug ingestion, hypertension or toxemia, ITP, autoimmune disease, infection during pregnancy, or a placental abnormality may explain the thrombocytopenia.

An abnormal maternal platelet count then will suggest a maternal origin for the thrombocytopenia. In such cases, an immunologic work-up, including autoimmune and alloimmune disorders, is indicated. An inherited thrombocytopenia also must be considered. A normal maternal platelet count does not exclude these disorders, but the infant's examination will then help to identify any abnormalities associated with thrombocytopenia. The presence of congenital abnormalities will suggest one of the disorders listed in Fig. 10.7. The presence of hepatosplenomegaly will suggest infection or an infiltrative process (neoplasm or granuloma).

Normal findings on physical examination suggest immune thrombocytopenia. Maternal and infant sera should be tested for antiplatelet antibodies, and platelet antigen typing for both parents should be performed. If an antiplatelet antibody is present, then its specificity should be determined. Immune thrombocytopenia caused by drug ingestion by the mother and thrombocytopenia associated with autoimmune disorders also need to be considered, in addition to congenital thrombocytopenias, metabolic disorders, and drug-induced thrombocytopenia. This approach also should be used in a sick infant with persistent thrombocytopenia. In spite of a thorough investigation, in many sick newborns, thrombocytopenia will remain unexplained [259, 262].

Thrombocytosis

Thrombocytosis in newborns has received little attention. Premature newborns have a wider range of normal platelet count at birth, with an upper limit as high as 682 000/mm^3 [175, 180]. The platelet count in full-term infants rarely rises above 400 000/mm^3 [175, 631]. Low-birth-weight infants from two weeks to six months of age may have rising platelet counts, which can reach as high as 800 000/mm^3 [632]. Matsubara and colleagues followed platelet counts in 24 healthy low-birth-weight infants, appropriate for gestational age, for 28–30 days. The cord-blood mean platelet count was 265 000 $\pm$ 63 000/mm^3. The platelet count rose over the next two weeks to a mean platelet count of 473 000 $\pm$ 140 000/mm^3 (range 246 000–828 000) and remained stable thereafter. Nine of 24 infants had platelet counts above 500 000/mm^3. TPO levels rose by day two, decreased by day five, and remained stable but elevated as compared with children and adults. Thrombocytotic

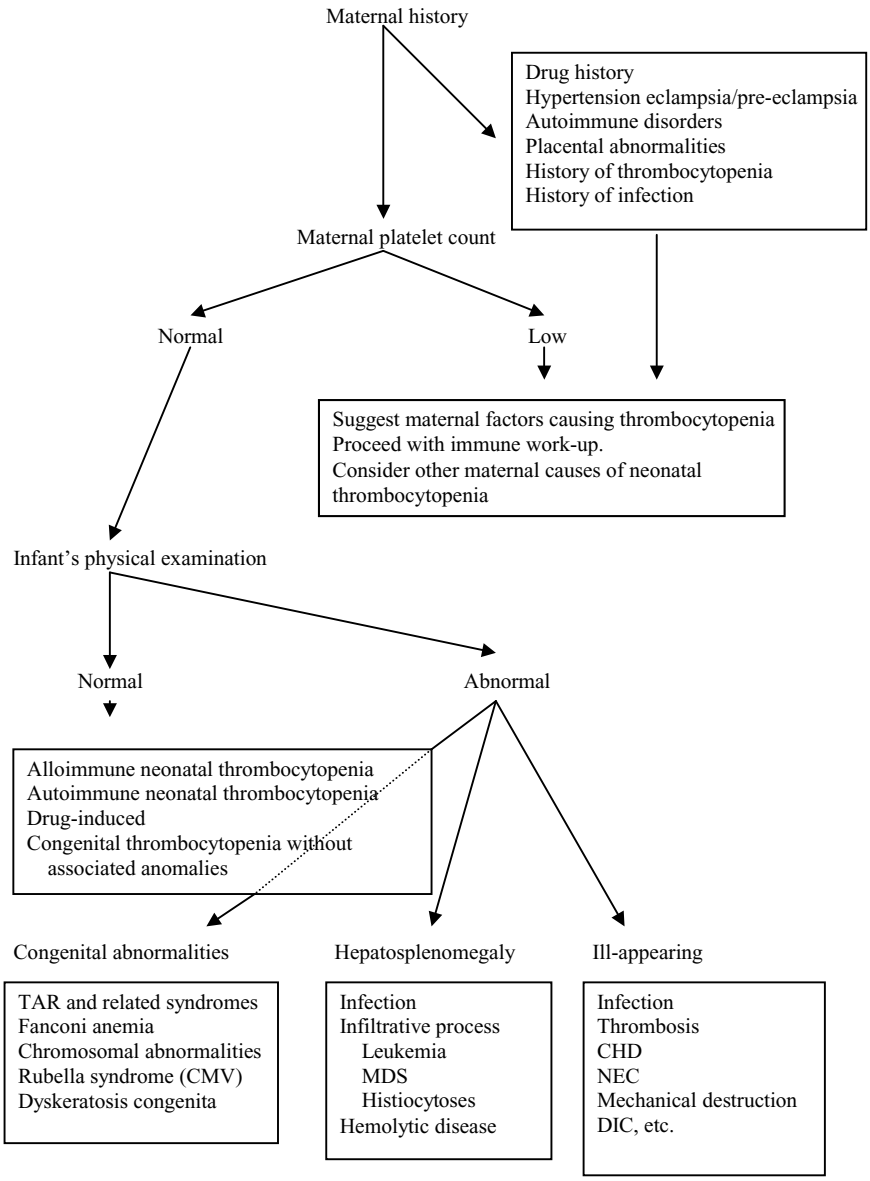

Fig. 10.7 Diagnostic approach to an infant with thrombocytopenia.
CHD, congenital heart diseases; MDS, myelodysplastic syndrome; NEC, necrotizing enterocolitis.

infants had higher TPO levels than nonthrombocytotic infants [633]. Whether this increased platelet count also can occur in full-term babies is not clear, and further studies are needed.

Thrombocytosis in the newborn has been associated with administration of erythropoietin to premature infants [634], parenteral nutrition [635], maternal polydrug abuse [636, 637], maternal methadone

use [638], maternal antischizophrenic drugs [639], vitamin K deficiency [640, 641], the myeloproliferative syndrome associated with Down's syndrome [642], acute megakaryoblastic leukemia [643, 644], granulomatous giant cell arteritis [645], congenital adrenal hyperplasia [646, 647], hyposplenism [648], vitamin E deficiency [649, 650], Caffey disease [650], Diamond–Blackfan anemia [651], subcutaneous fat necrosis [652], familial cholestatic syndrome [653], and administration of antimicrobials to the neonate. The latter include moxolactam, ceftriaxone, aztrionam, imipenum, meropenem, and zidovudine [639, 654–660]. Administration of monoclonal antibody against respiratory syncytial virus (RSV) to neonates also has been associated with thrombocytosis [661]. Thrombocytosis in infants and children has been reviewed by Addiego et al. [662], Vora and Lilleyman [663], Chan et al. [664], Heng and Tan [665], and Sutor [666]. Thrombocytosis in children is reactive in nature. Essential thrombocytosis is rare in children [667, 668]. Thrombocytosis is a common finding in hospitalized children, with an incidence of about 10% [662–664, 666]. As many as 25% of the infants with thrombocytosis are under two months of age and 2% are premature newborns [666]. Most affected children have mild thrombocytosis. Only about 7–8% have platelet counts above 900 000/mm^3, and 1–3% are platelet millionaires [662, 663, 666]. Overall infection is the most common cause of thrombocytosis. Other causes include surgery and trauma, hypoxia, autoimmune diseases, gastrointestinal disorders, renal disease, cancer (e.g. neuroblastoma), medications, anemia (e.g. iron deficiency, hemolytic anemia, blood loss), and splenectomy or asplenia. Many of the diseases associated with thrombocytosis in infants and children can be seen in the newborn period, but sufficient information on newborns is not available to conclude that these disorders cause newborn thrombocytosis. In children thrombocytosis is usually reactive and no therapy is necessary. Most infants recover quickly [664].

Essential thrombocytosis, a myeloproliferative disease in adults, may be different in childhood. One of two children we have studied with essential thrombocytosis did not have the increase in autonomous in vitro colony-forming cells seen in adults [669]. Instead, the child had an increased level of serum Mk-CSA. This may represent a congenital form of thrombocytosis (P. A. de Alarcón, unpublished data). Randi and colleagues described five cases of essential thrombocytosis in children, the youngest being nine months old. Their course was benign, suggesting that this disorder is not the same as the myeloproliferative disease of adults [670]. Most of these children were not treated and did well. There is only one other reported case of a five-month-old infant with essential thrombocytosis [671], and there are no reported cases of newborns with essential thrombocytosis.

Information on thrombocytosis in newborns is sparse. More studies, both clinical and experimental, are needed to better define newborn thrombocytosis and the mechanisms involved in its production.

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11

Neutrophil function and disorders of neutrophils in the newborn

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Introduction

The phagocytes comprise a small group of hematopoietically derived cells that play diverse roles in human host defense. The name, coined by Elie Metchnikoff over a century ago [1], refers to one of their most prominent specializations – the ability to ingest particulate targets. However, Metchnikoff's "microphages" and "macrophages" do much more than simply ingest foreign materials. They are now appreciated to perform multiple roles in inflammation and host defense, in both the innate and adaptive arms of immunity. This chapter will address phagocytic cells in their host-defense roles as they relate to innate immunity, using the polymorphonuclear leukocyte (PMN) and its functions in the human neonate as the major focus of discussion.

Neonatal phagocyte production

Hematopoiesis in the fetus is initiated in the yolk sac, with the formation of "blood islands" from primitive blood progenitor cells. By the second month of gestation, the fetal liver becomes the major site of blood-cell production, which then extends to include the spleen by five months' gestation. At about the same time, the earliest components of marrow-based blood production appear and continue to become more prominent over the subsequent months leading up to term, while splenic and hepatic hematopoiesis diminish. During this time, pluripotent hematopoietic stem cells are present in the circulation [2], presumably in transit from the hepatic and splenic hematopoiesis sites to the marrow to populate the latter for subsequent blood-cell production during extrauterine life. The proliferative potential of the cells in the circulation at the time of term birth is actually greater than that of adult bone-marrow cells [3], based upon in vitro studies, suggesting that the neonate's myeloid blood-cell production capabilities should be normal. However, at the time of delivery and during the neonatal period, myeloid cell production and kinetics differ from those of older children and adults. The greatest differences are the smaller size and lesser mobilization of the mature PMN storage pool in the neonate [4, 5]. In the infant, the storage pool comprises approximately twice the number of PMNs as are in the circulation, while in older children and adults, the storage pool is approximately ten times the circulating PMN number. While the smaller storage-pool size at term may reflect smaller storage-pool sizes during intrauterine development [6], because cord-blood hematopoietic progenitor cells obtained at term have in vitro expansion capabilities superior to those of adult bone marrow, the smaller storage-pool size is not due simply to diminished proliferative potential.

Instead, it has been suggested that lower production of myeloid growth factors during fetal [6] and neonatal life results in the smaller PMN storage pool of the newborn. When measured in umbilical cord blood from term infants, however, both granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor

(GM-CSF) levels are high [7], leading some authors to hypothesize that, at term, neonatal myelopoiesis is occurring at a near-maximum rate, an impression that has been confirmed in animals [8]. Such accelerated proliferative activity in neonates would predictably affect both neonatal PMN numbers and content in specific situations. With proliferation at near-maximal levels, the neonate's capability to further increase PMN production when stressed would be limited, i.e. marrow PMN storage-pool exhaustion might occur in some situations. Alternatively, because of the rapid production of PMNs, each cell's passage through its sequential differentiational stages might be accelerated, leaving less time for differentiational events to occur, or additional cell divisions at specific maturational stages might lead to diminished content of intracellular organelles. In the latter case, the neonatal PMN's contents of both primary and secondary granules would be lower than those of adult PMNs [8, 9], which could result in deficiencies of function that depend on granule content or mobilization. Although production of mononuclear phagocytes (monocytes) in the fetus and neonate have not been studied extensively, these cells differentiate from colony-forming units common to both PMNs and monocytes (the CFU-GM) and, therefore, would likely have production kinetics similar to those of neonatal PMN, at least through this stage. Regarding fetal and neonatal monocyte differentiation pathways specifically, little is known.

Normal polymorphonuclear leukocyte function

The functional capabilities of PMNs from normal adults are complex and highly integrated (Fig. 11.1). Following production of mature PMNs in the marrow, they are transported into the circulation, where they segregate into either the circulating pool or the marginated pool of cells within the circulation. Normally, these pools contain approximately the same numbers of cells and there is free exchange of cells between them. Marginated-pool PMNs are associated loosely with vessel walls and can be readily mobilized into the circulating pool by adrenergic stimuli [10, 11]. This process, demargination, is a component of the "fight-or-flight" response to threat, providing a rapid increase in the numbers of circulating PMNs capable of participating in any inflammatory responses to injury that might occur. Within the circulation, the PMN has a half-life in the order of six to seven hours under normal conditions [11], but these kinetics can be shortened [12] or prolonged [13] depending upon the clinical situation. Other PMN subpopulations can be identified based upon morphologic, functional, or antigenic criteria [14]. Although the existence of these subpopulations is documented clearly, their physiologic significance is less clear [14]. The marginated pool contains both cells associated loosely with the endothelial surface of the vessels [11] and a second subpopulation of cells that "roll" across the vascular endothelial cell surface. The rolling behavior of PMNs, first appreciated about 15 years ago [15], is mediated by loose interactions between L-selectin on the PMN surface and a variety of glycosylated antigens [16] on the endothelial cell surface. Viewed microscopically, these cells literally roll across the endothelial cell surfaces, slowly propelled by the flow of blood, in a manner that facilitates intimate contact between the PMN and the endothelial cell surface. This contact allows the PMN to closely examine the pattern of surface-antigen expression on endothelial cells (which becomes altered following endothelial cell exposure to a variety of inflammation-related cytokines). Upregulation of chemokine molecules and/or chemokine receptors by cytokine-exposed endothelial cells is sensed by the rolling PMN, resulting in upregulation of leukocyte integrins (predominantly CD11b/CD18) on the PMN surface via secretion of secondary granules. These newly expressed surface integrins interact with their receptors, which include intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2), on both the endothelial cell surface (ICAM-1 and -2) and the surfaces of other rolling, marginated, or circulating PMNs (ICAM-1). This interaction triggers stronger adhesion of PMNs to endothelial cells, stopping their rolling behavior, and also allows PMN–PMN adhesion, referred to as aggregation. When rolling stops, the PMN initiates

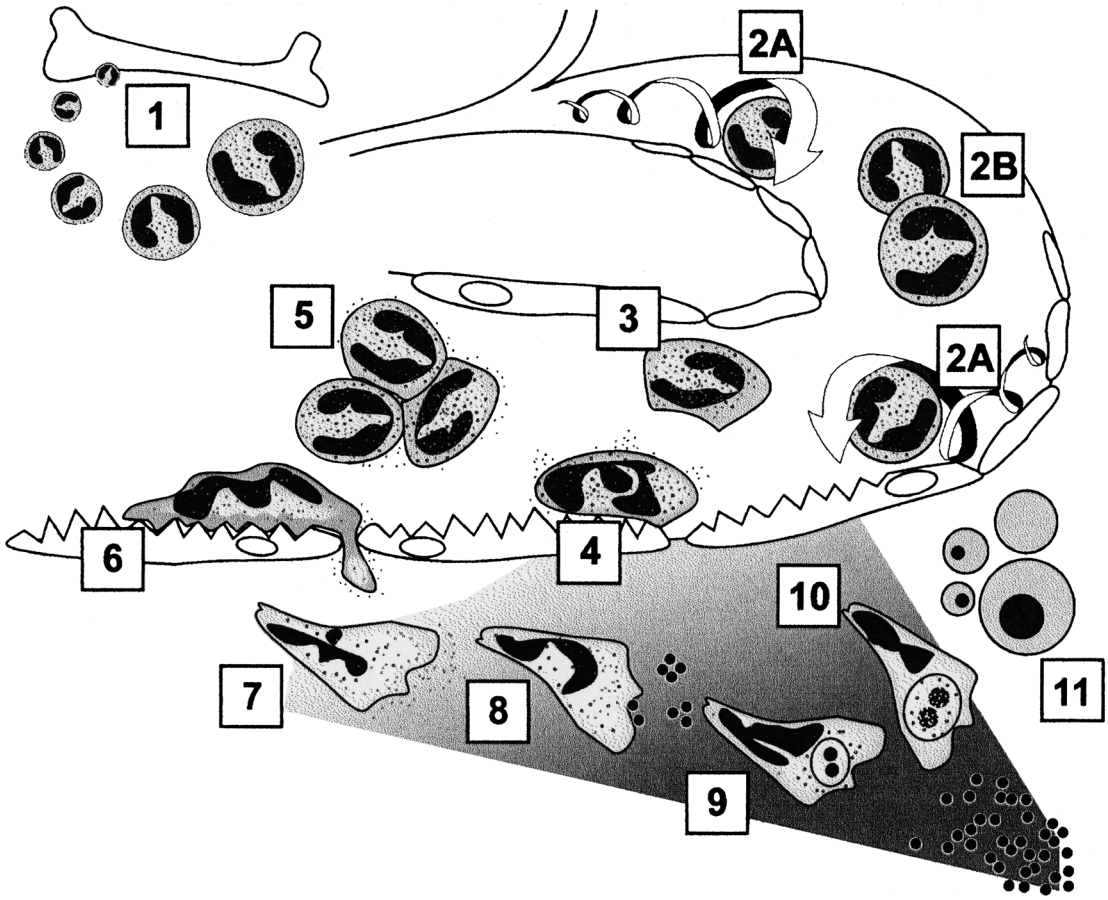

Fig. 11.1 Schematic illustration of components necessary for normal polymorphonuclear leukocyte (PMN) function.
Production in the bone marrow and passage from the marrow into the circulation (1).
Entering either the marginated (2A) or circulating (2B) pools in the circulation.
Signal recognition in the circulation (3) or on the endothelial surface (4).
Intravascular aggregation (5) with secondary granule secretion.
Firm adherence to the endothelial surface and initiation of exudation (6).
Chemotaxis up the gradient of chemoattractants generated by microorganisms, tissue injury or inflammation and continued secondary granule secretion (7).
Recognition of and binding to targets to be phagocytosed (8).
Phagocytosis of targets (9) with activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and degranulation into the phagosome.
Destruction of phagocytosed targets (10).
Apoptosis to clear cells from inflammatory sites following completion of their roles in acute inflammation (11).

movement over the endothelial cell surface (presumably due to signals generated by integrin ligation with ICAM-1) to locate the junctions between endothelial cells. At these junctions, PMN exudation (crossing the endothelial cell layer into the extravascular space) is initiated via extension of pseudopodia through junctions between endothelial cells. These initial probings are followed by extensive deformation by the PMN to allow the entire cell to pass through paraendothelial cell sites, opened by the PMN, that are typically 3 μm or less in diameter. In the same timeframe, aggregated PMNs disaggregate, presumably to follow PMNs already in contact with the vessel surface through the endothelial layer and on to the area where production of inflammation-related cytokines was responsible for the upregulation of endothelial cell chemokine that initiated the process.

Passage from the circulation to the extravascular tissues is accompanied by significant changes in PMN physiology, which reflects their "activation." Limited secretion of secondary granules occurs [17], which causes increased surface expression of opsonin receptors, chemoattractant receptors, low-level assembly of the nicotinamide adenine dinucleotide phosphate (NADP) oxidase complex, shape change, and increased adhesion. Once in the extravascular tissues, exposure to gradients of inflammatory mediators, cytokines, bacterial products, or complement-degradation products diffusing from the site of injury (all of which function as PMN chemoattractants) cause orientation of the PMNs to the gradient and chemotaxis towards the site of injury. Binding of chemoattractants to specific receptors on the PMN surface results in release of Ca^{++} from intracellular stores, producing sharp but transient rises in the free cytosolic Ca^{++} levels. These Ca^{++} transients initiate/modulate intracellular signal transduction via activation of Ca^{++}-dependent enzyme systems or signaling paths to trigger subsequent cellular responses. Closely related to these transient rises in intracellular Ca^{++} is surface-membrane depolarization (loss of surface-membrane potential). Among other effects, membrane depolarization likely alters the functional state of membrane potential-sensitive ion channels in the PMN surface membrane, facilitating influx, efflux, or retention of ions crucial to intracellular function. With exposure to increasing concentrations of chemoattractants as the PMNs move towards the inflammatory site, continued secretion of secondary granules upregulates surface expression of chemoattractant and opsonin receptors, releases signaling molecules and extracellularly active enzymes into the environment, and assembles the NADPH oxidase. The rate of PMN movement increases as the cells sense increasing chemoattractant concentrations; however, rather than plateauing at a maximum rate of movement as concentrations increase towards the site of chemoattractant generation (as would be expected with typical S-shaped dose–response curves), increasing chemoattractant concentrations result in diminished rates of movement (corresponding to a peaked dose–response effect). In high-concentration chemoattractant environments, cellular responses shift away from movement to responses related to microbicidal function (NADPH oxidase activity) and modulation of inflammation (secondary granule release), presumably because the cells have arrived at or near the site of injury. At this site, they will play their roles as microbial killers or modulators of acute inflammation.

To identify and bind to the targets that they will phagocytose, PMNs at the site use opsonins on the surface of the target (deposited via complement activation, immunoglobulin binding, or natural surface expression of lectins). Ingestion of targets small enough to be enclosed by a phagosome, or attachment to and "frustrated phagocytosis" of targets too large to be engulfed, is associated with fusion of lysosomal primary granules with the phagosome and massive assembly and activation of the NADPH oxidase to produce microbicidal reactive oxygen metabolites within the phagosome [18]. Both oxygen-dependent (requiring either reactive oxygen metabolite production or H_2O_2 production by the NADPH oxidase) and oxygen-independent (based on acidification of the phagosome and the activities of digestive, enzymatic, or microbicidal components released from the primary granules)

microbicidal systems are active within the phagosome and work cooperatively to kill or degrade the phagocytosed target. Efficient destruction of ingested targets by PMNs continuously mobilized to the site of injury by propagation of the acute inflammatory response ultimately controls progressive injury. Achieving this control necessitates a shift in the host's response away from the largely defensive innate responses of acute inflammation to both the protective responses of adaptive immunity and the healing-by-scar responses that follow injury. This shift is facilitated by decreasing PMN mobilization to the site through suppression of synthesis of acute inflammatory mediators and active degradation of existing mediators by PMN products [19], and clearance of PMNs already at the inflammatory site via apoptosis [20]. Once acute inflammation wanes, subacute inflammation and its associated healing processes proceed, ultimately repairing damage that occurred at the site of injury and achieving complete resolution of inflammation.

From this summary, it can be appreciated that the inflammation and host-defense-related roles of the PMN are both highly complex and closely integrated with one another. With this understanding, the unusual features and altered behavior of PMNs from neonates can be placed in perspective within host-defense and inflammatory responses.

Normal mononuclear phagocyte function

Monocytes are derived from a precursor colony-forming unit shared with the PMN and develop into monocytes under the influence of colony-stimulating factors other than G-CSF, including interleukin 3 (IL-3), GM-CSF, and macrophage colony-stimulating factor (M-CSF). While it is not known whether a functional monocyte storage pool is present in the marrow, it appears that once monocytes enter the circulation, they manifest many of the same characteristics as PMNs regarding subpopulations within the circulation, circulatory half-life, cellular responses to agonist stimulation, exudation from the circulation, and functional capabilities. In general, monocytes perform the same phagocytic cell functions as PMNs (adherence, chemotaxis, phagocytosis, respiratory burst activity, microbial killing, etc.), but cell for cell, they are quantitatively less capable than PMNs. The greatest difference between monocytes and PMNs is the stage of differentiation at which they enter the circulation from the marrow. At this point, the monocyte is a precursor cell with the potential for further differentiation into a long-lived macrophage/histiocyte once it exits the circulation, while the PMN is a terminally differentiated cell that enters the tissues and dies after it completes its functions there.

Neonatal polymorphonuclear leukocyte function

Neonatal PMN morphology is not grossly different from that of PMNs from older children and adults. However, numerous in vitro functional abnormalities exist in these cells (Fig. 11.2), and although no single abnormality would be considered severe in its degree of effect (relative to recognized PMN dysfunction syndromes), when these abnormalities occur together, they result in significantly altered PMN-mediated host defenses in the neonate.

Adhesion

Neonatal PMNs have abnormalities that alter the initial events necessary for PMN exudation from the circulation at both PMN–endothelial cell and PMN–PMN levels. Normal PMN "rolling" over endothelial cells occurs via leukocyte L-selectin interaction with multiple carbohydrate-bearing antigens [16] on the endothelial cell surface and, conversely, via interactions between E-selectin and P-selectin on endothelial cells and their glycoprotein counter-receptors on PMNs. In neonatal PMNs, the content [21–23], surface expression, and shedding of L-selectin following exposure to selected PMN agonists are all diminished. In addition, the total cell content of the CD11b/CD18 antigens that mediate strong adhesive interactions between both PMNs/endothelial cells (needed to initiate exudation) and PMNs/PMNs

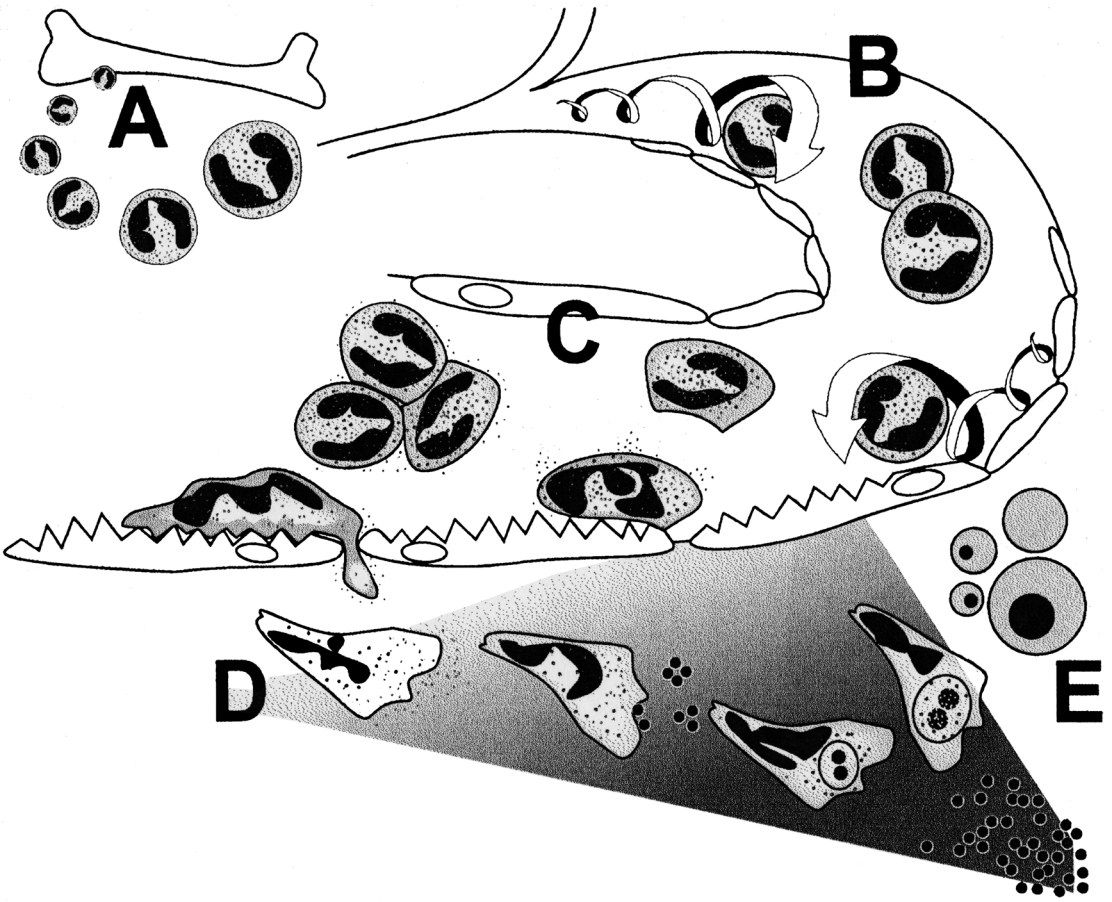

Fig. 11.2 Schematic illustration showing sites of abnormality in neonatal polymorphonuclear leukocyte (PMN) production and function.

A. Maximal stimulation of production by colony-stimulating factors (CSFs); fewer cell divisions during differentiation, resulting in diminished primary and secondary granule content; diminished mature PMN storage pool.
B. Diminished L-select in expression, possibly altering normal PMN-endothelial cell contacts during rolling.
C. Diminished leukocyte integrin expression and upregulation with stimulation, diminished aggregation responses; diminished assembly of actin cytoskeleton upon stimulation.
D. Diminished chemotactic activity; diminished microbicidal activity against selected microorganisms.
E. Altered PMN survival.

(that mediate aggregation) are decreased in both neonatal PMNs [23–28] and eosinophils [28] at term, and are even lower in neutrophils of premature infants [27]. Deficiencies in adhesive proteins would be expected to result in less efficient PMN rolling behavior over endothelial cells, altered aggregation responses, altered exudation, and possibly altered adherence responses in neonatal PMNs. Studies directly examining in vitro neonatal PMN aggregation, adherence, and exudation have shown abnormalities in all of these areas: neonatal PMN aggregation responses are slow and the usual deaggregation responses seen in PMNs from adults are absent [29]. Neonatal PMN exudation through

human umbilical-cord endothelial cell monolayers is poor [30], and neonatal PMN adherence to a variety of artificial and physiologic surfaces are all abnormal [30–32].

Surface-antigen heterogeneity, surface-receptor expression, and granule content

The fact that CD11 b/CD18 antigens are normally stored in the specific granules and are upregulated on the PMN surface when secondary granule secretion occurs suggests that the secondary granule content or function in neonatal PMNs may be deficient. Consistent with this hypothesis, neonatal PMN Fc receptor function is depressed [31] and, when examined specifically, the FcRIII content (another receptor contained in the secondary granule) of neonatal PMNs is low [25, 33]. The severity of this FcRIII content deficiency is related to gestational age [34]. When the content of each granule type has been examined in neonatal PMNs, abnormalities in all granule types have been reported. The myeloperoxidase content (primary granule marker) of neonatal PMNs (age 4–17 days), but not cord-blood PMNs, is low. This condition has been ascribed to the possibility that an additional cell division at the myelocyte stage occurs before progressing to the metamyelocyte stage, leaving each daughter cell with 50% of the normal myeloperoxidase content [35]. Both secretory vesicles (tertiary granules) and secondary granules also are deficient in neonatal PMNs [36], and both the content [37] and the release [38] of lactoferrin (secondary granule marker) are deficient in neonatal PMNs. These granule content and function deficiencies would be expected to adversely affect PMN functions requiring granule mobilization. Primary granules normally participate in microbicidal activity of PMNs within the phagosome, while both secondary granules and secretory vesicles function at the PMN surface and extracellularly via upregulation of surface receptors/membrane components and release of signaling and inflammation-regulating components, respectively. The functions most clearly dependent on secondary granule secretion are adherence (for upregulation of adhesive proteins, as noted above) and chemotaxis (for upregulation of chemoattractant receptors). Considering this, deficient chemotaxis responses in neonatal PMNs would not be surprising.

Chemotaxis

Chemotaxis by adult PMNs requires multiple cellular activities to be performed in a coordinated fashion. The cell must sense the chemoattractant gradient to determine the direction for movement, transduce this information to the locomotive machinery to initiate movement, precisely disassemble/reassemble the cytoskeleton to allow orientation within the gradient and movement of the cell, fluidize and deform its surface membrane to accommodate cellular shape change during movement, and continuously secrete granules to upregulate receptors for gradient sensing. With this level of complexity, it is not surprising that, when examined as an in vitro cellular function, assays for chemotaxis must be appropriate for its measurement.

Most studies of neonatal PMN chemotactic responses have reported lower responses than are usually observed in older children and adults [39–45]. One report notes normal locomotive responses in neonatal PMNs and monocytes at birth [46], but used an assay method (polycarbonate filter method) more appropriate for assessing monocyte chemotaxis than PMN chemotaxis [47]. While these authors found normal chemotaxis responses at birth, decreased neonatal PMN chemotaxis was observed by the sixth postpartum day and persisted to at least six months. Deficient chemotactic responses in neonatal PMNs are present regardless of the chemoattractant used [41, 43], implying a cellular rather than a stimulus-related abnormality. When temporal evolution of PMN chemotaxis from birth through six years of age [45] was examined, normal (adult) PMN chemotactic responses were achieved between two and five years of age. The basis for abnormal locomotion in neonatal PMNs is likely multifactorial. There do not appear to be differences in adult versus neonatal PMN deformability [48].

Studies using PMN subclass-specific monoclonal antibodies have shown that preparations of neonatal PMNs contain populations of viable, but relatively immotile, PMNs [49]. Membrane fluidity in neonatal PMNs differs from that of adult PMNs, but reports of both greater and lesser fluidity exist. Both excessively [50] and insufficiently [51] fluid surface membranes have been reported in neonatal PMNs, with both abnormalities affecting neonatal cell movement negatively. No resolution of these disparate results has been made.

Cytoskeleton

Cytoskeletal dynamics in neonatal PMNs are markedly different from those of adult PMNs. Actin polymerization responses are defective to chemoattractants [52] but are normal to nonphysiologic stimuli [53]. Microtubule assembly is defective compared with that in PMNs from adults [54] and is associated with abnormal neonatal PMN orientation in chemoattractant gradients. This abnormality in microtubule assembly is likely the cause of the defective concanavalin-A capping present in neonatal PMNs [55].

Signal transduction

An additional area of abnormality in neonatal PMNs that could contribute to abnormal chemotactic responses, as well as other altered cellular functions, is alteration of intracellular signal transduction. Studies with neonatal cells have shown that in response to chemoattractant stimulation, neonatal PMNs have impaired synthesis of inositol triphosphate second messengers [56] and strikingly deficient intracellular free Ca^{++} release and defective membrane-potential responses following stimulation [57]. However, defective membrane-potential responses were not confirmed by other authors [44], and Ca^{++} uptake following stimulation of neonatal PMN has been reported as normal [58], leaving open the question of whether abnormal Ca^{++} and membrane-potential responses to chemoattractant stimulation are present in neonatal PMNs.

Phagocytosis and bacterial killing

Microbicidal functions of neonatal PMNs have been scrutinized closely, with most emphasis being placed on the phagocytic and respiratory burst components of this function. Because effective phagocytosis is heavily dependent on sufficient opsonization of the target, the opsonic capabilities of neonatal serum also have received attention. The phagocytic and microbicidal activity of PMNs from healthy neonates are normal [59, 60]; however, in ill infants, phagocytosis and killing may be compromised due to opsonic defects in these infants. Confirmatory studies have shown that stressed neonates have bactericidal defects [61]; also, altering either the phagocytosis or the microbicidal assay conditions used (e.g. changing the PMN : target ratio, target organism, or opsonin source) can have dramatic effects on the assay results [62]. When 100 organisms/neonatal PMNs were used, phagocytic and microbicidal defects were observed, while lower PMN : target ratios produced normal results. In other experiments, type III *Streptococcus agalactiae* [63] and *Staphylococcus aureus* [64] were killed normally by neonatal PMNs, while use of type I *S. agalactiae* [65] demonstrated a microbicidal defect. Use of cord-blood serum rather than adult serum as the source of opsonin is often associated with defective phagocytosis [66], with both the classical pathway [67] and alternative pathways of complement [68] implicated as responsible under different conditions. Microbicidal activity in PMNs from preterm infants has been examined less extensively but appears to be abnormal [69]. Studies of neonatal monocyte phagocytosis and killing also have shown abnormalities [64, 70] in this cell type.

Stress effects

The observation that phagocytosis and microbicidal activity in ill or stressed neonates is deficient because of lower opsonic activity in the infant's plasma prompted examination of whether stress altered other neonatal PMN functional capabilities. Although production of chemiluminescence by PMNs is based on a number of events in the

PMN, including respiratory burst activity, myeloperoxidase activity, and oxidation of membrane lipids [71], this nonspecific measure of PMN "function" has been used to examine the effects of mode of delivery on neonatal PMN activity [72]. Lower chemiluminescence responses were observed in stressed neonates. When other functions were looked at subsequently, chemotaxis [73] was also depressed in stressed infants compared with normal term infants. Interest in whether the mode of delivery (Cesarian section with or without labor versus labor with vaginal delivery) might contribute to the stress of the infant and, therefore, lead to alteration of neonatal PMN function led to a series of studies with confusing results. Chemotaxis responses of Cesarian-delivered infants were better than those from vaginally delivered infants [74]. Infants exposed to labor followed by either Cesarian section or vaginal delivery had higher rates of resting oxygen consumption, spontaneous nitroblue tetrazolium (NBT) reduction, and hexose monophosphate shunt activity than infants born by Cesarian section without labor [75]. PMNs from infants delivered by spontaneous vaginal delivery had greater adherence to glass than PMNs obtained from infants born by Cesarian section [76]. These observations are consistent with labor-induced stress altering neonatal PMN functions. However, a fourth study [77] was unable to find any effect of labor or mode of delivery on PMN function in healthy term neonates. Other factors, e.g. acidosis, hypoperfusion, and/or infection, were thought to influence neonatal PMN function to a larger extent than the mode of delivery. Consistent with this concept, circulating PMNs obtained from infants with idiopathic respiratory distress syndrome show evidence of cellular "activation" [78], which would be consistent with the observation that PMNs from stressed infants are altered relative to PMNs in the normal newborn.

Respiratory burst activity

In parallel with examination of microbicidal activity in neonatal PMN, respiratory burst activity of these cells also has been examined by numerous authors. Depending upon the methodologies used, variable results have been reported. Using chemiluminescence methods to measure oxidative "function" (despite this being comprised of several distinct components [71]), PMNs from stressed neonates and severely preterm infants have low chemiluminescence responses [21, 79, 80]. However, when specific products of the respiratory burst have been measured, both fetal and neonatal PMN production of superoxide is normal or increased [80–83] while production of hydroxyl radicals is reduced significantly [80]. In addition, neonatal PMNs have low levels of superoxide dismutase [84], which could place them at risk for oxidative injury during respiratory burst activity. The presence of an intact respiratory burst in term infants, perhaps with diminished activity in stressed or preterm infants [85], is consistent with intact microbicidal activity of PMNs from term infants, with diminished activity in stressed or preterm infants.

Viability

With phagocytosis and killing of the target organism, the functional role of the individual PMN is completed, and this effete cell then either lyses at the inflammatory site to contribute to and further propagate the local acute inflammatory response or is cleared from the site of injury. As the acute inflammatory cell, clearance and/or removal of the PMN presents a problem because of its inflammation-propagating and tissue-injury potentials. It appears likely that removal of PMNs from inflammatory sites occurs via induction of apoptosis to utilize a noninflammatory mechanism for cell clearance [20]. Although not studied as intensely as other aspects of neonatal PMN function, two studies of neonatal PMN viability have been published and present conflicting results. The earlier report documents decreased viability in neonatal PMNs [86], while the second notes resistance to apoptosis [87] in neonatal PMNs. To date, the subject is unresolved.

Antiviral and cytotoxic activity

Antiviral and cytotoxic activities of neonatal PMNs have been examined in several different systems, although clinically significant viricidal or cytotoxic activities usually are not ascribed to PMNs. Antibody-dependent cellular cytotoxicity of neonatal PMNs against herpes simplex virus (HSV)-infected cells in vitro is equal to that of adult PMNs [88], as is direct cytotoxic activity [89]. However, in vitro antiviral activity against HSV [90] is said to be depressed.

Drug effects on neonatal polymorphonuclear leukocytes

The functional abnormalities present in neonatal PMNs are assumed to contribute significantly to the increased severity and frequency of bacterial infections in the newborn and, therefore, have been the focus of studies examining whether the functional defects can be reversed pharmacologically. The immunomodulatory methylxanthine derivative pentoxifylline, an agent that enhances erythrocyte deformability, has been examined in vitro by two groups for its effects on neonatal PMN function and was found to ameliorate neonatal PMN functional defects. In studies examining a variety of neonatal PMN functions [91, 92], pentoxifylline exposures in the 40–100-μg/ml range in vitro improved chemotaxis and membrane-potential responses and decreased F-actin assembly and H_2O_2 production in neonatal PMNs. In a study examining clinical use of pentoxifylline in neonatal sepsis, the drug had beneficial effects on both survival and a variety of physiological parameters that become abnormal in neonatal sepsis [93]. To date, pentoxifylline has not been examined in vivo for its effects on PMN function. The effects of several cytokines on neonatal PMN function also have been examined. In vitro interferon gamma (IFN-γ) exposure improves neonatal PMN chemotaxis responses and intracellular free Ca^{++} responses to adult PMN levels [94]. When colony-stimulating factor exposure effects were examined by these same authors, no effect of either GM-CSF or G-CSF was observed, in contrast to other studies [44, 95], which observed that in vitro GM-CSF exposure significantly improved neonatal PMN chemotaxis and superoxide production.

Other agents to which newborn infants are often exposed also have been examined for their effects on neonatal PMN function, including indomethacin and intralipid. Indomethacin is often used to achieve closure of patent ductus arteriosus. Indomethacin exposure in vitro results in dose-related suppression of spontaneous movement and chemotaxis in preterm, term, and adult PMNs [96]. Whether indomethacin has a significant effect on PMN function when used in vivo in the newborn is unknown. Lipid emulsions, because of their caloric densities, are used frequently in vivo to facilitate parenteral alimentation of the sick neonate; their use has been associated with increased incidences of bacteremia, particularly those caused by coagulase-negative staphylococci [97]. The mechanism proposed for this effect is ingestion of lipid by blood phagocytes, which blocks their normal phagocytic and bactericidal functions [98].

Neonatal mononuclear phagocytes

Compared with neonatal PMNs, mononuclear phagocytes of newborn infants have been examined less extensively. Deformability characteristics of neonatal monocytes are not different from those of adult monocytes [48]. Surface expression of selected antigens on cord-blood monocytes varies. Total Fc and C3b receptor expression on neonatal monocytes approximates that of adult monocytes [68], although recent studies have suggested that the FcRII class is expressed on lower percentages of cord-blood monocytes than on adult blood monocytes [99]. Surface L-selectin expression is normal [23], while upregulation of CD11b (Mac-1) expression is defective [23], similar to neonatal PMNs.

In the face of acute infection, neonatal monocytes show low expression of L-selectin, also similar to neonatal PMNs [21]. Monocyte chemotaxis is most depressed at birth [45]. It then improves slowly across early childhood, remaining abnormal through age six years. Phagocytosis and microbicidal abilities of neonatal monocytes vary. Phagocytosis of polystyrene beads by adherent neonatal monocytes is significantly less than that of adult monocytes [100], while phagocytosis of many physiologic particulates is normal; uptake of *Escherichia coli*, *Streptococcus pyogenes*, and *S. aureus* is equal to that by monocytes of adults [68], and killing of *E. coli* and *S. pyogenes* is equivalent. However, killing of *S. aureus* is slower than in adult monocytes, and both uptake and killing of *S. agalactiae* are seriously suppressed [68]. Respiratory burst activity in both cord-blood monocytes and monocyte-derived macrophages and random movement, chemotaxis, and ingestion/killing of *S. aureus* by neonatal monocyte-derived macrophages from cord blood all are comparable to the functional capabilities of adult monocytes [101, 102]. When effects of prematurity on neonatal monocyte surface antigen expression were examined, fewer monocytes expressing FcRII, FcRI, and CD14 were found in premature infants compared with adults. At the same time, larger proportions of premature monocytes express FcRIII and CD67 than do monocytes from adults [34]. Functionally, adherent monocytes from premature infants have significantly lower degranulation responses, superoxide production, surface CD11b/CD18 expression, and tumor necrosis factor α (TNFα) production compared with adherent monocytes from term infants [103].

The ability of blood monocytes to develop into monocyte-derived macrophages during in vitro cultivation has allowed selected neonatal macrophage functional capabilities to be examined. Killing of *Candida albicans* by neonatal in vitro derived macrophages is equivalent to that of adult in vitro derived macrophages, but neonatal cells do not respond to activation by IFN-γ either with enhanced candidacidal activity or increased superoxide production [104]. Other functions of neonatal monocyte-derived macrophages are equivalent to the capabilities of the same cell type derived from adult monocytes [101, 102].

Conditions altering neonatal leukocyte kinetics

Normal neutrophil numbers

The number of PMNs normally in the circulation of the newborn infant varies by birth weight and postpartum age [105]. At birth, absolute numbers of PMNs are 1800–5500/mm^3, which increases three- to five-fold over the next 12–18 hours of life. By 24 hours of life, the absolute PMN count begins to fall in the normal newborn, decreasing steadily to 1800–7200/mm^3 at five days, from whence it falls to and remains at 1800–5400 cells/mm^3 through 28 days of age. The mode of delivery, prolonged rupture of the fetal membranes without maternal fever, fetal bradycardia, meconium staining without lung disease, and uncomplicated transient tachypnea of the newborn have no effect on these dynamics [105]. In very-low-birth-weight (VLBW) infants, the normal ranges for leukocyte counts have lower minimum values [106]. From birth to 18–20 hours of age, normal absolute numbers of PMNs are 500–2200/mm^3; by 60 hours of age, this decreases to 1100/mm^3. From 60 hours to 28 days of age, the normal range for absolute numbers of PMNs in VLBW infants is 1100–6000/mm^3. These lower normal ranges must be considered when neutropenia is considered as a criterion for either therapeutic or diagnostic decision-making in premature infants.

Neutropenia

Table 11.1 lists causes of neutropenia in the newborn. Peculiar to the newborn are conditions in which maternal antibodies that recognize neonatal PMN antigens, or placental factors that alter neonatal PMN production, are passed to the infant. In alloimmune and autoimmune neutropenias, transplacentally passed maternal antibodies destroy PMNs via mechanisms similar to those occurring in hemolytic

Table 11.1 Conditions associated with neutropenia in the newborn (see Manroe *et al.* [105] and Mouzinho *et al.* [106] for definitions of neutropenia)

Immune
Autoimmune neutropenia in the mother
Alloimmune/isoimmune neutropenia
Non-immune
Maternal pre-eclampsia
Infection
Bacterial
Perinatal viral
Periventricular hemorrhage
Asphyxia
Drug reaction
Metabolic disorders
Isovaleric acidemia
Propionic aciduria
Methylmalonic aciduria
Glycogen storage disease 1B
Orotic aciduria
Hyperglycinemia
Ineffective granulopoiesis
Nutritional
Hyperalimentation-associated
Kostmann's disease (severe congenital agranulocytosis)
Pure white-cell aplasia
Reticular dysgenesis
Cartilage-hair hypoplasia
Hyper-IgM syndrome
Schwachman–Diamond–Oski syndrome
Dyskeratosis congenita
Component of congenital aplastic anemia syndrome

IgM, immunoglobulin M.

disease of the newborn. The antibodies reactive with neonatal PMNs are always present in the maternal serum, providing a method for diagnosing these conditions, and the neutropenia may last up to six months while maternal antibody clears from the infant's plasma. Immune neutropenias occur in approximately 3 in 1000 live births and are often responsive to IVIg or G-CSF [107].

A second cause of neonatal neutropenia related to the maternal–fetal relationship is pregnancy-associated maternal hypertension. In comparison with infants of normotensive mothers who develop neonatal neutropenia, the neutropenia in these infants persists significantly longer [108], apparently due to placental production of an inhibitor of myelopoiesis [109].

Inherited congenital neutropenic conditions are a second group of disorders that can present during the neonatal period. These congenital syndromes are rare, usually involving decreased PMN production. Kostmann disease (severe congenital agranulocytosis) is a severe neutropenic condition present from birth in which the bone marrow shows either absence of myeloid progenitors or maturation arrest at the promyelocyte/myelocyte stages. Abnormalities of the G-CSF receptor are present in some patients with this condition [110]. Pure white-cell aplasia is, as the name implies, a condition in which the myeloid lineage is completely absent from the marrow while other lineages are represented adequately. Reticular dysgenesis, the triad of severe neutropenia, lymphoid dysplasia/lymphopenia, and agammaglobulinemia, appears to be the result of defective hematopoietic stem-cell development. Several other congenital immunologic syndromes (cartilage-hair hypoplasia, hyper-immunoglobulin M (IgM) syndrome, X-linked agammaglobulinemia) also may have neutropenia associated with them.

Schwachman–Diamond–Oski syndrome (neutropenia, metaphyseal chondrodyplasia, dwarfism, and exocrine pancreatic insufficiency) and dyskeratosis congenita (reticulated hyperpigmentation of the skin with nail dystrophy) are conditions in which neutropenia occurs in association with other phenotypic abnormalities and present in the neonatal period. Identification of their accompanying abnormalities establishes the diagnosis.

Acquired forms of neutropenia also occur in the neonatal period. Certain conditions, including infection, periventricular hemorrhage, and asphyxia, can either depress or elevate PMN numbers. Drug sensitivities, metabolic disorders, and ineffective granulopoiesis also can result in acquired neutropenia.

Neutropenia in the newborn can either predispose to infection [111] or be the result of infection. Because infection-induced neutropenia can be

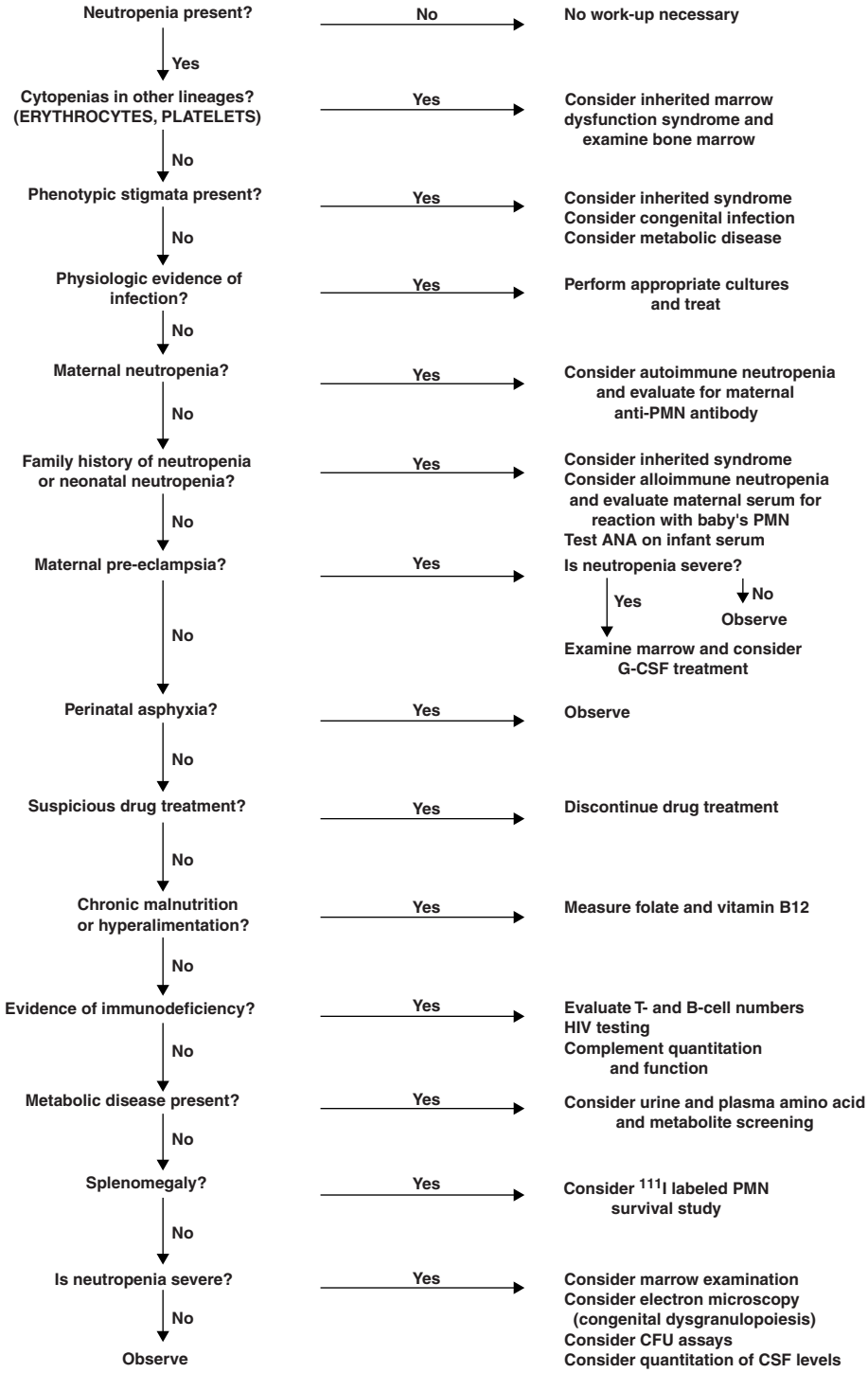

Fig. 11.3 Algorithm for evaluation of neonatal neutropenia. ANA, antineutrophil antibody; CFU, colony-forming unit; CSF, colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; HIV, human immunodeficiency virus; PMN, polymorphonuclear leukocyte. Infection should be considered in any acutely ill neonate with neutropenia.

associated with marrow PMN storage-pool depletion [112], the ratio of immature to total PMNs (I/T ratio) seen in the peripheral blood is suggested to be a useful predictor of infection. At birth, this ratio is 0.16 or less and slowly falls to 0.12 or less by five days in the healthy infant [105]. When examined simultaneously in septic infants, the I/T ratio in the peripheral blood correlates well with depletion of the marrow storage pool [113], such that an I/T greater than 0.75 predicts a neutrophil storage pool of less than 10% (normal 22–57%) in these infants. However, studies of the I/T ratio as a predictor of sepsis in neonates have shown it to have variable sensitivity (34–86%) and specificity (47–85%) when used alone [114–117], most likely because infection is not the only stimulus for mobilization of immature neutrophil forms in the neonate. An algorithm for the evaluation of neonatal neutropenia is given in Fig. 11.3.

Neutrophilia

Elevation of the numbers of neutrophils, neutrophilia, typically is a nonspecific response to stress in the neonate and can occur with many different conditions (Table 11.2). Of note, some conditions (e.g. infection) can result in either neutropenia or neutrophilia, depending on the individual patient, and this must be considered if either neutropenia or neutrophilia is encountered.

Absence of monocytes in the peripheral blood of neonates may be normal through 120 hours of age [117]. Through 28 days of age, the absolute numbers of monocytes remain relatively constant in the normal infant (0–1900/mm^3) but may become elevated during the early phases of ABO incompatibility or the recovery phase following sepsis, as often occurs in older children [117].

Table 11.2 Conditions associated with neutrophilia in the newborn (infants: >7000 cells/ μl; premature infants: >13 000 cells/μl)

Infection
Periventricular hemorrhage
Stressful labor
Asphyxia
Seizures
Pneumothorax
Meconium aspiration
Hemolytic disease
ABO incompatability
Symptomatic hypoglycemia
Leukemoid reaction
Congenital anomalies
Tetrology of Fallot
Dextrocardia/absent radii
Rudimentary little toes
Amegakaryocytic thrombocytopenia
Leukocyte adhesion deficiency

Eosinophilia

Eosinophilia is common in both preterm and term infants, with estimates of frequency varying from 13% to 75% [118, 119]. Despite age-related changes in blood eosinophil content in the neonatal period [120], eosinophilia is most frequently defined as more than 700 eosinophils/mm^3. As production of eosinophils is controlled largely by interleukin 5 (IL-5), interleukin 3 (IL-3), and GM-CSF, eosinophilia usually is independent of neutrophilia/neutropenia. Under basal conditions, eosinophils comprise less than 4% of peripheral blood leukocytes [121]. Over the first 12 hours following birth, mean eosinophil numbers are 250–300 cells/mm^3; over the ensuing five days, eosinophil numbers rise, but can vary widely from 100 to 2500/mm^3 [121]. Premature infants may have a higher incidence, greater severity, and longer duration of eosinophilia than term infants [119], but this has not been observed in all studies [120, 122]. Conditions associated with eosinophilia in the newborn are listed in Table 11.3. Of note, neonatal eosinophilia has been proposed as a marker for infant growth, as it may appear close to the time that birth weight is reattained [122]. An association with infection has also been reported in 78–100% of eosinophilic infants [118]. Pre-existing eosinophilia often decreases at the onset of infection [119] and may appear or increase over several days following the onset of infection [118], suggesting

Table 11.3 Conditions associated with eosinophilia in the newborn infant (modified from Sullivan and Calhoun [121]).

Most common
Infection (bacterial, viral, fungal)
Days of antibiotic therapy
Necrotizing enterocolitis
Anabolic state
Drug reactions (L-tryptophan, ceftriaxone)
Parenteral alimentation
Congenital heart disease
Chronic lung disease
Prolonged length of hospital stay
Uncommon
Hematologic condition
Hypereosinophilic syndrome
Eosinophilic leukemoid reaction
Eosinophilic leukemia
Thrombocytopenia/absent radii (TAR) syndrome
Congenital neutropenia with eosinophilia
Congenital immunodeficiency disorders
Hyper-IgE syndrome
Omenn syndrome
Familial eosinophilia
Cows' milk allergy

IgE, immunoglobulin E.

that it is responsive to infection rather than predictive of or predisposing to it. Because in most instances neonatal eosinophilia is more a curiosity than a significant clinical problem, in the absence of associated symptoms, the medical evaluation of eosinophilia should usually be limited to follow-up physical examinations and following the absolute eosinophil count.

Neonatal disorders with phagocytic cell dysfunction

Because neonates have inherent functional defects in their PMNs and monocytes, and also have higher rates of infection than older infants and children, the clinical manifestations that are the hallmark of phagocytic cell-dysfunction syndromes in older children may be obscured by the normally elevated background rates of infection in the normal neonate. Therefore, as many of the inherited phagocytic cell disorders can present during the neonatal period, it is necessary to have a relatively low threshold for specific evaluation for these conditions, as early recognition of these conditions facilitates their management and/or palliation/treatment. The diagnosis of inherited phagocytic cell-dysfunction syndromes during the neonatal period is also more difficult than in older children or adults because of the limited blood volume that can be taken from the neonate for diagnostic studies.

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a rare syndrome related to deficient or absent NADPH oxidase function in phagocytic cells. Multiple variants of the disorder exist because of the complex composition of the NADPH oxidase complex, with about 60% of cases showing an X-linked recessive inheritance pattern [123]. Overall, approximately 80% of cases occur in males. Intrauterine diagnosis by either molecular or functional methods is possible when the condition is known to occur in a kindred [124]; more frequently, however, the diagnosis is suspected because of a clinical presentation of severe, often recalcitrant infections with catalase-positive bacteria or fungi, which can begin during the neonatal period. Because of the inherent abnormalities in phagocyte function present in neonates, recognition of the clinical presentation of CGD during the neonatal period is difficult. Definitive diagnosis is based on demonstration of absent or severely deficient respiratory burst activity in phagocytes (quantitative superoxide or hydrogen peroxide assay), but because of the small size of the infant, a presumptive diagnosis based upon absent NBT reduction or flow-cytometric demonstration of deficient oxidant production by blood phagocytes may need to suffice as the diagnostic criteria during the neonatal period. In a neonate, a slide test format for assessing phorbol myristate acetate (PMA)-stimulated NBT reduction can be performed on small volumes (0.1 ml) of

Table 11.4 Method for performing a slide nitroblue tetrazolium (NBT) test

1. 0.1–0.5 ml of fresh whole blood is placed on one end of a glass microscope slide. The slide is held in a humidity chamber for 30–45 minutes at 37°C. Samples from a normal control and the patient are processed in parallel. (A simple humidity chamber can be constructed from a Petri dish with a circle of moist paper towel on the floor of the Petri dish, placing a length of wooden applicator stick on the moist paper towel, and placing the blood-loaded microscope slide into the Petri dish with the nonblood-bearing end supported on the applicator stick to keep the blood pooled at the opposite (lower) end of the slide while it clots. Allowing the blood droplet to spread out or dry on the slide during this incubation make interpretation of test results difficult and impossible, respectively.)
2. After the blood is clotted, the edge of the clot is loosened from the slide with a wooden applicator stick and the clot gently worked free of the slide (taking care to minimize scraping of the slide surface where the clot has formed) and removed. Normal saline is used to rinse loose erythrocytes from the slide, leaving a layer of adherent phagocytes on the glass where the blood has clotted.
3. The layer of adherent phagocytes is gently flooded with NBT solution (0.1% NBT in saline, with 20 ng/ml of phorbal myristate acetate (PMA) added to the NBT), taking care to keep the NBT/PMA solution from draining off the slide. The slide is placed in its humidity chamber at 37°C for 15 minutes.
4. At the end of this incubation, the NBT/PMA solution is drained on to the moist towel disk on the bottom of the humidity chamber. The slides are rinsed briefly with saline and allowed to air-dry. After drying, safranine (from a Gram stain solution set) is used to stain the adherent cells and the slide is examined microscopically. NBT reduction by adherent PMN appears as blue-purple formazan staining of individual cells, which is absent in chronic granulomatous disease.

whole blood obtained by venipuncture or even heel-stick (Table 11.4). Because of the absent respiratory burst activity in CGD phagocytes, microbial killing of catalase-positive organisms is deficient, and inflammatory responses are abnormal due to failure to normally extinguish acute inflammation. The latter is thought to underlie the tendency towards development of granulomatous inflammation in CGD and to explain the poor healing responses typically seen in these patients.

Leukocyte adhesion deficiency

Leukocyte adhesion deficiency (LAD) exists in two forms, LAD-1 (Mac-1 deficiency, leukocyte integrin deficiency, CD11/CD18 deficiency), which is due to deficiency or dysfunction of leukocyte integrins, and LAD-2, which is due to congenital deficiency of selectin function. LAD-1 is a rare inherited phagocytic cell disorder that can present in the neonatal period with severe infections, delayed separation of the umbilical stump, and leukocytosis. The biochemical abnormalities responsible are significant deficiency or total absence of leukocyte integrins (CD11b/CD18) from phagocytic cells or expression of dysfunctional leukocyte integrins, all of which result in deficiencies of adhesion-related functions in phagocytic cells. Two predominant phenotypes of LAD-1 exist, moderate and severe [125], representing deficiency and absence of the leukocyte integrins, respectively. LAD-2 is a more recently described, but less frequent, condition in which absence of selectin receptors on leukocytes and endothelial cells is due to an inability to form fucose from glucose or mannose [126]. The neonatal presentation of this condition may include intrauterine growth retardation, failure to thrive, and leukocytosis, with ultimate development of characteristic facies, severe periodontal disease, and leukocytosis. One study suggests that dietary supplementation with fucose may ameliorate these findings [127]. As noted above for CGD, recognition of an infectious presentation of LAD-1 or -2 may be difficult because of the frequency of infection in the normal neonate. In addition, because normal neonatal PMNs and monocytes both have relative deficiencies of leukocyte integrins and selectins, the genetic disorders of LAD-1 and -2 can almost be considered further extensions

of the phagocytic cell abnormalities present in the normal neonate. Noninfectious clues are likely to be most helpful in identifying LAD-1 and -2 during the neonatal period. Presumably because of the deficient adhesion-related functions in LAD PMNs, the marginated pool size in LAD patients is minimal, resulting in continuous leukocytosis and neutrophilia. Delayed separation of the umbilical stump, sometimes requiring surgical removal, is a frequent finding in LAD-1, while intrauterine growth retardation, failure to thrive, developmental delay, and the Bombay phenotype (H-null type) for erythrocytes are seen in LAD-2. Diagnosis is based upon demonstration of moderate or severe deficiency of leukocyte integrins (using anti-CD11b or anti-CD18 antibodies) on blood phagocytic cells of LAD-1, and of absence of sialylated CD15 leukocyte antigens in LAD-2, using flow-cytometric methods.

While delayed separation of the umbilical stump is seen commonly in LAD, it can also occur in normal infants whose umbilical-stump hygiene is excessive. Recent studies have confirmed that repeated daily applications of triple dye [128] and use of isopropyl alcohol [129] significantly delay separation of the cord; anecdotally, combined use of these agents by compulsive mothers can lead to umbilical-stump "mummification." In these instances, less aggressive local care of the stump usually results in subsequent separation. Of note, two other conditions also have been associated with delayed separation of the umbilical stump: alloimmune neutropenia [130] and subclinical omphalocele [131].

Myeloperoxidase deficiency

Myeloperoxidase (MPO) deficiency is the most common phagocytic cell-deficiency condition [132]. However, because it is usually clinically silent, it often remains unrecognized. Diagnosis is based upon demonstration of absent peroxidase activity in PMNs, an uncommon laboratory test except when automated differential count equipment is in use [132]. The clinical silence of MPO deficiency appears to be related to the function of MPO in PMNs: catalytic destruction of H_2O_2 in the presence of chloride to produce hypochlorous acid in the phagolysosome. In MPO deficiency, production of reactive oxygen metabolites, including H_2O_2, is enhanced because MPO activity appears to be important for normal termination of the respiratory burst [133]. It is this enhanced respiratory burst that mediates killing of most microorganisms, even in the absence of MPO. The exceptions to this rule are *Candida* species, which require MPO activity for their efficient killing by phagocytes [132]. Therefore, severe, recalcitrant or overwhelming infection with *Candida* species in the neonate may be the presenting manifestation of this phagocytic cell-deficiency syndrome.

Chediak–Higashi(–Steinbrinck) syndrome

Chediak–Higashi(–Steinbrinck) syndrome (CHS) is a rare disorder of intracellular packaging functions that manifests clinically as recurrent pyogenic infections, oculocutaneous albinism, and giant intracellular granules, often first appreciated in blood leukocytes. Inherited as an autosomal recessive condition, it occurs in at least four other animal species beside humans (mink, cow, mouse, orca). While a series of characteristic biochemical abnormalities are present in CHS phagocytes (delayed granule–phagosome fusion, elevated intracellular cyclic adenosine monophosphate (cAMP), delayed microbicidal activity), diagnosis usually is based upon the presence of giant intracellular granules on blood leukocytes.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency

Severe glucose-6-phosphate dehydrogenase (G6PD) deficiency is an uncommon form of the more typical G6PD deficiency. In this form, profound G6PD deficiency in leukocytes depresses the hexose monophosphate shunt activity necessary to replenish NADPH from nicotinic adenine diphosphate (NADP), thus compromising NADPH oxidase-based production of reactive oxygen metabolites and H_2O_2 by phagocytes. Suppression of phagocyte respiratory burst activity in severe G6PD deficiency results

in a CGD-like phenotype with recurrent infections, poor wound healing, and granulomatous inflammation [134]. NBT reduction is poor or absent, and the diagnosis is confirmed by demonstration of severe G6PD deficiency in leukocytes [135]. Of note, neutrophils from patients with "routine" G6PD deficiency have normal in vitro function [136] but when the incidence of sepsis in infants with "routine" G6PD deficiency was examined, the rate in G6PD-deficient infants was approximately twice that in normal infants [137].

α-Mannosidosis

α-Mannosidosis is a rare, autosomal recessive lysosomal storage disorder resulting from deficient activity of lysosomal α-mannosidase. Occurring in several animal species as well as humans, its principal manifestation is progressive mental deterioration due to neuronal storage of undegraded mannose-rich oligosaccharides. Neutrophil functional abnormalities occur in patients with α-mannosidosis – primarily depressed chemotactic responses and bacterial killing – resulting in recurrent bacterial infections. Phenotypic forms vary from severe infantile presentations [138] to milder juvenile-adult forms, and heterogeneity in disease severity occurs. Diagnosis is established by demonstration of deficient leukocyte or fibroblast α-mannosidase activity.

Defective glutathione metabolism

Whether defective glutathione metabolism in phagocytic cells results in significant functional abnormalities is unclear. Although rare cases of glutathione reductase deficiency, glutathione synthase deficiency, and glutathione peroxidase deficiency have been described in the past, and the roles of the glutathione system (protection against intracellular oxidants and replenishing NADPH from NADP) make it plausible that deficiency states could well result in PMN dysfunction, the data supporting these suppositions are scarce. Re-examination of the kindreds responsible for the first report of glutathione peroxidase deficiency as an unusual form of CGD has shown that both kindreds carry abnormalities of the NADPH oxidase [139], i.e. truly they are cases of CGD rather than deficiency of glutathione peroxidase.

Galactosemia

Galactosemia results from several autosomal recessive abnormalities of carbohydrate-metabolizing enzymes to produce elevated levels of galactose in the blood of affected infants. There is a predilection for development of neonatal sepsis with *E. coli* in this condition [140], possibly related to inhibitory effects of galactose on neonatal PMN chemiluminescence and chemotaxis responses [141]. Diagnosis is established by demonstrating elevated blood levels of galactose.

Glycogen storage disease B

This results from deficiency of glucose-6-phosphatase. The clinical manifestations include neutropenia, recurrent infections, and altered phagocytic cell function [142]. Abnormal chemotactic and respiratory burst activities are most commonly reported, but these are usually difficult to assess because of the associated neutropenia. Diagnosis is based upon demonstration of deficient glucose-6-phosphatase activity in hepatic tissue.

Trisomy 21

Patients with trisomy 21 have higher rates of infection and more severe infections than normal individuals. Their phagocytic cells show depressed chemotactic activity [143], while other functions (phagocytic, respiratory burst, and CD11b/CD18 expression) are normal. PMNs from patients with trisomy 21 also have shortened survival times during in vitro storage, which is associated with increased apoptosis [144]. In addition, both acute nonlymphocytic leukemia and self-limited myeloproliferative syndromes occur with higher incidences in infants with trisomy 21 [145], underscoring the presence of bone-marrow abnormalities in this condition.

Management options for abnormal phagocyte number and function

Leukocyte transfusions

Because of the roles the PMNs play in both host defense and acute inflammation, hypofunction in neonatal PMNs would be expected to result in decreased host defenses against pyogenic bacterial infections and depressed inflammatory responses. The clinical results of impaired host defenses in the neonate are more severe and frequent bacterial infections, which, because of the neonate's altered PMN production kinetics and storage pools, sometimes are accompanied by neutropenia. In neutropenic neonatal sepsis, mortality rates range from 60% to 90%, resulting in examination of the clinical effectiveness of fresh blood transfusions [146], exchange transfusions [147], and granulocyte [148–150] or buffy-coat transfusions [151, 152] and intravenous immunoglobulin treatment of septic/neutropenic neonates. Based on small case series, both fresh whole-blood transfusions and exchange transfusions are effective at lowering mortality in neutropenic septic neonates. In three different clinical trials, granulocyte transfusions collected by continuous flow filtration, intermittent flow centrifugation, or continuous flow centrifugation leukapheresis all decreased mortality to the range 0–10% [148–150]. However, two studies examining buffy-coat transfusions in septic neonates did not show significant lowering of mortality [151, 152], suggesting that the method used for cell preparation is critical for beneficial clinical effects. One retrospective case series comparing infants with severe *S. agalactiae* sepsis and neutropenia treated with or without intravenous immunoglobulins observed better survival of infants treated with immunoglobulin [153]. Because the technology needed for leukapheresis collection of PMNs is sophisticated and specialized, and recombinant human colony-stimulating factors have become available as therapeutic agents over the past decade, this approach to treatment of severe neonatal sepsis, although apparently effective, has been largely abandoned.

In the event that exchange or granulocyte transfusions are used in the neutropenic septic neonate, the potential for subsequent development of graft-versus-host disease in the recipient infant must be considered [154] because of the large numbers of nonPMN leukocytes present in each granulocyte transfusion. Although radiation doses of up to 5000 rads may be required to reduce lymphocyte mitogen responsiveness by 97–98% [155], doses in the range of 1500–2000 rads typically are used to treat granulocytes collected for transfusion, since at least one study has shown that these dose levels do not affect the chemotaxis or chemiluminescence responses of leukocytes collected by buffy-coat centrifugation [156]. However, when granulocyte preparations prepared by continuous centrifugation leukapheresis for administration to patients with phagocytic cell dysfunction syndromes were examined, irradiation with 1500 rads had variable effects on PMN respiratory burst responses, as assessed by NBT slide testing [157], suggesting that irradiation can alter the function of some preparations of leukocytes collected for transfusion. Current recommendations for irradiation of blood products include all lymphocyte-enriched products to be administered to premature infants in the first six months of life, unless time constraints interfere [154]. The role of granulocyte transfusions in the newborn and the technical aspects are further discussed in Chapter 14.

Cytokine treatments

The identification and production of lineage-specific, human colony-stimulating factors over the past two decades has resulted in their production as recombinant proteins and use as therapeutic agents. While used in many hematopoietic and oncologic conditions in adults and children, their proven applicability to conditions in neonates is limited but under active evaluation, with some investigators being very enthusiastic about their therapeutic potential in the neonate [107, 158]. Based on in vitro studies that have shown that GM-CSF exposure partially ameliorates the locomotive abnormalities

present in neonatal PMNs, a phase I–II trial of GM-CSF treatment in VLBW infants has shown that this cytokine induces increased PMN and monocyte production in the neonate [159]. A second case–control study has examined the use of G-CSF in neutropenic septic neonates [160]. In this study, G-CSF treatment was effective at increasing blood PMN counts and increased survival of treated infants (13 of 14 infants survived) compared with concurrently treated (but retrospectively selected) control infants with neutropenia and sepsis (5 of 11 infants survived). However, a recent randomized trial of G-CSF treatment in neutropenic infants with clinical evidence of early-onset sepsis showed no significant effect of this treatment [161]. G-CSF treatment of infants with eclampsia-associated neutropenia also has been reported to result in reversal of neutropenia [162, 163] and may reduce the incidence of sepsis in these infants [164]. As this is an area of enormous interest in neonatal medicine, clinical trials in neonates with these and other cytokines are ongoing. As the effectiveness of these agents as proliferative and/or PMN function-enhancing treatments becomes more clear, they will likely assume places of importance in treatments related to the inherent production and functional abnormalities of neonatal phagocytes.

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12

Immunodeficiency diseases of the neonate

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Introduction

Significant progress has been made towards the understanding of the clinical implications of the neonatal immune system and its immaturity. With the increasing survival of extremely premature infants, neonatologists and other physicians caring for these patients must be aware of the vulnerability of this select patient population. It is also important for neonatologists and pediatricians to be able to differentiate between immune immaturity and a true primary immunodeficiency that may present during the neonatal period. Failure to identify properly primary immunodeficiency diseases can result in delayed diagnosis and treatment, which can significantly affect the outcome of the disease. This chapter defines the immune immaturity of the neonate and how it impacts susceptibility to neonatal infection. It will describe the specific immune deficiency syndromes that may present during the neonatal period. Finally, the diagnosis and management of neonatal immunodeficiency diseases will be discussed in length in order to provide the reader with the proper approach and management guidelines to care adequately for these individuals.

Immaturity of the neonatal immune system

The immaturity of a neonate's immune response places the neonate at an increased risk for serious infection. An understanding of the development of the neonatal immune system is essential in order to be able to differentiate the clinical manifestations of infection associated with immaturity from those that identify a specific acquired or primary immunodeficiency disease. The focus of this section is to define how the immune immaturity of the neonate impacts susceptibility to infection. The immune defects associated with immaturity and their associated susceptibility to specific types of infections are summarized in Table 12.1.

The primary components of the immune response can be classified into innate and adaptive responses. Innate immunity is antigen-nonspecific and is composed of phagocytic cells, the complement system, and other soluble components of inflammation.

Soluble immunity and phagocytosis are the first line of defense against infection. Adaptive immunity, composed of T-cells and antibody, provides immunologic specificity and memory. Because adaptive immunity is not developed fully in term and preterm infants, soluble inflammatory mediators, phagocytic cells, and passively acquired maternal immunoglobulin play a critical role in protecting the newborn from infection.

Innate immunity

Phagocytic cells play a major role in the host-defense mechanism by the ingestion and killing of bacteria and other microorganisms. Leukocyte phagocytosis is enhanced when pathogens are opsonized, or coated by soluble proteins of the innate immune system.

Table 12.1 Defects in the immune response as a result of immaturity

Defect	Associated susceptibility	Age group impacted
Opsonization (e.g. complement, Ig, fibronectin)	Gram-negative and gram-positive bacteria	Preterm
		Term
T-cell-independent Ig production	Encapsulated bacteria	Preterm
		Term
		Infant
Antibody-directed cellular cytotoxicity	Viral infections	Preterm
		Term
Cytokine deficiencies	Viral infections (HSV)	Preterm
		Term

HSV, herpes simplex virus; Ig, immunoglobulin.

These components consist of the complement proteins, inflammatory proteins such as fibronectin, C-reactive protein (CRP), lactoferrins, and other proteins that enhance opsonization and microbial clearance. Opsonization activity is particularly reduced in preterm infants, principally due to their low serum antibody and complement levels [1–4].

Complement

The complement system is a major, multifaceted effector mechanism involved in the clearance of immune complexes, cytolysis, opsonization, chemotaxis, and nonspecific inflammatory responses [5]. It is subdivided into the classical and alternative pathways. These pathways converge at the third complement protein, C3, creating a membrane-attack complex comprised of the terminal complement proteins, C5b6789. Activation of the terminal complement components, C3–9, results in the production of serum opsonic, anaphylatoxic, chemotactic, and bacteriocidal activities. It also plays an important role in the host's defense against bacterial infection. The classical complement cascade is dependent on antibody for optimal activation through the binding of C1 to immunoglobulin Fc receptors. Antibody deficiencies in term and preterm infants limit the utility of complement, particularly C3 opsonization [6]. Studies have demonstrated that some bacteria cause defective activation of C3 in newborn sera, whereas other bacteria are able to activate C3 normally [7].

Syntheses of complement proteins are regulated developmentally and can be detected as early as the eighth week of gestation [8]. However, levels of these proteins remain low until the third trimester and, because there is no placental transfer of maternal complement, all of the components are derived from the fetus [9]. Concentrations of C3 increase rapidly late in gestation so that by the end of pregnancy, levels reach 60–80% of adult values [10]. Despite this late rise, terminal complement components may remain quite low, especially in preterm infants. Normal values for term and preterm complement levels compared with adults are displayed in Table 12.2. The functional capacities at term, as measured by the total hemolytic complement activity assay, are similar to adult values as reflected by classical pathway activity [10]. Alternative pathway activity and its individual components are deficient in both term and preterm infants and reach only 35–70% of adult levels [10, 11]. Despite classical pathway activity that is similar to adults, the limited diversity of transplacental antibody in newborns, especially preterm, produces a classical pathway that is of little value in the immediate postnatal period [11]. The alternative and classical complement pathways are important defense mechanisms for neonates; however, their immaturity contributes to decreased levels and activity in term and preterm

Table 12.2 Complement systems of preterm and term infants compared with adult controls (mean ± 1 SD)

Complement component	Preterm newborn (GA 28–33 weeks)	Preterm newborn (GA 34–36 weeks)	Full-term newborn (GA 37–42 weeks)	Adult
AP50	49.2 ± 15.9	53.3 ± 16.6	59.9 ± 21.3	93.1 ± 8.8
CH50	57.5 ± 25	75.1 ± 30.7	68.9 ± 25.8	96.1 ± 10.3
P	38.8 ± 15.1	39.4 ± 16	57.4 ± 15.2	99.3 ± 11
B	52.8 ± 17.1	47.7 ± 19.1	63.8 ± 25.7	99.3 ± 13.1
C1q	36.8 ± 16	35.1 ± 19	52.6 ± 16.7	103 ± 10.2
C1r	45.7 ± 18.1	60.6 ± 21.2	70.1 ± 20.6	93.9 ± 8.9
C1s	48.7 ± 16	61.7 ± 13.3	78.5 ± 13.6	95.6 ± 7.4
C2	62.5 ± 19.1	73.2 ± 22.8	79.4 ± 19.1	101.9 ± 11.3
C3	48.5 ± 19.8	63.1 ± 27	71.5 ± 25.3	96.4 ± 10.7
C4	56.3 ± 16.1	56.6 ± 23.8	72.7 ± 22	99.4 ± 13.8
C5	59.6 ± 16.5	70.4 ± 23	70.1 ± 36.7	96.6 ± 10.9
C6	38.7 ± 19.3	36.3 ± 12.9	57.6 ± 14.3	103.2 ± 11
C7	72.7 ± 15.5	75.2 ± 18.5	94.8 ± 13.2	102.5 ± 16.6
C8	28.7 ± 8	29.1 ± 10.7	36.2 ± 8.1	99.8 ± 11.3
C9	32.6 ± 28	52.8 ± 35	54.5 ± 6.7	104.4 ± 18.3

GA, gestational age; SD, standard deviation.

From Wolach, B., Dolfin, T., Regev, R., *et al*. The development of the complement system after 28 weeks' gestation. *Acta Paediatr* 1997; 86: 523, with permission.

infants, increasing susceptibility to bacterial infections. This deficiency is accentuated in premature infants, who display diminished activity of both pathways due to lower levels of C1q, C3, C4, and C9 and are therefore more susceptible to infection than term neonates [12, 13]. In both premature and term infants, the complement deficiencies resolve by 6–18 months of age.

Fibronectin, C-reactive protein, and lactoferrin

Term and preterm infants rely heavily on other soluble components of innate immunity, which are less effective in microbial clearance, increasing susceptibility to bacterial and fungal infections. Fibronectins are a class of high-molecular-weight inflammatory glycoproteins that enhance leukocyte adherence and migration. They also mediate hemostasis and hematopoiesis and play a role in wound healing through their augmentation of phagocytic function [11, 14]. Fibronectins are derived from macrophages and can be acquired passively through breast milk. Mean circulating adult plasma levels of fibronectin are 330 μg/ml, term infant values are 220 μg/ml, and preterm infants of 30–31 weeks' gestation have values of 152 μg/ml [11, 15]. Fibronectin levels are diminished further in states such as sepsis, asphyxia, and respiratory distress syndrome (RDS) [14–16].

CRP, an acute-phase reactant produced primarily in the liver, also plays a role in bacterial opsonization through its ability to bind C1q and activate the classical complement pathway [11]. Production by both the fetus and the term newborn is similar to adults, and thus elevated levels can serve as a surrogate marker of inflammation in the infant because maternally derived CRP does not cross the placenta [8, 11]. However, many infections, including group B *Streptococcus* (GBS), often do not elicit elevated CRP levels during the first 12–24 hours of infection [17].

Lactoferrin is a neutrophil-derived iron-binding glycoprotein and bacterial inhibitor that is acquired predominantly through breast milk. Production of

lactoferrin is low in neonatal neutrophils; however, nonspecific granulocyte stimulation with agents such as f-met-leu-phe results in degranulation and release of lactoferrin that is quantitatively similar to adult neutrophils [11, 18]. Other proteins, such as mannose and lipopolysaccharide-binding proteins, play an auxiliary role in host defenses against bacteria and fungi and have similar levels in both adults and neonates. Their relevance to protection from infection is uncertain because 5–7% of normal adults are deficient in these proteins [19]. However, there is a growing body of evidence indicating an association between the risk of severe bacterial infections and inherited deficiencies in the mannose-binding proteins [20, 21].

Cytokines

Cytokines are hormone-like proteins produced by lymphocytes and macrophages. They play an essential role in the differentiation, activation, and migration of the cellular components of the immune response [22]. Monocytes and macrophages are the primary cells that produce type 1 interferons (interferon alfa (IFN-α) and interferon beta (IFN-β)); and proinflammatory cytokines, including interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6). Deficiencies of neonatal cytokine production and activity may result from delayed maturation of cells responsible for cytokine production, developmentally induced insensitivity of the cytokine's receptor, or maturational impairment of the intracellular pathways responsible for cytokine activity [22]. TNF-α plays a role in host protection against gram-negative bacteria and is the principal mediator of septic shock. Neonatal monocytes migrate less well than those of adults, leading to slower accumulation at sites of infection [23]. Activated cord-blood monocytes from term infants produce similar, or slightly lower, levels of IL-1, TNF-α, IL-6, and interleukin 8 (IL-8) compared with monocytes from adults [24]. Production of IL-1 by preterm infants is similar to that in term infants, but other proinflammatory cytokines are diminished [25].

Interferons play a central role in resistance against viral infection through their activation of natural killer (NK) cells and by inhibiting intracellular viral replication. Interferon gamma (IFN-γ), produced by neonatal T-cells, is critical to the control of herpes simplex virus (HSV). However, production of IFN-γ by resting neonatal T-cells is at least five-fold lower than by adult cells and ten-fold less following activation [22]. Diminished production of IFN-γ primarily is the result of diminished IFN-γ gene transcription by T-cells [22]. The low interferon levels in neonates contributes to decreased TNF-α production, another cytokine that inhibits HSV replication and augments lysis of HSV-infected cells in vitro [26]. Neonatal T-cells also are deficient in their capacity to produce interleukin 4 (IL-4) [22]. Both IFN-γ and IL-4 are produced by memory T-cells, an absent cellular phenotype in neonates. Immaturity of this component of the cellular immune response appears to correlate with greater susceptibility to infection with viruses such as HSV in neonates [22]. This also contributes to impaired killing of other intracellular organisms, such as *Listeria monocytogenes*.

Placenta

The placenta is an active immune organ that modulates the fetal-maternal axis by producing cytokines that are critical to maintaining fetal homeostasis. During fetal life, these cytokines regulate hematopoiesis, modulate host defense against infection, protect the fetus against maternal rejection, and act as extrahematopoietic growth factors [27]. The mouse placenta produces the cytokines IL-4 and IL-10, which help to promote humoral immunity and suppress cellular immunity [28, 29]. Levels of placental cytokines are increased during fetal distress, specifically granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, IL-1, IL-6, and IL-8, which increase by 100-fold in the amniotic fluid of mothers with chorioamnionitis [30]. Placentally derived type I interferons impact the fetus by enhancing fetal NK cell function, which attenuate viral infections.

Chemokines

Chemokines are a family of structurally related proteins that cause leukocytes to move to sites of inflammation. Diminished chemotactic activity may be a factor in the slow influx of monocytes to inflammatory sites and the poor delayed hypersensitivity responses seen in neonates. Several differences in chemokine levels have been observed when comparing neonates with adults. Expression of RANTES (i.e. regulated on activation, normally T-cell-expressed and -secreted), a chemokine that acts on CD4 T-cells and monocytes, is increased in various fetal tissues, but it is present in adults only during disease states [31]. The clinical implications of this are unknown. IL-8 attracts neutrophils to inflammatory sites and can be found in high levels in the cord blood of babies with chorioamnionitis and those with increased gestational age [27]. Its expression is decreased in infants of mothers who received prenatal corticosteroids. Ex vivo administration of chemokines to neonatal neutrophils does not correct their defects in neonatal chemotaxis [32]. IL-16, produced by activated CD8+ lymphocytes and eosinophils, is a strong chemoattractant for lymphocytes and eosinophils [33, 34]. The precise role of IL-16 in the development of the immune system has not been established [27].

Humoral immunity

The ability to mount an effective humoral response against antigens is not fully mature until well after infancy, contributing to a neonate's already increased risk of infection [35, 36]. Humoral immunity consists of B-cells committed to the production of antigen-specific immunoglobulin molecules [10]. The ability of B-lymphocytes to generate a heterogeneous immunoglobulin repertoire provides antibody production to the diverse array of potential antigens found in nature [36]. B-cells and antibody production can be detected early in fetal development. B-cell precursors are defined by the surface expression of one or more components of the immunoglobulin molecules, while pre-B-cells have a genetic commitment to the B-cell lineage but lack immunoglobulin on their cell surface [10]. Pre-B-cells can be detected initially in the fetal liver by seven to eight weeks of gestation, and by the fifteenth week circulating B-lymphocyte numbers are equal to or higher than those of adults [11]. They can be found in the highest proportions in the spleen (30%), blood (35%), and lymph nodes (13%) [11, 37–40]. However, germinal centers are absent and do not develop until the fetus is exposed to antigenic stimulation [11]. Immature B-lymphocytes, detected as early as ten weeks of gestation, produce heavy and light chains and are characterized by the expression of immunoglobulin M (IgM) but not immunoglobulin G (IgG) or immunoglobulin A (IgA) on their cell surface [10, 41]. Fully differentiated plasma cells can secrete IgM by the fifteenth week and are capable of isotype switching to IgG, IgA, and immunoglobulin E (IgE) by 20 weeks [42].

Immunoglobulin G

The immunoglobulin diversity in the fetus is limited due to delayed maturation of the cellular components that control immunoglobulin genetic recombination. By early to mid gestation, diversity at the joining region of the variable, diversity, and joining gene segments, which generate the highest level of genetic diversity in the heavy chain of immunoglobulin, is markedly restricted compared with B-cells from adults [43, 44]. Restrictions of genetic diversity along with functional deficiencies in antibody production contributes to the decreased capacity of the fetus and neonate to respond to a complete array of antigens. Early in gestation, the fetus acquires the ability to produce serum immunoglobulins, but in the absence of intrauterine infection, the fetus makes very little antibody before birth [45]. Therefore, maternally derived immunoglobulin is essential to the protection of the fetus and newborn from infection, because most of the circulating antibodies at the time of birth are maternal IgG that have been transported across the placenta. Infants born to mothers with antibody deficiencies will have low

levels of IgG and are at increased risk for bacterial infections [46]. Furthermore, the fetus and newborn will be highly susceptible to pathogens to which the mother has not been exposed previously. At 17 weeks' gestation, the active transport of maternal IgG across the placenta begins and increases proportionally with gestational age [10]. Very prematurely born infants (24–26 weeks) usually have acquired little maternal antibody before delivery [11]. By 33 weeks, IgG levels in the fetus are equal to those of the mother [47]. Small-for-gestational-age infants have lower IgG levels than appropriate-for-gestational-age infants at any gestational age [11]. All IgG subclasses are able to cross the placenta, and studies have demonstrated that the amounts of IgG1, IgG2, IgG3, and IgG4 in cord serum are similar to those in maternal serum [8]. Normal-term infants have IgG levels that are 20–25% higher than maternal IgG levels and are generally greater than 1000 mg/dl [48].

Maternal antibody has a half-life in the fetus of approximately 30 days but continues to be detected in the infant during the first 18 months of life. Following birth, maternally derived IgG falls and there is a nadir between three and four months of age due to delayed IgG production by the newborn [8]. IgG values are approximately 400 mg/dl during this period. Decreased levels of IgG in premature infants persist for the first year of life. It is typical for a 28–32-week premature infant to have IgG levels of less than 100 mg/dl by three months of age. IgG loss is accelerated in chronically ill preterm infants, as they have smaller antibody pools that are depleted by repeated phlebotomy [10]. In general, preterm infants achieve adult IgG levels by four to six years of age [11]. Normal immunoglobulin levels for preterm and term infants, normal infants, and adults can be found in Table 12.3.

Table 12.3a Normal values for immunoglobulins for term infants to age three years

Age	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Newborn	598–1672	0–5	5–15
1–3 months	218–610	20–53	11–51
4–6 months	228–636	27–72	25–60
7–9 months	292–816	27–73	12–124
10–18 months	383–1070	27–169	28–113
2 years	423–1184	35–222	32–131
3 years	477–1334	40–251	28–113

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

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Table 12.3b Plasma immunoglobulin concentrations in premature infants 25–28 weeks' gestation

Age	IgG[a] (mg/dl)	IgM* (mg/dl)	IgA* (mg/dl)
1 week	251 (114–552)[b]	7.6 (1.3–43.3)	1.2 (0.07–20.8)
2 weeks	202 (91–446)	14.1 (3.5–56.1)	3.1 (0.09–10.7)
1 month	158 (57–437)	12.7 (3.0–53.3)	4.5 (0.65–30.9)
1.5 months	134 (59–307)	16.2 (4.4–59.2)	4.3 (0.9–20.9)
2 months	89 (58–136)	16.0 (5.3–48.9)	4.1 (1.5–11.1)
3 months	60 (23–156)	13.8 (5.3–36.1)	3.0 (0.6–15.6)
4 months	82 (32–210)	22.2 (11.2–43.9)	6.8 (1.0–47.8)
6 months	159 (56–455)	41.3 (8.3–205)	9.7 (3.0–31.2)
8–10 months	273 (94–794)	41.8 (31.1–56.1)	9.5 (0.9–98.6)

[a]Geometric mean.

[b]Normal ranges determined by taking antilog of (mean logarithm ± 2 SD of the logarithms).

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; SD, standard deviation.

From Ballow *et al.* [45], with permission.

Other immunoglobulins

All other immunoglobulin isotypes are produced endogenously by the fetus or infant because only IgG crosses the placenta. Although the fetus is capable of producing IgM in utero, levels generally remain low, and average levels of 11 mg/dl in term infants and 6 mg/dl in neonates less than 28 weeks' gestation are found [49]. IgM levels rise rapidly over the first month of life and increase gradually thereafter [8]. Premature infants have proportionately lower levels for the first six months of life compared with term

Table 12.3c Plasma immunoglobulin concentrations in premature infants 29–32 weeks' gestation

Age	IgG (mg/dl)[a]	IgM (mg/dl)*	IgA (mg/dl)*
1 week	368 (186–728)[b]	9.1 (2.1–39.4)	0.6 (0.04–1.0)
2 weeks	275 (119–637)	13.9 (4.7–41)	0.9 (0.01–7.5)
1 month	209 (97–452)	14.4 (6.3–33)	1.9 (0.3–12.0)
1.5 months	156 (69–352)	15.4 (5.5–43.2)	2.2 (0.7–6.5)
2 months	123 (64–237)	15.2 (4.9–46.7)	3.0 (1.1–8.3)
3 months	104 (41–268)	16.3 (7.1–37.2)	3.6 (0.8–15.4)
4 months	128 (39–425)	26.5 (7.7–91.2)	9.8 (2.5–39.3)
6 months	179 (51–634)	29.3 (10.5–81.5)	12.3 (2.7–57.1)
8–10 months	280 (140–561)	34.7 (17–70.8)	20.9 (8.3–53)

[a]Geometric mean.

[b]Normal ranges determined by taking antilog of (mean logarithm ± 2 SD of the logarithms).

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; SD, standard deviation.

From Ballow *et al.* Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. *Pediatric Research* 1986; 20: 901, with permission.

infants. Exposure to intrauterine infections such as cytomegalovirus (CMV), toxoplasmosis, and rubella may elicit elevated IgM levels (more than 20 mg/dl) at birth in some infants [50, 51]. However, exposure to intrauterine infections also can result in tolerance and absent IgM levels rather than specific antibody production. For example, in three series, over 33% of infants with intrauterine rubella infection, 19% of infants with toxoplasmosis infection, and 11% of infants with CMV infection did not produce an IgM response [51–53].

Because IgA is not able to cross the placenta, levels of only 0.1–0.5 mg/dl are found in the cord blood of term and preterm infants [49]. Adult levels are not achieved until puberty. Increased cord-blood IgA levels are observed in some infants with intrauterine infections [50]. The only source of secretory IgA for the newborn is from the colostrum in breast milk. Immunoglobulin D (IgD) and IgE are very low or undetectable in normal infants at birth [54]. The lack of isotype production other than IgM in the neonate is due in part to the inability of the neonate's naive T-cells to provide contact-dependent T-cell help for immunoglobulin class switch through the CD40/CD40 ligand pathway rather than an inherent defect in neonatal B-cells to switch to other classes of immunoglobulins [8]. This defect is apparent especially in neonatal group B streptococcal infections [55, 56]. Newborn B-lymphocytes also demonstrate deficiencies in IgG subclass production. Production of IgG1 and IgG3 exists, while that of IgG2 and IgG4 is absent [10]. Further compromise of the neonate's capacity to produce functional antibody is due to delayed maturation of T-cell-independent antibody production. Infants less than two years of age are particularly susceptible to encapsulated bacteria due to a defect in B-cell maturation that results in an inability to produce immunoglobulin to polysaccharide antigens within bacterial cell walls [10]. The limited capacity of naive neonatal T-cells to produce IL-4 and IFN-γ also contributes to this defect in antibody response.

Cellular immunity

T-lymphocytes have multiple roles in the immune response. Similar to B-cells, T-cells undergo extensive developmental regulation before they become effector T-lymphocytes. Early T-cells expressing CD4 or CD8 T-cell-lineage markers can be found in the fetal thymic cortex as early as the tenth week of gestation and in the fetal liver and spleen by 14 weeks, indicating that thymic maturation and emigration have occurred [57, 58]. During the third trimester, thymic cellularity increases and continues to increase postnatally, reaching a peak by ten years of age [8]. Maternal T-cells can cross the placenta during maternal-fetal hemorrhage, but there is no evidence for transfer of maternal cell-mediated immunity to the fetus. The percentage of T-cells in cord blood is significantly lower than that for adult peripheral blood (46% versus 72%) [59]. There is a significant difference in the proportion of naive to memory T-cells in newborns compared with children and adults. The relative absence of memory

Table 12.4a Blood lymphocyte subpopulations in newborns and infants[a]

Lymphocyte subpopulation	Neonatal	1 week–2 months	2–5 months	5–9 months	9–15 months	15–24 months	Adult
CD19+ B-lymphocyte	12% (5–22)	15% (4–26)	24% (14–39)	21% (13–35)	25% (15–39)	28% (17–41)	12% (6–19)
CD3+ T-lymphocytes	62% (28–76)	72% (60–85)	63% (48–75)	66% (50–77)	65% (54–76)	64% (39–73)	72% (55–83)
CD3+/CD4+ T-lymphocytes	41% (17–52)	55% (41–68)	45% (33–58)	45% (33–58)	44% (31–54)	41% (25–50)	44% (28–57)
CD3+/CD8+ T-lymphocytes	24% (10–41)	16% (9–23)	17% (11–25)	18% (13–26)	18% (12–28)	20% (11–32)	24% (10–39)
CD4/CD8 ratio per CD3+	1.8 (1–2.6)	3.8 (1.3–6.3)	2.7 (1.7–3.9)	2.5 (1.6–3.8)	2.4 (1.3–3.9)	1.9 (0.9–3.7)	1.9 (1.91–3.6)
CD3+/HLA-DR+ T-lymphocytes	2% (1–6)	5% (1–38)	3% (1–9)	3% (1–7)	4% (2–8)	6% (3–12)	5% (2–12)
CD3-/CD16–56+ NK cells	20% (6–58)	8% (3–23)	6% (2–14)	5% (2–13)	7% (3–17)	8% (3–16)	13% (7–31)

[a]Relative frequencies are expressed within the lymphocyte population; median and percentiles (5th to 95th percentiles).

NK, natural killer.

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T-cells, due to the lack of antigen activation, contributes to impaired cellular immunity. This is one of the major reasons why infants infected with viruses, such as HSV, have such a high probability for serious, life-threatening infection. T-cell dysfunction in neonates is demonstrated further by both diminished delayed hypersensitivity reactions and delayed acquisition of antigen-specific antibody [10]. These deficiencies explain why neonates are vulnerable to overwhelming group B streptococcal infections and tuberculosis. The normal distributions of lymphocyte subsets in normal newborns are displayed in Table 12.4.

Similar to immunoglobulin, T-cell receptor diversity is generated through genetic recombination of the variable, diversity, and joining gene segments that form the antigen binding or CDR3 region of the T-cell receptor. This process is regulated developmentally, does not begin until the third trimester, and requires approximately five months to complete. This stepwise regulation of T-cell receptor development does not appear to impact T-cell function or to contribute to immunodeficiency [60]. Neonatal T-cells from term and preterm infants are capable of rejecting foreign allografts and proliferating in response to protein antigens. As a result, graft-versus-host disease (GVHD) following blood transfusions is a rare event and newborns benefit from immunization with protein antigens such as hepatitis B [61].

Over 90% of T-cells within the term or preterm neonate have a naive phenotype and have emigrated recently from the thymus. Neonatal T-cells are similar to adult T-cells in their capacity to produce interleukin 2 (IL-2) and proliferate following mitogen activation. However, they have a greater requirement for antigen-presenting cells and signal-transduction molecules to achieve IL-2 transcription levels that are similar to those in adults [10]. Neonatal T-cells produce significantly diminished amounts of IL-4, leading to a lower capacity to provide T-cell help for B-cell differentiation when compared with adult T-cells [62, 63]. Studies have demonstrated that neonatal T-cells require greater activation signals

Table 12.4b Absolute size of lymphocyte subpopulations in blood[a]

Lymphocyte subpopulation	Neonatal	1 week–2 months	2–5 months	5–9 months	9–15 months	15–24 months	Adult
Lymphocytes	4.8	6.7	5.9	6	5.5	5.6	1.8
	(0.7–7.3)	(3.5–13.1)	(3.7–9.6)	(3.8–9.9)	(2.6–10.4)	(2.7–11.9)	(1–2.8)
CD19+ B-lymphocytes	0.6	1.0	1.3	1.3	1.4	1.3	0.2
	(0.04–1.1)	(0.6–1.9)	(0.6–3)	(0.7–2.5)	(0.6–2.7)	(0.6–3.1)	(0.1–0.5)
CD3+ T-lymphocytes	2.8	4.6	3.6	3.8	3.4	3.5	1.2
	(0.6–5)	(2.3–7)	(2.3–6.5)	(2.4–6.9)	(1.6–6.7)	(1.4–8)	(0.7–2.1)
CD3+/CD4+ T-lymphocytes	1.9	3.5	2.5	2.8	2.3	2.2	0.7
	(0.4–3.5)	(1.7–5.3)	(1.5–5)	(1.4–5.1)	(1–4.6)	(0.9–5.5)	(0.3–1.4)
CD3+/CD8+ T-lymphocytes	1.1	1.0	1.0	1.1	1.1	1.2	0.4
	(0.2–1.9)	(0.4–1.7)	(0.5–1.6)	(0.6–2.2)	(0.4–2.1)	(0.4–2.3)	(0.2–0.9)
CD3+/HLA-DR+ T-lymphocytes	0.09	0.3	0.2	0.2	0.2	0.3	0.09
	(0.03–0.4)	(0.03–3.4)	(0.07–0.5)	(0.07–0.5)	(0.1–0.6)	(0.1–0.7)	(0.03–0.2)
CD3-/CD16–56+ NK cells	1.0	0.5	0.3	0.3	0.4	0.4	0.3
	(0.1–1.9)	(0.2–1.4)	(0.1–1.3)	(0.1–1)	(0.2–1.2)	(0.1–1.4)	(0.09–0.6)

[a]Absolute counts ($\times 10^9$/l): median and percentiles (5th to 95th percentiles).

NK, natural killer.

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to express adult levels of CD40 ligand, a critical molecule in inducing immunoglobulin class switching through CD40 expressed on B-cells [64–66].

The cytolytic activity of NK and CD8 T-cells is decreased in the newborn. Insufficient cytokine signals, particularly interleukin 12 (IL-12) and IFN-γ, are the key factors responsible for this observation [10]. The delayed development of antigen-specific cellular immunity in the neonate may account for the severity of clinical diseases (HSV and other perinatally acquired viral and intracellular pathogens). This deficiency also impacts the ability of mononuclear phagocytes to effectively process and present antigen, a critical step in T-cell activation. IFN-γ also augments the activity of TNF-α, leading to its deficiency in neonates. IFN-γ plays an important role in the containment phase of infection. The mechanism for the decreased IFN-γ is due primarily to the lack of memory T-cells, the principal source of IFN-γ [67]. Neonatal T-cells produce decreased amounts of IL-2, interleukin 3 (IL-3), IL-4, interleukin 5 (IL-5), IL-6, GM-CSF, migration inhibition factor (MIF), IFN-α, and lymphocyte-derived chemotactic factor (LDCF) [11]. In summary, impaired T-cell function in the fetus and neonate is a result of decreased lymphokine production, low cellular cytotoxicity, and defective help for B-cell differentiation. In general, neonatal T-cells are skewed towards a TH2 (T-helper type 2) phenotype characterized by the elaboration of IL-4 and IL-5 rather than TH1 (T-helper type 1) cytokines that are characterized by the elaboration of IL-2 and γ-interferon.

Specific immune deficiency disorders presenting in the neonatal period

The majority of primary immune deficiency disorders are not clinically apparent during the neonatal period. The diagnosis is most commonly made during infancy and childhood on the basis of recurrent infections and clinical signs associated with

the particular immune disorder. Increased susceptibility to infections during the neonatal period is more likely the result of secondary immune deficiencies, such as immune immaturity, breakdown of integument barriers, and compromising medical procedures, including prolonged mechanical ventilation, indwelling urinary, venous, and arterial catheters, and changes in microbial flora associated with prolonged use of antibiotics. However, there are signs and symptoms present in the neonatal period that point to defects in one of the arms of the immune response, which, when present, warrant consideration and evaluation for a specific immune disorder. Examples of specific T-cell and B-cell disorders that are recognizable during the first year of life are listed in Table 12.5 and discussed below.

Table 12.5 Classification of immune disorders that manifest in neonates

Component of the immune system	Immune disorder
Predominant antibody defects	X-linked agammaglobulinemia
	Hyper IgM syndrome
	Transient hypogammaglobulinemia of infancy
Predominant defects in cell-mediated immunity	DiGeorge anomaly
	MHC class II deficiency
	Neonatal HIV infection
Combined antibody and cellular immunodeficiencies	Wiscott–Aldrich syndrome
Severe combined immunodeficiencies	X-linked SCID
	Zap-70 deficiency
	Adenosine deaminase deficiency
	Omenn syndrome

Modified from WHO Scientific Group. Primary immunodeficiency diseases. *Clinical and Experimental Immunology* 1999; 109 (supplement 1): 1. HIV, human immunodeficiency virus; IgM, immunoglobulin M; MHC, major histocompatibility complex; SCID, severe combined immunodeficiency diseases.

Predominant antibody defects

Clinical manifestations of disorders in antibody production

Deficiencies in B-cell function are characterized by recurrent sinopulmonary infections, principally with encapsulated bacteria such as *Streptococcus pneumoniae* or *Haemophilus influenzae*, persistent enteroviral infections of the gastrointestinal tract or central nervous system, and bacterial sepsis and meningitis [68]. Antibody deficiencies are often unrecognized during the neonatal period because of the protective effect of passively acquired maternal antibody. The similarity between the susceptibility to infection due to immunoglobulin deficiency secondary to immaturity versus a primary antibody deficiency contributes to the difficult recognition of primary antibody deficiencies in the neonatal period. Catabolism of passively acquired antibody occurs with a half-life of approximately 30 days, although maternal antibody can be detected throughout the first 18 months of life. As the levels of maternal antibody decline, immunoglobulin deficiency in the neonate becomes apparent and generally can be documented after age six months or earlier in preterm infants.

X-linked agammaglobulinemia

X-linked agammaglobulinemia (XLA) results in profoundly diminished levels of all immunoglobulin isotypes, which remain depressed for life. The defect in XLA is due to a mutation within a B-cell-specific src-associated tyrosine kinase (Bruton's tyrosine kinase, Btk), which plays a pivotal role in early B-cell development [69]. In its complete form, B-cell development is arrested in the bone marrow at the pre-B-cell stage and there is an absence of B-cells in blood and lymphoid tissues [70]. T-cell development, numbers, and function are normal. Clinical manifestations that can be observed during the neonatal period include dysplastic lymph nodes and tonsillar tissues due to absent germinal centers. Plasma cells are absent in the lamina propria of the gastrointestinal tract and IgM levels are undetectable, even if infection is present. Generally, XLA is diagnosed after the first year of life

due to recurrent sinopulmonary infections, bacterial sepsis, and chronic enteroviral infections associated with this disorder. However, overwhelming enteroviral sepsis syndromes and paralytic polio following immunization with the live polio vaccine have been observed in neonates and infants with XLA [71]. The disorder can be diagnosed in the neonatal period by the absence of circulating B-lymphocytes (no CD19 or CD20 bearing cells) using flow-cytometric analysis of peripheral blood mononuclear cells [72, 73].

Hyper-immunoglobulin M syndrome

Hyper-IgM (HIM) syndrome is characterized by low levels of IgG, IgA, and IgE with normal to elevated levels of IgM [74]. During the neonatal period, the immunoglobulin profiles can appear very similar to those of normal infants or infants with an intrauterine infection. B-cell numbers are normal, but infections with encapsulated organisms and other manifestations suggestive of B-cell deficiency are common. Two-thirds of infants with HIM syndrome have neutropenia, with the associated findings of perirectal abscesses and oral ulcers. In addition, pneumonitis due to infection with *Pneumocystis carinii* is frequent and cholangitis due to *Cryptosporidium* has been reported [75]. There is an increased susceptibility to diarrhea due to *Salmonella*, *Giardia*, and *Entamoeba* species. The disorder has also been reported in association with congenital rubella. Nodular lymphoid hyperplasia of the intestinal tract and generalized lymphadenopathy and splenomegaly can occur in young infants.

The disorder results from abnormal expression of CD40 ligand (CD154) on CD4 T-cells [76–79], which leads to a disruption of cell-to-cell signaling between T- and B-lymphocytes. As a result, the immunoglobulin class switch of the cells from IgM to other isotypes is impaired. In addition, the CD40/CD154 system plays a crucial role in cell-mediated immunity through its impact on signaling between T-cells and macrophages [80]. As a result, IL-12 production and IFN-γ production are abnormal, which contributes to increased susceptibility to *Pneumocystis* and other opportunistic organisms. The disorder is most commonly inherited as a X-linked primary immune deficiency, but autosomal recessive forms also have been recognized. The abnormal gene in the X-linked form has been mapped to Xq26 [81, 82].

Transient hypogammaglobulinemia of infancy

There is controversy as to whether transient hypogammaglobulinemia (THI) of infancy is a true primary immune deficiency or an extension of the physiologic nadir that occurs as maternal antibody disappears and the infant begins its own antibody production [83]. Normal IgG nadirs occur between three and six months of age in term infants, with lower values seen in premature infants. By definition, in this condition IgG levels are more than two standard deviations (SD) below normal age-related ranges and all immunoglobulin isotypes can be affected [83–85]. This phenomenon should be considered in chronically ill infants who remain hospitalized for several months, because recurrent infection, malnutrition, and repeated phlebotomy accentuate antibody loss [86]. There is evidence that infants with THI have a transient defect in CD4 T-helper cell function when compared with normal infants [87]. However, B-cell numbers in these children are normal, and they are able to generate specific antibody responses following immunization with T-dependent antigens such as tetanus. Increased susceptibility to infection has been observed in these children if total antibody falls below 50 mg/dl.

Antibody deficiency associated with secondary immune disorders

Many conditions seen in neonates have antibody deficiency as part of the clinical manifestations. Several congenital chromosomal disorders have been recognized as having immunoglobulin deficiency as part of their clinical spectrum [88]. Turner syndrome is often associated with decreased IgG and IgM levels. Immunodeficiency, centromeric heterochomatin, and facial abnormalities (ICF) syndrome results in

the instability of chromosomes 1, 9, 16, and 20, with associated findings of low total immunoglobulin levels, absent isohemagglutinins, and dysgammaglobulinemia. Hypogammaglobulinemia is a common associated finding in trisomy 21, monosomy 22, trisomy 8, and chromosome 18q syndrome. Not surprisingly, sickle cell disease and congenital asplenia syndromes have increased susceptibility to sepsis due to encapsulated bacteria. The functional asplenia observed in sickle cell disease actually begins during the first year of life. Congenital infections due to rubella, CMV, human immunodeficiency virus (HIV), Epstein–Barr virus (EBV), and toxoplasmosis can impair both antibody production and function. Antibody deficiency can occur due to protein-losing enteropathies and other conditions that result in excessive loss of protein.

Predominant defects in cell-mediated immunity

Clinical manifestations of disorders in cell-mediated or combined antibody and cellular immunodeficiencies

Due to its pivotal role in the immune response and the lack of maternally acquired cellular immunity, T-cell and combined immunodeficiency disorders commonly have their initial clinical manifestations during the neonatal period [88]. Signs and symptoms suggestive of a disorder in cell-mediated immunity include opportunistic infections such as *Pneumocystis carinii* pneumonia (PCP), *Mycobacterium tuberculosis* infection, fungal infections (most commonly due to candida), disseminated viral infections, and GVHD due to either maternally derived T-cells or transfusions with non-irradiated blood products. Clinical manifestations of GVHD include a macular erythematous rash, hepatitis, and chronic diarrhea. Many of these disorders have clinical and physical features that enhance their recognition during the newborn period [89].

DiGeorge anomaly

The DiGeorge anomaly is an embryopathy resulting from a field defect in neural crest cell migration [90]. The structures formed from the third and fourth pharyngeal pouches are impacted, particularly the thymus and parathyroid glands. Congenital heart defects are seen in the majority of children with the DiGeorge anomaly and include conotruncal, atrial, and ventricular septal defects [89]. Absence of the parathyroid glands with resulting deficiency in parathyroid hormone can lead to severe hypocalcemia, with tetany and seizures within a few days after birth. Facial abnormalities are frequently demonstrated and include bifid uvula, short philtrum of the upper lip, hypertelorism, antimongoloid slant to the eyes, mandibular hypoplasia, and low-set notched ears [91]. The absence of the thymus results in T-cell lymphopenia, placing the infant at risk for viral and fungal infections, failure to thrive, chronic diarrhea, and blood-transfusion-associated GVHD. In more than 95% of patients, there is a microdeletion in chromosome 22q11.2, which can be detected using fluorescent in situ hybridization (FISH) [92]. T-cell numbers and function are impaired to varying degrees, ranging from profound lymphopenia with few T-cells present to an increase in T-cells expressing immature markers with elevated CD4 : CD8 T-cell ratios. However, most patients with DiGeorge anomaly are only mildly lymphopenic but have a decreased percentage of CD3+ T-cells [89, 93, 94]. The most commonly recognized features during the neonatal period are hypocalcemia and congenital heart defects. However, the risk for GVHD following blood transfusions needs to be considered in neonates suspected of having DiGeorge anomaly [89]. All neonates with congenital heart lesions that may be associated with DiGeorge anomaly should have irradiated blood products until the diagnosis is ruled out.

Major histocompatibility complex class II deficiency

This rare T-cell defect is also known as bare lymphocyte syndrome. It results from a lack of major histocompatibility complex (MHC) class II on antigen-presenting cells and activated T-cells [95]. It has an autosomal recessive inheritance. Clinical

characteristics manifest themselves during early infancy and include recurrent bacterial infections, chronic diarrhea, chronic hepatitis with cholangitis, and symptoms suggestive of autoimmune disease. Viral infections with meningoencephalitis, enteritis, and hepatitis are commonly due to enteroviruses, adenovirus, and herpes viruses. The mechanism of decreased MHC II expression is the result of mutations within the genes that regulate MHC II transcription rather than within the MHC locus itself [96].

Laboratory findings show no evidence of MHC II (DR and DQ) expression on macrophages, B-cells, or activated T-cells. Total T-cell numbers may be normal, but there are decreased numbers and percentages of CD4 T-cells. T-cell responses to specific antigens are diminished in vivo and in vitro, but allogeneic response in vitro can be detected. Immunoglobulin profiles vary due to deficiency in T-helper cell function. Many patients have low IgG and IgA, and there is functional dysgammaglobulinemia.

Perinatal human immunodeficiency virus 1 infection

The most common global form of combined immune deficiency in neonates is maternally acquired HIV-1 infection. Without antiretroviral intervention for mother and child, about one-third of HIV-infected mothers transmit the virus to their infants [97]. Transmission can occur in utero, perinatally, or postnatally through breast feeding. Of infected infants, only about 20% show evidence of viremia at birth. These infants are thought to have become infected in utero and tend to show clinical manifestations at birth and more rapid disease progression [98]. The majority (80%) of infected infants have no detectable virus at birth and have acquired the virus near the time of delivery. In these infants, disease progression is slower and clinical manifestations are not apparent at birth. All infants born to HIV-infected women will have detectable HIV antibodies due to transplacental transfer of IgG. Clinical manifestations of HIV infection during the neonatal period include mucocutaneous candidiasis, splenomegaly, lymphadenopathy, and thrombocytopenia. Intrauterine coinfections with CMV and toxoplasmosis are common. PCP is rare in infants under two months of age [99]. Laboratory findings include hypergammaglobulinemia, lymphopenia, and inverted CD4 : CD8 T-cell ratios. Decreased numbers of T-cells with a naive phenotype denotes intrathymic infection and is a poor prognostic sign. Defects in cellular immunity due to HIV infection are multifaceted. HIV infection in neonates commonly results in decreased thymic output and lower T-cell numbers. HIV induces apoptosis of CD4 T-lymphocytes, resulting in chronic CD4 T-cell attrition. Viremia induces a state of T-cell anergy, which impairs the antigen-specific responses of both CD8 and CD4 T-cells. Finally, a unique aspect of HIV infection in children is viral-induced B-cell dysfunction, resulting in dysgammaglobulinemia and increased susceptibility to encapsulated bacteria.

Combined antibody and cellular immunodeficiencies

Wiskott–Aldrich syndrome

The principal clinical manifestations of Wiskott–Aldrich syndrome (WAS) are eczema, thrombocytopenia, and increased susceptibility to infection [100]. It is an X-linked recessive disorder and several hematopoietic lineages are impacted, primarily lymphocytes and platelets. Presentation in the newborn period consists of prolonged bleeding from circumcision, bloody diarrhea, petechiae, excessive bruising, and/or eczema [89]. Platelets are small, defective, and abnormally shaped, leading to sequestration in the spleen [101,102].

The immune deficiency seen in WAS results from aberrant cell-to-cell cognate interactions in immune regulation. Lymphocyte numbers are decreased and T-cell function is abnormal. Cytokine elaboration of TH2-type cytokines such as IL-4 is elevated, resulting in a skewed pattern of immunoglobulin production and subsequent eczema and an increased incidence of autoimmune cytopenias and rheumatologic conditions, such as juvenile rheumatoid arthritis [103]. The syndrome is associated

with a characteristic immunoglobulin profile of depressed IgM production, elevations in IgA and IgE, and IgG production that is normal or low. However, immunoglobulin profiles are often normal in the first few months of life [103]. Antigen-specific antibody production to polysaccharide antigens is impaired, contributing to increased susceptibility and poor response to these immunizations. The T-cell, immunoglobulin, and autoimmune abnormalities that are associated with WAS generally occur later in infancy and childhood.

The defect is due to mutations in the Wiskott–Aldrich associated protein (WASp), a cellular protein mapped to Xp11.22–11.2384 that plays an important role in the cellular cytoskeleton [104–106]. There is a large and varied number of mutations in the WASP gene, with possible severity of infection susceptibility or other problems corresponding to the site of the mutation [107–111].

Severe combined immunodeficiency diseases

Severe combined immunodeficiency diseases (SCID) can result from a variety of molecular mechanisms that lead to a deficiency of both antibody and cell-mediated immunity [112]. Presentation during the first few months of life consists of recurrent episodes of diarrhea, pneumonia, otitis, sepsis, and cutaneous infections [89]. Common to all forms of SCID is a high risk for severe or chronic infection with CMV, PCP, gram-negative sepsis, and mucocutaneous candidiasis that extends beyond the oral cavity. GVHD also can occur following transfusions with non-irradiated blood. However, more commonly, there is a 20–30% incidence of maternally derived GVHD due to maternal-to-fetal hemorrhage, with the acquisition of maternal lymphocytes [113]. Global impairment of T-cell function is evidenced by the failure of lymphocytes to demonstrate in vitro proliferation to mitogens. In vivo antigen-specific T-cell responses are impaired, as demonstrated by the absence of delayed hypersensitivity skin testing (DHST) to exposed antigens such as candida. The common clinical findings may be the result of impaired development of lymphoid stem cells, defects in lymphocyte-specific signal-transduction pathways, or enzymatic defects that result in lymphocyte destruction.

X-linked and other forms of severe combined immunodeficiency diseases resulting from defects in cellular signal-transduction pathways

X-linked SCID, the most common subtype of SCID, accounting for approximately 44% of US cases [114, 115], is due to mutations in the gamma chain of the IL-2 receptor complex located on chromosome Xq13.1 [116, 117]. This receptor is critical for T-cell activation and differentiation mediated through the JAK/Stat signal transduction pathway and is a common receptor pathway for signals mediated through interleukins 2, 4, 7, 9, 15 and 21 [117–119]. The defect causes a maturation arrest in T-cell development within the thymic cortex [120]. Although B-cell numbers are normal, immunoglobulin production is impaired due to both absent T-cell help and an intrinsic defect in B-cell activation. A similar but rarer autosomal recessive form of the disorder has been reported as a result of mutations in the JAK3 pathway [89]. Another form of SCID due to defective signal transduction is ZAP-70 deficiency [121]. This rare autosomal recessive disorder is due to a defect in the signal-transduction pathways generated through the T-cell receptor activation cascade. The mutations are found within the genes encoding T-cell-specific kinases downstream from the T-cell receptor. The result is a failure of antigen-specific activation through the CD3/T-cell receptor complex to induce cellular activation and proliferation, with subsequent loss of T-cell function. Abnormal T-cell development is manifest by CD8 T-cell lymphopenia, with an elevated ratio of CD4 : CD8 T-cells.

Severe combined immunodeficiency disease due to abnormal purine salvage pathway enzymes

Adenosine deaminase (ADA) deficiency is a relatively common autosomal recessive form of SCID (observed in approximately 16% of patients with SCID [114, 115, 122, 123]) that leads to severe

lymphopenia impacting T-cells, B-cells, and NK-cells [124]. The gene encoding ADA is on chromosome 20q13-ter [123]. ADA is a purine-salvage-pathway enzyme that transforms deoxyadenosine to deoxyinosine. The absence of the enzyme results in an accumulation of deoxyadenosine, a cellular toxin, within T-cells and B-cells. Immature lymphocytes are particularly sensitive. The most severe clinical variants of ADA deficiency are manifested during the neonatal period with opportunistic infections and GVHD typical of SCID. Unique clinical features of ADA deficiency include skeletal abnormalities, consisting of cupping and flaring of the costochondral junction of the ribs and pelvic dysplasia. The diagnosis can be made based on the assessment of ADA levels in red blood cells, but it can also be made in utero based on the assessment of ADA activity in fibroblasts obtained from amniotic fluid [124].

Severe combined immunodeficiency disease with hypereosinophilia (Omenn syndrome)

This rare subtype of SCID commonly has its clinical presentation during the neonatal period, with manifestations including diffuse erythroderma, pachyderma, alopecia involving the scalp and eyebrows, lymphadenopathy, hepatosplenomegaly, and diarrhea [125]. Laboratory findings include lymphocytosis and elevated blood eosinophils. T-cell infiltration can be found in the skin and gastrointestinal tract. The enlarged lymph nodes demonstrate macrophages but few lymphocytes. The thymus is also hypoplastic. Blood T-lymphocytes show high expression of activation markers, including CD25, HLA-DR, and oligoclonal T-cell expansions. There are few circulating B-lymphocytes and significant depression of all immunoglobulin isotypes. However, IgE is elevated. A small kindred of children with Omenn syndrome have demonstrated a mutation in the recombination-activating genes (RAG) [126]. It has been postulated that this defect allows for the escape of a few autoreactive T-cell clones, which can infiltrate the skin and gastrointestinal tract and induce a severe TH2 shift in cytokine production, particularly IL-4 and IL-5 [127, 128].

Other combined immune deficiencies seen in neonates

SCID phenotypes have been described in infants with multiple gastrointestinal atresias and cartilage-hair hypoplasia (short-limbed dwarfism). T-cell deficiencies are associated with several metabolic disorders, including orotic aciduria, methionine synthase deficiency, and biotin-dependent multiple carboxylase deficiency [129]. The early recognition of these disorders is essential due to the fact that these metabolic diseases are treatable [89, 122].

Complement deficiencies

Inherited disorders of the complement system are rarely recognizable during the neonatal period. Common clinical manifestations associated with complement deficiency include autoimmunity, angioedema, and recurrent bacterial infections with pathogens such as *Neiserria* [130]. The diagnosis of complement deficiencies during the neonatal period is complicated by the transient deficiencies within both the classical and the alternative complement components as a result of immaturity [9]. However, inherited complement deficiencies may have early manifestations with respect to increased susceptibility and severity to bacterial pathogens [131]. Thus far, the clinical manifestations of inherited complement deficiencies during the neonatal period have not been described.

Diagnosis and management of neonatal immunodeficiencies

General principles

Primary and secondary immune deficiencies may be recognized and treated during the neonatal period [8]. The pattern of clinical signs and symptoms often can be attributed to a specific defect within a particular component of immunity allowing for a rationale and stepwise diagnostic approach. Once recognized, the treatment and long-term management can be implemented [132]. Neonates, especially those in an intensive care unit setting, have multiple risk

Table 12.6 Immunoglobulin and lymphocyte profiles in immunodeficiency disorders

Disorder	IgG	IgA	IgM	IgE	CD3	CD4	CD8	CD19[a]
XLA	Low/nl[b]	Absent	Absent	Absent	Normal	Normal	Normal	Absent
HIM	Low/nl[b]	Low	Elevated	Low	Normal	Normal	Normal	Normal
THI	Low	Low	Normal	Normal	Normal	Normal	Normal	Normal
DiGeorge anomaly	Low/nl[b]	Low/nl[c]	Normal	Normal	Low	Elevated	Low	Normal
MHC II deficiency	Low	Low	Normal	Normal	Low	Low	Normal	Normal
HIV	Normal	Elevated	Normal	Normal	Normal	Low	Elevated	Normal
WAS	Normal	Elevated	Normal[d]	Elevated	Low	Low	Normal	Normal
XSCID	Normal	Low	Low	Normal	Absent	Absent	Absent	Normal
ZAP-70 deficiency	Low/nl[b]	Low	Low	Low	Low	Normal	Absent	Normal
ADA deficiency	Low/nl[b]	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Omenn syndrome	Low/nl[b]	Absent	Low	Elevated	Low[e]	Low	Low	Low

[a]B-cell enumeration using either CD19 or CD20 cell markers.

[b]All normal values are compared with age-matched healthy infants.

[c]Findings variable in DiGeorge anomaly.

[d]IgM values are often normal in WAS and decline after the first 12 months.

[e]T-cell and B-cell subset analysis results vary from patient to patient.

ADA, adenosine deaminase; HIM, hyper-immunoglobulin M; HIV, human immunodeficiency virus; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; MHC II, major histocompatibility complex class II; THI, transient hypogammaglobulinemia; WAS, Wiskott–Aldrich syndrome; XLA, X-linked agammaglobulinemia; XSCID, X-linked severe combined immunodeficiency diseases.

factors beyond immune immaturity that predispose them to infection. Furthermore, primary immune deficiency diseases are rare. As a result, they are often unrecognized or attributed to other, more common etiologies. For example, X-linked SCID occurs in only 1 in 50 000 live births [70]. Therefore, it is far more likely that opportunistic infections in the neonate are the result of a secondary rather than a primary immunodeficiency. It is an important but difficult task for the neonatologist or pediatrician to be able to identify and properly investigate a neonate suspected of having a primary immunodeficiency disorder.

Diagnostic approach to a neonate with a suspected defect in B-cell function

Antibody deficiency diseases are occult during the neonatal period and infancy because of the presence of passive maternal antibody and the overlap between the clinical manifestations associated with prematurity and those of primary antibody defects. Clinical signs and symptoms of B-cell deficiency observed in infants include recurrent or persistent bacterial pneumonia, sepsis, and meningitis, as well as chronic enteroviral meningitis and/or enteritis [5]. Laboratory signs associated with antibody deficiency include low serum total protein/albumin ratios and neutropenia [132]. The approach to a neonate with suspected B-cell deficiency as well as the associated clinical and laboratory findings characteristic of specific B-cell deficiency diseases are summarized in Table 12.6 and Fig. 12.1. Although helpful when present, a family history of immune deficiency is uncommon. Most B-cell deficiencies seen in infants and children such as XLA and X-linked HIM syndrome are X-linked recessive [133]. In addition, up to 40% of patients represent new spontaneous mutations [134].

Initial screening tests for the evaluation of a suspected antibody deficiency disease should include a complete blood count (CBC), with differential and quantitative immunoglobulins (IgG, IgA, IgM). Immunoglobulin profiles associated with specific

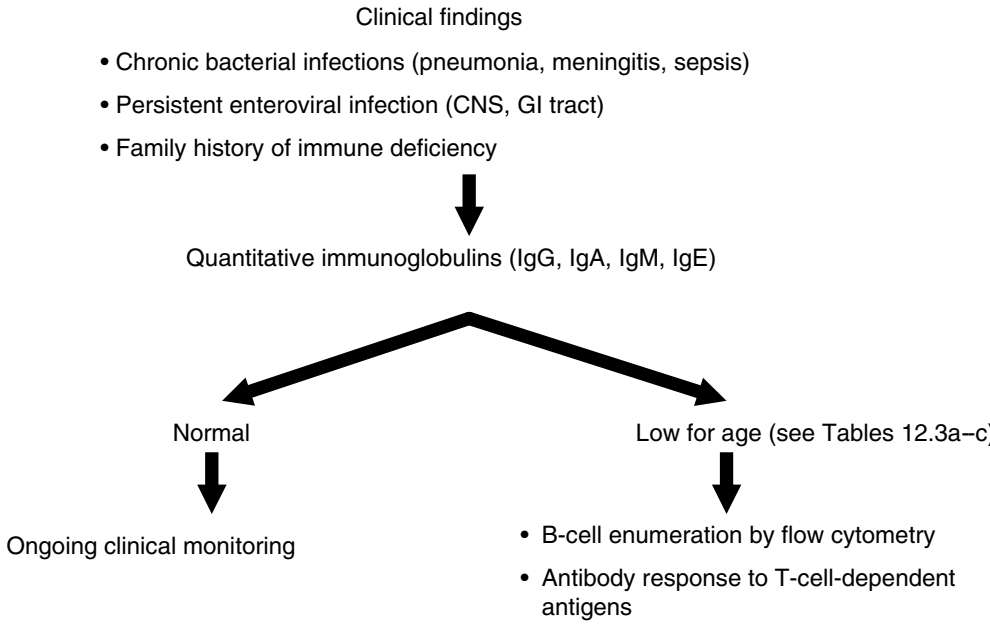

Fig. 12.1 Stepwise approach to the neonate with suspected B-cell deficiency. If clinical findings and abnormal quantitative levels of immunoglobulins are present, then B-cell enumeration by flow cytometry and antibody response to T-dependent antigens is recommended. Pneumococcal titers, serum protein electrophoresis, and immunoglobulin G (IgG) subclass determination are not recommended during the neonatal period.
CNS, central nervous system; GI, gastrointestinal; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M.

immune disorders are shown in Table 12.6. If abnormal or suspicion is still high despite normal values, then B-cell enumeration should be performed. Results of these assays should be compared with normal values of age- and gestational-age-matched healthy children (see Tables 12.4a–b). Assessing B-cell numbers is performed using flow-cytometry analysis of lymphocytes to enumerate lymphocytes bearing the B-cell-specific markers, CD19 and CD20 [5]. These laboratory tests are simple, rapid, and readily available and require only small volumes of blood.

A simple test evaluating functional antibody is to measure isohemaglutinin titers to ABO blood-group antigens [132]. However, many infants with normal B-cell function may have negative isohemagglutinin test results during the first year of life, making negative results to this test unreliable.

THI can be confirmed in a child with low total immunoglobulin levels, normal B-cells numbers, and normal increases in antibody titers following routine immunizations with tetanus or diphtheria [83]. Pre- and post-immunization titers are optimally performed before and 30 days after immunization.

Elevated or abnormal immunoglobulin profiles can indicate particular disease states. Increased immunoglobulin levels for age, particularly of IgA, is an early sign of HIV infection [135, 136]. An elevation in total serum IgM suggests a congenital infection [11]. Children with WAS have a characteristic immunoglobulin profile consisting of low total IgM, elevated IgA and IgE, and normal IgG levels. However, these values tend to be normal at birth [103]. Low total IgG levels at birth suggests a maternal antibody deficiency disease [46].

Laboratory testing that is generally not helpful in the diagnosis of antibody deficiency diseases during the newborn period includes serum electrophoresis, chromosome analysis, and pneumococcal titers. Antibody responses to T-independent polysaccharide antigens, as induced by the standard pneumococcal vaccine, do not become measurable in normal children until after the second year of life. Most inherited antibody deficiencies result from single gene mutations, making chromosomal analysis generally unrevealing.

Hypogammaglobulinemia associated with prematurity and THI are common events that do not require antibody replacement therapy or extensive immune evaluations [8]. These patients have normal B-cell numbers and make functional antibody to immunizations. XLA can be diagnosed by flow cytometry, showing absent CD20 and CD19 expressing B-lymphocytes and normal T-cell enumeration. Infants with autosomal or X-linked recessive forms of HIM syndrome have low or absent expression of CD40 ligand [80]. Assays for CD40 ligand (CD154) deficiency are available through several human immunology research laboratories. Polymerase chain reaction (PCR) amplification and sequencing of the specific genetic mutations enable precise molecular definition of single gene disorders and the detection of carrier states (see Table 12.7).

Table 12.7 Primary immune deficiency diseases that can be diagnosed based on analysis of single gene defects [89, 137]

Immune deficiency	Inheritance	Genetic defect
ADA SCID	Autosomal recessive	20q13-ter
RAG1 or RAG2 deficiencies	Autosomal recessive	11p13
DiGeorge anomaly*	Autosomal recessive	22q11.2
ZAP-70 deficiency	Autosomal recessive	2q12.zap70
XSCID	X-linked recessive	Xq13 region
WAS	X-linked recessive	Xp11–11.3
XLA	X-linked recessive	Xq22
X-linked HIM	X-linked recessive	Xq26

Modified from Buckley, R. H. Breakthroughs in the understanding and therapy of primary immunodeficiency. *Pediatric Clinics of North America* 1994; 666: 41, with permission from Elsevier.

ADA, adenosine deaminase; RAG, recombination activating gene; WAS, Wiskott–Aldrich syndrome; XLA, X-linked agammaglobulinemia; XSCID, X-linked severe combined immunodeficiency diseases.

* chromosomal deletion.

Management of infants with antibody deficiency disorders

Providing intravenous immunoglobulin (IVIG) therapy can effectively treat antibody deficiency. An IVIG infusion consists of pooled human gamma globulin from thousands of plasma donors and therefore contains a broad array of antibodies to multiple antigens [137]. For the most part, IgA and IgM have been removed and the solution is enriched for monomeric human IgG. IVIG comes from a variety of donor sources, and many different products are available. Unlike other blood products, the risk of infusion-associated infections is exceedingly low, because the fractionation process inactivates viral and bacterial pathogens [137]. There is a risk for infusion-related reactions such as fever and rigors, but this risk is decreased in infants when compared with children and adults. IVIG is given as a slow intravenous infusion over two to four hours. It is given as a 5%, 6%, 10%, or 12% solution, depending on the specific product. The usual dose for replacement therapy is 200–600 mg/kg [137–139]. Antibody replacement is given every three to four weeks in order to maintain IgG trough levels above 500 mg/dl. IVIG is indicated in the treatment of XLA, HIM, and SCID, but not for selective IgA deficiency or THI of infancy.

The use of IVIG for preventing infections in preterm infants is controversial, and a review evaluating 19 studies demonstrated a 3–4% reduction

in nosocomial infections without a reduction in mortality or other important clinical outcomes [138]. Thus, the use of IVIG for prophylactic use in premature infants should be at the physician's discretion. IVIG also may be used for suspected or proven infection in neonates. However, a review of six studies stated that there was insufficient evidence to support the use of IVIG to prevent mortality in neonates with suspected or proven infection [140]. Prior clinical studies did demonstrate improvement in neutrophil counts, with no evidence of toxicity [141, 142].

Even with the effective implementation of IVIG, patients are still susceptible to infections because of ongoing deficiency in IgA and IgM, which are not provided by IVIG. IVIG does not cross the blood–brain barrier well; nor does it penetrate into mucosal surfaces [143]. However, chronic viral meningoencephalitis has been managed successfully using intraventricular gamma globulin, and chronic viral gastroenteritis can be treated with oral gamma globulin or human colostrum [143]. In addition to IVIG, infants with antibody deficiency disorders require the aggressive use of antibiotics to treat infections.

Long-term management of patients with known or suspected antibody deficiency is dependent on the clinical scenario. Patients with THI should have follow-up evaluations three to four months from their diagnosis, to ensure that functional and quantitative antibody is restored. The long-term management of XLA includes lifelong IVIG and aggressive management of infections [69]. Infants with persistent antibody deficiency should not receive immunizations with live viruses such as polio, measles, mumps, rubella, or varicella [144]. They may benefit from immunizations that enhance cell-mediated immunity, such as hepatitis B, pertussis, and diphtheria. Household contacts should receive only the inactivated polio vaccination and should be quarantined following varicella immunization [144]. Recommendations for vaccination should be part of the discharge plan for infants with antibody defects. Clinicians caring for children with immune deficiency should stay informed of the changes in vaccine recommendations (see www.aap.org) [144]. Families should be counseled on the lifelong need for immunoglobulin replacement therapy and receive genetic counseling. Infants with HIM are at high risk for PCP, cryptosporidium, and other opportunistic infections. As a result, PCP prophylaxis with trimethoprim-sulfamethoxazole (co-trimoxazole) is recommended for these patients [144].

Management of mothers with antibody deficiency conditions

Pregnant women with antibody deficiency, such as common variable immune deficiency (CVI), require additional antibody replacement during pregnancy to compensate for increased catabolism during pregnancy and to ensure adequate transplacental transfer of IgG to the fetus. Without IVIG therapy, their infants are at risk for life-threatening infections [46]. Gamma globulin has been administered to pregnant women without adverse effects on either the mother or the fetus [145]. However, doses of up to 600 mg/kg every three weeks over the third trimester are required to maintain adequate levels for both mother and child [146]. In addition, the breast milk and colostrum of mothers with IgA deficiency are deficient in IgA, compromising the mucosal immunity of their children [147].

Diagnostic approach to the neonate with a suspected defect in cell-mediated immunity

Compared with B-cell and other primary immune deficiency diseases, defects in cell-mediated immunity are more likely to have their initial clinical manifestations during the neonatal period [148]. The approach to the neonate with suspected T-cell deficiency and the associated clinical and laboratory findings characteristic of specific T-cell deficiency diseases are summarized in Table 12.6 and Fig. 12.2. Examples of clinical findings include facial abnormalities, as seen in DiGeorge anomaly and immunodeficiency, centromeric region instability, and facial anomalies syndrome (ICF), and the skeletal anomalies seen in ADA and short-limbed dwarfism. Opportunistic infections associated with many cell-mediated disorders include PCP, persistent fungal infections, and CMV. Chronic rash, diarrhea, unexplained hepatitis, splenomegaly, and

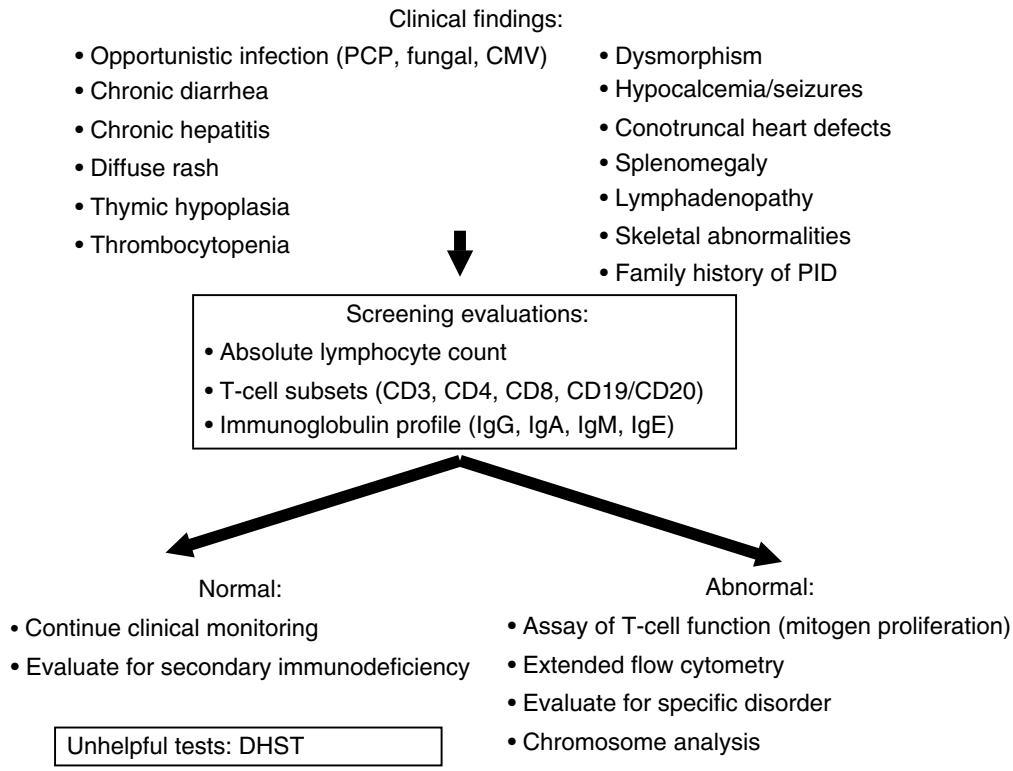

Fig. 12.2 Stepwise approach to the neonate with suspected defect in cell-mediated immunity. Numerous clinical findings are displayed but demonstrate the variety of presentation. Screening evaluations (center) include absolute lymphocyte count routinely obtained on complete blood count testing. Other screening evaluations include T-cell subsets via flow cytometry and immunoglobulin profile analysis. When clinical findings are present and/or screening evaluations are abnormal, then mitogen proliferation, extended flow cytometry, specific disorder evaluation, and chromosomal analysis are recommended. Delayed hypersensitivity skin testing (DHST) is not recommended during the neonatal period.
CMV, cytomegalovirus; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; PCP, *Pneumocystis carinii* pneumonia; PID, primary immune deficiency.

generalized lymphadenopathy may be caused by GVHD or Omenn syndrome [89]. Thymic hypoplasia may be present on a chest radiograph, but both anteroposterior and lateral films are needed for detection. While many infants (especially preterm infants with a history of maternal pre-eclampsia or prenatal stress, or those whose mothers received prenatal corticosteroids) will have a small thymic silhouette on the chest radiograph, the thymus should increase in size after several weeks of life [86]. Persistence of a decreased thymus is an indication of a possible T-cell deficiency [149]. Like B-cell deficiencies, many defects in cell-mediated immunity are inherited as autosomal recessive or X-linked recessive traits [133]. When positive, family history is helpful in establishing the diagnosis. However, a high percentage of cases represent new mutations.

Associated laboratory findings suggestive of specific T-cell immunodeficiency include thrombocytopenia with small platelet volumes in WAS, hypocalcemia in DiGeorge anomaly, and eosinophilia in Omenn syndrome [89]. Cytogenetics can be helpful in confirming the diagnosis of ICF syndrome, through which testing will show elongation

of the secondary constrictions with associated multi-branched configurations in chromosomes 1, 9, 16, and, occasionally, 2 [88]. FISH analysis will demonstrate the interstitial microdeletion in chromosome 22q11.2 seen in the majority of patients with DiGeorge anomaly [150–152]. Another deletion associated with DiGeorge anomaly has been identified on chromosome 10p13 [153–155].

When a deficiency in cell-mediated immunity is suspected during the neonatal period, absolute lymphocyte counts with T-cell subset analysis should be performed. The normal range for the cord-blood absolute lymphocyte count is 2000–11 000/mm [3], and values below this range should alert the physician to perform more extensive T-cell studies [115, 122, 156]. Patients with DiGeorge anomaly or WAS will have low lymphocyte numbers in addition to their associated clinical findings. SCID subtypes such as ADA, X-linked SCID, and Zap-70 deficiency will have characteristic T-cell subset distributions that should prompt further assessment of T-cell function. Similar to the assessment of immunoglobulin levels, total lymphocyte counts and subset distributions should be compared with age-matched standards, shown in Table 12.4.

Based on the findings from the initial T-cell subset analysis, the next step in pursuing the diagnosis of a suspected T-cell immune deficiency includes an assessment of T-cell function by measuring in vitro lymphocyte proliferation to mitogens. Unfortunately, delayed hypersensitivity skin testing (DHST) is not a reliable measure of T-cell function in the neonate due to the limited exposure to recall antigens [157]. However, neonates that are exposed to candidiasis for longer than one week generally have positive DHST to *Candida* testing [5].

Extremely low numbers of both B- and T-lymphocyte subpopulations and absent lymphocyte proliferation supports the diagnosis of ADA deficiency and other SCID syndromes. The diagnosis of ADA deficiency can be confirmed through the measurement of ADA in red blood cells or through specific chromosomal analysis [123]. Infants with X-linked SCID have absent T-cells and NK cells, but B-cell numbers are often normal [114]. Lymphocyte proliferation assays are depressed and T-cell help for B-cells is absent, resulting in abnormal immunoglobulin levels. ZAP-70 deficiency has characteristic T-cell subset distributions consisting of normal numbers of CD4 T-cells but very low numbers of CD8 T-lymphocytes [121]. Lymphocyte stimulation through the T-cell receptor complex is abnormal, resulting in depressed mitogen proliferation in vitro. The in vitro defect can be corrected using phorbol esters, which phosphorylate protein kinase C (PKC), thus confirming the diagnosis.

The diagnosis of DiGeorge anomaly through T-cell subset analysis demonstrates low total T-cell numbers, increases in early T-cell markers on peripheral blood lymphocytes, and elevated (above three) ratios of CD4 to CD8 T-cells.

Flow-cytometric characteristics of Omenn syndrome include low total B- and T-cell numbers. There is an increase in T-cells expressing activation markers such as HLADR and oligoclonal expansion of activated memory T-cells expressing CD45RO [158]. These observations support the use of extended flow cytometry in making the diagnosis of Omenn syndrome.

Management of T-cell immune deficiency

Disorders in cell-mediated immunity should be considered life-threatening conditions that require immediate medical intervention [148]. These infants are extremely susceptible to severe, often fatal opportunistic infections. They are also at risk for iatrogenic conditions such as GVHD following blood transfusions with nonirradiated blood products [89]. Early recognition leads to the appropriate initiation of prophylactic therapies and allows initiation of definitive treatment to correct the defect [89, 137, 159]. Long-term outcomes are improved if therapy such as bone-marrow transplantation or enzyme-replacement therapy is initiated early in life [89, 112, 148, 160]. The steps in the management of the neonate with suspected disorders in cell-mediated immunity are shown in Table 12.8.

Infants with suspected cell-mediated or combined immunodeficiency should receive only irradiated blood products. Leukocyte-reduced blood products are not an acceptable alternative. Notification of

Table 12.8 Management of infants with cellular and combined immunodeficiencies

1) Blood transfusions with irradiated CMV-negative blood.
2) PCP prophylaxis[a].
3) IVIG for antibody replacement[b].
4) No live immunizations[c].
5) Initiation of definitive therapy early (BMT, enzyme replacement).

[a]Trimethoprim (5 mg/kg/day) and sulfamethoxazole (25 mg/kg/day) divided twice daily, three times per week.
[b]Usual dose is 200–600 mg/kg for replacement given every three to four weeks. Infuse over four to six hours.
[c]For complete recommendations, visit www.aap.org and www.cdc.gov.
BMT, bone-marrow transplantation; CMV, cytomegalovirus; IVIG, intravenous immunoglobulin; PCP, *Pneumocystis carinii* pneumonia.

support staff and especially blood-bank personnel may help to ensure that this is accomplished. CMV-negative blood products also are recommended. Mothers who are seropositive for CMV, HIV, or human T-cell lymphotropic virus 1 (HTLV-1) should be cautioned against breast feeding infants who have defects in cell-mediated immunity. Children with SCID or SCID variants and who have hypogammaglobulinemia or dysgammaglobulinemia should receive IVIG replacement therapy, even in certain cases when IgG levels are normal.

Antibody deficiency also should be considered in the treatment of infants with DiGeorge anomaly and HIV infection. PCP prophylaxis with trimethoprim (5 mg/kg/day) and sulfamethoxazole (25 mg/kg/day) divided to be given twice daily, three times per week, should be administered beginning in the second month of life [5, 89]; it can be given intravenously if necessary. Prophylaxis for candida and mycobacterium infections should also be considered in infants and children with very low (fewer than 100 cells/μl) T-cell counts. Vaccinations with live viruses or bacille Calmette–Guerin (BCG) should not be given [144]. Household contacts should not be given live polio, varicella, or smallpox vaccinations [144]. Clinicians should consult the latest recommendations when vaccinating children with immune deficiency (see www.aap.org and www.cdc.gov) [144].

Genetic counseling and detection of carriers within the patient's family are necessary components towards the effective management of inherited disorders of cell-mediated immunity. Several of these disorders result from single gene mutations that can be confirmed by PCR amplification of the target sequences. A list of known chromosomal locations for primary immunodeficiencies is given in Table 12.7. Most mutations identified represent unique mutations within the impacted gene or gene family [133]. Molecular-based tools and reagents to identify these genes are becoming more easily available to allow genetic confirmation of the disorder and to identify carriers within the kindred. Examples of several known single gene immune deficiency diseases include XLA, X-linked HIM, X-linked SCID, and ZAP-70 deficiency [133, 134].

The treatment for individuals with DiGeorge anomaly usually involves prophylaxis alone, as most patients have the partial form and show a gradual improvement in T-cell development. Thrombocytopenia and the risk of hemorrhage is the most common complication of WAS in infancy [103]. Thrombocytopenia often improves after the first year of age, and can be improved with splenectomy (see Chapter 10). However, because of the long-term complications of immunodeficiency and malignancy, bone-marrow transplantation has been used to treat WAS, and numerous patients have had complete corrections of both platelet and immune abnormalities [161].

Definitive therapy for SCID varies with the specific condition. Children with SCID generally succumb to infection or malignancy unless the condition is corrected with bone-marrow transplantation before the age of two years. The best prognosis is for those infants transplanted before six months of age. However, enzyme replacement therapy with polyethylene glycol (PEG) modified bovine ADA for ADA deficiency, given by subcutaneous injection once weekly, has resulted in clinical and immunologic improvement [160, 162–164]. Trials of gene therapy are currently under way to treat both ADA deficiency and X-linked SCID [165, 166]. Because

of the high complexity and rapid changes in the management of infants with T-cell deficiency and SCID, rapid referral to a tertiary care center is recommended.

Diagnostic approach and management of infants with suspected human immunodeficiency virus infection

Screening all pregnant women for HIV-1 infection is standard medical care in the USA [167]. Nearly all infants born to HIV-infected women have positive HIV enzyme-linked immunosorbent assay (ELISA) and western blot test results due to passively acquired maternal antibody that persists for the first 12–18 months of life. If both the mother and the infant (for the first six weeks of life) receive zidovudine (AZT) antiretroviral therapy, then the risk of infection for the infant is reduced to less than 10% [168]. Without prophylaxis, the risk for infection is 30–50%. If the mother's status is unknown, the infant can be screened effectively with an HIV ELISA. If the mother is HIV-infected, then HIV antigen testing of the infant using qualitative PCR amplification of whole blood should be performed in order to determine whether the child is infected. This assay should be carried out at birth and at follow-up evaluations at one, four, and six months after birth. If these tests are all negative, then repeat HIV ELISA should be performed at 18 months in order to confirm seroconversion [169].

Before seroconversion, infants born to HIV-infected mothers are considered exposed and therefore at risk for infection. They should receive trimethoprim-sulfamethoxazole (co-trimoxazole) for PCP prophylaxis after one month of age and should not be given live viral immunizations. Because of the risk of transmission, these mothers should be counseled against breast feeding their infants. Duration of AZT treatment to prevent infection in exposed infants is six weeks. Therapy should be started within 12 hours of birth at a dose of 2 mg/kg every six hours for term infants. If given intravenously, then the dose is 1.5 mg/kg every six hours. Premature infants less than 34 weeks' gestation should be dosed at 1.5 mg/kg every 12 hours for two weeks; then the dosage should be increased to 2 mg/kg every eight hours for the next four weeks [170–172]. Premature infants should receive equivalent doses orally or intravenously [170–172]. The principal adverse effect of AZT is bone-marrow suppression. If clinically significant anemia or neutropenia occurs, then AZT dosing should be reduced by 30% [170].

Diagnosis of complement deficiency

Clinical manifestations of complement deficiencies are observed rarely during the neonatal period. The prevalence of inherited diseases of complement is 0.03% [173]. Recognition and diagnosis of these disorders are hampered largely by transient deficiencies of both the classical and alternative complement components due to immune immaturity. In older infants and children, unexplained angioedema, recurrent bacterial infections, and autoimmune disease all are associated with deficiency in either the classical or the alternative complement cascade. In the past, a condition in neonates that was linked to a complement defect was Leiner's disease. Severe seborrheic-like dermatitis, intractable diarrhea, and recurrent infections with gram-negative bacteria and candida characterized this disease [174]. These patients demonstrated a deficiency in the opsonic activity of the fifth complement component. However, subsequent patients with these symptoms have not had these results duplicated, leaving the precise etiology of the disorder in doubt [8, 173].

Evaluation for deficiency in the classical complement pathway can be carried out easily using the total hemolytic complement (CH_{50}). A homozygous deficiency in any single complement component results in the compete absence of activity. Partial deficiencies as a result of inherited defects, immaturity, or increased consumption associated with infection lead to a depressed but not absent CH_{50}. If there is absent CH_{50} activity, then the next step is to define the missing component through measurement of individual complement components. The approach

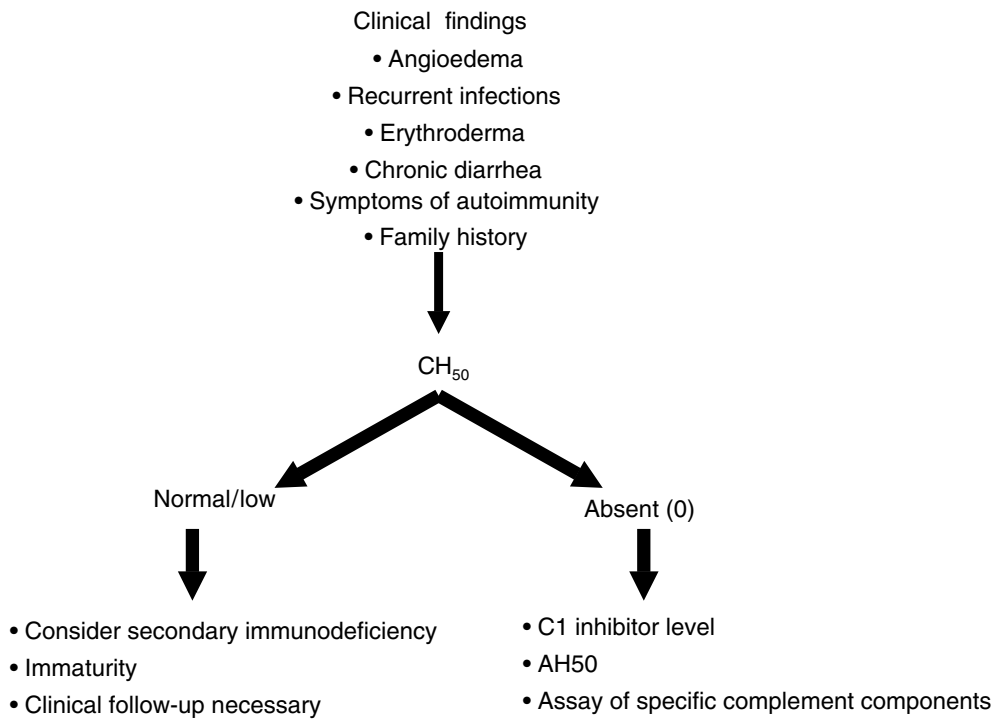

Fig. 12.3 Stepwise approach to the neonate with suspected complement deficiency. Clinical findings are observed rarely during the neonatal period but, when present, warrant a total hemolytic complement (CH_{50}) evaluation. If absent, then C1 inhibitor levels, alternative hemolytic complement (AH50), and assay for specific complement components are recommended. If normal/low, then close follow-up and consideration of other causes are recommended.
AH50, alternative hemolytic complement; $CH_{50,}$ total hemolytic complement.

to evaluating a neonate with suspected complement deficiency is displayed in Fig. 12.3.

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13

Hemostatic abnormalities

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Introduction

Hemostatic abnormalities can be considered as either congenital or acquired and can be classified as hemorrhagic or thromboembolic disorders. In newborns, the clinical presentation, diagnosis, and management of hemorrhagic and thromboembolic disorders differ from those in older infants and children, likely reflecting profound differences of the hemostatic system at birth. While severe congenital hemostatic defects usually present in the first hours to days of life with distinct symptoms in otherwise well newborns, acquired hemostatic disorders usually present in sick newborns with a variety of presentations and distinct etiologies that differ from older children and adults. In newborns, the diagnosis of hemostatic abnormalities based upon plasma concentrations of components of the hemostatic system requires age-appropriate reference ranges, because plasma concentrations of several procoagulant and inhibitor proteins are physiologically decreased at birth. The aim of this chapter is to discuss the clinical presentation, diagnosis, and management of the most common congenital and acquired hemostatic disorders in newborns.

Hemorrhagic disorders

Congenital hemorrhagic disorders

General information

Hemostatic proteins

For congenital deficiencies of components of the hemostatic system, both a severe and a milder form occur. Severe congenital deficiencies of prothrombin, factor V (FV), factor VII (FVII), factor VIII (FVIII), factor IX (FIX), factor X (FX), factor XI (FXI), fibrinogen, factor XIII (FXIII) and alpha$_2$-antiplasmin (α_2AP) all can present with bleeding in the first days of life [1–4]. Mild congenital deficiencies of these proteins usually do not cause bleeding at birth in otherwise healthy full-term newborns. Although von Willebrand disease (vWD) is the most common bleeding disorder during childhood, only rare severe forms of von Willebrand factor (vWF) deficiency present with bleeding in newborns. Deficiencies of FXII, high-molecular-weight kininogen, and prekallikrein do not cause bleeding and are not discussed further here.

Inheritance

Congenital deficiencies of prothrombin, FV, FVII, FX, FXI, FXIII, fibrinogen, and α_2AP are inherited as autosomal recessive traits, with consanguinity present in many families. Deficiencies of FVIII (hemophilia A) and FIX (hemophilia B), the most common congenital bleeding disorders present in newborns, are inherited as sex-linked recessive traits. While vWF deficiency type 1 and 2 are inherited predominantly as an autosomal dominant pattern, type 3 is transmitted as an autosomal recessive trait [5, 6]. Rarely, combined deficiencies of prothrombin, FVII, FIX, and FX, or of FV and FVIII, present in newborns [7, 8].

Clinical presentation

While heterozygous factor deficiencies rarely manifest with bleeding complications at birth,

unexplained bleeding in otherwise healthy full-term newborns should be investigated carefully because it may reflect the presence of a homozygous or severe congenital factor deficiency. Bleeding manifestations include persistent oozing from the umbilical stump, bleeding into the scalp resulting in large cephalohematomas, bruising, bleeding following circumcision, and persistent bleeding from peripheral sites from which blood samples have been obtained. A small but important proportion of newborns present with an intracranial hemorrhage (ICH) as the first manifestation of their bleeding tendency [1, 9–14]. Bleeding into joints, which is typical for older infants with severe forms of FVIII or FIX deficiency, occurs rarely in newborns. Congenital factor deficiencies also can be associated with other disorders, such as FVII deficiency in infants with Dubin–Johnson syndrome or Gilbert syndrome [15, 16].

Diagnosis

Unexpected congenital factor deficiencies usually are diagnosed with abnormal results of coagulation screening tests (activated partial thromboplastin time (APTT), prothrombin time, fibrinogen) and subsequent specific coagulation protein assays. In newborns, the diagnosis of many congenital factor deficiencies based upon plasma concentrations can be difficult, because plasma concentrations of several coagulation proteins are physiologically decreased at birth (Table 13.1) [17–19]. Mild to moderate congenital deficiencies of prothrombin, FV, FVIII, FIX, FX, and FXI result in plasma concentrations that may overlap with neonatal physiological values. By contrast, plasma concentrations resulting from either mild to moderate FVII deficiency or severe deficiency of FV, FVII, FVIII, FIX, FX, or FXIII can be distinguished easily from physiologic values (Table 13.1). Prenatal diagnosis of most congenital factor deficiencies is performed by amniocentesis or chorionic villus biopsy. The prenatal diagnosis of congenital deficiencies of specific coagulation proteins is confined largely to severe hemophilia A and B, although deficiencies of FV, FVII, FXIII, and vWF also have been diagnosed prenatally [20–23]. Prenatal diagnosis of vWF deficiency is indicated only for type 3 or severe vWD [24]. Early diagnosis permits either termination of the pregnancy or early intervention when indicated.

Table 13.1 Plasma concentrations of coagulation proteins in full-term healthy newborns and in congenital factor deficiencies

	Plasma concentration (U/ml)		
		By factor deficiency	
Factor	At birth	Severe	Mild–moderate
II	0.27–0.64	–	<0.01–0.20
V	0.34–1.45	<0.01	0.02–0.20
VII	0.28–1.43	<0.01	0.01–0.15
VIII	0.50–1.78	<0.01	0.01–0.50
IX	0.15–0.91	<0.01	0.01–0.50
X	0.12–0.87	<0.01	0.01–0.15
XI	0.10–0.87	–	<0.01–0.70
XIII	0.11–0.93	<0.01	–

Treatment

The fundamental principle of treatment is to increase plasma concentrations of the deficient coagulation protein to a minimal hemostatic level when a newborn is bleeding or when a hemostatic challenge is planned. A minimal hemostatic plasma concentration of a particular coagulation protein varies and is dependent upon the protein and the nature of the hemostatic challenge (Table 13.2). Replacement therapy can be achieved with plasma, cryoprecipitate or factor concentrates (Tables 13.2 and 13.3).

Congenital factor deficiencies

The following provides specific information on the presentation and management of congenital factor deficiencies with an emphasis on severe forms.

Table 13.2 Properties of coagulation proteins

Factor	Chromosome	Molecular weight	Plasma concentration (μg/ml)	Half-life (hours)	Minimal hemostatic level	Replacement therapy
I	4q26–q28	330 000	3000	120	0.5–1.0 g/l	FFP/cryoprecipitate Fibrinogen concentrate
II	11p11–q12	72 000	100	72	0.15–0.40 U/ml	FFP/PCC
V	1q21–q25	330 000	10	12–36	0.10–0.25 U/ml	FFP
VII	13q34	50 000	0.5	4–6	0.05–0.10 U/ml	FVII concentrate PCC/FFP
VIII	Xq28	330 000	0.1	12–15	0.30–0.50 U/ml	FVIIIC
IX	Xq27	56 000	5	18–30	0.20–0.50 U/ml	FIX concentrate PCC
X	13q34	58 800	10	65	0.10–0.20 U/ml	PCC/FFP
XI	4q32–q35	160 000	5	65	0.10–0.30 U/ml	FXI concentrate FFP
XIII	(a) 6p24p25 (b) 1q31–q32	320 000	60	72–240	0.10–0.50 U/ml	FXIII concentrate Cryoprecipitate FFP
vWF	12pter–p12	309 000	5–10	12	0.30–0.50 U/ml	FVIII-vWF-concentrate Cryoprecipitate

(a), subunit of FXIII; (b), subunit of FXIII; FFP, fresh frozen plasma; FVII, factor VII; FVIII, factor VIII; FIX, factor IX; FXI, factor XI; PCC, prothrombin complex concentrate; vWF, von Willebrand factor.
Adapted with permission [377].

Factor VIII deficiency

FVIII is synthesized as a single-chain glycoprotein with a molecular weight of 330 000. The gene for FVIII is located on the long arm of the human X chromosome in the most distal band Xq28 (Table 13.2). Following activation by FXa or thrombin, FVIIIa acts as a cofactor for the FIXa-mediated activation of FX, which subsequently converts prothrombin to thrombin in the presence of FVa, negatively charged phospholipids, and calcium.

Severe FVIII deficiency (FVIII <1%) is the most common congenital coagulation disorder to present in newborns. A small percentage of newborns with moderate (FVIII 1–5%) or mild (FVIII 5–50%) FVIII deficiency also may present following an acquired hemostatic challenge [10, 14, 25–29]. Based upon recent cohort studies, 40–60% of children with severe hemophilia A are clinically symptomatic as newborns. A further 40% present by one year of age, and 50% have had a major bleed by 1.5 years. Common presentations are ICH, muscle and mouth bleeding, cephalic hematoma, bleeding from the umbilical stump, and bleeding following circumcision [30–32]. Rarer reported clinical presentations include bleeding in the adrenal gland, hematoma of the liver, splenic rupture, and radial-artery pseudoaneurysm following radial artery puncture [33–35]. Although rare, severe hemophilia A may occur in females and present at birth. The plasma concentrations of FVIII in these female newborns are, in general, below 0.01 U/ml.

Treatment

Currently, if available recombinant factor FVIII concentrates are generally considered the treatment of choice in the newly diagnosed patient with hemophilia A with highly purified derived factor VIII concentrates as the next best alternative (Table 13.3).

Table 13.3 Common factor concentrates available in Europe and/or North America in 2004

		Availability	
Name	Manufacturer	Europe	North America
Factor VIII			
High purity			
Haemofil M	Baxter	Yes	Yes
Monoclate P	ZLB Behring	Yes	Yes
Recombinant			
Helixate (NexGen or FS)	ZLB Behring	Yes	Yes
Kogenate FS	Bayer	Yes	–
Recombinate	Baxter	Yes	Yes
Refacto	Wyeth	Yes	Yes
Advate	Baxter	Yes	Yes
Factor IX			
High purity			
Alphanine SD	Alpha	Yes	Yes
Berinin	ZLB Behring	Yes	–
Immunine	Baxter	Yes	Yes (Canada only)
Mononine	ZLB Behring	Yes	Yes
Recombinant			
BeneFix	Genetics Institute[a]	Yes	Yes
Fibrinogen			
Haemocomplettan HS	ZLB Behring	Yes	–
Factor VII			
Factor VII	Baxter	Yes	Yes (Canada only)
Recombinant			
Novoseven	NovoNordisk	Yes	Yes
Factor XI			
Factor XI	BioProducts Laboratory	Yes	–
Hemoleven	LFB France	Yes	–
Factor XIII			
Fibrogammin HS	ZLB Behring	Yes	–
Factor XIII	BioProducts Laboratory	Yes	–

[a]Distributed in Europe by Baxter.

Guidelines for FVIII replacement therapy for specific acute hemorrhages in infants and children with severe hemophilia A are provided in Table 13.4 [36]. Alternative therapy to FVIII replacement has been used in newborns undergoing circumcision. In one study of ten patients with severe hemophilia A, local fibrin glue was used instead of infusion of FVIII concentrate. Only two of three patients who bled postoperatively required FVIII concentrate [37]. Insufficient data are available to support the benefit of prophylactic doses of FVIII intrauterine or shortly after birth in newborns suspected or confirmed to have hemophilia in order to offset the trauma of labor and reduce the risk of ICH.

Factor IX deficiency

Human FIX is a vitamin K-dependent single-chain glycoprotein with a molecular weight of 56 000. The gene for FIX is located on the tip of the long arm of the

Table 13.4 Factor VIII (FVIII) and factor IX (FIX) replacement therapy in children with severe hemophilia A and B

Type of bleed	Minimum hemostatic level	Hemophilia A (FVIII)	Hemophilia B (FIX)[a]
Joint	30–50%	20–40 U/kg day usually for 2–4 days	30–40 U/kg day usually for 2 days
Muscle	40%–50%	20–40 U/kg daily	40–60 U/kg daily
Oral mucosa	50%	25 U/kg	50 U/kg
Epistaxis	Initially 80–100%, then 30% until resolution	40–50 U/kg, then 30–40 U/kg/day	80–100 U/kg, then 70–80 U/kg/day
Gastrointestinal	Initially 100%, then 30% until resolution	40–50 U/kg, then 30–40 U/kg/day	80–100 U/kg daily, then 70–80 U/kg/day
Hematuria	Initially 100%, then 30% until resolution	40–50 U/kg, then 30–40 U/kg day	80–100 U/kg, then 70–80 U/kg daily
Central nervous system	Initially 100%, then 50–100% for 10–14 days	50 U/kg, then 25 U/kg every 12 hours or continuous infusion	100 U/kg, then 50 U/kg every 24 hours or continuous infusion[b]
Surgery	Initially 100%, then 50% until wound healing begins, then 30% until wound healing is complete	50 U/kg, then dose every 12 hours or by continuous infusion	100 U/kg, then dose every 24 hours or by continuous infusion[b]

[a]Recoveries with recombinant FIX is variable, and higher doses may be necessary to achieve target hemostatic levels.
[b]Continuous infusion of high-purity product may be possible.
Adapted with permission [36].

X chromosome at position Xq27 (Table 13.2). Following activation by FXIa or FVIIa complexed with tissue factor, FIXa complexed with FVIIIa on membrane surfaces activates FX, which subsequently converts prothrombin to thrombin.

Bleeding due to severe FIX deficiency (FIX <1%) in newborns occurs at the same sites as for FVIII deficiency [10, 14, 25, 38–44]. The diagnosis of mild FIX deficiency (FIX 1–5%) in newborns may be difficult because of physiologically decreased FIX levels as low as 0.15 U/ml and the potential for concurrent vitamin K deficiency in these patients (Table 13.1).

Treatment

As with factor VIII, if available, recombinant factor IX concentrate may be the treatment of choice in the newly diagnosed patient with hemophilia B, although variable recovery has been reported with this product. Factor IX levels should be measured with initial use of the product. Again highly purified plasma derived factor IX concentrates are an alternative (Table 13.3). Guidelines for FIX replacement therapy for specific acute hemorrhages in infants and children with severe haemophilia B are provided in Table 13.4.

Afibrinogenemia/hypofibrinogenemia

The fibrinogen molecule is a dimer with a molecular weight of 330 000 consisting of three pairs of polypeptide chains named Aα, Bβ, and γ. These three chains are encoded by three separate genes located on chromosome 4 (Table 13.2). Following enzymatic cleavage of fibrinopeptides by thrombin, fibrinogen is converted into insoluble fibrin, inducing clot formation.

Fibrinogen deficiency is rare. Bleeding due to afibrinogenemia has been reported in newborns following circumcision and as umbilical stump bleeding and soft-tissue hemorrhage [45–48].

Treatment

Although fresh frozen plasma (FFP) can be used as initial therapy, cryoprecipitate or fibrinogen concentrates are preferable. FFP at a dose of 10–20 ml/kg

or cryoprecipitate at a dose of 1 U/5 kg of body weight usually raises the plasma fibrinogen level 50–100 mg/dl and lasts for four to five days, reflecting the long plasma half-life of fibrinogen (Table 13.2). One fibrinogen concentrate (Haemocomplettan HS, ZLB Behring) is only available in Europe (Table 13.3). The dose required for children depends on the type of bleeding and on the weight of the patient.

Prothrombin deficiency

Prothrombin is a vitamin K-dependent plasma glycoprotein with a molecular weight of 72 000. The gene for prothrombin is located on chromosome 11 at position 11p11–q21 (Table 13.2). Prothrombin is converted to thrombin by FXa in the presence of FVa, phospholipids, and calcium.

Deficiency of prothrombin is very rare. Bleeding complications due to prothrombin deficiency have been reported in two newborns and consisted of gastrointestinal bleeding and ICH [49, 50]. Adult patients with homozygous prothrombin deficiency may experience easy bruising, epistaxis, menorrhagia, or bleed following invasive procedures such as circumcision, venepunctures, and intramuscular injections. Hemarthroses are uncommon but have been described [46].

Treatment

Bleeding secondary to prothrombin deficiency can be treated with FFP at a loading dose of 10–20 ml/kg followed by 3 ml/kg every 12–24 hours. Although PCC contains significant amounts of prothrombin, administration of PCC should be monitored carefully because the presence of other vitamin K-dependent factors in PCC may activate the coagulation cascade, leading to thrombotic complications (Table 13.2).

Factor V deficiency

FV is synthesized as a single-chain glycoprotein with a molecular weight of 330 000. The gene for FV is located on chromosome 1 at position 1q21–25 (Table 13.2). Following activation by thrombin or FXa, FVa acts as a cofactor for the FXa-mediated activation of prothrombin.

Bleeding due to severe FV deficiency has been reported in newborns. The clinical presentations include ICH, subdural hematoma, bleeding from the umbilical stump, gastric hemorrhage, and soft-tissue hemorrhage [51–55]. Antenatal intraventricular hemorrhage (IVH) has been reported in two newborns [56, 57].

Treatment

Currently, only FFP is available as a replacement source for FV. When bleeding occurs, infusions of FFP at an initial dose of 10–20 ml/kg, followed by infusions of 3–6 ml/kg, can correct the bleeding defect. One infusion of FFP per day is usually sufficient to achieve the minimal hemostatic FV level (Table 13.2).

Factor VII deficiency

FVII is a vitamin K-dependent single-chain zymogen with a molecular weight of 50 000. The gene for FVII is located on the long arm of chromosome 13 at position 13q34 (Table 13.3). FVII is a component of the extrinsic pathway of blood coagulation. Following activation by binding to its cofactor, tissue factor, FVIIa catalyzes activation of additional FVII and activates both FX and FIX.

Severe FVII deficiency (FVII <1%) usually causes significant bleeding equivalent to that seen in patients with severe hemophilia. Patients with FVII levels greater than 5% generally have mild hemorrhagic episodes. The most common reported bleeding complication in newborns with congenital FVII deficiency is ICH [58–60]. In a review of 75 patients with FVII deficiency, ICH was observed in 12 (16%) patients. In 5 (41%) of these 12 patients, ICH occurred in the first week of life, with fatal outcome [61]. Congenital FVII deficiency may occur in infants with Dubin–Johnson syndrome [16, 62] or Gilbert syndrome [15].

Treatment

Bleeding episodes due to FVII deficiency can be treated with FFP, PCC, or FVII concentrates (Tables 13.2 and 13.3). For severe hemorrhage, FFP should be administered at an initial dose of 20 ml/kg

followed by a dose of 3–6 ml/kg, repeated every 8–12 hours until healing occurs. Although FFP or PCC can be used as initial therapy, FVIIa concentrate has become the replacement product of choice. However, careful monitoring is required because of the risk of thromboembolic events (TEs) [63]. A recovery study of recombinant FVIIa has been reported for a newborn with severe FVII deficiency and ICH. The drug was administered intravenously every four hours at doses of 15, 22, and 30 μg/kg. Factor VII coagulant activity was over 100% between 30 and 180 minutes after each infusion, with mean trough levels over 25% for all three doses. Infusions were tolerated well and maintained effective hemostasis with a good clinical outcome [64]. In 1988, Daffos and colleagues used fetal blood sampling to diagnose FVII deficiency at 24 weeks' gestational age; the fetus was transfused in utero at 37 weeks' gestational age with 200 U of FVIIa concentrate. Subsequently, the baby was born without hemorrhagic complications and did not bleed as a newborn [52].

Von Willebrand factor deficiency

vWF is an adhesive multimeric glycoprotein with a molecular weight of 309 000. The gene for vWF is located on the short arm of chromosome 12 at position 12pter–p12 (Table 13.2). Following simultaneous binding of vWF to the glycoprotein Ib receptor on platelet surfaces and to collagen in the subendothelium, vWF mediates the adhesion of platelets to the injured vascular wall. Platelet adhesion is mediated by the highest-molecular-weight multimers of vWF. In addition, vWF acts as a plasma carrier and stabilizing protein for FVIII.

vWD includes partial or complete deficiency of vWF (types 1 and 3), qualitative variants with decreased platelet-dependent function (types 2A and 2M), qualitative variants with increased affinity for platelet glycoprotein Ib (type 2B), and qualitative variants with markedly decreased affinity for FVIII (type 2N). Although vWD is the most common congenital bleeding disorder, patients rarely present as newborns because plasma concentrations of vWF are increased at birth with an increased proportion of high-molecular-weight multimers [65, 66]. Bleeding due to vWF deficiency is reported rarely in newborns. Two infants with type 2B vWD presented with bleeding at blood sampling and soft-tissue sites. Repeated platelet counts were less than 50×10^9/l, and the bleeding time and APTT were prolonged. The vWF multimeric structure was characterized by the absence of the high-molecular-weight forms, and the patients' platelets aggregated at much lower ristocetin concentrations than did those of controls [67]. One infant with type 2A vWD presented with bleeding from the umbilical stump and later with life-threatening epistaxis [68]. Another newborn presented at the age of seven days with ICH and subdural hemorrhage. The FVIII level was 0.03 U/ml and both the vWF antigen and ristocetin cofactor activity (RCO) levels were undetectable [69].

Treatment

The goal of therapy for vWD is to normalize platelet adhesion and increase plasma concentrations of FVIII. Although partially purified FVIII concentrates contain large amounts of vWF, not all of them are able to normalize platelet adhesion because they contain only low- and middle-molecular-weight multimers [70]. Partially purified FVIII concentrates containing some of the high-molecular-weight vWF multimers that have been used successfully to treat bleeding in patients with vWD are listed in Table 13.5. Both type 2 and type 3 vWD can present in newborns and may require treatment with FVIII-vWF concentrates at a dose varying from 5 to 40 U/ml initially administered every 12–24 hours.

Factor X deficiency

FX is a vitamin K-dependent two-polypeptide-chain molecule with a molecular weight of 58 800. The gene for FX is located on the long arm of chromosome 13 at position 13q34 (Table 13.2). Following activation by the FVIIa–tissue factor complex or the FIXa–FVIIIa complex, FXa converts prothrombin to thrombin in the presence of FVa, phospholipids, and calcium.

Table 13.5 Factor VIII–von Willebrand concentrates

		Availability			
Name	Manufacturer	Europe	North America	Method of purification	Method of viral inactivation
Alphanate	Alpha	Yes	Yes	Affinity chromatography	Solvent detergent and dry heat at 80°C for 72 hours
Haemate HS	ZLB Behring	Yes	–	Multiple precipitation	Pasteurization at 60°C for 10 hours
Humate P	ZLB Behring	–	Yes[a]	Multiple precipitation	Pasteurization at 60°C for 10 hours
Koate-DVI	Bayer	Yes	Yes	Multiple precipitation	Solvent detergent and dry heat at 80°C for 72 hours
Immunate	Baxter	Yes	–	Aniono-exchange chromatography	Vapor heat at 60°C for 10 hours

[a]Only Humate P has Food and Drug Administration (FDA) approval for use in von Willebrand disease in the USA.

Bleeding complications due to severe FX deficiency have been reported in newborns. The most common clinical presentation is ICH, but other sites of bleeding have also been reported, including bleeding from the umbilical stump and from heel-prick sites, gastrointestinal bleeding, scrotal hemorrhage, and spontaneous bruising [71–74]. Subdural hemorrhage has been diagnosed antenatally in one newborn [75].

Treatment

Uncomplicated bleeding episodes can be treated with FFP at an initial dose of 20 ml/kg followed by a dose of 5–10 ml/kg every 24 hours until hemostasis occurs. For severe bleeding episodes, PCC also can be used, at doses that depend upon the concentration of FX contained in the concentrate (Table 13.2). However, repeated administration of PCC should be avoided because of the risk of TEs and disseminated intravascular coagulation (DIC). For similar reasons, high doses of PCC that increase FX levels by more than 50% of normal should be avoided.

Factor XI deficiency

FXI is a homodimer composed of two identical chains bound together by disulfide bonds and with a molecular weight of 160 000. The gene for FXI is located on chromosome 4 at position 4q32–35 (Table 13.2). Following activation by FXIIa, FXIa mediates the activation of FIX in the presence of calcium.

Deficiency of FXI is rare and is different from other coagulation protein deficiencies in that bleeding symptoms do not necessarily correlate with plasma concentrations of FXI; nor do all patients bleed [76]. Bleeding complications due to severe FXI deficiency have been reported in two newborns. One newborn bled after circumcision at the age of three days, while another newborn was diagnosed prenatally with bilateral subdural hemorrhage [77, 78].

Treatment

Bleeding episodes requiring treatment can be controlled with either FFP at a dose of 10–20 ml/kg followed by a dose of 3–6 ml/kg every 24 hours until hemostasis is achieved or by FXI concentrates (Tables 13.2 and 13.3).

Factor XIII deficiency

FXIII is a tetramer consisting of two A subunits and two B subunits and with a molecular weight of 320 000. The gene for the A subunit of FXIII is located on chromosome 6 at 6p24–25, while the gene for the B subunit is located on chromosome 1 at position

1q31–32 (Table 13.2). The catalytic activity of FXIII resides in the A subunit. After binding to calcium, FXIII is converted to its activated form by thrombin during fibrin formation. Activated FXIII catalyzes the formation of peptide bonds between adjacent molecules of fibrin monomers, leading to a mechanical and chemical stability of the fibrin clot.

Severe FXIII deficiency typically manifests at birth with bleeding from the umbilical stump or ICH [11, 79–90]. Other clinical presentations of homozygous FXIII deficiency include delayed wound healing, abnormal scar formation, and recurrent soft-tissue hemorrhage, with tendency to form hemorrhagic cysts. ICH occurs even in the absence of a trauma in approximately one-third of all affected patients [91]. Heterozygous newborns are not clinically affected.

Treatment

FFP, cryoprecipitate, or FXIII concentrates can be used for the treatment of FXIII deficient patients. Newborns with FXIII deficiency should be placed on a prophylactic regimen of FXIII because of the high incidence of ICH. Plasma concentrations of FXIII over 1% are effective, and the very long half-life of FXIII permits once-a-month therapy. Therefore, prophylactic replacement therapy is achieved either with low doses of FFP (2–3 ml/kg) administered every four to six weeks, cryoprecipitate at a dose of 1 bag/10–20 kg every three to six weeks, or, preferably, FXIII concentrate at a dose of 10–20 U/kg every four to six weeks, depending on the clinical situation and the preinfusion plasma concentration of FXIII (Tables 13.2 and 13.3) [92].

Familial multiple factor deficiencies

Congenital deficiencies of two or more coagulation proteins have been reported for 16 different combinations of coagulation factors [93]. Bleeding in the neonatal period has been reported for only two infants with combined factor deficiencies. One infant with FII, FVII, FIX, and FX deficiencies presented with spontaneous bruising and bleeding from the umbilical stump, which persisted until three months of age, when the infant was treated with FFP [8]. A second infant with FV and FVIII deficiencies presented with serious bleeding (undescribed) in the first week of life [7]. Multiple factor deficiencies, including combined FXI and FXII, combined FXI and FVIII, and combined FVIII, FXI, and FXII deficiencies, have been observed in patients with Noonan's syndrome [94].

Treatment

Initial therapy is usually with FFP. Subsequent treatment will vary depending upon the specific factors affected.

Alpha$_2$-antiplasmin deficiency

α_2-AP is a single-chain glycoprotein with a molecular weight of 63 000 and a plasma half-life of approximately 60 hours. The gene for α_2-AP is located on chromosome 18 at position p11.1–q11.2. α_2-AP is a major inhibitor of the fibrinolytic cascade by forming an irreversible inactive complex with plasmin.

α_2-AP deficiency is a rare congenital disorder that presents with normal coagulation screening tests. A common presentation is bleeding from the umbilical stump [95]. Reports of older children and adults describe bleeding in the central nervous system, epistaxis, hemarthrosis, spontaneous intramedullary hematoma, and excessive bleeding during surgery or following minor trauma [96–101].

Treatment

Successful administration of antifibrinolytic agents, including tranexamic acid and epsilon-aminocaproic acid, have been reported to treat bleeding episodes or as prophylaxis during surgical procedures in adult patients with α_2-AP deficiency [95, 98, 102]. The recommended doses of tranexamic acid for surgical procedures in adults are 4 g/day by continuous infusion or either 4 × 1 g in a one-hour infusion or orally for 14 days [103]. No data on dose requirements of antifibrinolytic agents in newborns and children with α_2-AP deficiency are available.

Acquired hemorrhagic disorders

The most common causes of acquired hemorrhagic disorders are vitamin K deficiency, DIC, and liver disease.

Vitamin K deficiency

Mechanism of action of vitamin K

Vitamin K is a cofactor essential for the γ-carboxylation process of the vitamin K-dependent factors FII, FVII, FIX, FX and inhibitor proteins C and S. These proteins bind calcium through the gamma carboxyl groups, permitting subsequent concentration on negatively charged phospholipid surfaces. The lack of functional activity for vitamin K-dependent coagulation proteins causes bleeding, which is reversible with vitamin K therapy.

Sources of vitamin K

Sources of vitamin K in humans are the diet that provides vitamin K_1 (phytonadione), which is particularly present in leafy green vegetables, and intestinal bacteria that synthesize vitamin K_2 (menaquinone). Newborns are at increased risk of vitamin K deficiency due to poor placental transfer of vitamin K (10%) during pregnancy, insufficient bacterial colonization of the colon at birth, and inadequate dietary intake of vitamin K in breast-fed babies [104–108]. Finally, certain drugs, including oral anticoagulants, anticonvulsants, rifampicin, and isoniazid, administered to mothers during pregnancy can interfere with either vitamin K storage pool or vitamin K function in the fetus and the newborn [109–112].

Clinical presentation of vitamin K deficiency

Bleeding due to vitamin K deficiency can be classified into three patterns based on the timing and type of complications [113]. A first, early pattern of vitamin K deficiency bleeding (VKDB) presents in the first 24 hours of life with serious bleeding, including ICH. A second, classical pattern of VKDB presents on days one to seven of life in breast-fed infants, with gastrointestinal bleeding, widespread ecchymoses, sometimes ICH, or bleeding from puncture sites. The third, late pattern of VKDB presents beyond the first week of life in breast-fed infants and usually is associated with a variety of diseases that compromise the supply of vitamin K, such as diarrhea, cystic fibrosis, alpha$_1$-antitrypsin (α_1AT) deficiency, hepatitis, celiac disease, and other rare disorders (Table 13.6).

Laboratory evaluation of suspected vitamin K deficiency

The laboratory tests for detecting the presence of vitamin K deficiency include screening tests (prothrombin time and APTT) and specific factor assays (FII, FVII, FIX, FX). Patients with vitamin K deficiency produce decarboxylated forms of the vitamin K-dependent factors (protein induced by vitamin K absence, PIVKA), which can be measured directly or as a discrepancy between coagulant activity and immunological concentration [114–124].

Treatment of vitamin K deficiency

Any patient suspected to have vitamin K deficiency should be treated immediately with vitamin K while awaiting laboratory confirmation. The route and specific type of therapy are dictated by the urgency of the clinical situation and the potential side effects of therapy. Severe anaphylactoid reactions, although rare, have complicated intravenous vitamin K administration, even when the solution is diluted and infused slowly. The preferred systemic route is subcutaneously, because it is safe and effective. Oral vitamin K is effective if absorption is unimpaired, but this route produces a slower correction of the prothrombin time (six to eight hours) compared with parenteral vitamin K administration (two to six hours).

Bleeding due to vitamin K deficiency should be treated with subcutaneous vitamin K at doses appropriate for age. Depending on the severity of bleeding, 10–20 ml/kg or FFP should be given. FFP is particularly useful when the precise nature of the coagulopathy is unknown. Prothrombin complex

Table 13.6 Pattern of vitamin K-deficiency bleeding (VKDB) in infants

	Early VKDB	Classical VKDB	Late VKDB
Age at onset	<24 hours	Days 1–7	Week 2 to 6 months
Etiology	Drugs administered to mother during pregnancy	Marginal vitamin K content in breast milk Inadequate milk intake	Marginal vitamin K content in breast milk Malabsorption of vitamin K (liver or bowel disease) Commoner in boys than girls and in summer than winter
Location	Cephalohematoma, umbilicus, intracranial, intraabdominal, intrathoracic, gastrointestinal	Gastrointestinal tract, umbilicus, nose, needle-prick sites, circumcision, intracranial	Intracranial (30–60%), skin, nose, gastrointestinal tract, needle-pick sites, umbilicus, urogenital tract, intrathoracic
Frequency without prophylaxis	<5% in high-risk groups	0.01–1.5%	4–10/100 000 births
Avoidance measures	Stop or replace offending drugs Give vitamin K prophylaxis to mother during pregnancy	Adequate vitamin K supply by early and adequate breast feeding Vitamin K prophylaxis	Vitamin K prophylaxis Early recognition of predisposing conditions

Adapted with permission [113].

concentrates can be administered to newborns with life-threatening bleeding or ICH as an alternative to FFP. However, administration of PCCs requires careful monitoring because of the risk of thromboembolic complications. The amount of plasma required to totally correct a severe vitamin K deficiency is so large that it may result in volume overload.

Prophylactic vitamin K administration

Several trials have assessed the benefit of prophylactic vitamin K in neonates and have provided different support depending upon the strength of the study design. Two major randomized controlled trials assessed the benefits of vitamin K prophylaxis using clinical bleeding as the outcome measure [125, 126]. In the first study, 3338 full-term infants were randomized to receive either placebo or menadione (100 μg or 5 mg) intramuscularly. Healthcare personnel, who were unaware of the type of therapy, assessed minor and major bleeding outcomes. The incidence of ICH as well as minor bleeding was increased significantly in the placebo group compared with both treatment arms. This clinical result was supported by laboratory tests showing that the prothrombin time was prolonged in infants with hemorrhagic complications and corrected when vitamin K was given. In the second study, 470 infants were randomized to receive vitamin K or nothing. The study population consisted of male infants who were undergoing circumcision. Bleeding following circumcision was observed in 32 of 230 infants not receiving vitamin K compared with six of 240 infants treated with vitamin K. The same investigators reproduced these results in a subsequent, smaller, nonblinded trial [127].

In studies comparing infants treated with prophylactic vitamin K at birth versus concurrent untreated infants, biochemical evidence of vitamin K

deficiency was observed less frequently in infants who received vitamin K prophylaxis [118, 128–130]. Other studies in large newborn populations in which vitamin K prophylaxis was instituted and then withdrawn or never instituted, bleeding occurred rarely in infants receiving prophylactic vitamin K but was observed when prophylactic vitamin K was withdrawn [131, 132]. Numerous studies report a beneficial effect (either clinically or biochemically) following administration of vitamin K to mothers [130].

In addition to general prophylaxis at birth, certain risk groups require additional vitamin K prophylaxis. This population includes infants with α_1-AT deficiency, chronic diarrhea, cystic fibrosis, and celiac disease. Pregnant women receiving oral anticonvulsant therapy should receive vitamin K in the third trimester to prevent overt vitamin K deficiency in their infants at birth.

Route of prophylactic vitamin K administration

While the benefit of vitamin K prophylaxis in newborns is recognized uniformly, some controversy still exists with regard to the route of administration and dosing. Although intramuscular administration of 1 mg vitamin K at birth provides complete protection against VKDB, several disadvantages have limited the acceptance of this form of prophylaxis. These disadvantages include local trauma, poor acceptance by parents, relatively high costs, and the potential risk of cancer [133]. Oral administration of a single dose of 1 mg vitamin K at birth protects against early and classical VKDB but is not effective in preventing late VKDB [134, 135]. Repeated or continuous oral vitamin K supplementation has been shown to be as effective as intramuscular prophylaxis, but it requires appropriate compliance and may not fully protect infants with cholestatic disease from late VKDB [136, 137]. The efficacy of oral vitamin K prophylaxis may be improved by the use of a new mixed-micellar water-soluble vitamin K formulation [138, 139]. Current recommendations for vitamin K prophylaxis in healthy newborns in different countries are listed in Table 13.7.

Table 13.7 Current recommendations for vitamin K prophylaxis in healthy newborns in different countries

Country	Recommended vitamin K prophylaxis
Australia	All newborns: 1 mg IM on day 1
Canada	All newborns: 1 mg IM on day 1
France	Formula-fed newborns: 2 mg oral on day 1 and 2 mg orally between days 2 and 7 Breast-fed newborns: 2 mg oral on day 1 and 2 mg orally weekly, or 25 μg orally daily until completion of breast feeding
Germany	All newborns: 2 mg orally on day 1, days 4–10, and days 28–42
Switzerland	All newborns: 2 mg[a] orally on days 1, 4, and 30
The Netherlands	Formula-fed newborns: 1 mg orally on day 1 Breast-fed newborns: 1 mg orally on day 1 and 25 μg oral daily from week 1 to 13
USA	All newborns: 1–2 mg IM on day 1

[a]Mixed-micellar vitamin K preparation.
IM, intramuscular.

Disseminated intravascular coagulation

Etiology

DIC is not a disorder in itself but a process that is secondary to a variety of age-specific underlying diseases [140]. In premature newborns, DIC usually develops secondary to respiratory distress syndrome, congenital viral infections, serious bacterial infections, hypothermia, and meconium or amniotic-fluid aspiration syndromes. In full-term infants, DIC usually occurs in association with adverse events affecting the fetoplacental unit, resulting in asphyxia, shock, and release of tissue thromboplastin at the time of birth [141–144].

Pathogenetic mechanism

The pathogenetic mechanism of DIC is a systemic activation of coagulation mediated by several cytokines, particularly interleukin 6 (IL-6). This

process leads to a tissue-factor-mediated increased generation of thrombin and a decreased generation of natural anticoagulants, including antithrombin, protein C, and tissue-factor-pathway inhibitor, and consequently, to extensive intravascular deposition of fibrin. The simultaneous inhibition of the fibrinolytic pathway, due to increased plasma concentration of plasminogen-activator inhibitor 1 (PAI-1), prevents adequate removal of fibrin from small vessels, resulting in extensive thrombosis and organ failure. The process also leads to consumption of platelets and coagulation factors, which may cause severe hemorrhage [140].

Clinical presentation and diagnosis

Clinically affected infants usually present with bleeding complications, which include diffuse oozing from mucous membranes or sites of invasive procedures, hematuria, hemoptysis, bruising, hematomas, and, in some cases, ICH. The diagnosis of DIC is based upon compatible clinical features in conjunction with abnormalities of specific coagulation tests. The classic laboratory abnormalities that reflect clinically significant DIC include prolongation of screening tests (prothrombin time, APTT), thrombocytopenia, decreased plasma concentrations of specific coagulation factors (fibrinogen, FV, FVIII) and inhibitors (antithrombin, heparin cofactor II (HCII), protein C), and increased concentrations of fibrinogen degradation products (FDP) [142–144]. The diagnosis of DIC can be facilitated by very sensitive tests that measure the in vivo effects of thrombin and/or plasmin generation. These tests include prothrombin activation fragment 1 and 2 and thrombin–antithrombin (TAT) complexes. However, abnormalities in these tests alone do not necessarily signify the presence of, nor the need to treat, DIC. For example, increased plasma concentrations of TAT complexes are present in cord plasma from healthy infants and likely reflect activation of the coagulation system during the normal birth process [145–147].

Treatment

DIC is a secondary disease. The cornerstone of treatment of DIC in all patients remains the successful treatment of the underlying disease. Treatment of the secondary hemostatic disorder may be helpful but has to be considered as an adjuvant therapy. Since decreased concentrations of platelets and coagulation factors may cause serious bleeding, replacement therapy with platelet concentrates and plasma in the form of FPP or cryoprecipitate may be beneficial in clinically symptomatic infants. Transfusion of factor concentrates generally is not recommended because of contamination with activated coagulation factors, which could, at least theoretically, augment the already activated coagulation system. Clinically practical goals when treating infants who are bleeding from DIC are to maintain the platelet count above 50×10^9/l, fibrinogen concentrations above 1.0 g/l, and prothrombin time and APTT values near normal values for age. Although replacement of natural inhibitors of coagulation, including antithrombin and protein C, may be appropriate therapy in patients with DIC, the lack of large clinical trials cannot support a general recommendation to use these [148, 149]. Another attractive therapy option in patients with DIC is to inhibit the activation of the coagulation system by using heparin. However, heparin may increase the risk of bleeding, and there are no trials showing that it is helpful. The use of heparin usually is limited to patients with large-vessel thrombosis [150–153].

Several uncontrolled trials and two randomized clinical trials in newborns with DIC have been published [144, 154–162]. Gobel and colleagues [163] randomized 36 infants to either unfractionated heparin (UFH) or placebo, while Gross and colleagues [164] randomized 33 newborns to exchange transfusion, administration of FFP and platelets, or no specific therapy. Although no beneficial effect of therapy was shown, both trials were not conclusive because the sample sizes were insufficient to detect a 50% reduction in mortality.

Liver disease

Etiology

The causes of liver disease associated with an increased risk of bleeding in the neonatal period include congenital heart disease with severe cardiac low output, hypoxia, extrahepatic biliary atresia, inherited metabolic disorders, and viral hepatitis.

Pathogenetic mechanism

The mechanisms responsible for hemostatic impairment in liver disease include decreased synthesis of coagulation factors, activation of both the coagulation and the fibrinolytic systems, poor clearance of activated hemostatic components, loss of coagulation proteins into ascitic fluid, thrombocytopenia, platelet dysfunction, and concomitant vitamin K deficiency. Frequently, this condition presents in association with DIC.

Clinical presentation

The clinical presentation is variable and usually dependent on the underlying disorder. Symptoms include ecchymoses and petechiae, mucous-membrane bleeding, hemorrhage from gastrointestinal varices or into the abdomen, and ICH.

Laboratory evaluation

Laboratory abnormalities include prolongation of screening tests, prothrombin time, APTT, thrombocytopenia, and prolonged bleeding time. Plasma concentrations of FVII, FV, fibrinogen, and plasminogen are decreased, while FDP and D-dimer frequently are increased. A normal concentration of FVIII, reflecting significant extrahepatic synthesis, can help distinguish severe liver disease from DIC.

Treatment

Therapy modalities include factor replacement in the form of FFP and cryoprecipitate, platelet transfusions, and vitamin K administration. Factor VIIa concentrate has been demonstrated to normalize the prolonged prothrombin time in an infant with fulminant hepatitis and in patients with liver cirrhosis undergoing invasive procedures [165, 166]. The feasibility and effectiveness of these treatment options depend on the severity of the liver disease, the hemostatic impairment, and other clinical features.

Other clinical conditions

Cyanotic congenital heart disease

Cyanotic congenital heart disease in newborns may be associated with hemostatic disorders, including coagulation defects as well as quantitative and qualitative platelet defects [167–170].

Cardiopulmonary bypass

Cardiopulmonary bypass (CPB) is associated with a significant risk of bleeding, either intra- or postoperatively. The increased risk of bleeding is due mostly to the use of heparin in combination with hemostatic defects occurring during and following CPB. Hemostatic defects include important decreases in plasma concentrations of all coagulation proteins, primarily by hemodilution and secondarily by consumption, activation of the fibrinolytic system, and significant quantitative and qualitative platelet defects [171].

Extracorporeal membrane oxygenation

Extracorporeal membrane oxygenation (ECMO) is an increasingly used technique that permits oxygen transfer through a semipermeable membrane into blood of critically sick newborns with refractory hypoxemia. Causes of neonatal life-threatening respiratory failure requiring treatment with ECMO include meconium aspiration, sepsis, congenital diaphragmatic hernia, and pulmonary hypertension. Hemorrhage, particularly ICH, is one of the

most serious complications of this technique. ICH occurs with an incidence of 25–50% in treated newborns and is associated with increased mortality and long-term neurologic morbidity [172–175]. Similarly to CPB, the increased risk of bleeding is due mostly to the use of heparin in combination with hemostatic defects occurring during ECMO and including significantly decreased plasma concentrations of coagulation proteins and platelet dysfunction [176, 177]. Other recognized factors associated with an increased risk of ICH during ECMO include prolonged hypoxia, ischemia, prematurity, acidosis, sepsis, and treatment with epinephrine [178].

Intracranial hemorrhage

General information

ICH in newborns is particularly concerning because of the potential for a poor neurologic outcome. The incidence of ICH in newborns is unknown and is likely changing as a reflection of improving perinatal care. The widespread use of ultrasound during pregnancy has resulted in the detection of ICH in utero and provided a safe modality for monitoring fetuses at risk.

Clinical presentation

Unfortunately, the diagnosis of ICH may be delayed due to the nonspecific nature of the early clinical presentation, which includes lethargy, apnea, vomiting, and irritability. By contrast, the more extreme clinical presentation of ICH is usually recognized early and is characterized by seizures, meningismus, and a tense fontanelle. ICH can occur both in full-term and in premature newborns and in a variety of locations.

Full-term newborns

ICH is rare in full-term newborns and usually occurs either spontaneously or secondary to a variety of insults such as birth asphyxia, trauma, vitamin K deficiency, and several congenital factor deficiencies. Other risk factors include small birth weight, young gestational age, and race [179–188]. In a review of 75 cases of FVII deficiency, 12 (16%) patients suffered an ICH. ICH occurred in the first week of life in 5 of these 12 patients and in the first year of life in 9 of the 12. The risk of ICH in children with severe hemophilia A or B ranges from 2% to 8%. The location of the ICH is most commonly subarachnoid, but subdural and parenchymal bleeding also occurs. Some infants require surgical intervention, and many have long-term neurological deficits.

Premature newborns

The characteristic form of ICH in premature newborns is an IVH. This is characterized by bleeding from the fragile microvasculature of the subependymal germinal matrix that may extend into the lateral ventricles and/or brain parenchyma [179–185, 189–192]. Approximately 20–40% of premature infants born before 32 weeks' gestational age or with a birth weight below 1500 g develop an ICH [193]. Most IVHs occur in the first 24 hours of life, with almost all developing by 72 hours of life [185, 191, 192, 194–196]. In more than half of the affected newborns, IVH is clinically silent [197]. Ultrasonography allows accurate bedside diagnosis and follow-up of IVH [195–200]. Four severity grades of IVH have been defined using ultrasonography. Grade I defines a bleeding in the subependymal matrix, grade II indicates intraventricular bleeding, grade III indicates intraventricular bleeding and dilation, and grade IV includes intraventricular bleeding with extension in the parenchyma [201].

The pathophysiologic mechanism of IVH in premature infants is incompletely understood and is likely multifactorial [202–206]. Abnormalities of cerebral blood flow resulting in ischemia, and subsequent reperfusion of brain tissue, are the most likely causes [207–213]. Other potential contributing mechanisms include the fragility of the germinal matrix capillaries, oxidative damage to the endothelium, and concurrent impairment of the coagulation system, including decreased plasma concentrations of some coagulant proteins, thrombocytopenia, and

enhanced local fibrinolytic activity [154, 207, 214, 215]. Factors such as vaginal delivery, labor, intrapartum asphyxia, respiratory distress syndrome, increased mean, diastolic, and systolic blood pressures, and decreased superior vena cava flow due to an immature myocardium have been associated with IVH in premature newborns [216–218]. The role of vitamin K deficiency in the development of IVH has been assessed in three randomized controlled trials evaluating the role of vitamin K administration to mothers during pregnancy in preventing IVH in newborns. Two trials demonstrated benefit, but one did not [219–221].

Currently, no valid recommendations can be provided for the treatment of IVH. Treatment modalities include correction of underlying hemostatic defects using blood products, inhibition of fibrinolysis, stabilization of capillary membranes, and regulation of cerebral blood flow. Clinical trials assessing the role of FFP, concentrates of FXIII, antithrombin, and platelets, antifibrinolytic agents (tranexamic acid), and prostaglandin inhibitors (ethamsylate or indomethacin) for the prevention of IVH have documented some benefit in the use of these products; however, the results are, in part, conflicting [160, 222–230].

Thromboembolic disorders

General information

Incidence

The overall incidence of TEs in newborns admitted to neonatal intensive care units is 2.4 per 1000 admissions, which contrasts with an incidence of 2.5–5% of all adults [231].

Etiology

Almost 90% of TEs in newborns are associated with arterial or venous catheters, particularly umbilical artery catheters (UACs) and central venous lines (CVLs). Idiopathic TEs represent less than 1% of newborn TEs as compared with approximately 40% of adult TEs. Rarely, but usually with catastrophic results, TEs are secondary to severe congenital deficiencies of coagulation inhibitors [231].

Congenital prothrombotic disorders

General information

Congenital deficiencies of coagulation inhibitors are autosomally inherited, with adult heterozygotes having plasma concentrations at approximately 50% of normal values. Although newborns with heterozygous inhibitor deficiencies rarely develop TEs, up to 20% of newborns with TEs are heterozygous for a congenital prothrombotic disorder [232]. In addition, an increased prevalence of heterozygous inhibitor deficiencies has been described in newborns with porencephaly and ICH [233, 234].

Common congenital prothrombotic disorders

Activated protein C resistance/factor V Leiden

Activated protein C (APC) is a natural anticoagulant that proteolyzes two cofactors, FVIIIa and FVa [235–237]. Activated protein C resistance (APCR) is due to a mutant of FV, FV Leiden, in 95% of all patients. The risk of TEs is increased three- to eightfold in adult heterozygous, and 80-fold increased in homozygous, carriers of the FV Leiden mutation [238–240].

Individuals with homozygous or heterozygous FV Leiden usually have their first thrombotic event following puberty and rarely at birth [241–243]. When TEs occur in newborns, either multiple prothrombotic disorders or, more commonly, acquired risk factors usually are present. The contribution of APCR to TEs in newborns is likely dependent on the underlying disorder and the type of TEs [244]. Several cases of severe TEs in newborns with FV Leiden are reported. One infant presented shortly after birth with progressive purpuric skin lesions and microvascular hemorrhagic thrombosis in the brain [245]. One infant had a life-threatening cerebellar TE during the second day of life [246]. Another infant

with heterozygous FV Leiden mutation developed life-threatening inferior vena cava TEs during the first 24 hours of life, while another newborn developed a renal-vein thrombosis (RVT) [247, 248]. Bilateral RVT and sinovenous thrombosis (SVT) were diagnosed within three weeks of birth in a full-term neonate [249]. The overall thrombogenicity of the FV Leiden mutation seems to be aggravated by additional thrombogenic risk factors, including protein C or protein S deficiency, and acquired risk factors, including infection and anoxia at birth [246].

Prothrombin gene G20210A

As described previously, prothrombin is the precursor molecule of thrombin, a critical enzyme in hemostasis, which has several coagulant but also anticoagulant activities (Table 13.2). A mutation in the 3′-untranslated region of the prothrombin gene at position 20210A is associated with an increased risk of thrombosis. In a population-based patient control study (Leiden Thrombophilia Study), heterozygosity for the prothrombin G20210A allele conferred a relative risk for thrombosis of 2.8% (95% confidence interval (CI), 1.4 to 5.6), making prothrombin G20210A the second most commonly inherited defect linked to TEs [250].

Children with heterozygous prothrombin 20210A mutation and TEs are reported rarely in the literature, and none in the literature were newborns [251, 252]. Similarly, no newborns have been reported with homozygous prothrombin G20210A mutation and TEs.

Antithrombin deficiency

Antithrombin is a serine protease inhibitor with a molecular weight of 58 000 and a plasma half-life of 72 hours. The gene for antithrombin is located on chromosome 1 at position 1q23–27. Antithrombin inactivates thrombin as well as FXa, FIXa, FXIa, and FXIIa [253]. The risk for heterozygous adults to develop TEs by mid-adult life is approximately 50% [254]. Additional genetic and clinical risk factors are likely important codeterminants for the development of TEs.

Heterozygous antithrombin deficiency in newborns with TEs has been reported. In these reports the clinical presentation was variable, reflected the site of the thrombi, and did not include purpura fulminans. TEs occurred in arterial or venous vessels and in a variety of unusual locations, including the central nervous system and coronary arteries. One infant developed a fatal aortic and vena cava thrombosis [255–262].

Homozygous antithrombin deficiency is extremely rare, presents within the first ten years of life, and causes severe TEs, which may be venous or arterial [263–267]. One of two infants reported in the literature developed TEs involving a deep leg vein and the inferior vena cava at the age of 1 week and recurrent TEs in both legs at ages 3 and 13 years [264].

Treatment of TEs caused by antithrombin deficiency includes anticoagulation therapy with UFH or low-molecular-weight heparin (LMWH) and, usually, transfusion with antithrombin concentrates to provide a substrate for heparin. The infusion of 50 U of antithrombin concentrate per kilogram usually increases the plasma concentration of antithrombin to approximately 120% in an individual with a baseline level of 50%. A detailed assessment of the response to antithrombin concentrate has been described in one infant with heterozygous antithrombin deficiency and TEs [268]. A bolus of 52 U/kg of antithrombin concentrate increased the plasma concentration of antithrombin from 0.10 U/ml to 0.75 U/ml at 1 hour and to 0.18 U/ml at 36 hours. A second bolus of 104 U/kg of antithrombin concentrate resulted in a 90-minute value of 1.48 U/ml and a 24-hour value of 0.19 U/ml. The infant was then treated with a continuous infusion of 2.1 U/kg/hour of antithrombin concentrate, which maintained a plasma concentration of antithrombin of 0.40–0.50 U/ml [268]. Antithrombin deficiency in newborns may be acquired, e.g. secondary to congenital nephrotic syndrome.

Protein C deficiency

Protein C is a vitamin K-dependent serine proteinase inhibitor with a molecular weight of 62 000 and a plasma half-life of six hours. The gene for protein C is located on chromosome 2 at position q13–q14. Following activation by thrombin and thrombomodulin in the presence of calcium, APC inactivates FV, FVa, and FVIIIa. Adult patients with a congenital deficiency of protein C have a ten-fold increased risk of developing TEs compared with the general population [269, 270]. A number of large studies have confirmed that heterozygous protein C deficiency does not usually present with thrombosis during childhood [243, 271, 272]. Despite the low absolute risk, there are numerous case series and cohort studies suggesting that there is an increased prevalence of heterozygous protein C deficiency in infants and children with TEs [233, 273–277].

Homozygous protein C deficiency is a rare but life-threatening disorder, usually manifesting within hours of birth, with purpura fulminans, cerebral and/or ophthalmic damage occurring in utero, and, less commonly, large-vessel thrombosis. Purpura fulminans is an acute, rapidly progressive hemorrhagic necrosis of the skin due to dermal vascular thrombosis [278–280]. The skin lesions start as small ecchymotic sites that increase in a radial fashion, become purplish-black with bullae, and then become necrotic and gangrenous [279, 280]. Lesions occur mainly on the extremities, but they can occur on the buttocks, abdomen, scrotum, and scalp as well as at pressure points, at sites of previous punctures, and at previously affected sites. The neurologic complications have resulted in mental retardation and delayed psychomotor development. The eye involvement consists of vitreous or retinal hemorrhage secondary to thrombosis, resulting in partial or complete blindness. These newborns usually have severe diffuse DIC with secondary hemorrhagic complications [281]. The diagnosis of homozygous protein C deficiency in these patients depends upon the appropriate clinical picture, a protein C level that is essentially not measurable, and confirmation of a heterozygous state in the parents. The presence of decreased plasma concentrations of protein C in the absence of clinical manifestations and family history cannot be considered diagnostic of homozygous protein C deficiency because physiologic levels in newborns can be as low as 0.12 U/ml.

Neonatal purpura fulminans due to homozygous protein C deficiency is treated with an infusion of 10–20 ml/kg FFP every 12 hours, which increases plasma concentrations of protein C from 15% to 32% at 30 minutes and from 4% to 10% at 12 hours after infusion [282]. When available, protein C concentrates are preferable over FFP because they avoid infusions of large volumes and are safer with regard to viral transmission. Doses of protein C concentrate range from 20 to 60 U/kg, where doses of 60 U/kg increase plasma concentrations of protein C over 0.60 U/ml [283]. Replacement therapy with FFP or protein C concentrates is continued until the clinical lesions are resolved (usually six to eight weeks). Following resolution of the skin lesions, and under cover of replacement therapy to avoid skin necrosis, lifelong administration of oral anticoagulants can be started with target international normalized ratios (INR) values ranging from 3 to 4.5. Recurrent skin lesions should be treated with FFP infusions or protein C concentrates.

Successful administration of long-term prophylactic doses of LMWH has been reported in two sisters with homozygous protein C deficiency and decreased but measurable plasma concentrations of protein C [284]. Homozygous protein C deficiency was diagnosed in one sister at nine years of age, when she presented with recurrent TEs and repeated episodes of severe skin necrosis during therapy with oral anticoagulants, whenever the INR decreased below 3.5. Family testing showed a similar homozygous protein C mutation in her asymptomatic six-year-old sister, while both parents were heterozygous. Long-term LMWH therapy was started in the older sister at therapeutic doses of 1.0 mg/kg every 12 hours during the first six months

followed by prophylactic doses of 1.0 mg/kg once daily for two years and ten months. During this time, no further episodes of TEs or skin necrosis occurred. Treatment with prophylactic doses (1.0 mg/kg once daily) of LMWH successfully prevented TEs in the asymptomatic sister.

Protein S deficiency

Protein S is a vitamin K-dependent single-chain glycoprotein with a molecular weight of 69 000 and a plasma half-life of 60 hours. The gene for protein S is located near the centromer of chromosome 3 at position p11.1–11.2. Protein S functions by enhancing the proteolytic activity of APC against FVa and FVIIIa [285]. Protein S circulates in two forms, a free active form and an inactive form that circulates bound to complement 4B (C_4B). In newborns, protein S circulates completely in the free active form because of the absence of C_4B binding protein [286, 287]. Although the prevalence of protein S deficiency in the general population is uncertain, protein S deficiency is a clinically important risk factor for venous or arterial thrombosis, which usually occurs spontaneously or in the presence of acquired risk factors [288]. While heterozygous protein S deficiency rarely presents with TEs during childhood, homozygous protein S deficiency usually presents at birth with purpura fulminans [271, 272, 289–299].

Homozygous protein S deficiency and compound heterozygous protein S mutation have been described in a small number of newborns [291–299]. The majority of these patients presented with purpura fulminans similar to that seen in homozygous protein C deficiency. The ophthalmic complications have been described in detail and are similar to severe retinopathy of prematurity [298]. The plasma concentration of protein S in infants presenting with purpura fulminans is less than 1%.

Since no specific protein S concentrates are available, treatment of purpura fulminans secondary to homozygous protein S deficiency requires either 10–20 ml/kg FFP every 12 hours or cryoprecipitate at a dose of 1 bag/5 kg of body weight [294]. In a pharmacokinetic study, recovery of protein S following the infusion of 10 ml/kg of FFP was 0.23 U/ml at 2 hours and 0.14 U/ml at 24 hours. The approximate half-life of protein S in this infant was 36 hours [294]. Long-term treatment strategies are similar to those of infants with protein C deficiency.

ADDENDUM

Heparin cofactor II deficiency

Heparin cofactor II (HC II) is single-chain glycoprotein with a molecular weight of 66 000 and a plasma half-life of approximately 2.5 days. The gene for HC II is located on chromosome 22q11. Heparin cofactor II inhibits only thrombin, and has no activity against other proteases generated during coagulation or fibrinolysis[1]. Thrombin inhibition by HC II is increased more than 1000-fold in the presence of heparin, heparan sulfate, or dermatan sulfate[2]. While history of TEs has been documented in members of 15 families, in which inherited deficiency of HC II has been described, deficiency of HC II alone or in combination with other prothrombotic defects is not considered a strong risk factor for TEs in adults at the present time[3].

The role of HC II deficiency in the development of TEs during childhood is unclear. In one study, only 1 out of 285 infants and children with TEs showed inherited deficiency of HC II[4].

Alpha-2 macroglobulin

Alpha-2 macroglobulin (α2-M) is a glycoprotein with a molecular weight of 725 000. The gene for α2-M is located on chromosome 12[5]. Alpha-2 macroglobulin inhibits a number of serine protease molecules including FXa, thrombin, tPA, kallikrein, and plasmin by irreversible complex formation[6]. No data on both the incidence of α2-M deficiency in the general population and the possible role of α2-M deficiency in the development of TEs in adults are available. Only one case of arterial thrombosis in an adult with congenital α2-M deficiency has been reported so far[7].

No single patient with α2-M deficiency presenting during infancy or childhood with TE has been described in the literature.

Plasminogen deficiency

Plasminogen is a single-chain glycoprotein with a molecular weight of 92 000. The native form of plasminogen, Glu-plasminogen, has a plasma half-life of 60 hours. This form is easily converted to a second form, Lys plasminogen, that has a plasma half-life of 18 hours. The gene for plasminogen is located on the long arm of chromosome 6 at band q26–27[8]. Plasminogen is the central component of the fibrinolytic system responsible for the degradation of fibrin. Although plasminogen deficiency is found in 0.5% to 2.0% of adult patients with TEs, there is insufficient evidence to conclude that heterozygous or homozygous deficiency of plasminogen constitutes a significant risk factor for thrombotic disease[9].

Heterozygous plasminogen deficiency has been occasionally reported in infants and children with TEs or stroke[10–12]. Homozygous plasminogen deficiency type 1 has been associated with the development of ligneous conjunctivitis, which is characterized by the formation of recurrent proliferative pseudomembranes on the conjunctival tissue and other mucosal sites. Patients with homozygous plasminogen deficiency may develop ligneous conjunctivitis within days of birth or later during childhood in association with other clinical symptoms including airway obstructions, vulvovaginitis, defective wound healing and congenital occlusive hydrocephalus[13, 14]. Although favorable response to systemic plasminogen replacement therapy and topical plasminogen eye drops has been reported, this treatment option is problematic because plasminogen concentrates are no longer commercially available[15, 16]. Other treatment modalities including topical antibiotics, corticosteroids, hyaluronidase, cyclosporin, disodium cromoglycate, and heparin were unsatisfactory and not successful in all cases.

Catheter-related thromboembolic events

Arterial catheter

Umbilical arterial catheter

UACs are used in sick newborns to monitor oxygen saturation and blood pressure and to facilitate repetitive blood sampling. The tip of the UAC is positioned either high (level of T5–T10) or low (level of L3–L5). Both of these positions may influence the frequency of TEs and ICH [300–302]. TEs associated with UACs include celiac, mesenteric, and renal-artery occlusions and embolic events in the lower limb as well as in the central nervous system, when a right-to-left shunt is present. While the majority of children with UAC-related TEs are clinically asymptomatic or present with minor symptoms, a small percentage (1–5%) present with severe ischemia to the legs and selected organ dysfunction. The most sensitive test for the diagnosis of UAC-related TEs is contrast angiography [303]. The validity of noninvasive techniques such as Doppler ultrasound has not been established.

Venous catheter

Umbilical venous catheter

Umbilical venous catheters (UVCs) are placed through the umbilical vein into the inferior vena cava. The appropriate placement of a UVC is critical to the prevention of serious organ impairment [304]. Portal-vein thrombosis (PVT) and hepatic necrosis can be caused by UVCs not placed beyond the ductus venosus [305]. Predisposition to hepatic necrosis also has been observed when hyperosmolar solutions are injected directly into the portal or hepatic system. Long-term sequelae of UVCs include PVT with portal hypertension, splenomegaly, gastric and esophageal varices, major bleeding related to the varices, and hypertension [306–310].

Central venous line

Central venous lines (CVL) in newborns are placed into the upper venous system either through

peripheral veins in the arm or through the major vessels, usually the jugular vein, or into the lower system through the femoral vein [311]. The clinical presentation of acute CVL-related TEs includes swelling and discoloration of the related limb, swelling of the face, pulmonary embolism, chylothorax, and superior vena cava syndrome [312–316]. More commonly, CVL-related TEs manifest with chronic symptoms, including repeated loss of patency, CVL-related sepsis, and prominent collateral circulation in the skin over the chest, back, neck, and face [231, 317, 318].

Diagnosis of venous catheter-related thrombosis

The diagnostic tests used to investigate venous catheter-related TEs include lineograms, venography, and compression ultrasound. While lineograms are useful only for delineating the location of the tips and clots present at the tips of CVLs, TEs along the intravascular length of venous catheters are detected by venography or ultrasound. With the exception of TEs in the jugular and axillary veins, diagnosis of TEs in the upper central venous system using ultrasound can yield false-negative results for several reasons: (i) the clavicles can hinder the view on the distal subclavian veins; (ii) large collaterals cannot be distinguished from large deep veins in the upper system; and (iii) compression of veins in a central location is not feasible due to the presence of the thoracic cage. When possible, venography is preferred over ultrasound for the upper central veins.

Non-catheter-related thromboembolic events

Renal-vein thrombosis

The majority of non-catheter-related TEs are RVTs, which occur primarily in newborns and young infants. Of the 268 cases described in the literature, 79% presented within the first month, usually within the first week of life. Bilateral RVTs occurred in 24% of patients [249, 319–336]. Some newborns developed RVTs in utero [337–340]. The pathophysiologic mechanism of RVT includes reduced renal blood flow, increased blood viscosity, hyperosmolality, and hypercoagulability secondary to primary disorders, such as perinatal asphyxia, shock, polycythemia, cyanotic congenital heart disease, diabetic fetopathy, dehydration, and septicemia [334, 341–343]. The clinical presentation of RVT in newborns includes a flank mass, hematuria, proteinuria, thrombocytopenia, and nonfunction of the involved kidney. The role of antithrombotic therapy in RVT remains uncertain. In the absence of controlled clinical trials, one approach is to use supportive care for unilateral RVT in the absence of uremia and extension into the IVC. Anticoagulation therapy should be considered for unilateral RVT that does extend into the IVC or bilateral RVT because of the risk of pulmonary embolism and complete renal failure. Thrombolytic therapy should be considered in the presence of bilateral RVT and impending renal failure.

Sinovenous thrombosis

Approximately 50% of sinovenous thromboses (SVT) in children occur in the neonatal period or in early infancy [273]. Important pathophysiologic mechanisms causing SVT in newborns include perturbation of the blood flow during the birth process, increased blood viscosity, hyperosmolality, and hypercoagulability in conjunction with the relatively slow blood flow in the venous sinuses [344]. SVT in newborns frequently presents with seizures, as well as lethargy, jitteriness, full tense fontanel, dilated scalp veins, and, rarely, swelling of the eyelids [345–349]. While magnetic resonance imaging (MRI) is the radiographic technique of choice for the diagnosis of SVT in children, ultrasound through an open fontanel offers a valid alternative for critically ill newborns. When this technique is used, Doppler ultrasound is preferred over conventional cranial ultrasound because it can detect abnormalities of sinovenous blood flow [350–353]. Treatment of SVT in newborns, including the administration of UFH or LMWH, is discussed later in this chapter.

Anticoagulant and thrombolytic therapy in newborns

General information

The effective treatment of TEs in newborns is problematic for a variety of reasons. Because of the absence of completed, well-designed clinical trials assessing the use of antithrombotic drugs in newborns, optimal doses of anticoagulants are unknown and treatment guidelines are extrapolated from studies in adults. This is likely unsatisfactory due to the marked differences in the coagulation system as well as the etiology and pathogenesis of neonatal TEs. The risk of bleeding during anticoagulant or thrombolytic therapy is also unknown, but potentially increased, especially in premature newborns.

Options for the treatment of TEs in newborns include a short course (10–14 days) of UFH or LMWH to achieve APTT or heparin concentrations, respectively, at the low end of adult therapeutic ranges. If the thrombus extends following discontinuation of short-term heparin or LMWH therapy, then administration of LMWH for three months should be considered. In general, oral anticoagulants are to be avoided in this population. Close monitoring for evidence of thrombus extension without anticoagulation therapy is an option for initial management in some circumstances. The following section briefly describes some of the important issues pertaining to anticoagulant and thrombolytic therapy in newborns.

Anticoagulant therapy

Unfractionated heparin

The anticoagulant activity of UFH is dependent on catalyzing the ability of antithrombin to inhibit thrombin and FXa. At birth, plasma concentrations of antithrombin and the capacity to generate thrombin are physiologically decreased compared with adults [17–19]. Sick premature newborns frequently have plasma levels of antithrombin below 0.30 U/ml [18]. In addition, UFH is cleared more quickly in the young compared with in the adult both in animal models and in humans [354–356]. These physiological differences likely explain the increased requirements of UFH in newborns to maintain therapeutic levels.

Therapy with UFH usually is monitored by the APTT. The recommended therapeutic range for the treatment of TEs in children is an APTT value between 60 and 85 seconds. This range should reflect a heparin level by protamine titration of 0.2–0.4 U/ml or an anti-FXa level of 0.35–0.7 U/ml [357]. In pediatric patients, APTT values have been shown to predict correctly whether concentrations of UFH are therapeutic approximately 70% of the time [358]. When APTT values and heparin concentrations are discordant, monitoring with heparin levels is preferred. A nomogram, initially used in adults, and then adapted, tested, and modified for children, is shown in Table 13.8 [358, 359].

Low-molecular-weight heparin

Like UFH, anticoagulant activities of LMWHs are mediated by catalysis of antithrombin. In contrast to UFH, which possesses equal activity against FXa and thrombin, LMWHs have increased specific activity against FXa and less activity against thrombin in vitro [360]. The potential advantages of LMWH for infants include predictable pharmacokinetics that result in minimal monitoring, subcutaneous administration, reduced risk of heparin-induced thrombocytopenia, and probable reduced risk of osteoporosis [361].

Pharmacokinetic studies have shown that newborns and young infants require increased doses of LMWH to achieve target anti-FXa levels compared with older children [362]. The guideline for therapeutic and prophylactic LMWHs is an anti-FXa level of 0.50–1.0 U/ml and 0.10–0.30 U/ml, respectively, in a sample taken four hours following subcutaneous injection (Tables 13.9 and 13.10). A nomogram for dose adjustments of LMWH is shown in Table 13.11 [363, 364].

Table 13.8 Protocol for systemic unfractionated heparin (UFH) administration and adjustment in pediatric patients

I. Loading dose: UFH 75 U/kg intravenous over 10 minutes.
II. Initial maintenance dose: 28 U/kg/hour for infants <1 year of age.
20 U/kg/hour for children >1 year of age.
III. Adjust UFH to maintain an APTT of 60–85 seconds (assuming this reflects an anti-factor Xa level of 0.35–0.70 U/ml).

APTT (seconds)	Bolus (U/kg)	Hold (minutes)	Rate change (%)	Repeat APTT
<50	50	0	+10	4 hours
50–59	0	0	+10	4 hours
60–85	0	0	0	Next day
86–95	0	0	−10	4 hours
96–120	0	30	−10	4 hours
>120	0	60	−15	4 hours

IV. Obtain blood for APTT 4 hours after administration of the UFH loading dose and 4 hours after every change in the infusion rate.
V. When APTT values are therapeutic, take a daily complete blood count and APTT.

APTT, activated partial thromboplastin time.
Adapted with permission [378].

Table 13.9 Therapeutic doses of reviparin-sodium and enoxaparin in pediatric patients

	Dose	Interval (hours)
Reviparin-sodium		
Weight <5 kg	150 U/kg/dose	12
Weight ≥5 kg	100 U/kg/dose	12
Enoxaparin		
Age <2 months	1.5 mg/kg/dose	12
Age ≥2 months	1.0 mg/kg/dose	12

Enoxaparin has an anti-factor Xa of 110 U/mg.
Adapted with permission [378].

Table 13.10 Prophylactic doses of reviparin-sodium and enoxaparin in pediatric patents

	Dose	Interval (hours)
Reviparin-sodium		
Weight <5 kg	50 U/kg/dose	12
Weight ≥5 kg	30 U/kg/dose	12
Enoxaparin		
Age <2 months	0.75 mg/kg/dose	12
Age ≥2 months	0.50 mg/kg/dose	12

Enoxaparin has an anti-factor Xa of 110 U/mg.
Adapted with permission [378].

Oral anticoagulants

Oral anticoagulants function by reducing plasma concentrations of the vitamin K-dependent proteins. At birth, levels of the vitamin K-dependent coagulant factors and inhibitors are approximately 50% of adult values [17–19, 365]. These levels are similar to those found in adults receiving oral anticoagulants for the treatment of TEs [366]. Because of the potential risk of bleeding from further anticoagulation and the presence of borderline vitamin K status, oral anticoagulant therapy is avoided when possible during the first month of life [367, 368].

Breast-fed newborns remain very sensitive to oral anticoagulants due to the low concentrations of vitamin K in breast milk [105, 106, 369, 370]. In contrast, some children are resistant to oral anticoagulants due to impaired absorption, requirements for total parenteral nutrition, or nutrient formulae, all of which are supplemented with vitamin K to

Table 13.11 Nomogram for monitoring reviparin and enoxaparin in pediatric patients

Anti-factor Xa level (U/ml)	Hold next dose	Dose change	Repeat anti-factor Xa level
<0.35	–	Increase by 25%	4 hours after next morning dose
0.35–0.49	–	Increase by 10%	4 hours after next morning dose
0.5–1.0	–	–	Next day, then 1 week later, then monthly thereafter, at 4 hours after morning dose
1.1–1.5	–	Decrease by 20%	Before next morning dose
1.6–2.0	3 hours	Decrease by 30%	Before next morning dose
>2.0	Until anti-factor Xa is 0.5 U/ml	Decrease by 40%	Before next dose

Adapted with permission [379].

protect against hemorrhagic disease of the newborn [369, 371]. These factors likely cause the observed increased dose requirements of oral anticoagulants in infants. The most commonly used test for monitoring oral anticoagulant therapy is the prothrombin time, reported as an international normalized ratio (INR). Currently, therapeutic INR ranges for children are extrapolated directly from recommendations for adult patients. The recommended therapeutic range for the treatment of TEs in children is an INR between 2.0 and 3.0. However, the available biological and clinical data suggest that optimal therapeutic INR ranges may be lower in infants. A nomogram for dose adjustments of oral anticoagulants is shown in Table 13.12.

Table 13.12 Protocol for oral anticoagulation therapy to maintain an international normalized ratio (INR) between 2 and 3 in pediatric patients

I. Day 1: if the baseline INR is 1.0–1.3, then loading dose of 0.2 mg/kg orally.

II. Days 2–4: adjust loading dose as follows:

INR	Action
1.1–1.3	Repeat initial loading dose
1.4–1.9	50% of initial loading dose
2.0–3.0	50% of initial loading dose
3.1–3.5	25% of loading dose
>3.5	Hold dosing until INR <3.5, then restart according to stage III guidelines

III. Maintenance dose guidelines:

INR	Action
1.1–1.4	Increase by 20% of dose
1.5–1.9	Increase by 10% of dose
2.0–3.0	No change
3.1–3.5	Decrease by 10% of dose
>3.5	Hold until INR <3.5, then restart at 20% less than the previous dose

Adapted with permission [378].

Thrombolytic therapy

The actions of thrombolytic agents are mediated by converting endogenous plasminogen to plasmin. At birth, plasma concentrations of plasminogen are reduced to 50% of adult values [17, 19]. The decreased levels of plasminogen in newborns slow the generation of plasmin and reduce the effect of thrombolytic agents, including streptokinase, urokinase, and tissue plasminogen activator (tPA) in an in vitro fibrin clot system [372, 373]. Supplementation of plasmas with plasminogen increases the thrombolytic effect of all three agents in vitro [373, 374]. Although more information on the use of streptokinase and urokinase is available, tPA, as a recombinant protein, is currently the thrombolytic agent of choice for several reasons: (i) in vitro experiments suggest that tPA provides increased clot lysis compared with urokinase and streptokinase; (ii) the use of urokinase has been limited since 1999, as a

Table 13.13 Systemic thrombolytic therapy in pediatric patients

	Loading dose	Maintenance dose	Monitoring
Urokinase	4400 U/kg	4400 U/kg/hour for 6–12 hours	Fibrinogen, TCT, prothrombin time, APTT
Streptokinase	2000 U/kg	2000 U/kg/hour for 6–12 hours	As above
Tissue plasminogen activator	None	0.1–0.6 mg/kg/hour for 6 hours	As above

Note: start heparin therapy either during, or immediately upon completion of, thrombolytic therapy. A loading dose of heparin may be omitted. The length of time for optimal maintenance is uncertain. Values provided are starting suggestions; some patients may respond to longer or shorter courses of therapy.
APTT, activated partial thromboplastin time; TCT, thrombin clotting time.
Adapted with permission [378].

consequence of a warning from the Food and Drug Administration (FDA) concerning inadequate infectious screening of potential donors; and (iii) tPA has an increased fibrin specificity and low immunogenicity compared with streptokinase. The most commonly used dose regimens for thrombolytic therapy in pediatric patients with arterial or venous TEs are presented in Table 13.13.

The most important adverse effect of thrombolytic therapy is bleeding. A recent review of the literature specifically examined the incidence of ICH during thrombolytic therapy in children [375]. ICH was found in 14 of 929 (1.5%) patients analyzed. According to age, ICH was identified in 11 of 86 (13.8%) preterm infants, 1 of 83 (1.2%) term infants, and 2 of 468 (0.4%) children after the neonatal period. However, in the largest study of premature infants included in this review, the incidence of ICH in patients receiving thrombolytic therapy was similar to the incidence of ICH in the control arm, which did not receive thrombolytic therapy [375]. To reduce the risk of bleeding, concurrent hemostatic problems such as thrombocytopenia or vitamin K deficiency, as well as hypertension, should be corrected before starting thrombolytic therapy if possible.

There is no therapeutic range for thrombolytic agents. The correlation between hemostatic parameters and the efficacy and safety of thrombolytic therapy is too weak to have useful clinical predictive value [376]. However, in patients with bleeding, the choice and doses of blood products used can be guided by appropriate hemostatic monitoring. The most useful single assay is the fibrinogen level, which usually can be obtained rapidly and helps to determine the need for cryoprecipitate and/or plasma replacement. A commonly used lower limit for fibrinogen level is 100 mg/dl. The APTT may not be helpful in the presence of low fibrinogen levels, concurrent therapy with UFH, or the presence of FDPs [376]. Measurements of FDPs are helpful in determining whether a fibrinolytic effect is present.

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14

Transfusion practices

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Transfusion therapy in the neonatal population requires an understanding of the dynamic interactions of the fetomaternal unit, the physiologic changes that accompany the transition from fetus to neonate and from neonate to infant, and the underlying pathophysiology of different hematologic disorders. Blood products utilized in neonates include packed red blood cells (PRBC), platelet concentrates, granulocyte concentrates, fresh frozen plasma (FFP), and cryoprecipitate, but modifications of the components are often required to compensate for the small blood volume, immunologic immaturity, and compromised organ function of the transfusion recipients, who may be premature and/or sick. Extremely low-birth-weight infants (birth weight <1000 g) invariably receive one or more RBC transfusions, especially in the first few weeks of life [1]. Intensive blood-bank support with PRBCs, platelets, and FFP is vital for neonates undergoing extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass [2, 3].

Although the transfusion of blood products has been an integral part of supportive care of critically ill neonates for decades, guidelines for transfusions remain controversial, since most have been extrapolated from evidence in adults or based on small studies in neonates with marginal statistical validity. The growing awareness of the hazards of blood transfusion, both among medical professionals and in the lay public, has led to a re-evaluation of this hitherto commonly accepted practice, with the development of strategies to minimize risk and improve benefits.

Pretransfusion testing

A sample of cord blood should be collected in all newborn infants at the time of delivery, but routine testing of cord blood for ABO group and Rh type is not necessary for healthy newborn infants unless the mother is Rh-negative and/or has a positive antibody screen [4]. ABO and Rh type should be determined on samples obtained from both mother and baby for sick infants. Cord blood may be used for initial testing. The baby's blood group is determined from the red cells alone, since the corresponding isoagglutinins anti-A and anti-B in the serum/plasma are usually weak or absent. Screening for atypical antibodies may be performed on maternal blood if available, or in the neonatal serum/plasma. A conventional cross-match is unnecessary if there are no atypical antibodies demonstrable. Since the formation of alloantibodies in the first four months of life is extremely rare, further compatibility testing for repeated small-volume transfusions can be omitted [5]. However, if the infant is supported with plasma and platelets, then passive acquisition of antibody may occur. In this circumstance, cross-matching is indicated, particularly for major surgical procedures, where large volumes of blood may be transfused. Compatible blood may be low anti-A, B titer group O, Rh-negative, of the infant's own ABO and Rh or blood group, or an alternative compatible ABO and RH group. If the antibody screen is positive, then serological investigation to identify the antibody is necessary. Full compatibility testing should be performed

with appropriately selected blood, negative for the antigen(s) to which the antibody is directed.

Red blood cell transfusions

Anemia in the neonatal period may be secondary to blood loss, hemolysis, or impaired production of RBCs. The usual postnatal decline in RBC mass that occurs in all newborn infants is more pronounced in preterm infants, resulting in hemoglobin levels that drop to 8 g/dl in infants weighing 1000–1500 g, and to 7 g/dl in infants weighing less than 1000 g, by four to eight weeks of age [6]. This phenomenon, termed anemia of prematurity, is due primarily to delayed and diminished synthesis of endogenous erythropoietin (EPO), together with hemodilution secondary to rapid expansion of blood volume associated with postnatal growth and shortened RBC survival in neonates. In sick newborn infants requiring intensive care, frequent blood sampling for laboratory tests and monitoring of blood gases results in significant iatrogenic anemia and may be correlated directly to transfusion requirements [7, 8]. Ninety percent of infants who weigh less than 1000 g or are less than 28 weeks' gestation at birth require multiple small volume PRBC transfusions [1, 8–10]. Although sampling losses and therefore transfusion requirements may be related to the degree of prematurity and severity of illness [10, 11], it should be noted that even when adjusted for these factors, there is considerable variation in transfusion practice in different centers, with a significant impact on the number and volume of RBC transfusions, indicating a large discretionary element in the utilization of RBC transfusions [12–15]. This is not surprising, given the paucity of convincing scientific data to guide clinicians.

The hemoglobin levels at which blood transfusion would be indicated and the definition of "nonphysiologic" versus "physiologic" anemia in preterm neonates is fraught with controversy. Tissue oxygen delivery is determined not only by the hemoglobin concentration but also by the proportion of fetal to adult hemoglobin, level of RBC 2,3-diphosphoglycerate (2,3-DPG), cardiac output, and arterial oxygen tension [6, 16, 17]. Tachypnea, periodic breathing or apnea, tachycardia, poor feeding, poor growth, decreased activity, and anaerobic metabolism resulting in lactic acidosis all are considered to be indicators of significant anemia. However, many of these symptoms are nonspecific and do not correlate well to hemoglobin levels or respond consistently to RBC transfusions [18]. Attempts to identify accurate indicators of peripheral oxygen delivery have not been successful. One group used a nonradioactive measure of circulating blood volume, which correlated with venous hematocrit, especially in older very low-birth-weight (VLBW) infants [19]. Another group utilized near-infrared spectroscopy to measure fractional oxygen extraction as compared with conventional clinical criteria [20]. Neither unique method proved to be a useful predictor of transfusion, based at least in part on study design and complexity of methodology.

The absence of clear evidence of benefit of RBC transfusions in certain situations has led to re-evaluation of commonly accepted indications and to the generation of increasingly stringent, although still largely empirically derived, guidelines. Table 14.1 is a summary of guidelines published by the Pediatric Hemotherapy Committee of the American Association of Blood Banks (AABB), the Fetus and Newborn Committee of the Canadian Pediatric Society (CPS), and most recently the British Committee for Standards in Haematology Blood Transfusion Task Force (BCSH) [21–23]. The committees recognized the difficulty in defining clinical manifestations of anemia, such as tachypnea, tachycardia, apnea, poor feeding, and poor weight gain, to determine the need for transfusions in stable preterm infants. These parameters were defined in detail in the stricter guidelines used by the US Multicenter Recombinant Human Erythropoietin (rHuEPO), trial conducted between 1991 and 1993 [24]. The trial demonstrated that there was no adverse effect, in the short term, of conservative transfusion practices in VLBW infants, including those on moderate ventilatory support or supplementary oxygen. However, infants with severe cardiorespiratory compromise were excluded from the

Table 14.1 Guidelines for red blood cell transfusions in neonates

	AABB 1994 [21]	CPS 1992 [22]	BCSH 2004 [23]
Severe pulmonary or cyanotic heart disease or heart failure	Hb <130 g/l	Hb <130 g/l	Hb <120 g/l in neonate requiring intensive care
Acute blood loss	>10% blood volume	Hypovolemic shock	10% blood volume
Phlebotomy loss	Cumulative loss >10% blood volume within 1 week	Cumulative loss >10% blood volume within 72 hours when further blood sampling is expected	Cumulative loss; >10% blood volume in 1 week in neonate requiring intensive care
Stable newborn with clinical manifestations of anemia	Hb <80 g/l	Hb <80–100 g/l	Hb < 70 g/l in stable patient Hb < 11 g/l in chronic oxygen dependency

Source: Ramasethu *et al.* [18].

AABB, American Association of Blood Banks; BCSH, British Committee for Standards in Haematology Blood Transfusion Task Force; CPS, Canadian Pediatric Society.

Table 14.2 Comparison of the US Multicenter Recombinant Human Erythropoietin (HuEPO) trial [24] and College of American Pathologists (CAP) [25] guidelines for red blood cell transfusions in neonates

	US rHuEPO	CAP
Asymptomatic	Hct ≤20% retics <100 000/μl	Hct ≤20% retics <100 000/μl
Weight gain <10 g/day while receiving ≥100 kcal/kg/day	Hct ≤30%	Hct ≤25%
Significant apnea or bradycardia	Hct ≤30%	Hct ≤25%
Tachypnea or tachycardia	Hct ≤30%	Hct ≤25%
<35% hood oxygen CPAP/IMV with Paw <6 cm H_2O	Hct ≤30%	Hct ≤25%
>35% hood oxygen	Hct ≤35%	Hct ≤30%
CPAP/IMV with Paw ≥6–8 cm H_2O	Hct ≤35%	Hct ≤30%
IMV with Paw >8 cm H_2O	Not in trial	Hct ≤35%
Severe congenital heart disease	Not in trial	Hct ≤35%
Surgery	Hct ≤30%	No recommendation
Phlebotomy loss alone	No transfusion	No transfusion

CPAP, continuous positive airway pressure; Hct, hematocrit; IMV, intermittent mechanical ventilation; Paw, mean airway pressure; Retic, absolute reticulocyte count.

trial. The guidelines published by the Red Blood Cell Administration Practice Guideline Development Task Force of the College of American Pathologists (CAP) in 1998 are the most stringent, stipulating that RBC transfusion is required only when the hematocrit drops to 30% or less in an infant with moderate respiratory distress needing ventilatory support with mean airway pressures of 6–8 cm of water and up to 35% oxygen [25]. However, hematocrit levels of at least 30–35% are recommended in infants with bronchopulmonary dysplasia with clinical evidence of decreased oxygen delivery. Table 14.2 compares the US rHuEPO and CAP guidelines. The long-term safety of adopting these largely empirically derived guidelines in the care of moderately or acutely ill infants has not been established.

Anticoagulant-preservative solutions

RBCs are stored in one of several anticoagulant-preservative solutions to improve red cell viability and to extend storage. RBC units collected in

citrate-phosphate-dextrose adenine (CPDA-1) usually have a hematocrit of approximately 70% and a shelf life of 35 days. Citrate serves as the anticoagulant by chelating calcium, phosphate acts as buffer and a substrate for organic phosphocompounds, dextrose is a source of energy, and adenine maintains intraerythrocytic adenosine triphosphate (ATP), which is reflected in maintenance of red-cell membrane integrity and contributes to improve in vivo survival. The shelf life of PRBC may be extended to 42 days in additive-preservative (A-P) solutions, such as adenine-saline 1, adenine-saline 3 and adenine-saline 5, which contain additional sodium chloride and adenine. AS-1 and AS-5 also contain mannitol to prevent hemolysis. The hematocrit of the PRBCs in additive-containing media is approximately 60%, producing a less viscous product with a faster flow rate than standard PRBCs.

Clinicians have been wary of using older PRBCs for neonates because of the marked decline in intraerythrocytic 2,3-DPG levels, with possible effects on oxygen dissociation and a substantial increase in the K^+ concentration of the supernatant plasma of stored units [26–28]. However, intraerythrocytic 2,3-DPG regenerates rapidly in transfused red cells in adults and is assumed to do the same in infants [29]. Inhibition of the red-cell membrane-bound ATP pump during extended storage, particularly in blood that is irradiated before storage, causes leakage of intracellular potassium, resulting in K^+ levels greater than 30–50 mEq/l in the plasma. In infants receiving small-volume transfusions of 10–15 ml/kg of PRBC, the amount of K^+ delivered by the transfusion is estimated to be only about 0.15 mEq/kg in about 3 ml of the supernatant and is presumed to not pose a significant risk [30], but there are occasional case reports of life-threatening hyperkalemia [31].

Several studies have addressed questions about metabolic and physiologic effects of the high concentrations of glucose, sodium, and mannitol in additive anticoagulant-preservative solutions [32–37]. In a randomized controlled trial in 29 neonates, Strauss and colleagues found no significant differences in serum potassium, sodium, or osmolality in neonates transfused AS-1 units stored to outdate, as compared with neonates transfused with CPDA-1 units less than seven days old [33]. The AS-1 units were packed to remove additive with a resultant hematocrit of 85–87%; 47% of the AS-1 transfusions were more than 14 days old. It is interesting to note that post-transfusion serum potassium levels increased in 60% of infants after the transfusion of fresh CPDA-1 RBCs but decreased in 62% of neonates after the transfusion of stored AS-1 RBCs. This has been attributed to the fact that reactivation of the sodium/potassium ATP pump in transfused RBCs may cause them to behave as a potassium sink. Weekly analysis of infant 2,3-DPG concentrations demonstrated similar levels in infants transfused with either CPDA-1 or AS-1. Goodstein and colleagues studied 12 premature infants (birth weight less than 1250 g), each of whom received 17 ml/kg transfusions of AS-1 preserved blood packed to a hematocrit of 63% [34]. A battery of pre- and post-transfusion tests revealed no clinically relevant biochemical abnormalities, although increases in ammonia and lactic dehydrogenase (LDH) levels were noted, as well as small decreases in bicarbonate concentration, despite maintenance of pH. The safety of red blood cells stored in AS-3 for up to 42 days for small-volume neonatal transfusions has been reported, with no significant post-transfusion changes in laboratory values in 33 neonates receiving 120 AS-3 RBC transfusions [37]. Seventy-eight of the 120 transfusions were less than 21 days old, and 42 were 22–42 days old (Strauss, R. G. personal communication).

For massive transfusion such as ECMO, exchange transfusion, or cardiopulmonary bypass, the safety and efficacy of additive solutions have not been studied with the same scrutiny. Theoretical calculations, based on the constituents of the additives, indicate that hyperosmolality, hyperglycemia, hypernatremia, hyperkalemia, and hyperphosphatemia are likely to occur with massive transfusions in neonates [26, 36]. Given the risk of toxicity, RBCs stored in additive solutions should be avoided for massive transfusions, or the additive solutions should be removed by centrifugation or by washing.

Infusion issues

Infusion of PRBCs is possible through needles and short catheters as small as 24, 25, and 27 gauge, although increased hemolysis has been reported when older units are infused [38–40]. Additive RBCs infused through a hand-held device demonstrated hemolysis when 25 gauge or smaller needles were used even in fresh units [41]. PRBCs cannot be infused through 27 or 28 gauge central venous catheters [38, 42].

The necessity of warming small-volume RBC aliquots before transfusion is questionable, particularly if the transfusion is given slowly over two to three hours. A controlled blood warmer should be used for large-volume transfusions, particularly exchange transfusions. Blood components dispensed in syringes cannot be warmed in water baths because of the risk of contamination, but they may be warmed adequately when placed in warm-air incubators for 30 minutes before transfusion [43, 44]. Overheating, with resultant hemolysis, may occur when syringe aliquots are placed under radiant warmers or phototherapy lights [43, 44]. Shielding the blood components from excessive infrared energy by using aluminum foil abrogates these abnormalities. At the other extreme, freezing and lysis may occur if RBC products are stored in unmonitored refrigerators or freezers.

All blood components must be filtered before transfusion. The standard 120–170-μ pore-size filter is adequate for red cells, plasma, and platelets. Microaggregate filters with pore size of 10–20 μ can screen out 20–120-μ particles of clumped platelets, fibrin, and nonviable granulocytes that develop during storage, but their use for small-volume transfusions is controversial. The manufacturer's instructions should be followed to prevent hemolysis. Third-generation leukodepletion filters with "ultrafiltration" capability of removing 99.9% of white blood cells by mechanical sieving and cell adhesion have been recommended to decrease transmission of cytomegalovirus (CMV) and other viruses harbored in leukocytes and to decrease the incidence of alloimmunization [45]. In order to guarantee quality assurance, leukodepletion preferably is carried out in the laboratory within a few hours of blood collection, rather than by using bedside filters [46]. Microaggregate filters are not needed when leukodepleted components are used.

There is little information on the optimal volume of PRBCs to be transfused to correct anemia. Most infants are transfused 10 or 15 ml/kg of PRBCs, depending upon the cardiovascular status. One small study indicates that transfusion of 20 ml/kg of PRBCs results in a significantly greater rise in hematocrit in VLBW infants, as compared with transfusion of 10 ml/kg, without any detrimental effects on pulmonary function or vital signs [47]. Some of the formulae that may be used to calculate volumes for transfusions are indicated in Table 14.3.

Whole-blood transfusion

Whole blood or reconstituted whole blood, prepared by combining a unit of RBCs with an appropriate volume of compatible FFP, is the product of choice in the setting of massive transfusion or acute blood loss, where restoration of oxygen-carrying capacity and blood volume are needed simultaneously. Uncross-matched O Rh-negative whole blood may be required for transfusion in infants with shock secondary to acute blood loss in emergencies such as splenic rupture, massive subgaleal hemorrhage, or ruptured vasa previa. Often, the acute resuscitation of the neonate is carried out with crystalloid or colloid solutions and packed RBCs. The levels of coagulation factors V and VIII are low and platelet function is grossly abnormal in stored whole blood, which offers little advantage in the emergency situation over packed RBCs, since other blood components may need to be transfused subsequently. In children under the age of two years, transfusion of fresh (less than 48 hours old) whole blood has been associated with significantly less postoperative blood loss following bypass surgery for complex congenital heart disease compared with the transfusion of multiple blood components; this has been attributed to better platelet function in fresh

Table 14.3 Formulae for transfusion of red cell products

I. Blood volumes
- Preterm ≅ 100–120 ml/kg
- Full-term ≅ 80–85 ml/kg
- Adult ≅ 70–75 ml/kg

II. Calculation of volume of packed RBC transfusion

$$\text{PRBC volume} = \text{EBV} \times \frac{(\text{Hct desired} - \text{Hct observed})}{\text{Hct of packed red cell unit}}$$

III. Calculation of blood needed for exchange transfusion

Severe anemia

$$\text{Exchange volume of PRBC} = \frac{\text{EBV} \times \text{desired Hb rise}}{\text{Hb of PRBC} - \text{Hbw}}$$

$$\text{Hbw} = (\text{initial Hb} + \text{desired Hb})/2$$

Polycythemia

$$\text{Exchange volume of plasma/5\% albumin/saline} = \frac{\text{EBV} \times \text{desired Hct change}}{\text{starting Hct}}$$

III. Formula for reconstitution of blood to a specific hematocrit for exchange transfusion

$$\text{Volume of packed cells} = \frac{\text{total exchange volume} \times \text{desired Hct of new unit}}{\text{Hct of PRBC}}$$

$$\text{Volume of FFP} = \text{total exchange volume} - \text{volume of packed cells}$$

EBV, estimated patient's blood volume; FFP, fresh frozen plasma; Hb, hemoglobin; Hbw, calculated per formula; Hct, hematocrit; PRBC, packed red blood cells; RBC, red blood cells.

blood [48]. Other indications for transfusion of whole blood or reconstituted whole blood in the newborn include exchange transfusions, cardiopulmonary bypass, ECMO, and continuous hemofiltration [49].

Donor exposure

In the 1980s, 80% of VLBW (birth weight 1500 g or less) infants received multiple transfusions, often from different donors [50]. Over the past decade, a concerted effort has been under way to minimize the potential risks of transfusions by reducing the number of transfusions and donor exposures. A retrospective study of transfusion practices in VLBW infants admitted to one tertiary-level nursery found that the mean number of transfusions per infant declined from seven in 1982, to five in 1989, to 2.3 in 1993, before the use of recombinant erythropoietin. Concomitantly, donor exposures per infant also decreased from 4.8 and 5.0 in 1982 and 1983, to 2.2 in 1993, although only PRBCs stored in CPDA for less than seven days were used [1]. The decline in transfusions and consequent decrease in donor exposures were attributed to improvements in clinical care, laboratory micromethods, noninvasive monitoring techniques, and the adoption of conservative transfusion guidelines.

Several studies have documented the safety of using RBCs stored in additive anticoagulant-preservative solutions until the expiration date of 35–42 days [32–37]. One or two preterm infants for whom multiple PRBC transfusions are anticipated are assigned to dedicated units of freshly collected PRBCs. Small aliquots of RBCs may be obtained repeatedly from the dedicated units, using a sterile connecting device to transfer the red cells into a separate bag, without compromising the integrity of the primary storage bag [51–53]. A closed-system filter-syringe set may be used instead of a transfer bag to prepare small aliquots for neonatal transfusions [54]. The use of dedicated units until

expiration date, with appropriate inventory management, can effectively reduce the number of donor exposures in neonates requiring multiple small transfusions, without undue wastage of blood and without adversely affecting costs [51–55]. Most limited donor programs report the reduction of donor exposures to approximately 2.0 per VLBW infant. Neonates born before 28 weeks' gestation and those between 28 and 31 weeks with intrauterine growth retardation are the infants at highest risk of needing more than one donor [56].

Directed donor transfusions

Directed donations from first- and second-degree relatives rather than from unknown blood donors are perceived by the lay public to have a lower risk of transmitting viral infections, although there are no scientific data to support this contention. The transfusion of blood from biologic parents poses unique immunologic and serologic risks to the neonate [30, 57, 58]. Transfusion-associated graft-versus-host disease (TAGVHD), a well-recognized complication of the use of familial blood, is described later in this chapter. Maternal plasma may contain antibodies directed against paternal red cell, leukocyte, and platelet antigens that also may be expressed on neonatal cells. Transfusion of maternal blood components containing these antibodies theoretically could result in hemolysis, thrombocytopenia, or transfusion-related acute lung injury (TRALI), since current pretransfusion testing may not detect these incompatibilities. It is recommended that mothers should not provide blood components containing plasma, while maternal red cells and platelets should be given as washed concentrates [57]. Paternal red cells, white cells, and platelets may express antigens that are not inherited by the infant but to which the mother may have produced an immunoglobulin G (IgG) antibody that crosses the placenta. Transfusion of paternal cells to the infant could interact with transplacentally transmitted maternal IgG antibody in the infant's plasma. Fathers and paternal blood relatives should not serve as donors for blood components containing cellular elements; a full antiglobulin cross-match should be performed to detect red-cell incompatibilities if their use is unavoidable. In addition, all blood components obtained from first- and second-degree relatives should be irradiated before transfusion. One study showed that although biological parents were interested in donating for their infants, many were found to be ineligible for serological and medical reasons. However, those eligible were able to supply all small-volume RBC transfusions for their infants using a single-donor system in which RBCs were stored in AS-3 for 42 days or less [59].

Recombinant erythropoietin

The benefit of exogenous EPO to stimulate RBC production and overcome anemia of prematurity is still being debated, despite numerous multicenter trials [62–64]. Earlier randomized controlled trials in the USA, Europe, and South Africa demonstrated that treatment with recombinant human erythropoietin (rHuEpo) was associated with modest reductions in the number and volume of RBC transfusions in VLBW preterm infants, with maximum benefit being seen in larger, more stable preterm infants [24, 62, 63]. However, these trials excluded infants with significant cardiorespiratory compromise, the very infants likely to experience the greatest phlebotomy losses and require the most transfusions. One trial on a small number of extremely low-birth-weight infants (birth weight less than 750 g) who were mechanically ventilated showed that early administration of EPO and parenteral iron resulted in fewer transfusions in the first three weeks of life [64]. The study was limited by the small number of patients and the exclusion of extremely ill infants and was too brief in duration to determine whether the benefits of early EPO therapy lasted through the hospitalization of these fragile infants. A recent multicenter controlled trial in the USA addressed this specific issue and found that the administration of EPO and supplemental iron to preterm infants starting in the first 96 hours after birth and continuing until 35 weeks postmenstrual

age did not decrease the number of transfusions in infants with birth weight between 400 and 1000 g. In larger infants weighing 1001–1200 g, early EPO therapy did not reduce the percentage of infants who received any transfusion [60]. The European Multicenter Erythropoietin β Study Group found only a modest statistical difference in transfusion requirements in infants with birth weights below 1000 g and who received early erythropoietin compared with controls [61]. A meta-analyis of 21 controlled clinical trials suggests that the use of conservative transfusion criteria and other methods to reduce iatrogenic blood loss and improve protein and iron nutrition are critical to decreasing transfusions [65]. rHuEpo may be useful to reduce the need for late transfusions in preterm infants after 15 days of age and also for the management of late hyporegenerative anemia in rhesus hemolytic disease [66–68]. This topic is discussed further in chapter 4.

Autologous transfusion

The placenta contains 75–125 ml of blood at birth, a substantial volume of fetal blood that could be an ideal source of autologous blood for the neonate, eliminating the potential risks of transfusion transmitted diseases and TAGVHD. The simple measure of delayed cord clamping at birth has a significant impact on red-cell mass, hematocrit, and transfusion requirement in preterm infants, but it may also lead to circulatory overload [69, 70]. Heparinized placental blood has been used in the delivery-room resuscitation of term infants with shock and profound anemia, and placental blood stored in anticoagulant media has been used for neonatal transfusion successfully, albeit infrequently [71–73]. Protocols for ensuring proper collection without bacterial contamination and adequate anticoagulation are still being refined [74]. Although in vitro storage characteristics of placental blood stored for 28 days in anticoagulant media are reported to be similar to those of adult blood, there is no information about the post-transfusion red-cell survival and other biochemical parameters of transfused autologous placental blood [75]. Technical issues in collection [76, 77] and the rarity of use [78] raise questions regarding the usefulness of autologous cord-blood storage.

Exchange transfusion

The majority of exchange transfusions are performed for the treatment of hemolytic disease of the newborn (HDN) [79]. The procedure also has been used successfully for other indications, such as correction of life-threatening hyperkalemia [80], treatment of drug overdose or toxicity [81, 82], disseminated intravascular coagulation (DIC) [83], and for neonatal sepsis unresponsive to other therapy [84–86].

When performed for alloimmune HDN, exchange transfusions correct anemia, replace the infant's antibody coated RBCs with antigen-negative RBCs that should have normal in vivo survival, remove free maternal antibody in the plasma, and decrease bilirubin levels. The commonly used criteria for early exchange transfusion in HDN, performed within 9–12 hours of birth, include cord hemoglobin levels of 10–11 g/dl or less, cord bilirubin levels of 5–5.5 mg/dl or greater, and rapidly rising bilirubin concentrations of 0.5–1 mg/dl/hour or greater, despite phototherapy. Late exchange transfusions are performed when serum bilirubin concentrations threaten to exceed 20 mg/dl in term infants, the level at which the risk of bilirubin encephalopathy or kernicterus is approximately 10%. Prematurity, hypoxemia, acidosis, hypothermia, and sepsis predispose to kernicterus at lower levels of bilirubin.

A double-volume exchange transfusion (calculated as 2×80 ml/kg in a full-term infant) replaces 87–90% of the infant's blood volume but eliminates only about 50% of the intravascular bilirubin because of a slowly equilibrating tissue-bound pool. The use of albumin before exchange transfusion in an effort to mobilize tissue bilirubin is controversial. Equilibration of extravascular and intravascular bilirubin and continued breakdown of sensitized and newly formed red cells by persisting maternal antibodies results in a rebound of bilirubin following

initial exchange transfusion, often necessitating repeated exchange transfusion in severe hemolytic disease.

Blood chosen for the exchange should be as fresh as possible (preferably less than 5 to 7 days old) in order to maximize in vivo red-cell survival. If the delivery of an infant with severe HDN is anticipated, then O Rh-negative blood cross-matched against the mother may be prepared before the infant is born. Blood prepared after the infant is delivered should be negative for the antigen(s) responsible for the hemolytic disease and may be cross-matched against the infant. In ABO HDN, the blood must be type O and either Rh-negative or Rh-compatible with the mother and infant. In addition, the blood should be hard-packed or washed free of plasma or have a low titer of anti-A or anti-B antibodies. Usually, type O cells are used with AB plasma to ensure that no anti-A or anti-B antibodies are present, but this results in two donor exposures per exchange transfusion. Blood prepared for exchange transfusion for nonimmune indications such as nonimmune hyperbilirubinemia, drug overdose, and sepsis, needs to be cross-matched against the infant only. Irradiated citrate-phosphate-dextrose blood is used as whole blood or reconstituted whole blood (red cells suspended in saline, albumin, or plasma) with a hematocrit of 40–50%. Additive-containing anticoagulant-preservatives are avoided. Hypoxemic or acidotic infants should receive blood known to lack hemoglobin S [49].

The blood is warmed through a temperature-controlled in-line blood warmer, and the exchange transfusion is performed by the traditional push–pull method with a single vascular access, usually the umbilical vein, or by isovolumetric techniques utilizing two access sites for simultaneous removal of the infant's blood and administration of new blood [87]. Aliquots of 5–20 ml with a maximum of 5 ml/kg are withdrawn or infused in the discontinuous method, at a rate not exceeding 5 ml/kg every three minutes to avoid rapid fluctuations in arterial pressure, which are accompanied by changes in intracranial pressure [88]. When an isovolumetric exchange is being done, volumes to be removed/reinfused should not exceed 2 ml/kg/min. The duration of the exchange is usually one to two hours.

Potential complications of exchange transfusion include hypocalcemia, hyper- and hypoglycemia, hyperkalemia, thrombocytopenia, dilutional coagulopathy, neutropenia, DIC, umbilical venous and/or arterial thrombosis, necrotizing enterocolitis, and infection. Despite advances in the management of critically ill newborn infants, morbidity and mortality associated with exchange transfusions remains high, particularly in infants who are premature or sick, or both. The risk of death or permanent serious sequelae has been estimated to be as high as 12% in sick infants, compared with less than 1% in healthy infants, in one study [89]. The potential risk of adverse events from exchange transfusion needs to be balanced against the risk of bilirubin encephalopathy in ill infants.

Exchange transfusions have improved survival rates in neonates with gram-negative sepsis and neutropenia or sclerema, conditions ordinarily associated with extremely high mortality [84–86]. The rationale for the use of exchange transfusion in neonatal sepsis includes removal of bacteria and inflammatory mediators, correction of acidosis, and improvement of tissue oxygenation by the correction of anemia. Exchange transfusions enhance IgG, immunoglobulin A (IgA), and immunoglobulin M (IgM) levels, clear endotoxin, increase neutrophil counts, and enhance neutrophil function when fresh whole blood, stored for less than 12 hours, is used [84–86, 90]. It should be noted that the requirement for "fresh" blood does not usually permit the necessary pretransfusion serologic testing and is not available in most institutions in the USA.

Partial exchange transfusion

Partial exchange transfusion using packed RBCs is used to correct severe anemia and improve oxygenation in critically ill hydropic infants with severe HDN until they can be stabilized sufficiently to undergo a complete double-volume exchange transfusion.

Partial exchange transfusion is used more often to decrease the hematocrit in neonates with

polycythemia-hyperviscosity syndrome, diagnosed in infants with a hematocrit above 65–70% and a combination of symptoms attributed to polycythemia, such as hypoglycemia, tachypnea, congestive cardiac failure, hypotonia, tremors, seizures, abnormal renal function, and necrotizing enterocolitis [91]. Partial exchange transfusion to reduce the hematocrit to about 55% causes rapid amelioration in the clinical manifestations of polycythemia and is associated with reversal of cerebral blood-flow abnormalities in symptomatic infants [92], but it is uncertain whether the long-term outcome, particularly in asymptomatic infants, is improved by this procedure [93, 94]. The indications for treatment include infants with hematocrit of 70% or above and symptomatic infants with hematocrit between 65 and 70%, but these criteria are still debated. Isotonic saline has been found to be as effective as 5% albumin or plasma protein solutions utilized as replacement solutions [95]. Peripheral vessels are preferred for withdrawing and infusing replacement fluid in polycythemic infants [96]; the umbilical vein may be used if peripheral access is difficult. An increased incidence of necrotizing enterocolitis has been described with the use of FFP administered through the umbilical vein, but it is difficult to decipher whether this was part of the syndrome or due to the procedure or the nature of the replacement fluid [97].

Intrauterine fetal transfusion

Intrauterine transfusion has been used for the correction of critical anemia secondary to alloimmune hemolytic disease, fetal parvovirus infection, twin-to-twin transfusion, fetomaternal hemorrhage, and homozygous alpha-thalassemia. Fetal transfusions may be administered by several routes: intraperitoneal, intravascular by direct funipuncture, intrahepatic venous puncture, combinations of intravascular with intraperitoneal transfusions, and even intracardiac transfusion as a last resort [98]. Intraperitoneal fetal transfusion has been largely replaced by direct intravascular fetal transfusion (IVT), since it circumvents the problem of poor absorption of RBCs from the peritoneal cavity in the most severely affected hydropic fetuses. The intravascular technique also allows for sampling and testing of fetal blood for precise diagnostic evaluation of the fetal status. Intraperitoneal transfusions may be necessary when intravascular access is difficult, as in early pregnancy when the umbilical vessels are narrow, or later when increased fetal size prevents access to the umbilical cord. The relative merits of direct simple IVT versus intravascular exchange transfusion have been debated, but the shorter procedure time with direct simple IVT has made it the procedure of choice at most centers. Transfusions are performed at hematocrits of 25–30% or less. Hematocrit levels fall rapidly in fetuses with severe alloimmune hemolytic disease, necessitating a second transfusion within 7–14 days; the interval between subsequent transfusions is usually 21–28 days. Very low pretransfusion fetal hematocrits, rapid large increases in post-transfusion hematocrits, and increases in umbilical venous pressure during IVT are associated with fetal death post-transfusion [99, 100].

Fetuses are at risk for both post-transfusion CMV and TAGVHD, so CMV seronegative or third-generation leukodepleted, irradiated blood is recommended. The blood should be as fresh as possible for maximal in vivo survival, antigen-negative for the offending antibody(ies), be indirect antiglobin test (IAT) cross match compatible with maternal serum, CPD or washed free of the anticoagulant citrate and other additives, warmed, and packed to a hematocrit of 70–85% in a volume calculated to increase the fetal hematocrit to between 40% and 45% [101]. Transfusions are provided up to 33–34 weeks, with delivery as soon as lung maturity is achieved by antenatal steroid therapy. The decision as to when to deliver the fetus is based on gestational age, fetal weight, lung maturity, severity of fetal disease and response to transfusions, and the ease of performing the transfusions combined with antenatal ultrasound and Doppler studies. Infants with alloimmune hemolytic disease and who have received multiple intrauterine transfusions are

delivered closer to term and often require less phototherapy and fewer exchange transfusions in the neonatal period [102]. However, some still have significant hemolytic anemia at birth requiring aggressive intervention, and many require additional simple transfusions for severe and prolonged hyporegenerative anemia secondary to suppression of fetal erythropoiesis [103]. Suppression of erythropoiesis that persists into the neonatal period may be greater when the last intrauterine transfusion occurs close to delivery and the cord-blood hemoglobin level is high [104]. It must be remembered that the circulating red cells in the neonate who has received multiple intrauterine transfusions are the donor cells, and this may lead to misleading results on initial blood grouping and a false-negative direct antiglobulin test (DAT).

Intrauterine platelet transfusions have been used to treat severely thrombocytopenic fetuses with fetomaternal alloimmune thrombocytopenia, to prevent antenatal intracranial hemorrhage [105, 106]. Repeated transfusions at weekly intervals often are necessary because of the short survival time of transfused platelets. Since each transfusion carries a 1–2% risk of fetal loss, the cumulative risk of fetal loss with multiple transfusions could be considerable. Washed maternal platelets resuspended in plasma may be used for single transfusions, but for repeated transfusions, a panel of selected platelet antigen-negative donors is required. Human leukocyte antigen (HLA)-incompatibility leading to poorer than expected responses to fetal platelet transfusions has been reported [107]. This complication may be rectified by using HLA-compatible platelet antigen-negative donors. Again, all blood products should be irradiated before transfusion to prevent TAGVHD.

Platelet transfusions

Thrombocytopenia, defined as a platelet count of less than 150×10^9/l, occurs in less than 1% of term infants but is a common complication encountered in the neonatal intensive care unit (NICU), occurring in 18–20% of low-birth-weight infants [108, 109]. In sick infants, low platelet counts usually are due to a combination of impaired production and increased peripheral destruction. One-fifth of infants with marked thrombocytopenia have evidence of DIC [108]. In addition, infants treated with ECMO are the other major group of neonates likely to require platelet support.

Isolated perinatal thrombocytopenia often is antibody-mediated, secondary to maternal idiopathic thrombocytopenia (ITP), in which antibodies are directed against both maternal and neonatal platelet antigens, or secondary to neonatal alloimmune thrombocytopenia (NAIT), a condition analogous to alloimmune hemolytic disease, in which transplacentally transmitted maternal antibodies are directed against fetal/neonatal platelet antigens inherited from the father [105, 106]. NAIT usually is more severe, with a 10–20% risk of intracranial hemorrhage (ICH), half occurring in utero because of severe fetal thrombocytopenia. Fetomaternal incompatibility for the PL^{A1} (HPA-1) antigen is responsible for 80% of cases, while HPA-5b is responsible for an additional 15% in the Caucasian population. Other antigens may be responsible for the condition in other racial groups, e.g. the Pen/Yuk (HPA-4) system in the Oriental population.

Quantitative and qualitative platelet disorders may cause significant bleeding with resulting morbidity, the most serious being ICH. The incidence and severity of intraventricular hemorrhage and serious neurological sequelae is greater in low-birth-weight infants with thrombocytopenia than in those with normal platelet counts [110]. However, one prospective controlled trial showed no difference in the incidence of new ICHs or extension of existing hemorrhages between infants given prophylactic platelet transfusions to maintain platelet counts of at least 150×10^9/l or to control infants who were not transfused until platelet counts dropped to less than 50×10^9/l or had evidence of bleeding [111]. The minimum level to which platelet counts may be allowed to fall before transfusion has to be individualized, since hemostatic competence is determined not only on the platelet count but also on platelet function, vascular integrity, and levels of coagulation factors. Sick infants may have prolonged bleeding times

Table 14.4 Guidelines for platelet transfusion support of neonates

Prophylactic platelet transfusions
Stable preterm neonates with platelet counts $<30 \times 10^9$/l
Stable term neonates with platelet counts $<20 \times 10^9$/l
Sick preterm neonates with platelet counts $<50 \times 10^9$/l
Sick term infants with platelet counts $<30 \times 10^9$/l
Preparation for an invasive procedure, e.g. lumbar puncture or minor surgery in neonates with platelet counts $<50 \times 10^9$/l, and for major surgery in neonates with platelet counts $<100 \times 10^9$/l
Platelet transfusions in neonates with clinically significant bleeding
Neonates with platelet counts $<50 \times 10^9$/l
Neonates with conditions that increase bleeding, e.g. DIC, platelet counts $<100 \times 10^9$/l
Neonates with documented significant platelet functional disorders, e.g. Glanzmann's thrombasthenia, irrespective of the circulating platelet count

"Sick" infants in this context are defined as those with a history of perinatal asphyxia, extremely low birth weight (<1000 g), need for ventilatory support with an inspired oxygen concentration >40%, clinically unstable, signs of sepsis, or those who require numerous invasive interventions.

DIC, disseminated intravascular coagulation.

Source: Blanchette *et al.* [113].

despite only moderately reduced platelet counts ($75–150 \times 10^9$/l). Two clinical conditions that predicted repetitive platelet transfusions in one study were liver disease and renal insufficiency [112]. Published indications for platelet transfusion have been derived by consensus and by extrapolation from studies in adults and older children (Table 14.4) [113].

The choice of platelet product is determined by the underlying diagnosis. Whenever possible, the platelets should be ABO- and Rh-compatible. A standard platelet unit from one donor contains no fewer than 5×10^{10} platelets in 40–70 ml of plasma and has a shelf life of five days from collection. In the absence of ongoing consumption, 15 ml/kg of a standard platelet unit raises the platelet count by 50 000/mm^3. The standard unit may be concentrated to a volume of 15–20 ml for patients with volume restrictions, but this is associated with some loss of platelets and may affect platelet function adversely. The shelf life of volume-reduced platelets is only four hours. Platelet apheresis units may be aliquoted into multiple small neonatal units by means of sterile connecting devices and are useful in order to reduce donor exposures in infants on ECMO who may require multiple platelet transfusions. Apheresis units are particularly useful in NAIT, when multiple platelet transfusions of a particular antigen specificity are required. Platelets should not be warmed or infused through umbilical arterial lines.

In NAIT, the risk of hemorrhage and serious neurological sequelae is highest at the time of delivery and for as long as the thrombocytopenia persists. If the HPA incompatibility is known, then antigen-negative platelets prepared from typed platelet donors provide a sustained increase in platelet count. If the HPA incompatibility is unknown, then the mother is likely to be the most readily available source of compatible platelets, since her platelets will be antigen-negative. Maternal platelets collected by whole-blood donation or by apheresis should be washed free of antibody-containing plasma and irradiated before transfusion [114]. If NAIT is anticipated, then arrangements may be made to collect maternal platelets well in advance and to cryopreserve them for use after the infant's birth [115]. In the event that there are no facilities for collecting and preparing maternal platelets, and laboratory

confirmation of the offending antigen is awaited, then transfusion of platelets negative for both HPA-1a and HPA-5b is likely to be successful in 95% of Caucasian infants [116]. Random donor platelets, 98% of which are PL^{A1} (HPA-1)-positive, generally are ineffective but have been advocated widely as supplementary treatment, with or without intravenous immunoglobulin (IVIG), for a possible hemostatic effect in a bleeding emergency until compatible antigen-negative platelets are available. The hemostatic effect of random donor platelets in this situation has not been proved.

Neonatal autoimmune thrombocytopenia (NITP) secondary to maternal ITP usually is less severe than NAIT. A review of the clinical course of 601 infants born to mothers with ITP revealed that severe neonatal thrombocytopenia (defined as a platelet count below 50×10^9/l mm) occurred in 72 (12%) infants with this condition, and ICH was reported in 6 (1%). The lowest platelet count in four of the six infants with ICH was less than 10×10^9/l [117]. The nadir of thrombocytopenia in NITP is on day three or four and usually responds to IVIG and/or corticosteroids [114, 118]. Platelet transfusions of any antigen type and maternal platelets are ineffective in these infants because the antibodies are reactive against a wide range of platelet antigens. Random donor platelets are advocated only in life-threatening bleeding emergencies, again for a possible hemostatic effect. Exchange transfusions may be performed to remove circulating antibody.

Fresh frozen plasma and cryoprecipitate transfusions

FFP is plasma separated from red cells and platelets of whole-blood donations and frozen to −18°C or below within eight hours of collection. FFP is available in volumes of approximately 200 ml if collected from whole-blood donations or of 600 ml if derived from apheresis; smaller aliquots may be prepared for neonatal use by using a sterile connecting device. Cross-matching is not performed, since type-specific or AB-negative product usually is issued. FFP contains all the blood coagulation factors, with each milliliter providing one unit of coagulation factor activity, and also contains components of the fibrinolytic system and complement systems, albumin, and globulins. Once thawed, FFP should be transfused within six hours if used for labile factor replacement. In other cases where repeated FFP transfusions are required, a thawed unit from a single donor may be divided into smaller aliquots and used within 24 hours if stored between 1 and 6°C.

The potential risk of transfusion transmitted viral infection from FFP has been mitigated at least in part by the use of donor-retested FFP, where FFP is placed in quarantine for 16 weeks and released for transfusion only after the donor is retested as negative at the end of that time period. Pooled solvent/detergent-treated plasma is a product currently in use in Europe and has been proposed as a superior alternative to standard FFP with a decreased risk of transmission of enveloped viruses [119]. This product has been withdrawn from use in the USA. The product has lower levels of high-molecular-weight multimers of von Willebrand factor. In addition, each unit is a pool of 2500 donors and once thawed must be used within four hours. This combination of factors makes its use in babies requiring small-volume repetitive transfusions problematic [120].

Cryoprecipitate is the protein fraction derived from FFP thawed at 1–6°C, which is then resuspended in a small volume of plasma and refrozen to −18°C for storage. Cryoprecipitate contains fibrinogen in a concentrated form as well as factor VIIIC, von Willebrand factor, and factor XIII.

The most common cause of disordered hemostasis in sick neonates is DIC, secondary to conditions such as asphyxia, sepsis, shock, respiratory distress syndrome, and necrotizing enterocolitis. Coagulopathy also may be secondary to acute or chronic liver disease. Bleeding due to vitamin K deficiency is rare, since most infants now routinely receive prophylactic vitamin K at birth. This condition occurs occasionally in breast-fed infants who have inadvertently missed prophylactic vitamin K and in infants with severe deficiency of vitamin K-dependent factors due to maternal anticonvulsant therapy.

FFP infusions are indicated for the treatment of clinically significant bleeding as well as for correction of hemostatic defects due to a decrease in one or more coagulation factors, before invasive procedures. The rationale for the use of FFP in neonates has been derived from evidence in adult patients [121–123]. It is preferable not to use FFP for the treatment of isolated coagulant factor deficiencies for which virus-inactivated plasma-derived or recombinant factor concentrates are available, but in practice, treatment of active bleeding due to suspected coagulation disorder often is initiated in the absence of a definitive diagnosis, since the small blood volumes of newborn infants may not permit blood sampling for elaborate laboratory testing beyond the basic screening tests. Commercial concentrates, some manufactured using recombinant technology, are available for factors VIIa, factor VIII, von Willebrand factor, and factor IX. Concentrates of fibrinogen, factor XIII, antithrombin, protein C, and the prothrombin complex are still undergoing clinical trials and are not as accessible, but they may be obtained from the manufacturers.

FFP is not indicated for the treatment of hypovolemic shock in the absence of coagulation abnormalities, for nutritional support, or for the treatment of immunodeficiency states. Although earlier studies demonstrated that the incidence of periventricular-intraventricular hemorrhage was reduced by prophylactic FFP, a large multicenter randomized controlled trial provided no evidence that the routine early use of FFP affected the rates of death or disability in preterm infants [124]. The use of FFP as a source of immunoglobulins for the treatment of neonatal sepsis is unjustified in the light of present evidence [125, 126].

The dose of FFP administered usually is 10–20 ml/kg, with further doses being determined by the clinical situation, underlying disease process, and laboratory monitoring. The amelioration of hemostatic problems by the transfusion of FFP in neonates with DIC is only temporary and, hence, multiple transfusions may be required until the underlying cause resolves. Indeed, the outcome of DIC has been found to be dependent on the success of treatment of the underlying pathologic process and aggressive supportive care and is not altered by therapy directed specifically at coagulopathy [83]. The correction of coagulopathy secondary to liver failure also is temporary in the absence of recovery of hepatic function. A single transfusion of FFP usually will control bleeding due to vitamin K deficiency; vitamin K should be administered as soon as possible. Cardiopulmonary bypass is associated with profound and global decreases in components of the coagulation and fibrinolytic systems in neonates, but there is no evidence that prophylactic replacement with FFP either prevents excessive bleeding or decreases transfusion requirements [127–129]. Bleeding in this situation often is related to platelet dysfunction and responds better to platelet administration. Cryoprecipitate, rather than FFP, has been shown to restore hemostasis in children with ongoing bleeding [129].

Cryoprecipitate is the product of choice for severe hypofibrinogenemia associated with DIC and for bleeding due to congenital fibrinogen or factor XIII deficiency, if commercial plasma-derived virus-inactivated concentrates are unavailable.

Extracorporeal membrane oxygenation

ECMO is the use of prolonged extracorporeal circulation and gas exchange through a membrane oxygenator to provide temporary life support in patients with profound cardiorespiratory failure who fail to respond to conventional therapy. The continuous contact of blood with the prosthetic surface of the ECMO circuit results in complex interactions of the plasma protein systems and blood cells, leading to activation of the coagulation cascade and simultaneous fibrinolysis. Blood proteins adhere instantly to the surface, creating a layer composed of fibrinogen, albumin, globulins, and other proteins. The thrombogenicity of the prosthetic surface is related to the amount of fibrinogen that adheres to it, since the fibrinogen activates Hageman factor, complement, and platelets. Priming the circuit with an albumin phase creates a protein monolayer that inhibits further

blood–prosthetic surface interaction. Fibrin formation is inhibited by systemic heparinization. Qualitative and quantitative changes in platelets have been described in infants treated with ECMO [130]. Platelet counts decrease by a mean of 26% from baseline counts within 15 minutes of initiating ECMO and continue to decline further over the next hour. Platelet aggregation is reduced considerably. Transfused platelets have a reduced survival rate and do not appear to significantly reverse platelet aggregation abnormalities in infants on ECMO.

The use of heparin to prevent clot formation in the circuit and the decrease in platelet number and function put the infant at risk for bleeding [131]. Infants placed on ECMO are critically ill and may already have altered hemostasis secondary to sepsis, shock, or profound hypoxia, predisposing them to devastating intracranial hemorrhage [132]. Maintaining the balance between prevention of thrombosis in the circuit and bleeding in the infant requires careful juggling of multiple parameters.

Blood-bank support is vital for infants on ECMO. Group- and type-specific PRBCs are required to prime the ECMO circuit. Some ECMO centers prefer to use FFP instead of albumin to prime the prosthetic surfaces. Multiple small-volume transfusions of PRBCs are subsequently administered to maintain the hematocrit at 40–45%, to optimize oxygen delivery. Platelet and FFP support is variable, with postsurgical infants, patients with sepsis, and patients on prolonged bypass requiring considerably more transfusions [132, 133]. Platelet counts generally are maintained between 100 and 200 $\times$ 10^9/l, depending on the assessed risk of hemorrhage in the individual infant. A minimum platelet count of 150 $\times$ 10^9/l is recommended for infants at high risk, and a count above 200 $\times$ 10^9/l is recommended for postoperative cases and for infants with active bleeding.

The necessity for multiple blood components for neonates on ECMO resulted in mean donor exposures as high as 22.8 $\pm$ 9.5 per ECMO run in the past [134]. Changes in transfusion practices and blood component management have now reduced donor exposures to seven to ten in many centers [2, 134, 135]. Strategies for limiting donor exposure include increasing the volume of red cells used for small-volume transfusions to 15 ml/kg, using a system of sequential aliquots from the same blood unit, using standard FFP units up to 24 hours after thawing, using single-donor apheresis platelet aliquots, and avoiding the use of volume-reduced platelets and the empirical use of FFP and cryoprecipitate.

Granulocyte transfusion

Quantitative and qualitative abnormalities in the neutrophil/phagocytic system and defects in humoral immunity make neonates, particularly those born prematurely, uniquely susceptible to developing sepsis with increased risk of mortality following infection. The neutrophil storage pool, consisting of polymorphonuclear neutrophils, bands, and metamyelocytes, is smaller in infants than in adults and is exhausted rapidly in the presence of bacterial infection, leading to significant neutropenia. Functional abnormalities of the phagocytic system, including decreased deformability, chemotaxis, phagocytosis, bactericidal killing, and oxidative metabolism, have been described in neonates with sepsis [136].

Neonates less than 14 days old and with bacterial sepsis and neutropenia (neutrophil plus band count less than 3×10^9/l), and older neonates with bacterial sepsis unresponsive to antibiotics with neutrophil plus band counts under 0.5×10^9/l, have been considered to be candidates for granulocyte transfusions [21]. Studies of the efficacy of granulocyte transfusions for the treatment of life-threatening infections in neonatal patients are difficult to evaluate because of small numbers of patients with varying degrees of illness and supportive care regimens, differing methods of harvesting neutrophils, and different transfusion protocols. In a retrospective analysis, neonates with culture-positive sepsis, predominantly resistant *Klebsiella* infection, and who were treated with 2–15 transfusions of leukopheresed granulocytes had a 10% mortality rate compared with 72% mortality in similarly infected neonates who did not

receive such transfusions [137]. A small prospective randomized study in septic neonates with positive cultures and neutrophil storage-pool depletion demonstrated 100% survival in seven neonates, each of whom received a single granulocyte transfusion, compared with only 11% survival in nine similar neonates who were not transfused with granulocytes [138]. In a larger prospective study involving 35 infants, the survival rate in infants receiving granulocyte transfusions was reported to be 95% compared with 64% survival in infants who received only supportive care [139]. However, a positive blood culture was not a selection criterion in this study, with 20% of both patients and controls presumed to have overwhelming bacterial sepsis based on clinical criteria. Granulocyte transfusions also have been shown to improve survival when compared with IVIG therapy [140]. Two studies reporting the use of stored buffy coat granulocytes in the treatment of neonatal sepsis showed no improvement in survival rates, but the dose of granulocytes delivered by this method was smaller than granulocytes obtained by leukopheresis [141, 142]. In a meta-analysis of clinical studies of granulocyte transfusions in the treatment of bacterial sepsis, the dose of transfused granulocytes emerged as a significant predictor of success, with transfusion of adequate doses (more than 0.5×10^9/kg) producing an 18-fold increase in the odds of survival in septic neutropenic neonates, as compared with controls ($P < 0.05$) [143].

Technical difficulties and the ability to collect granulocytes for transfusion in a timely manner, together with concerns about the transmission of viruses like cytomegalovirus harbored by leukocytes, have limited the more widespread use of white-blood-cell transfusions. In addition, there are occasional case reports of severe and fatal reactions to granulocyte transfusions in neonates attributed to pulmonary leukoagglutination [144, 145]. In an era where leukocyte-depleted blood products increasingly are being recommended for neonatal transfusions [46, 146], it is likely that granulocyte transfusions will be replaced by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factors (GM-CSF) for the treatment of neonatal sepsis associated with neutropenia [147, 148].

If a decision is made to use granulocytes, then daily transfusions are recommended until there is clinical improvement and evidence of recovery of neutrophil counts. Granulocyte concentrates for neonatal use should be prepared by automated leukopheresis and should contain $1.0–2.0 \times 10^9$/kg neutrophils in 10–15 ml/kg volume. Measures to improve the yield of neutrophils from donors have included stimulation with corticosteroids and, more recently, with G-CSF [149]. Use of stimulated granulocytes has not been reported in neonates. The component must be ABO- and Rh-compatible and cross-matched with the recipient, since the product usually contains a large number of RBCs. Donor-recipient compatibility is improved by HLA matching or by leukocyte cross-matching, but this issue has not been addressed in the neonatal population. Granulocyte concentrates should be CMV negative, irradiated and infused as soon as possible after collection, using standard 170-μm filters. The product should not be refrigerated or warmed above room temperature.

Adverse reactions to blood transfusion

The physiological immaturity of various organ systems in the newborn gives rise to significant differences in the incidence and types of adverse reactions when compared with older children and adults.

Hemolytic reactions

Acute hemolytic transfusion reactions are rare in neonates, due partly to the absence of naturally occurring anti-A and anti-B antibodies until the age of four months, and also due to the practice of transfusing neonates with group O blood. Despite multiple blood transfusions and exposure to multiple blood donors, post-transfusion red-cell alloimmunization has been reported infrequently in neonates, again a feature of immunologic immaturity [150].

However, severe immune-mediated hemolysis has been associated with transfusion of adult blood containing anti-T antibodies in neonates with activation of the normally masked Thompsen–Friedenreich antigen on the surface of the neonatal red-cell membrane. This phenomenon, known as T-activation, occurs by the removal of N-acetyl neuraminic acid residues from the cryptantigen by neuraminidases produced by bacteria, particularly *Clostridia*. T-activation has been reported mainly in neonates with necrotizing enterocolitis, especially in those with severe disease requiring surgical intervention, and also in clinically septic infants with other surgical problems [151–153]. T-activation should be suspected in neonates at risk who have evidence of intravascular hemolysis with hemoglobinuria and hemoglobinemia following transfusion of blood products or unexplained failure to achieve the expected post-transfusion hemoglobin increment. Routine cross-matching techniques will not detect the polyagglutination due to T-activation when monoclonal ABO antiserum is used. Minor cross-matching of neonatal T-activated red cells with donor anti-T-containing serum may show agglutination, but this is not performed routinely. Infants at risk and with discrepancies in forward and reverse blood grouping and evidence of hemolysis on smear should be suspected of T-antigen activation. The diagnosis is confirmed by specific agglutination tests using peanut lectin *Arachis hypogea* and *Glycine soja*. Further hemolysis may be prevented by using washed red cells and platelets, and low titer anti-T plasma [154]. Exchange transfusion with plasma-reduced components may be necessary for infants with severe ongoing hemolysis. A-P red cells, which are relatively plasma free are another alternative.

Numerous nonimmunologic causes of hemolysis leading to morbidity and mortality have been described in neonates, including excessive infusion pressure through 28 gauge or smaller needles, accidental overheating of the blood in the administration set, inappropriate storage of RBC products in freezers or unmonitored refrigerators leading to freezing and lysis, simultaneous infusion of incompatible fluids or drugs, and the transfusion of abnormal donor RBC (glucose-6-phosphate dehydrogenase deficiency, sickle cell trait, or paroxysmal nocturnal hemoglobinuria) [14].

Leukocyte alloimmunization

Transient white-blood-cell antibodies have been described in multiply transfused preterm infants [150], but their immunological effects either in the short or the long term are not clear at present. The passive transfusion of blood containing leukoagglutinins directed against the recipient's antigen initiates complement activation, microvascular lung injury, pulmonary edema, and severe hypoxemia, a condition termed transfusion-related acute lung injury (TRALI). This acute life-threatening complication is reported rarely in neonates, partly because of the difficulty in distinguishing the acute respiratory distress that accompanies the syndrome from other causes of respiratory deterioration [144, 145, 155].

Transfusion-associated graft-versus-host disease

TAGVHD occurs due to the proliferation and engraftment of viable donor lymphocytes in an immunosuppressed or immunodeficient transfusion recipient who does not recognize the cells as being foreign and is unable to reject them. Similarities in HLA antigens facilitate engraftment and, therefore, this phenomenon is much more likely to occur when family members serve as blood donors or when the population is relatively homogeneous. TAGVHD in infants has been reported following transfusions from blood relatives, following intrauterine fetal transfusion, after postnatal transfusions in infants who received intrauterine transfusions, in neonates with primary cell-mediated immunodeficiency disease, and, rarely, in preterm infants without a definitive diagnosis of underlying primary immunodeficiency [156–160]. Preterm infants as a group are the recipients of thousands of simple blood transfusions each year, but only five cases of TAGVHD have been reported in the English-language literature in

infants whose only risk factor appeared to be their immunological naivety secondary to prematurity. In spite of the large number of blood products that infants on ECMO receive, only one case of TAGVHD has been reported in this setting [161]. It is possible that the condition is underdiagnosed and underreported, since the manifestations, which include fever, rash, gastrointestinal symptoms, and bone-marrow and hepatic failure, are similar to many other medical conditions in sick newborn infants [162].

It has been suggested that lymphocytes transfused during intrauterine transfusions may induce a state of immune tolerance in the fetus, which then increases the propensity to develop TAGVHD following postnatal transfusion. TAGVHD following exchange transfusion in neonates who did not receive intrauterine transfusions has been attributed to the large number of viable lymphocytes transfused in relatively fresh blood during the procedure [163]. The threshold number of lymphocytes necessary to produce a graft-versus-host reaction may vary depending upon the immune status of the host and the antigenic similarity of the donor–recipient pair. No leukocyte-depletion filter or processing has yet been shown to uniformly decrease the lymphocyte count of a product below the critical threshold. Whole blood, RBCs, granulocyte and platelet concentrates, and fresh plasma all have been implicated in TAGVHD. Irradiation at a minimum of 2500 cGy to the midplane of the bag is currently the only acceptable way to abrogate the disease. Current guidelines recommend the use of irradiated blood components for neonates with known or suspected congenital cell-mediated immunodeficiency states, infants with congenital leukemia or malignancy undergoing chemotherapy, fetuses receiving intrauterine transfusions, postnatal transfusions in neonates who have received intrauterine transfusions, exchange transfusions, and for recipients of cellular blood products from first- and second-degree relatives and HLA-matched donations [164, 165]. TAGVHD has not been reported in children or adults with human immunodeficiency virus (HIV) infection despite the severe immunodeficiency associated with the disease, so irradiation of blood products is not recommended for this indication at present. Although preterm infants are known to be immunoincompetent, it is unclear whether all preterm infants should receive only irradiated blood products, in the absence of other factors that would predispose them to TAGVHD. Some institutions provide irradiated blood products to all neonates to avoid TAGVHD in patients with undiagnosed immunodeficiency.

Metabolic adverse reactions

Inhibition of the red-cell membrane-bound ATP pump during extended storage causes leakage of intracellular potassium, resulting in K^+ levels of more than 30–50 mEq/l in the supernatant plasma, particularly in blood that is irradiated before storage. Small-volume transfusions of RBCs stored to their expiration date of 35–42 days usually do not cause clinically significant elevations of serum potassium, but life-threatening hyperkalemia has been described in two infants following the transfusion of only 10 ml/kg of 19-day-old irradiated PRBC. The measured K^+ level in each aliquot was 120 mEq/l and 185 mEq/l [31]. Hyperkalemic arrest has been described in infants receiving exchange transfusions and rapid infusions of large volumes of stored packed RBCs in sick term and preterm infants [166]. Washed red cells are advisable in infants with hyperkalemia or renal failure or when large volumes of blood are infused rapidly.

Hypoglycemia may occur during transfusion of small volumes of CPDA-1 or AS-1 red cells if dextrose-containing intravenous infusion is interrupted. Rebound hypoglycemia frequently is detected after exchange transfusions in infants with erythroblastosis fetalis who often exhibit hyperinsulinemia. Large-volume transfusions, as may occur during surgical procedures, may lead to hyperglycemia in small premature neonates with tenuous glucose control mechanisms.

Hypocalcemia, secondary to the chelation of calcium by citrate in the anticoagulant, has been observed during exchange transfusions and

following massive transfusions in both term and preterm infants.

The plasticizer di-(2-ethylhexyl)-phthalate (DEHP), present in blood bags, intravenous sets, and ECMO circuits made of polyvinyl chloride, is lipophylic and leaches into almost all blood products. Significant exposure to plasticizer is reported in infants undergoing exchange transfusions and ECMO [167, 168]. A review has detailed the lack of knowledge of plasticizer toxicities in infant and children exposed during transfusion [169]. Although no short- or long-term complications have been determined in infants, studies in animals raise concerns about possible effects on hepatocellular, pulmonary, and reproductive function and potential carcinogenicity.

In addition to metabolic effects, blood transfusions may cause significant alterations in temperature, blood pressure, and pulmonary compliance in fragile preterm infants.

Transfusion-transmitted infections

The potential risk of transfusion transmitted viral infection from a blood donor with negative serological and nucleic-acid testing results in the USA is estimated to be 1 in 2 135 000 for HIV, 1 in 641 000 for human T-lymphotrophic virus (HTLV) I and II, 1 in 935 000 for hepatitis C virus (HCV), and 1 in 205 000 for hepatitis B virus (HBV) [170–172]. The addition of HIV-1 p24 antigen testing in March 1996 to detect infected seronegative donors in the window period of infection resulted in the detection of three antigen-positive, antibody-negative donations in an estimated 18 million tested donations, over 18 months, at a cost of US$90 million, unaffordable in most countries [173]. In countries with high HIV seroprevalence, the risk of HIV infection from a single unit of HIV seronegative blood may be as high as 5.4–10.6 per 1000 units [174]. Since 1999, the US blood supply has been screened for HIV and HCV with nucleic-acid testing (NAT). This has shortened the window-period risk for HCV from 70 to 10–30 days and for HIV from 22 to 10 days. The current estimated post-transfusion risks are noted in Table 14.5.

Table 14.5 Current risk estimates of viral transmission by transfusion

Virus/infection	Estimated risk in 2002
Human immunodeficiency virus	1 : 2 135 000
Hepatitis C virus	1 : 1 935 000
Hepatitis B virus	1 : 205 000
Human T-lymphotropic virus I/II	1 : 641 000

Donor deferral questions, geographical area deferrals, and additional testing for other viral diseases, e.g. West Nile virus by NAT, are added as there are real or perceived threats to transfusion transmissions.

Nearly all cases of HIV infection acquired from blood transfusions in the USA occurred before the implementation of routine blood-donor screening practices. The median symptom-free period from birth to symptomatic infection is reported to be significantly longer in neonatal transfusion-acquired infection compared with perinatally acquired infection [175]. Asymptomatic HIV-1 infection has been identified as late as 5–9.5 years post-transfusion in studies in which cohorts of neonates transfused before routine donor screening for HIV antibody were tested retrospectively [176]. Once symptomatic, the progression of transfusion-acquired HIV disease does not appear to be different from vertically acquired infection in neonates.

The hepatitis viruses may be transmitted by cellular and noncellular blood products. Post-transfusion hepatitis A is rare and usually asymptomatic in newborn infants. However, secondary horizontal spread has led to large outbreaks in NICUs, with symptomatic infection in nurses, physicians, parents, and relatives who have been in direct contact with infected neonates [177]. The outcome of transfusion-acquired hepatitis B in neonates is assumed to be similar to that of perinatally acquired infection, with a 70% risk of chronic carrier state. Improvements in donor screening and the use of more sensitive second-generation enzyme immunoassay (EIA) methods for the detection of antibody to HCV have reduced the incidence of post-transfusion hepatitis considerably. A follow-up study of a cohort

of children who received transfusions of blood screened by surrogate markers of hepatitis or by EIA-1 anti-HCV testing, during ECMO therapy in the neonatal period, found 7 of 83 children to be anti-HCV seropositive and significantly increased alanine aminotransferase levels in four of the seven [178]. Another retrospective cohort study of neonates transfused with blood from donors infected with HCV before the institution of routine screening indicated that asymptomatic infection was present in more than 60% of children at the median age of 6.3 years. Children who were HCV RNA-positive had mildly elevated transaminase levels; mild hepatitis was evident in those who underwent liver biopsy [179]. Preliminary data indicate that progression of liver disease due to transfusion-acquired hepatitis C is milder in children than in adults, but long-term follow-up studies are awaited [180]. Seventy percent of HCV-infected adults develop chronic hepatitis, 20% develop cirrhosis within two decades, and 1–5% may develop hepatocellular carcinoma after 20 years [181]. Persistent hepatitis G virus infection has been observed after neonatal transfusions, but its relevance is not clear since no clinical or biochemical signs of liver disease have been observed on follow-up [182].

CMV is ubiquitous in blood donors, with seropositivity rates ranging from 30% to 70%. Viral genome has been detected in the white blood cells of CMV-seropositive individuals, and even CMV-seronegative donors may harbor latent CMV infection in their mononuclear cells. Post-transfusion CMV infection in low-birth-weight infants born to seronegative mothers causes a serious clinical syndrome of fever, respiratory distress, hepatosplenomegaly, and cytopenias and may result in death [183]. Disseminated CMV infection has been described in a full-term infant following ECMO [184]. Risk factors for neonatal acquisition of CMV from transfusions include exposure to at least 50 ml of blood, birth weight less than 1200 g, and maternal CMV seronegativity. CMV-seronegative blood or leukocyte-reduced blood is recommended for transfusions in low-birth-weight infants born to seronegative mothers or those with unknown serostatus and for intrauterine transfusions [185]. Leukocyte reduction may be performed by saline washing or by using frozen deglycerolized red cells, but these products do not permit the use of multiple small-volume transfusions from one donor. Leukodepletion using third-generation filters is thought to provide an effective alternative to CMV-seronegative blood components [46]. However, residual white cells remain following leukodepletion and may result in CMV transmission in severely immunocompromised patients [186]. In hyperendemic areas, neonates may be infected with CMV despite the use of leukodepleted blood products, by perinatal transmission or from maternal breast milk [187].

Bacterial contamination of blood products, particularly platelet concentrates, continues to be a small but persistent problem, with serious consequences for the recipient [188]. The condition is recognized rarely in neonates, possibly because the organisms usually implicated are skin flora, which often are responsible for nosocomial infection in neonatal nurseries. Transmission of syphilis from blood transfusion also is rare, because donors are infectious only in the early stage of disease and the spirochetes do not survive for more than 120 hours at 4°C. Post-transfusion syphilis has been reported in an infant after an exchange transfusion with fresh whole blood [189].

Transfusion-acquired malaria is being reported more frequently and should be considered when fever develops following neonatal transfusions, even in nonendemic areas such as the USA [190, 191].

In 1996, the identification of ten cases of a new variant form of Creutzfeldt–Jakob disease (CJD), a prion with etiological links to bovine spongiform encephalopathy (BSE) raised the specter of the possibility of transmission of prion disease by blood transfusion [192]. Transmission of CJD by blood transfusion has been reported in several animal models. In 2004, two human cases were identified as likely transfusion transmitted by the World Health Organization (WHO). Most transfusion services have refused to construe the absence of evidence of transmission as an absence of risk and, since no suitable diagnostic tests are available, attempt to minimize the risk

by excluding donors considered to be at higher risk for acquiring the disease. Revised donor policies are expected to lead to the deferrals of a significant number of blood donors [192].

Advances in the past century have made transfusions safer than they ever have been, but the goal of zero-risk transfusion remains distant.

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15

Umbilical-cord stem-cell transplantation

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Overview

Over the past two decades, hematopoietic stem-cell transplantation has emerged as an effective approach to curative therapy for both pediatric and adult patients with aggressive or recurrent malignancies, congenital immunodeficiency diseases, some genetic diseases, including inborn errors of metabolism and hemoglobinopathies, and congenital and acquired bone-marrow-failure syndromes. Traditionally, autologous or allogeneic stem and progenitor cells have been obtained from bone marrow or mobilized peripheral blood. More recently, banked umbilical-cord blood (UCB) has emerged as an alternative source of stem and progenitor cells for transplantation. Cord blood is readily available and can be transplanted across partially mismatched human leukocyte antigen (HLA) barriers, increasing the availability of allogeneic stem-cell donors for patients lacking traditional HLA-matched related and unrelated donors. Additional applications of stem-cell therapies envisioned in the next decade, including regenerative therapies for non-hematopoietic tissues damaged by injury or disease, are currently unproven and the subject of ongoing preclinical research.

A major limitation to stem-cell transplantation therapy is donor availability. Only 20–25% of patients in need of a transplant will have an HLA-matched relative who can serve as their donor. Of those lacking a related donor, approximately 25% of Caucasian patients will identify an HLA-matched unrelated living bone-marrow donor through the National Marrow Donor Program and other donor registries, but less than 10% of patients of ethnic minority backgrounds will find a suitably matched unrelated marrow donor. For the remaining patients, a fully matched unrelated stem-cell donor cannot be identified.

Over the past decade, umbilical-cord blood, the baby's blood remaining in the placenta or afterbirth, has emerged as an alternative source of hematopoietic stem cells for use in hematopoietic stem-cell transplantation. Cord blood has several advantages over bone marrow, most importantly that it does not need to be fully HLA-matched for a successful transplant. It is also readily available, can be collected without risk to the mother or infant donor, and is significantly less likely to transmit infectious diseases transmissible through the blood. It also has been shown to be less likely to cause acute and chronic graft-versus-host disease (GVHD), a major obstacle to the success of allogeneic bone-marrow transplantation. Because cord blood can be collected without risk, mothers of ethnic minority backgrounds are more likely to donate to cord-blood banks than to volunteer to be listed on adult bone-marrow registries.

Cord-blood procurement and banking

Cord blood can be collected from the placenta either in situ during the third stage of labor or ex vivo within 10–15 minutes after delivery of the placenta. Cord blood is harvested into a closed sterile

collection container containing citrate phosphate dextrose (CPD) anticoagulant to prevent clotting. Generally, this is accomplished by sterilely puncturing one of the umbilical veins and allowing the cord blood to drain by gravity into a bag containing anticoagulant. CPD has been identified as the anticoagulant with the lowest toxicity to hematopoietic stem and progenitor cells, with activity over a range of collection volumes. During collection, care must be taken to avoid mixing of maternal and fetal blood, because this would lead to contamination of the cord blood unit with HLA-disparate, immunocompetent maternal T-cells, which could cause GVHD in an immunoincompetent transplant recipient [1]. After harvest from the placenta, the collected cord blood can be maintained at room temperature for up to 48–72 hours without appreciable cell losses. Most banks deplete red blood cells and reduce volume of the cord-blood collection with hetastarch separations. For long-term storage, cells are cryopreserved in 5–10% dimethylsulpoxide (DMSO) and stored under liquid nitrogen. Maximal storage time, or expiration date, is unknown, but under stable conditions cells are likely to remain viable for decades.

There are two major types of cord-blood donations, public and private [2, 3]. In general, public banks are not-for-profit and, with third-party federal or private financial support, public banks collect cord bloods from healthy term pregnancies after maternal informed consent. In granting consent, the infant's mother acknowledges that the donation is voluntary and that she gives up all rights to the cord blood for the public good. The mother also agrees to allow her medical records and the baby's newborn records to be reviewed, gives a detailed family medical history, and allows a sample of her own blood to be taken for infectious disease testing and HLA typing. The results of these tests are not routinely provided back to the baby's parents or physicians. However, if a disease potentially putting the infant donor at risk is identified, then the parent and the physician are both notified. Public cord-blood banks ensure confidentiality and do not allow any contact between the infant donor or the donor's family and the patient receiving the stem cells.

Private cord-blood banks are generally for-profit companies providing a service for a fee. Private donations are financed by the family of the donor or recipient and usually are directed to a specific recipient or their family. In the minority of private donations, a relative in need of a transplant donor is identified in advance of the baby's birth. The donation allows for greater mismatching between the donor and recipient and also spares the donor from the risks of a bone-marrow or peripheral stem-cell harvest. The remainder of private or directed donations are undertaken as an investment in the unknown potential for cord blood to be used to treat serious illnesses in the future. To date, although several thousand cord bloods have been banked, only 11 transplants have resulted from these private, speculative banking activities.

Private cord-blood banking is a service offered to new parents by for-profit companies in the USA. The majority of these companies provide a collection kit to families interested in private banking. There are no established medical indications for banking. The units are collected by the physician, midwife, or nurse delivering the baby and shipped back to the company's banking facility. Compensation for the collector is variable, ranging from none to monetary reimbursement based on effort expended. Limited testing is performed on the units, and minimal standards are used to determine whether a unit is eligible for processing and banking. Both collection and banking fees and yearly storage fees generally are charged. Units can be stored for autologous or directed donation to a relative in the family. Most obstetricians and pediatricians feel that routine cord-blood storage in healthy babies is unnecessary [3]. In this regard, it is important to point out that autologous cord blood would not be used for bone-marrow transplantation of a child with acute leukemia because of concern of contamination with malignant cells [4] as well as lack of graft-versus-leukemia effects. Directed donation of umbilical-cord blood for another family member is indicated when there is a first-degree relative with a high risk or refractory pediatric malignancy amenable to treatment with transplantation

therapy, a hemoglobinopathy or other transfusion-dependent anemia, congenital immunodeficiency, or inborn error of metabolism. There are insufficient data to address the question of whether related haploidentical cord blood will play a role in the treatment of adults with refractory malignancies or other diseases amenable to treatment with allogeneic stem-cell transplantation.

Over the past decade, with support from governmental and/or private sources, unrelated cord blood has been banked by various investigators for public use around the world [5–12]. It is estimated that approximately 100 000 units are listed in individual public banks around the world. Establishment of these banks was stimulated by the need for stem-cell donors for patients with malignant diseases and genetic conditions correctable by transplantation therapy and lacking a suitably matched related donor. Units stored in public banks are available for any patient in need and who is medically eligible for transplantation therapy. These units are tested and HLA-typed at the time of banking rendering them readily available for transplantation therapy when identified as the donor of choice for a patient in need.

The first public bank was established by Dr Pablo Rubinstein at the New York Blood Center, with grant support from the National Heart Lung and Blood Institute (NHLBI) of the National Institutes of Health [5]. This bank, now known as the National Cord Blood Program, has banked more than 16 000 units in the past 11 years and has provided more than 1400 units for transplantation worldwide. In 1996, the NHLBI funded three academic centers to establish a second public bank, the Cord Blood Transplantation Study (COBLT), with banking sites at Duke University Medical Center in collaboration with the American Red Cross (the Carolinas Cord Blood Bank), Georgetown University, and the University of California at Los Angeles [6]. Collection of units for the COBLT banks was supported through June of 2001, creating an inventory of approximately 8000 units. A public bank now listing approximately 6000 units was also established at Cardinal Glennon Hospital in St Louis, supported with philanthropic sources. Other smaller public banks have also been established throughout the USA, but none has collected more than 1000 units to date and many have closed due to lack of funding.

Most public banks employ a team of dedicated nurses and medical technologists who are responsible for donor recruitment, donor screening, obtaining maternal consents, medical histories and blood samples, harvesting the units, and transporting the units to the laboratory for banking. Units generally are collected from term babies born after an uncomplicated pregnancy, labor, and delivery. Units are cryopreserved within a finite time, usually 48–72 hours, from collection and placed in quarantine until test results are obtained. Units passing a screening algorithm designed to eliminate units at risk for transmitting genetic or infectious diseases are HLA-typed and placed in the search registry for patient access.

Maternal donors give consent for a voluntary donation of their baby's cord blood, testing of maternal and/or fetal blood for infectious and genetic diseases transmitted through blood and stem cells, testing of the cord blood for stem and progenitor cell content, and review of the family's medical history and the medical records of the infant and mother. Screening for homozygous hemoglobinopathies may be performed by direct testing of the cord blood or, in states or countries with mandatory screening, by obtaining the results of the infant donor from the state or country screening program. Donor confidentiality is preserved via labeling using barcodes, but most banks retain a link between the donor's demographic information and the barcode label. Maternal donors are excluded if they are known to be infected with hepatitis B or C viruses, human T-lymphotrophic virus (HTLV), or human immunodeficiency virus (HIV). They are also excluded if they have traveled to or lived in countries where HIV or malaria is endemic or if they practice high-risk behaviors or have positive viral serologies or nucleic acid testing (NAT) for syphilis, hepatitis B or C, HTLV I or II, or HIV 1 or 2.

Detection of cytomegalovirus (CMV) in cord-blood donors presents a unique problem. Between 40% and 90% of mothers are CMV immunoglobulin G (IgG)-positive, representing immunologic

conversion after routine infection earlier in life but not infection during pregnancy. Maternal IgG crosses the placenta into the fetal blood. Thus, if the mother is CMV IgG-positive, then the cord blood will test positive, reflecting passive transfer of the maternal IgG. However, this in no way indicates that the fetus was exposed to or infected with CMV. Since detection of CMV IgG does not correlate with CMV disease, alternative methods of CMV viral detection have been sought. The New York Blood Center Placental Blood Program routinely cultured donor infant saliva for CMV virus as well as assayed CMV IgG and immunoglobulin M (IgM) in these infants' mothers. The overall incidence of positive cultures was 0.3%, but this did not correlate reliably with titers of CMV IgM in mothers of babies with positive cultures. Techniques to detect CMV DNA in cord blood cells or plasma are under development but are not used routinely in clinical practice at this time. In one study, investigators at Duke University Medical Center and Emory University demonstrated that only 3% of UCB units from mothers with positive CMV IgMs contained CMV, as demonstrated by NAT testing [7].

Some banks employ look-forward programs, following infant donors for 0.5–2 years after donation to determine whether these infants develop any diseases that could be transmitted to and clinically expressed in a transplant recipient, but most collection centers and banks do not have the resources to effectively execute follow-up or look-forward programs. Mothers are counseled to inform the banks if their infant develops any serious illnesses during childhood (e.g. cancer, acquired immunodeficiency syndrome (AIDS), genetic diseases, immunodeficiency syndromes).

Publicly banked units are characterized in laboratories committed to excellence in stem-cell processing and cryopreservation. Cell counts, differentials, viability, ABO and Rh typing, CD34, CD3, and clonal hematopoietic progenitor cell content are enumerated routinely. Bacterial and fungal cultures are performed routinely, and positive units are excluded from the inventory. Screening for homozygous hemoglobinopathies or two heterozygous hemoglobinopathies is carried out on the infant donors or the cord-blood units directly. Positive units are excluded from the bank. Procedures for volume reduction, red-blood-cell depletion, and cryopreservation are standardized and validated [5, 6]. HLA typing is performed on the red-cell pellet by molecular techniques with a minimum of antigenic typing for HLA class I, A and B and at the allelic level for HLA class II, DRB1. Results are entered into electronic databases that can be accessed by transplant centers searching for donors for their patients. The COBLT project studied over 33 000 potential maternal donors and 18 000 collected cord-blood units. The main reason for donor exclusion was a risk factor for infectious-disease exposure in the mother or a positive infectious-disease screening test in the mother around the time of delivery. After unit collection, the main reason for exclusion for banking was low volume. In the 8000 units banked, the average number of total nucleated cells was 1×10^9 and the average number of CD34 cells was 2.6×10^6 [6]. This represents a 10–20-fold decrease from the average number of cells used in a typical bone-marrow transplant.

Currently, there is no centralized registry where units from all unrelated cord-blood banks list their inventories. The larger banks (e.g. the National Cord Blood Program at the New York Blood Center and the COBLT Banks) have independent search programs, while some of the smaller banks have networked through the National Marrow Donor Program and the American Red Cross. Netcord, an international collaborative allegiance of 14 banks, is developing an Internet-based search engine to facilitate searching. Thus, patients and transplant centers in search of a donor must search banks individually. There are also no formal regulations for cord-blood collection, processing, and banking by governmental agencies, although in the USA the Food and Drug Administration (FDA) has published proposed regulations that are expected to go into effect before the completion of 2004. Cord blood has not been licensed by the FDA as a product. Thus, public units used for transplantation can be obtained at no cost or with an FDA set fee for cost reimbursement from the major public banks in the USA and other countries.

Early transplantation experience with umbilical-cord blood

Before the demonstration of successful engraftment in a human patient transplanted with UCB, there was great skepticism about the feasibility of the procedure. Many investigators doubted that cord blood would contain sufficient numbers of cells for short-term or durable marrow reconstitution. Those believing that engraftment was possible thought that this would hold true only for young children of low weight and small stature. Many worried that the naivety of cord-blood lymphocytes would lead to post-transplant immunodeficiency and lack graft-versus-leukemia effects. The proof that cord-blood transplantation was feasible required a human experiment.

The first cord-blood transplant was performed by Eliane Gluckman at the L'Hospital St Louis in 1988 in a six-year-old boy with Fanconi anemia [13]. The patient remains well and durably engrafted 15 years later. The transplant was supported by a multidisciplinary international collaboration between several academic medical centers, a private insurer (BCBS), L'Hospital St Louis in Paris, and the first private cord-blood company (Biocyte). Unfortunately, to date, this altruism was not extended to support public banking or additional basic or clinical studies in the field. The success of this transplant paved the way for approximately 60 additional related UCB transplants over the subsequent five years. Outcomes of these transplants demonstrated the feasibility of engraftment in children, delayed times to engraftment of neutrophils and platelets, and a reduced incidence of acute and chronic GVHD, compared with results in allogeneic bone-marrow transplantation from matched related donors [13–19].

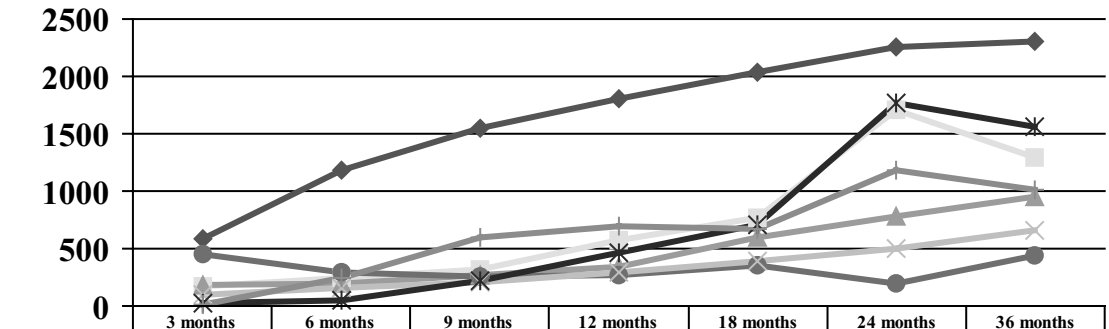
Unrelated umbilical-cord blood transplantation

The first unrelated cord-blood transplant was performed in August 1993 utilizing an HLA-mismatched unit from the New York Blood Center [20]. Since that time, more than 3000 unrelated cord-blood transplants have been performed worldwide [16, 20–41]. The majority of these transplants have been used to treat children with cancer or genetic diseases, with an overall success rate of 50–60% [20–35]. In patients with malignancies, younger children with earlier staged disease have an improved prognosis. Over the past four years, approximately 150 adults lacking traditional stem-cell donors and with high-risk malignancies have received unrelated cord-blood transplants, with an overall success rate comparable to unrelated bone-marrow transplantation in adults [27, 28].

After cord-blood transplantation, neutrophil engraftment occurs in a median of 25 days in 90% of children and in 80% of adults [20–41]. Cell dose, rather than HLA match, has been identified as the most important factor correlating with clinical outcomes after cord-blood transplantation [16, 20, 22, 34–36]. Patients receiving larger cell doses per kilogram of recipient body weight (more than 3×10^7 cells/kg) experience more rapid neutrophil and platelet engraftment, higher probabilities of overall engraftment, earlier immune reconstitution, and superior event-free survival. However, because of the biological differences in size and thymic function in babies versus children versus adults, it has not been possible to distinguish completely between the influence of cell dose versus age in analysis of clinical outcomes in UCB transplants.

The incidence of moderate to severe (grades II–IV) acute GVHD after unrelated partially HLA-mismatched UCB transplantation is approximately 40%, with 10% of patients experiencing severe (grades III–IV) disease [18, 20–22, 34, 35]. A major advantage of cord-blood stem cells as compared with bone-marrow stem cells is that cells derived from cord blood are much less likely to cause extensive chronic GVHD [20–22, 34]. The conventional wisdom dictates that UCB stem and T-cells are more tolerant of a new host than phenotypically identical cells from bone marrow or mobilized peripheral blood [42–51].

The major cause of failure after an unrelated cord blood transplant is opportunistic infections [20–22, 34–36, 52]. Fatal infections generally occur before 100 days post-transplant in children and 180 days



	3 months	6 months	9 months	12 months	18 months	24 months	36 months
PHA/100	586.56	1183.25	1547.03	1806.64	2040.59	2256.98	2300.62
CD4	165.20	239.50	317.00	569.00	764.50	1710.00	1292.5
CD8	177.00	196.00	267.00	339.00	592.00	775.00	954.5
CD56	453.00	294.00	253.00	265.00	356.00	201.00	443
CD45RO	99.00	155.00	213.00	288.00	387.00	504.50	657
CD45RA	27.00	46.00	214.00	468.00	707.00	1770.50	1560.5
CD19	18.00	249.00	594.00	691.00	672.00	1186.50	1012

Fig. 15.1 Immune reconstitution median absolute values.

post-transplant in adults. The tendency towards infection is felt to be due to several factors, including lower cell dosing leading to longer periods of neutropenia in the first several weeks post-transplant as well as defects in neutrophil function, since these cells recapitulate neonatal neutrophil function and ontogeny. Cord-blood-derived lymphocytes are naive, having never been exposed to common bacterial and viral pathogens. Cord blood lacks T-cells with memory of CMV, Epstein-Barr virus (EBV) or other common viruses that have the potential to reactivate in the transplant recipient.

The pace of immune reconstitution relates to patient age as well as dose of cells transplanted. The youngest patients (under two years of age) recover adequate T-cell numbers and function by three to four months post-transplant. Children between the ages of 2 and 17 years reach a similar endpoint by 12 months post-transplant. Adults may never fully recover thymic-dependent immune function and generally take up to three times longer than children to recover protective function (Fig. 15.1) [53]. This is felt to be due to the fact that the host's adult thymus has a lower capacity to contribute to the immune reconstitution process. To increase the success of cord-blood transplantation in adults, it is generally agreed that the cell dose of a single transplant needs to be increased. Current preclinical and clinical research focuses on using combined cord-blood transplants or ex vivo expansion of cord-blood cells to address this issue. Results of phase I clinical trials are encouraging, but additional studies are required to establish the utility of these approaches [54, 55]. Nonmyeloablative stem-cell transplant (NST) or "mini-transplants" are undergoing testing in adult patients with malignancies who are unable to tolerate full-dose myeloablative therapy. Limited trials using unrelated cord blood in this setting have demonstrated success in 50% of heavily pretreated older patients with malignant diagnoses [56, 57]. Similar approaches have been suggested for children with nonmalignant diagnoses; however, immune-mediated graft rejection and autologous marrow recovery have been obstacles to the success of this approach in younger and immunologically healthier patients.

Cord-blood transplantation has been especially successful in young infants and toddlers with inborn errors of metabolism. In these patients, the cord-blood stem cells provide a durable source of cells

capable of producing the defective or missing enzyme in the patient. When performed early in the course of disease, stem-cell transplantation prevents further progression and, in some cases, facilitates reversal of organ damage caused before transplantation therapy. In patients with leukodystrophies, cord-blood transplantation performed in the presymptomatic phase prevents demyelination in both the central and the peripheral nervous systems. In patients with mucopolysaccharidoses, corneal clouding, hepatocellular damage, and bony deformities will correct and central-nervous-system damage will be prevented if transplantation therapy is used before the age of two years. Most interestingly, cardiac disease, usually caused by atherosclerotic changes in coronary arteries in early childhood, also is ameliorated. These observations suggest that cord-blood stem cells are capable of transdifferentiation and cellular plasticity and raise optimism for the development of cord-blood-derived cellular therapies for hepatic, cardiac, and neurodegenerative diseases of adults [58–60].

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16

Neonatal oncology

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Oncology

Malignancies occurring in the newborn have some unique features that distinguish them from tumors occurring later in childhood. Neonatal tumors generally are not histologically distinct from those occurring later, but their incidence is different. Perinatal cancers also can vary in their clinical presentation, biology, course, and response to treatment. Some tumors of infancy are associated with inherited syndromes and chromosomal/gene abnormalities. This chapter will review malignant tumors that occur in the newborn, focusing on features that differ from tumors occurring later in childhood.

Epidemiology

Cancer in the newborn (defined for purposes of this chapter as cancer diagnosed before one month of age) is a rare event. Data from the Third National Cancer Survey (1969–71) reported an incidence of 3.65 cases diagnosed before 29 days of life per 100 000 live births [1]. Other population-based studies have yielded numbers within the same range (1.88–2.98/100 000 and 2.27/100 000) [2, 3]. More cancers are diagnosed in the first year of life than in any other year (up to age 18 years) according to data from the Surveillance, Epidemiology, and End Results program from 1976 to 1984 and from 1986 to 1994, with an incidence of 23.3/100 000/year [4]. Assuming an equal distribution over each four-week period, this translates to 1.8 cases/100 000/four-week period. It does not appear that neonatal cancers account for a disproportionate number of tumors diagnosed before one year of age.

The distribution of types of malignancies in the neonatal period is different from that found in later childhood. The results of several series are summarized in Table 16.1. Neuroblastoma is the most common malignancy found at birth, followed by leukemia. Brain tumors and sarcomas have a similar incidence, and order-of-occurrence rate varies with individual reports. When all neoplasms are considered (including nonmalignant tumors), teratoma usually is reported as the most common tumor [5, 6], followed by hemangiomas and lymphangiomas. For all children under 15 years of age, leukemia is the most common malignancy, followed by central-nervous-system (CNS) tumors, lymphomas, neuroblastomas, sarcomas, and renal tumors [7].

Exposure to maternal malignancy

An aspect of oncology unique to the neonate is the possibility of exposure of the fetus to maternal malignancy. The infant could be exposed to adverse effects of chemotherapy; additionally, the infant potentially could develop a cancer by metastatic spread from the mother. A review of the literature through 2001 found only 68 cases reported of metastatic involvement of the products of conception (placenta and/or fetus) and only 14 cases of tumor developing in the fetus (seven melanoma, seven leukemia/lymphoma) [11]. Since that series, there have been

Table 16.1 Summary of six series of cancers in infants (note: sum of numbers in each column is not equal to the number of cases in the series due to other tumors not being reported)

Series	UKCCSG in Broadbent [3]	Borch *et al.* [2]	Campbell *et al.* [8]	Parkes *et al.* [9]	Isaacs [10]	Isaacs [10]	Total (%)
Teratoma		6		46	62	25	139 (20)
Neuroblastoma	44	20	48	15	36	16	179 (25)
Leukemia	27	12	8	8	18	10	83 (12)
Brain tumors	19	8	9	5	15	13	69 (10)
Sarcomas	20	11	12	8	8	8	67 (9.5)
Retinoblastoma	8	2	17	2	4		43 (6.1)
Liver tumors	3		1	6	18	7	35 (5)
Renal tumors	3		4	7	6	4	24 (3.4)
Total (in series)	136	76	102	99	167	126	706

UKCCSG, United Kingdom Children's Cancer Study Group.

two additional cases reported of transfer of tumor to the fetus (lymphoma [12] and small-cell carcinoma of the lung [13]). It is estimated that 3500 new cases of cancer are diagnosed annually in pregnant women in the USA, which is equivalent to 1 case in every 1000 pregnancies [14]. Even allowing for some degree of underreporting of cases of maternal–fetal transmission in the literature, it is still clear that the vast majority of maternal malignancies do not result in development of cancer in the infant. This is true in spite of the repeated demonstration that single cells pass between mother and infant across the placenta [15–18]. It seems reasonable to assume, particularly in cases of maternal hematologic malignancy, that maternal malignant cells often cross the placenta and are eliminated by the fetal immune system. Fetal immunodeficiency or human leukocyte antigen (HLA) identity to the mother could allow the malignant clone to proliferate. In at least one reported case, the mother and fetus were not HLA-identical [12]. In another case, maternal leukemia cells were demonstrated in the cord blood of the infant but were not present in peripheral blood samples obtained at six weeks, three months, and six months [19]. Immune function of infants who have developed maternal malignancy has not been reported.

Specific tumors occurring in infancy

Teratoma

Teratomas in infants occur primarily in extragonadal locations along the midline, with sacrococcygeal location being the most common [20]. There is an approximately 3 : 1 ratio of girls to boys [21–23]. Most sacrococcygeal teratomas in infants are benign, but approximately 20% will have malignant elements [21, 22]. The most common malignant histologies identified are yolk-sac tumor and embryonal carcinoma [24, 25].

Sacrococcygeal teratomas usually present as a mass protruding between the coccyx and the rectum. Because the lesions are usually exophytic and visible for examination, they often are diagnosed in the first month of life [5, 6].

Treatment consists of early and complete surgical resection, including removal of the coccyx [21, 25]. Tumor markers (alpha-fetoprotein (AFP) and β-human chorionic gonadotrophin (HCG)) should be checked. Complete resection results in a high cure rate for benign lesions [25–27]. Recurrence rates of up to 20% have been reported, even with tumors felt to be benign at the time of initial resection, with both malignant and benign histologies reported

at recurrence [24, 25, 27–30]. Platinum-containing chemotherapy regimens are indicated for primary lesions that contain malignant elements, with event-free survival rates in excess of 80% [26, 31–34]. At least one large series has shown that a majority of patients with nonmetastatic malignant sacrococcygeal teratoma can be treated successfully with complete surgical resection alone, with chemotherapy reserved for those who suffer a recurrence [27, 35].

Recurrent disease is managed by repeat surgical resection alone for lesions that are benign at the time of recurrence [27]. The majority of malignant recurrences are local and may follow an initial diagnosis of either benign or malignant teratoma [27, 36]. Recurrences with malignant elements generally require platinum-based chemotherapy as well as surgical resection to achieve cure, although rare patients with recurrent disease can be cured with complete surgical resection alone [36]. The most important prognostic factor for patients with recurrent disease is completeness of relapse tumor resection [36].

Neuroblastoma

Neuroblastoma is the most common malignancy of the neonate (Table 16.1). Neuroblastomas occurring in early childhood are distinct in their clinical behavior and generally respond much better to therapy than those in older children. Autopsy series of infants who died from unrelated causes indicate an occurrence rate far greater than the reported incidence of neuroblastoma [37, 38]. The majority of these tumors are thought to regress spontaneously, consistent with the generally less aggressive behavior of these tumors in infants. The finding of occult neuroblastoma also raises the issue of screening in an attempt to identify tumors at an earlier stage to improve their prognosis, which will be discussed later in this section.

Presentation

Neuroblastoma arises from neural crest cells, which normally develop into the adrenal medulla and sympathetic ganglia, and can present anywhere along the sympathetic chain. The most common presentation is an abdominal mass arising from the adrenal gland, but primary lesions also can occur in the neck, posterior mediastinum, retroperitoneum, and pelvis. Symptoms are generally related to mass effect of the primary and/or metastatic tumor. Cervical tumors can be associated with Horner's syndrome, posterior mediastinal tumors can cause respiratory distress, and paravertebral tumors often invade the intervertebral foramina and cause symptoms of spinal-cord compression. Widespread use of prenatal ultrasonography has led to an increased prenatal detection rate [39–42]. Most prenatally diagnosed tumors are of the adrenal gland, and most frequently are of low stage. Increased urinary excretion of catecholamine metabolites is found in more than 90% of children with neuroblastoma [43] and can be associated with hypertension and diaphoresis in the child or, rarely, in the mother prenatally [44].

Metastatic disease often is present in neonates, most frequently to the liver, bone marrow, and skin [45]. Skin lesions usually present as bluish subcutaneous nodules ("blueberry muffin"), and are indistinguishable from those in congenital leukemia by visual appearance. Liver involvement often is extensive and can cause respiratory compromise due to abdominal distention. Bone-marrow involvement can cause cytopenias or leukoerythroblastosis but often is apparent only on bone-marrow examination, classically showing clumps of tumor cells. The unique pattern of metastatic disease restricted to the liver, bone marrow, and skin in infants is classified as stage 4S (see below) [46, 47].

Diagnostic evaluation

Diagnosis of neuroblastoma is made by biopsy of the primary or metastatic tumor. Concentrations of catecholamine metabolites (homovanillic acid (HVA), vanillylmandelic acid (VMA)) should be checked in the urine. Random (spot) samples of urine for catecholamine metabolites are as

Table 16.2 International neuroblastoma staging system

1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive).
2A	Localized tumor with incomplete gross resection; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.
2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.
3	Unresectable unilateral tumor infiltrating across the midline[a], with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement.
4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S).
4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver and/or bone marrow[b] (limited to infants under one year of age).

Note: multifocal primary tumors (e.g. bilateral adrenal primary tumors) should be staged according to the greatest extent of disease, as defined above and followed by a subscript letter M (e.g. 3_M).

[a] The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

[b] Marrow involvement in stage 4S should be minimal, i.e. less than 10% of the total nucleated cells identified as malignant on bone-marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The meta-iodobenzyl-guanine (MIBG) scan (if performed) should be negative in the bone marrow.

From Brodeur *et al.* [47] with permission.

sensitive and as specific as 24-hour collections [48, 49]. The diagnosis can be established in patients with high surgical risk without a biopsy of the primary lesion by demonstrating bone marrow involvement and elevated urinary catecholamine levels. Biopsy of abdominal lesions should be avoided in infants with massive liver involvement and where there is concern about wound closure. Shimada and colleagues [50] have developed a histopathologic classification system that has prognostic significance. Tissue samples also should be evaluated for amplification of the N-*myc* oncogene and for ploidy, both of which have an impact on prognosis [51–53]. Allelic loss of chromosome 1p also has been shown to be associated with aggressive tumor behavior [54].

Staging evaluation should include a computerized tomograghy (CT) scan or magnetic resonance imaging (MRI) scan of the primary lesion, as well as CT or MRI of suspected metastatic sites. A technetium bone scan should be obtained to evaluate for cortical bone metastases. Bilateral bone-marrow aspirates and biopsies should be performed to evaluate for bone-marrow involvement.

The current staging system, the International Staging System (INSS), has evolved from several earlier systems and is based on extent of disease as well as surgical resectability (Table 16.2) [47]. Stage 1 tumors are localized and surgically resectable, while stage 2 tumors are localized but have residual disease after surgery. Stage 3 tumors cross the midline and stage 4 tumors present with metastatic disease. As mentioned earlier, infants presenting at less than 12 months of age with metastatic disease restricted to the liver, skin, and/or bone marrow are classified as stage 4S.

Treatment

Stratification for therapy is based upon a combination of stage and biologic features. All infants with stage 1 or 2 disease are classified as low-risk, regardless of biologic features. Surgery alone is generally sufficient for disease control, and the few that grow after surgery are treated effectively with surgery [55–57]. Infants with stage 2 disease by definition have residual disease after surgery, yet most residual masses regress without further intervention.

Infants with stage 4S disease frequently have spontaneous regression of their disease [58, 59] but may be symptomatic from massive involvement of the liver, either initially or upon disease progression. Infants under two months of age are at particular risk for disease progression. Chemotherapy and/or radiation therapy should be used in these patients based upon severity of clinical symptoms to effect symptomatic relief [60, 61]. The intent of intervention is to allow time for spontaneous tumor regression. Once tumor regression begins, it generally continues without further therapy. Stage 3 and 4 tumors are relatively uncommon in infants. Infants with stage 3 tumors without *n-myc* amplification have an excellent prognosis (90% event-free survival) with a brief course of combination chemotherapy [62]. Infants with stage 4 disease without *n-myc* amplification do not fare as well, but survival rates greater than 50% have been reported [63]. Infants with stage 3 or 4 disease with *n-myc* amplification require more intensive therapy with high-dose chemotherapy and radiation therapy. For details of therapy of these high-risk patients, the reader is referred to more comprehensive textbooks.

Therapy for neuroblastomas detected by prenatal ultrasound is evolving. These tumors have been managed in the past as other tumors of similar clinical stage, with surgery and chemotherapy as indicated. These tumors are usually stage 1, 2, or 4S and usually have favorable biological characteristics. An almost universally favorable outcome has been documented [40, 42, 64]. Based on these findings, a clinical trial is under way that employs a conservative approach with observation alone, with intervention with surgery with or without chemotherapy reserved for patients with disease progression.

Screening

Based upon the high incidence of neuroblastomas in infants and the knowledge that patients with high-stage disease have a poor prognosis, mass screening programs have been mounted using urinary concentrations of HVA and VMA to detect neuroblastoma before it is clinically apparent. The expectation of these programs is that screening would lead to detection of tumors before they progressed to higher stages and thereby reduce the incidence of high-stage disease. These programs have resulted in a marked increase in the observed incidence of neuroblastoma but no decrease in the incidence of high-stage disease [65–68]. The tumors identified by screening almost always are biologically favorable, and nearly all cases identified by screening have a favorable outcome, many with no intervention [69]. Death rates from high-stage disease in screened populations have not been decreased significantly [66–68]. Additionally, screening results in exposure of infants with subclinical tumors to treatment-related morbidity [66]. Based on these findings, there appears to be little justification for such screening programs.

Leukemia

Leukemia usually is reported as the second most common malignancy in the neonate (Table 16.1). Leukemia occurring in the newborn period has some features that are different from those of leukemia occurring later in childhood. The majority of neonatal leukemia is acute myeloid leukemia, a reverse of the ratio in older children [1]. Neonatal leukemias present frequently with high white cell counts, and many have leukemic skin nodules (leukemia cutis). Leukemia occurring during the first few days of life has long been assumed to have been present in utero and, thus, has been classified as congenital, as opposed to leukemias that present later, which presumably developed postnatally. Recent studies of

twins with leukemia challenge this distinction (see below).

Presentation

Infants with leukemia present with signs and symptoms related to infiltration of leukemic blasts into tissues, bone-marrow failure, and leukocytosis. Skin infiltration (leukemia cutis) occurs in up to 50% of infants with leukemia and may be the first presenting sign [70]. It appears as multiple firm subcutaneous nodules, which are classically bluish to gray in color ("blueberry muffin") [71, 72]. Patients with leukemic skin infiltrates have, on rare occasions, been shown to have normal bone-marrow studies [73, 74]. Leukemia cutis has a very similar appearance to cutaneous neuroblastoma and dermal erythropoiesis, which occurs in severely anemic infants. Hepatosplenomegaly is a common finding.

Bone-marrow infiltration with failure of hematopoiesis leads to signs and symptoms associated with anemia, thrombocytopenia, and neutropenia. Anemia can cause pallor, lethargy, poor feeding, tachycardia, and flow murmurs. Thrombocytopenia can result in excessive bleeding and bruising. Neutropenia predisposes the child to bacterial sepsis and pneumonia.

Neurologic symptoms can be seen in infants with CNS involvement, including specific cranial nerve deficits as well as generalized symptoms such as lethargy and poor feeding. Profound leukocytosis can lead to hyperviscosity, with resulting cardiac, pulmonary, and CNS dysfunction.

Diagnostic evaluation

Peripheral blood counts should be obtained. The values can vary widely, occasionally being completely normal but usually showing leukocytosis with leukemic blasts. Anemia and thrombocytopenia also may be present. The possibility of CNS involvement should be investigated by performing a lumbar puncture. Leukemoid reaction, a common response to hypoxia, infection, or severe hemolysis, can mimic the peripheral blood findings of leukemia, with cytopenias and leukocytosis with immature forms. A bone-marrow aspirate can distinguish the conditions, as marrow samples in leukemia have blast infiltration whereas marrow samples from patients with leukemoid reaction do not. Transient myeloproliferative disorder associated with trisomy 21 can be difficult to distinguish from acute leukemia (see below).

Acute lymphoblastic leukemia

Infant acute lymphoblastic leukemia (ALL) is a biologically distinct entity from ALL in older children [75]. Infants with ALL historically have had a poor response to therapy, with disease-free survival rates of 20–30% [76–78]. More than 80% of infants with ALL have a balanced reciprocal translocation between the long arms of chromosomes 4 and 11, t(4;11), involving the MLL gene at chromosome band 11q23 [77, 79–81]. Infants lacking a rearrangement involving 11q23 appear to have a prognosis similar to that in older children [77, 81]. Infants with ALL with t(4;11) often exhibit unfavorable features at presentation, including high white cell count and CNS involvement [77]. Leukemic cells with t(4;11) usually express the B-cell-associated antigen CD19 on their surface but lack expression of CD10 [77].

Treatment of infants with t(4;11) ALL with chemotherapeutic regimens used successfully to treat older children with ALL has yielded disappointing results [77, 78, 80]. Newer approaches employing higher-dose therapy hopefully will be more successful.

In utero origins of infant acute lymphoblastic leukemia

There is a high risk of an identical sibling of a twin pair developing ALL if the other twin has infant ALL (reported in up to 25%) [82, 83]. This has long been thought to be due to a commonly inherited genetic risk factor. Recent data from several twin pairs challenge this hypothesis. Analysis of DNA breakpoints and immunoglobulin gene rearrangements from twins with infant ALL revealed the blasts to have identical acquired (nongermline) changes in their

DNA [84]. The most likely explanation for these findings is transfer of leukemic cells from one twin to the other through shared placental circulation. Because the twins are identical, there can be no immunologic elimination of the leukemic clone. These findings have been extended to include leukemias with translocations other than t(4;11), which occur later in childhood [85, 86].

Acute myeloid leukemia

Acute myeloid leukemia (AML) accounts for the majority of perinatal leukemias. Acute monocytic (FAB M5) and acute myelomonocytic (FAB M4) are the most common subtypes encountered in infants [87, 88]. Translocations involving chromosome 11q23 can be seen in infant AML but usually involves a different partner gene (and chromosome) than in ALL [89]. In contrast to infant ALL, appropriate therapy in perinatal AML has resulted in outcomes similar to those achieved in older children with AML [88].

Chronic leukemias

Rarely, infants can present with features of myelodysplasia. The syndrome is characterized by prominent hepatosplenomegaly, frequent skin involvement, leukocytosis with immature myeloid forms in the peripheral blood, monocytosis, and features of dysplasia in the bone marrow with less than 20% blasts [90, 91]. Elevated hemoglobin F also is seen frequently. The term "juvenile myelomonocytic leukemia" (JMML) has been proposed, and generally accepted, to describe this syndrome to include entities previously known as juvenile chronic myelogenous leukemia (JCML), chronic myelomonocytic leukemia (CMML), and monosomy 7 syndrome [90, 91]. Adult-type chronic myelogenous leukemia (CML) rarely can present in this age group with a similar clinical picture to JMML, but it can be distinguished by the presence of the t(9;22) chromosomal translocation. Care must be taken to distinguish this syndrome from infectious causes, particularly in infants, as there are case reports of (CMV), cytomegalovirus Epstein–Barr virus (EBV), and human herpes virus (HHV)-6 infections mimicking JMML in infants [92–95].

Prognosis in JMML is poor, with allogeneic bone-marrow tranplantation the only known effective therapy. Event-free survival in series of patients with JMML undergoing bone marrow transplantation (BMT) is in the range of 30–40% [90, 91].

Transient myeloproliferative disorder

Transient myeloproliferative disorder (TMD), also referred to as transient leukemia, transient abnormal myelopoiesis, and transient myeloproliferative syndrome, is a unique disorder indistinguishable from the acute megakaryocytic leukemia commonly seen in older Down syndrome (DS) children, except for its unusual propensity to spontaneously regress. Predominantly, this myeloid proliferative disorder occurs in newborns and infants less than 90 days of age with trisomy 21 [96], but it also has been reported in phenotypically normal children with mosaic DS and in children in whom the trisomy 21 is restricted to the hematopoietic cells and/or the TMD cells [97, 98]. Thus, constitutional trisomy 21 should be ruled out in all young infants with myeloid leukemias.

Newborns and young infants present with a varying severity of symptoms [99]. The most severe may die in utero due to hydrops fetalis. Hydrops, whether fatal or simply symptomatic at birth, initially was felt to be due to the hyperleukocytosis often seen in TMD. However, autopsy findings of severely affected newborns indicate that most often this is due to fibrosis of the liver and/or heart, resulting in severe hypoalbuminemia and/or compromised cardiac contractility [100–102]. The fibrosis is a result of organ infiltration by the TMD cells (see below). However, most patients with TMD have a mild course before spontaneous regression. Severe manifestations of TMD are found in approximately 10–19% of those diagnosed [100, 103]. Most often, patients are found to have elevated white blood counts and peripheral blasts, with many exhibiting concentrations well above 50 000/μl and 50%, respectively

[99, 104]. Hepatosplenomegaly is a common finding and needs to be monitored closely, both for its occasional association with hepatic dysfunction and its ability to compromise thoracic volume. Pulmonary, pericardial, and abdominal effusions occasionally are present at diagnosis. Recently, a vesiculopustular dermal eruption has been described in several TMD patients due to infiltration by leukemic blasts [105]. Examination of the bone marrow typically reveals fewer blasts than found in the peripheral blood [100]. Blasts are megakaryoblastic, appearing most often with cell-surface markers identified by flow cytometry consistent with a platelet-precursor derivation. Disseminated intravascular coagulation (DIC), hepatic insufficiency, cardiac failure, and renal failure all may be present or may develop during TMD. Symptoms either may regress from the initial time of diagnosis or may worsen for a period of time before spontaneous regression. It is therefore imperative that these patients be followed closely until TMD resolves. For patients with significantly severe symptoms, intervention may be necessary while awaiting spontaneous regression. Intervention may include exchange transfusion or leukopheresis for peripheral blast counts in excess of 100 000/μl, where concerns over vascular sludging and resulting CNS thrombosis exist. For patients with symptoms due to organ infiltration, low-dose chemotherapy, most often cytarabine, has been used, with varying success [103]. The decision in whom to begin intervention, and the timing of that intervention, particularly in patients with hepatic involvement, is uncertain. The degree of severity and the rapidity in worsening should be a guide. Time to spontaneous resolution varies considerably. For a small but significant percentage of TMD patients, the outcome is fatal, most often a result of severe hepatic fibrosis and hepatomegaly progressing to respiratory failure and/or renal failure [101, 103, 104]. The proportion of children with TMD who have a fatal outcome has been estimated as high as 67% but likely is in the 10% range [104]. The likely reason for the higher estimates is the use of retrospective surveys and literature reviews where the worst cases are more likely to be reported. Patients with hepatic dysfunction in addition to the presence of hepatomegaly appear to be at greatest risk of disease-related mortality. For those in whom hepatomegaly was present, it may persist for many months after all other signs and symptoms of TMD have resolved.

Children with a history of TMD are at significant risk of later developing acute leukemia, most often AML, particularly the megakaryocytic form (FAB M7). This risk is estimated to be 20–30%, with virtually all cases occurring in the first five years of life [106]. To date, there are no prognostic findings that identify which patients may be at greatest risk for developing AML. Frequent monitoring of former TMD patients is recommended during this period of risk, with serial physical examinations and complete blood counts. An early manifestation of development of acute leukemia in the former TMD patient is a slowly falling platelet count. Up to a third of those DS children developing a myeloid malignancy are found to have myelodysplasia most often associated with dysplastic megakaryocytes [107]. Fortunately, for the DS child with AML, the prognosis is markedly better than for children without DS, with recent cure rates in excess of 80% with conventional-dose chemotherapy [108, 109].

As noted above, the leukemic blasts in TMD are indistinguishable from megakaryoblasts seen in DS children with FAB M7 AML (which accounts for 70% of AML in DS) [110, 111]. Studies have determined that the TMD cells are clonal [112] and, therefore, neoplastic, as opposed to a polyclonal expansion of cells seen occasionally in lymphoproliferative disorders. Cytogenetics has not revealed a unique nonrandom event to identify patients with TMD. Recent molecular studies indicate that in all DS children with TMD who were analyzed, there was a mutation of the GATA1 locus (but not in DS children without TMD or acute megakaryocytic leukemia), which resulted in a dysfunctional truncated molecule [113–115]. GATA1 mutations also have been reported in DS patients with AMkL. It is known that GATA1 deficiency is associated with thrombocytopenia and hyperproliferation of megakaryocytes [116]. Based on this work, GATA1 is likely to play a critical role in the etiology of TMD, and mutagenesis of GATA1

represents a very early event in DS myeloid leukemogenesis [113–116].

The reason for the spontaneous regression is unknown, but several theories have been proposed [96]. It may be related to the cell of origin. It is noted that as opposed to AML, TMD has predominantly peripheral blood blasts. Bone-marrow fibrosis is a unique finding in older patients with AMkL. In TMD patients, however, the fibrosis is found predominantly in the liver, and patients often have massive hepatomegaly at diagnosis or progress to this in the ensuing weeks after diagnosis [100, 101]. As pointed out by several authors [101, 102], megakaryoblasts contain the cytokines platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β), both known to induce fibrosis.

It is hypothesized, therefore, that the cell of origin in TMD is present in the liver and that it is a remnant of fetal hepatic hematopoiesis. The natural regression of hepatic hematopoiesis may be an explanation for the spontaneous regression seen in TMD [96, 101]. Not conflicting with this theory but alternatively, another study noted that telomerase activity was elevated in acute megakaryotic leukemia patients and in TMD patients with severe manifestions, but not in TMD patients whose disease regressed spontaneously [117]. This could represent the reason for the regression in TMD versus the progressive nature of AMkL, or it could represent a normal part of spontaneous regression in hepatic hematopoiesis. Finally, it is unknown whether the original TMD cells remain quiescent, later evolving to AMkL, in that subset of patients who develop it or whether it is a new clone that arises. Cytogenetic analysis has suggested both possibilities [118, 119].

Brain tumors

Brain tumors are uncommon in neonates. Only 17 of 1296 children with brain tumors were symptomatic before two months of age in one series [120, 121]. Brain tumors in infants predominantly are supratentorial, in contrast to those in older children [122–125]. The neurologic immaturity of infants prevents many of the localized findings that can be associated with brain tumors in older children. The most common presenting sign is macrocephaly (50–67% of cases) [122, 124, 125]. Since the skull of a neonate can expand, symptoms of increased intracranial pressure may be less frequent. Vomiting (0.5–14%) and poor feeding (3.5–5.7%) have been reported [122, 124, 125]. Cranial-nerve deficits and seizures are uncommon [126].

Histologic diagnoses from reported case series have been summarized. The diagnoses encountered are the same as in older children, but their distribution is somewhat different. Teratoma is the most common tumor to present in the neonatal period, followed by astrocytomas [122, 124, 125, 127]. There appears to be an increased incidence of choroid plexus papillomas in neonates [122–125]. Other tumors reported in neonates include primitive neuroectodermal tumor (PNET), ependymoma, and craniopharyngioma.

Brain tumors have been diagnosed prenatally on routine ultrasonography [128–132]. For patients with macrocephaly, cranial ultrasound is a good initial study, as it is noninvasive and can be performed at the bedside. Once an intracranial mass is detected, CT or preferably MRI can be obtained for evaluation for surgery. Magnetic resonance angiography and venography also can be performed to define the relationship of the tumor to major vessels.

Diagnosis is established by biopsy. In general, total surgical resection is the goal. Some tumors, particularly PNET, are responsive to chemotherapy. Radiation therapy has been used in neonates, but because of the severe neuropsychological sequelae of radiation in very young children, attempts to either delay or avoid radiation generally are made. Prognosis varies with location, histology, and degree of surgical resection. Accurate data for outcomes are not available due to the rarity of the various tumors; however, outcome generally is poor [124]. Advances in radiation therapy techniques may allow delivery of treatment doses to the tumor bed with reduced dose, and therefore reduced damage, to surrounding tissue.

Sarcomas

Sarcomas in infants are rare, accounting for approximately 10% of neonatal cancers (Table 16.1). Because of the relative rarity of this group of tumors, the relative incidence of the various tumors is dependent on the case series. Among sarcomas, the most common tumors are fibrosarcoma, rhabdomyosarcoma, and PNET [133–135].

Rhabdomyosarcoma accounts for 50% of soft-tissue sarcomas in children but is somewhat less common in infants [136]. Rhabdomyosarcoma presents as an enlarging soft-tissue mass. Associated symptoms are due to mass effect and therefore are dependent on tumor location. Data from intergroup rhabdomyosarcoma (IRS) I and II showed that primary sites in infants less than 12 months old were genitourinary (26%), extremities (23%), and head and neck (16%) [136]. Metastatic disease can appear in the lungs, lymph nodes, liver, bone marrow, bone, and brain [137, 138]. Work-up therefore should include MRI or CT of the primary site, CT of the chest, bone scan, bone-marrow aspirate and biopsy, and CT of the head, as well as lumbar puncture for those with head and neck primaries.

Two histologic subtypes of rhabdomyosarcoma have been described: embryonal and alveolar. It was known that these histologic subtypes had differing clinical behaviors; they were subsequently demonstrated to be associated with distinct chromosomal translocations. Alveolar rhabdomyosarcoma is associated with either t(2;13) or t(1;13), resulting in fusion of the PAX3 and FHKR genes or the PAX7 and FHKR genes, respectively [139]. In contrast, embryonal rhabdomyosarcomas are associated with allelic loss of the 11p15 region [139].

Treatment of rhabdomyosarcoma consists of combined modality therapy with surgery and chemotherapy, with radiotherapy being reserved for cases where complete surgical resection is not possible. Complete resection of nonmetastatic primaries is recommended if it can be accomplished with acceptable morbidity. The most effective chemotherapeutic regimen includes a combination of vincristine, actinomycin D, and cyclophosphamide [140–142]. Gross or microscopic residual disease is treated with radiotherapy.

Prognosis is influenced by stage at presentation, histology, and location of the primary lesion. Patients who have initial complete surgical resection and embryonal histology, as well as those with primary tumors in the head and neck (particularly orbital) and genitourinary region (especially vagina and uterus), can expect to do well, with cure rates in excess of 90% [142]. Those with metastatic disease at diagnosis fare much more poorly, with long-term survival in the range of 25% [141].

PNET is now accepted as being on the same spectrum of disease as Ewing's sarcoma, and the two tumor types are now referred to collectively as the Ewing's sarcoma family of tumors (ESFT). The recognition that these tumors are related is based upon the similar pathologic appearance, clinical behavior, and the fact that they share a characteristic chromosomal translocation, t(11;22)(q24;q12) [143]. These tumors present as masses, either of the extremity or of the central axis. Determining whether a tumor arises primarily from bone or soft tissue can be difficult, as bony tumors often have a significant soft-tissue component and soft-tissue tumors can invade bone. The most common sites of metastatic disease are lung, bone, and bone marrow [144]. Diagnosis is made by biopsy of the primary lesion. Work-up should include CT or MRI of the primary site, CT of the chest, bone scan, and bone-marrow aspirates and biopsies. Current treatment consists of chemotherapy with ifosfamide, etoposide, vincristine, actnomycin-D, doxorubicin, and cyclophosphamide. After several courses of therapy, evaluation for local control is performed. Complete surgical resection is performed if it is feasible with acceptable morbidity, and radiation therapy is given to patients with microscopic residual disease and to patients for whom a surgical resection is not possible. Prognosis is correlated strongly to the presence of metastatic disease and, to a lesser extent, to success of surgical resection. Prognosis for infants appears to be similar to that for older children, with patients with localized disease having an event-free survival of about 60% [145].

Congenital fibrosarcoma (CFS) is a distinct entity from fibrosarcoma occurring later in childhood or adulthood. CFS samples contain a recurring characteristic t(12;15) chromosomal translocation involving the ETV6 and NTRK3 genes that is not detected in fibrosarcomas occurring later in childhood [146, 147]. CFS has a much better prognosis than fibrosarcoma in adults, with five-year disease-free survival greater than 90% [148–150]. The incidence of fibrosarcoma is higher in the first six months of life, with about one-third of fibrosarcomas diagnosed before five years of age being diagnosed shortly after birth [148, 151]. It occurs most commonly in the extremities, but it also can appear in the back, retroperitoneum, sacrococcyx, and head and neck [148, 151]. Metastatic disease is rare. Diagnosis is made by biopsy. Treatment is by surgical excision, which is usually curative [148–150]. Although local recurrence has been reported, repeat resection usually is curative [149]. For patients where surgical excision is not possible, there is some evidence that combination chemotherapy can be effective [152, 153].

Retinoblastoma

Retinoblastoma is a rare tumor that presents occasionally at birth. It deserves mention because of the implications of early detection. It can be detected by the lack of the normal red reflex when the infant's eyes are examined with an ophthalmoscope. Retinoblastoma is associated with mutations in the RB1 gene at chromosome band 13q14 [154–157]. It has been demonstrated that about 40% of cases of retinoblastoma result from inheritance of a germline mutation in the RB1 gene [158, 159]. This disease serves as a model for the "two-hit" hypothesis [158, 160]. Patients who inherit a germline mutation of one RB1 allele somatically acquire a mutation in the other allele. A significant number of patients with hereditary retinoblastoma will present with bilateral disease [158, 159]. There are occasional cases of sporadic bilateral disease. These appear to be due frequently to a new germline mutation in the paternal allele [161, 162]. Sporadic cases of retinoblastoma result from somatically acquired mutations in both RB1 alleles. Patients with germline mutation of one allele are at markedly increased risk for development of second malignancies, particularly osteogenic sarcomas [163]. All newborns should be examined for the presence of a red reflex, and any child with a family history of retinoblastoma should have a full ophthalmologic examination. The incidence of retinoblastoma appears to be higher in some developing nations than in developed countries, and is more frequently extraorbital [164–166]. The higher incidence of extraorbital disease correlates to a greater age at diagnosis [164, 167], emphasizing the importance of early detection by screening all newborns for the presence of a bilateral red reflex.

Most retinoblastomas in the USA are detected while they are still intraocular. The disease usually is curable when detected early [168–171]. Attempts are made to avoid enucleation by using cryotherapy or laser therapy with or without chemotherapy. Retinoblastoma is a radiosensitive tumor, but the use of external beam radiation is associated with a high rate of second malignancies in the radiation field [172, 173]. Newer radiotherapy techniques are being employed to reduce exposure of surrounding tissues, including plaque radiotherapy. Extensive intraocular disease can be managed with enucleation. Patients with metastatic disease require aggressive chemotherapy regimens [174–176].

Hepatic tumors

The most common causes of hepatic mass in infants is hemangioendothelioma [177]. Malignant disease in the liver is due most frequently to disseminated neuroblastoma or infiltration by leukemia. Hepatoblastoma is by far the most common primary liver malignancy in infants. The majority of hepatoblastomas occur later than infancy and have been seen in children < 1 year of age [178–180]. An association of hepatoblastoma with several syndromes has been noted, including familial adenomatous polyposis, Beckwith–Wiedemann syndrome, and Li–Fraumeni syndrome [181, 182]. There also is an increased incidence of hepatoblastoma among surviving

premature infants [183, 184], with risk increasing with lower birth weight [185].

Hepatoblastoma presents with abdominal enlargement and hepatomegaly. Lungs are the most common site of metastatic disease, and CT scan of the lungs as well as MRI or CT of the abdomen is indicated for staging work-up. AFP is elevated in the majority of patients and can be useful in monitoring disease status [186]. It is important to note that AFP is relatively elevated in infants without malignancy as compared with adults. It is, therefore, important to compare values with those of age-appropriate controls [187, 188].

Complete surgical resection with subsequent chemotherapy is the treatment of choice. For tumors that initially are unresectable, chemotherapy often can render them resectable [189–191]. Some authors recommend that all patients receive preoperative chemotherapy, regardless of initial resectability [191]. For patients in whom resection after chemotherapy still is not possible, liver transplant is an alternative [192]. Prognosis is correlated most strongly with resectability [193]. Approximately two-thirds of patients with initially unresectable disease can be cured with chemotherapy followed by surgical resection and postoperative chemotherapy [191, 193].

Renal tumors

Mesoblastic nephroma

The majority of renal masses that occur in the neonate are malformations, such as hydronephrosis and polycystic kidney disease. The most common tumor of the kidney in the neonate is mesoblastic nephroma, accounting for approximately three-quarters of the renal neoplasms in infants under 30 days of age [194]. The majority of children with mesoblastic nephroma present with an asymptomatic abdominal mass. Hematuria, hypertension, and vomiting also can be present. The majority of mesoblastic nephromas are confined to the renal capsule, and surgical excision alone is curative in the vast majority of cases [195]. The very rare cases in which there have been either local or distant recurrences have been managed with chemotherapy [196–198].

Wilms tumor

True Wilms tumor is unusual in the neonatal period [194]. Wilms tumor is believed to arise from blastemal elements, also known as nephrogenic rests, which persist beyond 36 weeks of gestation [199]. Several recognizable syndromes are associated with an increased risk of development of Wilms tumor, including WAGR (Wilms, aniridia, genitourinary tract abnormalities, mental retardation) and Beckwith–Weidemann syndrome (gigantism, omphalocele, macroglossia, hemihypertrophy). These syndromes are associated with a loss of function of the WT1 gene at chromosome band 11p13(WAGR) [200] or WT2 at chromosome band 11p15 (BWS) [201]. Among patients with Wilms tumor without an identifiable syndrome, approximately 40% have abnormalities in expression of WT1 and WT2 [202]. A minority (about 4%) of patients with Wilms tumor have a germline mutation of WT1 [203]. Wilms tumor occasionally is bilateral. There is no observed increase in either germline or acquired mutations in WT1 in patients with bilateral disease [204, 205]. These data paint an interesting, but obviously incomplete, picture of the molecular origins of Wilms tumor.

Like mesoblastic nephroma, Wilms tumor presents as a usually asymptomatic abdominal mass. Imaging studies including CT of the abdomen and chest are used for staging. Metastatic disease is most common in the lungs. It appears that most neonatal Wilms tumors are low-stage. Treatment for low-stage disease with complete surgical resection and chemotherapy results in cure rates in excess of 90% [206]. Infants with small (less than 550 g) stage I tumors have been treated with surgical resection alone, with salvage therapy reserved for patients suffering a recurrence [207]. The recurrence rate (11/75) in the current COG protocol exceeded the stopping rule of 10%, resulting in the early closure of the observation only arm. However, all patients who

suffered a recurrence were treated successfully with salvage therapy, and the remaining patients (64/75) were cured without exposure to chemotherapy [207]. This approach needs to be validated in further trials. Higher-stage disease requires the use of more intensive chemotherapy and radiation therapy [206].

Other renal neoplasms

Two renal tumors other than those described already are found occasionally in children. Rhabdoid tumor of the kidney was described originally as a histologic variant of Wilms tumor. Because of its very different response to therapy and different histology, it is now considered a distinct entity. Rhabdoid tumor can metastasize to the brain and unfortunately is associated with a poor prognosis. Clear-cell sarcoma of the kidney was also described as a Wilms tumor variant. Bone metastases are often found with this tumor. Combined chemotherapy and radiation therapy in clear-cell sarcoma results in an event-free survival rate over 80% [206].

Histiocytosis

Histiocytosis is a disease with a broad spectrum of clinical presentations. Langerhans cell histiocytosis (LCH) is a term that covers a spectrum of presentations formerly known as eosinophilic granuloma, Hand–Christian–Schuller disease, and Letterer–Siwe disease [208]. The disease is characterized by infiltration of tissues with tissue macrophages (Langerhans cells), which express S-100 and CD1a surface antigen, as well as cytoplasmic Birbeck granules on electron microscopy [209, 210]. The disease usually presents in later childhood, but occasionally it is present at birth. Multiple organ systems can be involved in LCH, most commonly the skin (especially scalp), bone (lytic lesions, especially skull), lung, liver, and spleen. Draining ears and lymphadenopathy are also commonly present. CNS involvement is seen most commonly as diabetes insipidus due to hypothalamic infiltration. Other manifestations of CNS disease include hyporeflexia, dysarthria, nystagmus, psychomotor retardation, and neuropsychological defects. These findings are associated with hypodense lesions on MRI, which have been shown not to contain Langerhans cells on biopsy [211–213]. This is a distinct problem from the well-recognized diabetes insipidus due to hypothalamic and pituitary infiltration, and to date it is poorly understood. LCH has been thought to represent an inflammatory process as opposed to a true cancer, but there is evidence that the infiltration is clonal [214]. The often disseminated presentation and sometimes fatal course make this distinction seem semantic. The diagnosis is established by biopsy of lesions with demonstration of Langerhans cells. Work-up includes imaging of suspected sites of disease.

The spectrum of presentation of LCH is broad. Many patients present with only skin and/or bony involvement, while others have involvement of almost all organ systems mentioned. Prognosis is related directly to the degree of organ system involvement, with involvement of the liver, lungs, and bone marrow predicting a poor outcome [215–218]. Patients without organ involvement usually have spontaneous resolution, while those with extensive organ involvement frequently have a fatal course. Fortunately, the majority of patients have limited disease, although children under two years of age more commonly have extensive disease. Disseminated disease is treated with combination chemotherapy, which often leads to an apparent initial response; patients who respond after six weeks of therapy with vinblastine, prednisone, and etoposide generally do well [219]. Conversely, lack of response predicts a fatal outcome in 66% of patients [219]. While there is near-universal consensus that treatment is appropriate for patients with disseminated disease, it is not clear that intervention has an impact on outcome, as there is a rate of spontaneous regression without intervention and trials have not contained a no-treatment arm. It is possible that early response identifies patients who would do well without intervention. Approaches for patients unresponsive to the above therapy include 2-chlorodeoxyadenosine [220] and bone-marrow transplantation [221–224].

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17

Normal values and laboratory methods

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Introduction

Profound changes in the normal values for blood components occur during gestation and the first few months of life. The developmental aspects of hematopoiesis must be considered in the evaluation of the neonatal blood picture. Additionally, the timing and site of blood sampling can affect the results. Because of a broad normal range for many factors, the clinician should be aware that the presence of an abnormal condition is not always excluded by a normal laboratory test.

Red blood cell measurements

Complete blood counts (CBC) are performed routinely on automated cell counters, which function by one of two principles: voltage pulse-impedance or light scatter. For the interested reader, the operation of these instruments has been described [1]. The red blood cell count (RBC) and mean cell volume (MCV) are measured directly by these instruments, while the hematocrit is calculated from these values. Alternatively, the hematocrit also can be measured by direct centrifugation of blood in a microhematocrit tube filled by capillary action. Hemoglobin (Hb) is measured directly by automated cell counters [1]. The mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) are calculated values. Most automated cell counters also create histograms of red-blood-cell size and determine the red-cell distribution of width (RDW), a quantitative measure of the variation in red-cell size.

Normal values for Hb, hematocrit (Hct), RBC, and indices for fetuses and infants on the first day of life are shown in Table 17.1 [2–4]. As shown in Table 17.2, Alur and colleagues [3] found that African-American preterm infants had lower red-cell values on the first postnatal day. Schmaier and colleagues [5] found the MCV and MCH for term African-American infants to be 106.4±5.7 fl and 34.5±2.2 pg, respectively. Two-thirds of the infants whose MCV was <94 fl had Barts Hb identified on electrophoresis. Hence, alpha thalassemia should be considered for newborns with an MCV below 94 fl. Table 17.3 shows serial red-cell values for term and preterm infants in the first year of life [6–9]. The hemoglobin, hematocrit, MCV, and MCH all fall significantly over the first two months of life. These values fall more dramatically in preterm infants [9].

Site of sampling is important in interpreting hemoglobin measurements in the newborn. As shown in Fig. 17.1, hematocrit levels obtained from capillary blood samples are consistently higher than simultaneously obtained venous blood samples [10]. This difference appears to persist for several weeks, with preterm infants of four to six weeks of age having 11–12% higher hemoglobin values when obtained by heel-stick as compared with venous samples [11]. This discrepancy is about 5% in term infants [12].

Timing of sampling affects the hematocrit results. Shohat and colleagues [13] found that hematocrit levels peak two hours after birth and decline

Table 17.1 Normal red-cell values in utero or on the first day of life in preterm infants

	Gestational age (weeks)	RBC ($\times 10^6$ per μl)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/l)	RDW (%)	Reticulocyte (%)
Fetal[a]	18–21	2.85 ± 0.36	11.69 ± 1.27	37.3 ± 4.32	131.11 ± 10.97				
	22–25	3.09 ± 0.34	12.2 ± 1.6	38.59 ± 3.94	125.1 ± 7.84				
	26–29	3.46 ± 0.41	12.91 ± 1.38	40.88 ± 4.4	118.5 ± 7.96				
	30	3.82 ± 0.64	13.64 ± 2.21	43.55 ± 7.2	114.38 ± 9.34				
Postnatal	23–25		14.5 ± 1.6	43.5 ± 4.2	115.6 ± 5.6	38.6 ± 2.2	33.4 ± 0.9	15.9 ± 1.4	
	26–28		15.1 ± 1.6	45 ± 4.5	114 ± 7.6	38.3 ± 2.9	33.6 ± 0.6	16.5 ± 1.9	
Central[b]	29–31		16.2 ± 1.7	48 ± 5	110.4 ± 6.6	37.3 ± 2.5	33.7 ± 0.7	16.4 ± 1.5	
Postnatal	24–25	4.65 ± 0.43	19.4 ± 1.5	63 ± 4	135 ± 0.2				6 ± 0.5
	26–27	4.73 ± 0.45	19 ± 2.5	62 ± 8	132 ± 14.4				9.6 ± 3.2
Capillary[c]	28–29	4.62 ± 0.75	19.3 ± 1.8	60 ± 7	131 ± 13.5				7.5 ± 2.5
	30–31	4.79 ± 0.74	19.1 ± 2.2	60 ± 8	127 ± 12.7				5.8 ± 2
	32–33	5.00 ± 0.76	18.5 ± 2	60 ± 8	123 ± 15.7				5 ± 1.9
	34–35	5.09 ± 0.5	19.6 ± 2.1	61 ± 7	122 ± 10				3.9 ± 1.6
	36–37	5.27 ± 0.68	19.2 ± 1.7	64 ± 7	121 ± 12.5				4.2 ± 1.8
	Term	5.14 ± 0.7	19.3 ± 2.2	61 ± 7.4	119 ± 9.4				3.2 ± 1.4

[a]Data adapted from Forestier *et al.* [2].

[b]Data adapted from Alur *et al.* [3].

[c]Data adapted from Zaizov and Matoth [4].

Data expressed as mean ± SD. Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; RBC, red blood cell count; RDW, red-cell distribution of width.

Table 17.2 Effect of race on red-blood-cell (RBC) parameters in preterm infants

	23–25 weeks' gestation		26–28 weeks' gestation		29–31 weeks' gestation	
	White	African-American	White	African-American	White	African-American
Hb	15.3±1.6	14.2±1.5	15.6±1.2	14.9±1.8	6.7±1.6	15.4±1.7
Hct	45.0±5.0	42.6±4.0	46.0±3.0	44.0±5.0	50.0±5.0	45.5±4.6
MCH (pg)	39.4±2.0	38.2±2.0	39.1±2.0	37.7±3.4	37.6±2.1	36.2±3.0
MCV (fl)	116±6	115±6	116±5	113±9	112±5	107±8
MCHC (g/l)	33.8±0.5	33.0±1.0	33.7±0.6	33.5±0.6	33.6±0.6	33.8±0.9
RDW	16.0±1.0	15.7±1.9	16.2±1.0	16.7±2.4	16.3±1.4	16.3±1.4

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Data from Alur *et al.* [3].

Data expressed as mean ± SD. Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; RDW, red-cell distribution of width.

Table 17.3 Red blood cell values in the first year of life

	RBC ($\times 10^6$ per μl)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/l)	Reticulocyte (%) or absolute reticulocyte count
Term infants							
Day[a]							
1	5.14 ± 0.7	19.3 ± 2.2	61 ± 7.4	119 ± 9.4		31.6 ± 1.9	3.2 ± 1.4
2	5.15 ± 0.8	19 ± 1.9	60 ± 6.4	115 ± 7.0		31.6 ± 1.4	3.2 ± 1.3
3	5.11 ± 0.7	18.8 ± 2	62 ± 9.3	116 ± 5.3		31.1 ± 2.8	2.8 ± 1.7
4	5. ± 0.6	18.6 ± 2.1	57 ± 8.1	114 ± 7.5		32.6 ± 1.5	1.8 ± 1.1
5	4.97 ± 0.4	17.6 ± 1.1	57 ± 7.3	114 ± 8.9		30.9 ± 2.2	1.2 ± 0.2
6	5.00 ± 0.7	17.4 ± 2.2	54 ± 7.2	113 ± 10		32.2 ± 1.6	0.6 ± 0.2
7	4.86 ± 0.6	17.9 ± 2.5	56 ± 9.4	118 ± 11.2		32 ± 1.6	0.5 ± 0.4
Week[a]							
1–2	4.80 ± 0.8	17.3 ± 2.3	54 ± 8.3	112 ± 19		32.1 ± 2.9	0.5 ± 0.3
2–3	4.2 ± 0.6	15.6 ± 2.6	46 ± 7.3	111 ± 8.2		33.9 ± 1.9	0.8 ± 0.6
3–4	4.00 ± 0.6	14.2 ± 2.1	43 ± 5.4	105 ± 7.5		33.5 ± 1.6	0.6 ± 0.3
4–5	3.60 ± 0.4	12.7 ± 1.6	36 ± 4.8	101 ± 8.1		34.9 ± 1.6	0.9 ± 0.8
5–6	3.55 ± 0.2	11.9 ± 1.5	36 ± 6.2	102 ± 10.2		34.1 ± 2.9	1 ± 0.7
6–7	3.4 ± 0.4	12 ± 1.5	36 ± 4.8	105 ± 12		33.8 ± 2.3	1.2 ± 0.7
7–8	3.4 ± 0.4	11.1 ± 1.1	33 ± 3.7	100 ± 13		33.7 ± 2.6	1.5 ± 0.7
8–9	3.4 ± 0.5	10.7 ± 0.9	31 ± 2.5	93 ± 12		34.1 ± 2.2	1.8 ± 1
9–10	3.6 ± 0.3	11.2 ± 0.9	32 ± 2.7	91 ± 9.3		34.3 ± 2.9	1.2 ± 0.6
10–11	3.7 ± 0.4	11.4 ± 0.9	34 ± 2.1	91 ± 7.7		33.2 ± 2.4	1.2 ± 0.7
11–12	3.7 ± 0.3	11.3 ± 0.9	33 ± 3.3	88 ± 7.9		34.8 ± 2.2	0.7 ± 0.3
Month[b]							
4	4.35 ± 0.38	11.6 ± 0.8	34.1 ± 2.2	78.2 ± 4.6	26.9 ± 1.5		44 000 ± 20 000[d] μl^{-1}
5	4.51 ± 0.27	11.9 ± 0.9	35.6 ± 2.2	77.8 ± 2.8	26.5 ± 1.3		34 000 ± 15 000 μl^{-1}
6	4.58 ± 0.32	11.5 ± 0.8	33.8 ± 2.2	74.7 ± 2.9	24.9 ± 1.4		34 000 ± 15 000 μl^{-1}
7–8	4.89 ± 0.31	12.8 ± 0.6	37.9 ± 3.8	77.5 ± 3.6	26.2 ± 2.0		36 000 ± 14 000 μl^{-1}
9–10	4.85 ± 0.22	12.5 ± 1	37.2 ± 3.7	76.8 ± 5.2	25.9 ± 1.7		
1 Year	5.00 ± 0.23	12.3 ± 1	37.3 ± 4.1	74.6 ± 5	24.8 ± 2.7		
Preterm infants							
Day[c]							
1	4.71 ± 0.75	18.2 ± 2.7		115 ± 5	38.9 ± 1.7	33.5 ± 1.2	
3	4.4 ± 0.83	16.2 ± 2.9		112 ± 4	39.0 ± 3.4	33.8 ± 1	
7	4.45 ± 0.83	16.3 ± 2.9		110 ± 5	37.3 ± 1.8	33.9 ± 1.3	
14	4.10 ± 0.69	14.5 ± 2.4		106 ± 5	36.3 ± 1.9	33.9 ± 1	
21	3.71 ± 0.59	12.9 ± 2		102 ± 5	35.3 ± 2.2	34.2 ± 1.1	
28	3.17 ± 0.6	10.9 ± 1.9		100 ± 5	35.1 ± 1.9	34.4 ± 1.0	
35	2.97 ± 0.45	10 ± 1.4		98 ± 5	34.4 ± 1.5	34.5 ± 0.7	
42	2.94 ± 0.49	9.5 ± 1.5		97 ± 5	32.2 ± 1.7	33.7 ± 0.9	
49	3.21 ± 0.59	10.1 ± 1.7		95 ± 5	32.1 ± 1.6	33.5 ± 1	

[a]Adapted from Matoth *et al.* [6].

[b]Adapted from Galanello *et al.* [7].

[c]Adapted from Stockman and Oski [9].

[d]Adapted from Kling *et al.* [8].

Data expressed as mean ± SD. Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; RBC, red blood cell count.

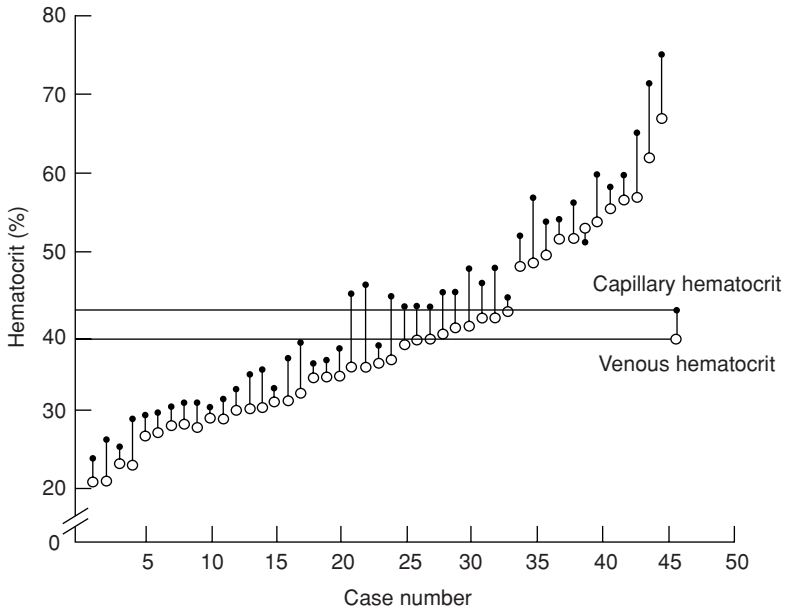


Fig. 17.1 Simultaneous capillary and venous hematocrit levels in term newborns. Reproduced with permission. *Clin Perinatol* 1984; 11: 489–510.

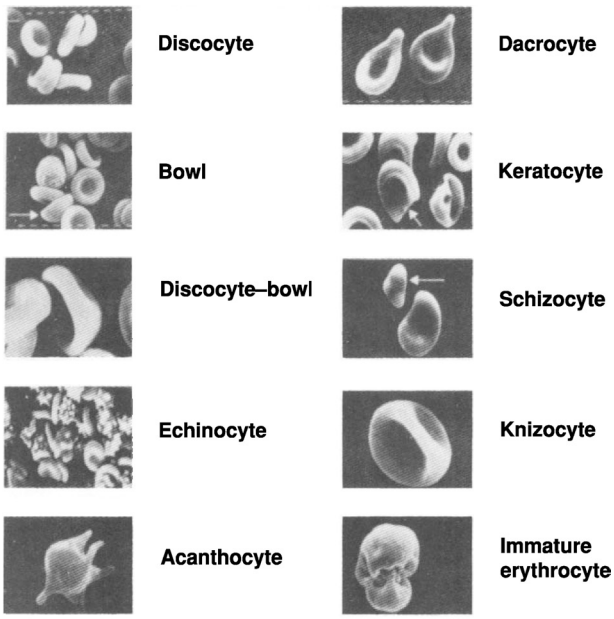
thereafter. Hematocrit values at 24 hours of age are similar to cord-blood levels.

The timing of umbilical-cord clamping after birth has a significant impact on the total neonatal blood volume. The combined blood volume of the fetus and placenta is approximately 105 ml/kg [14]. The blood volume and the red-cell volume of the infant increase significantly when clamping of the umbilical cord is delayed for one to three minutes after birth. Infants with delayed clamping of the umbilical cord have blood volumes that are roughly 25% higher than those of infants whose cords were clamped early [15].

Low and colleagues [16] studied blood of healthy term infants whose umbilical cords were clamped within one minute of delivery. These infants had an average hematocrit of 53.6%, with a plasma volume of 41.5 ml/kg and a total blood volume of 76 ml/kg. Infants studied at 24 hours of age had a blood volume of 83 ml/kg. The difference was accounted for by an increase in the plasma volume. In serial studies where the same infants were studied at birth and at 24 hours of age, the blood volume was unchanged in approximately half of the infants, 25% had a 7% increase in blood volume accounted for by an increase in the plasma volume, and the remainder had a 31% increase in blood volume. In contrast, infants with delayed clamping of the umbilical cord had blood volumes of 90 ml/kg at 4 hours and 90–95 ml/kg at 72 hours of life [14].

Morphology

Newborns have an increased percentage of abnormal erythroid shapes. Using wet preparations with 0.2% glutaraldehyde, Zipursky and colleagues [17, 18] compared the erythrocyte differential count from preterm and term infants with those of adults (Fig. 17.2) [17]. As demonstrated, newborns have significantly greater percentages of morphologic abnormalities. This may be due to decreased splenic function [18].



Erythrocyte differential count (median and 5–95% range)

Cell type	Premature infant	Term infant	Normal adult
Discocyte	39.5 (18–57)	43 (18–62)	78 (42–94)
Bowl	29.0 (13–53)	40 (14–58)	18 (4–50)
Discocyte–bowl	3.0 (0–10)	2 (0–5)	2 (0–4)
Spherocyte	0.0 (0–3)	0 (0–1)	0 (0–0)
Echinocyte	5.5 (1–23)	1 (0–4)	0 (0–3)
Acanthocyte	0.0 (0–2)	1 (0–2)	0 (0–1)
Dacrocyte	1.0 (0–5)	1 (0–3)	0 (0–1)
Keratocyte	3.0 (0–7)	2 (0–5)	0 (0–1)
Schizocyte	2.0 (0–5)	0 (0–2)	0 (0–1)
Knizocyte	3.0 (0–11)	3 (0–8)	1 (0–5)
Immature erythrocyte	1.0 (0–6)	0 (0–2)	0 (0–0)

Fig. 17.2 Differential count of erythroid shapes from newborn infants. Courtesy of Dr Alvin Zipursky. Reprinted with permission.

Reticulocyte count

Reticulocyte counts are high at birth and decrease rapidly over the first week of life, as shown in Table 17.3. This is likely due to the effect of increased oxygen levels on erythropoiesis, as discussed in Chapters 3 and 4. DeMarsh and colleagues found that infants whose umbilical cords were clamped early had significantly higher reticulocyte counts on the third day of life in infants than in infants whose umbilical cords were clamped late [19]. The traditional method for determining reticulocytes is to incubate the blood with dyes that stain for RNA and then to count manually the percentage of positively staining erythrocytes. This method is time-intensive and somewhat subjective. Because this value is expressed as a percentage of red blood cells, if the total red blood cell count is low, such as usually occurs with anemia, then the reticulocyte percentage may be within or above the normal range without accelerated erythropoiesis. The absolute reticulocyte count, determined by multiplying the RBC by the reticulocyte percentage, is less ambiguous. Modern automated cell counters can use flow-cytometric methods that accurately measure the absolute reticulocyte count [1].

Table 17.4 Postnatal values for hemoglobin (Hb) F and Hb A2 during the first six months of life in healthy term and preterm infants

	Full-term		Preterm	
	Median	Range	Median	Range
Hb F (fraction of total Hb)				
Day 1	0.68	0.55–0.78	0.75	0.67–0.84
Day 5	0.67	0.53–0.74	0.76	0.63–0.86
Day 30	0.59	0.34–0.72	0.73	0.45–0.86
Day 90	0.28	0.13–0.44	0.45	0.23–0.64
Day 180	0.05	0.01–0.14	0.09	0.05–0.22
Hb A2 (fraction of total Hb)				
Day 1	0.005	0.002–0.009	0.004	0.002–0.007
Day 5	0.005	0.002–0.01	0.004	0.002–0.006
Day 30	0.008	0.002–0.023	0.004	0.001–0.009
Day 90	0.021	0.014–0.027	0.016	0.008–0.021
Day 180	0.027	0.021–0.032	0.025	0.019–0.029

Reproduced with permission from Paes *et al.* [20, 21].

Abnormal hemoglobin

Structural Hb abnormalities are investigated using a number of techniques, as discussed in Chapter 8. Electrophoretic techniques separate Hb molecules

Table 17.5 Indices of erythropoiesis and iron status of infants 1–7 months of age (mean ± 1 SD)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
Hb concentration (g/l)	134±13	110±8.4	114±6.5 (22)	119±6.3	124±5.7	125±6.3	124±8.1
Plasma EPO level (U/l)	8.8±4	23.2±19	17.5±7.1	12.9±5.4	11.8±6	11.8±5.5	12.9±4.7
Absolute reticulocyte count ($\times 10^9$/l) (n)	25±17	85±26	83±35	44±20	34±15	34±15	36±14
Plasma transferrin level (g/l)	1.72±0.34	2.26±0.24	2.39±3.34	2.62±0.39	2.59±0.37	2.51±0.38	2.57±0.4
Total iron binding capacity (μmol/l) (n)	44±9	58±6	61±9	67±10	66±10	64±9	66±10
Plasma iron level (μmol/l) (n)	21.1±3.4	13.4±3.2	12.6±2.3	9.5±5.5	9.8±4	12.3±3.5	10.7±2.3
Transferrin saturation (%) (n)	49.9±15.6	23.5±6.3	20.9±4.5	13.8±7.8	14.9±6.3	18.9±5.4	16.3±3.9

EPO, erythropoietin; Hb, hemoglobin; SD, standard deviation.
Reproduced with permission from Kling *et al.* [8].

on the basis of differing charges and can be performed on citrate agar (acid pH) or cellulose acetate (alkaline pH) gels. For older children and adults, Hb electrophoresis is performed on cellulose acetate gels, but for the newborn it is advantageous to use both gels, because the high Hb F concentration obscures the diagnosis of certain variant hemoglobins. Isoelectric focusing is commonly used by newborn-screening programs because of its ability to identify a wide variety of abnormal hemoglobins using a small amount of blood that can be collected distantly on a filter paper. High-pressure liquid chromatography (HPLC) also can identify abnormal hemoglobins. Recent transfusion will complicate the interpretation of Hb electrophoresis studies. The relative percentages of Hb F and Hb A2 over the first six months of life are shown in Table 17.4 [20, 21].

Erythropoietin

Serum or plasma erythropoietin concentrations are measured by immunologic assays. Levels increase in response to hypoxia [22]. Cord-blood erythropoietin levels increase with gestational age, from 36 to 43 weeks (Fig. 17.3) [23]. Plasma erythropoietin levels for term infants from one to seven months of age are noted in Table 17.5 [8], while those of preterm infants for the first 60 days of age are shown in Fig. 17.4 [24]. Levels are significantly lower for infants 1–60 days of age than at birth or later in life. Levels are also elevated significantly in stressed newborns.

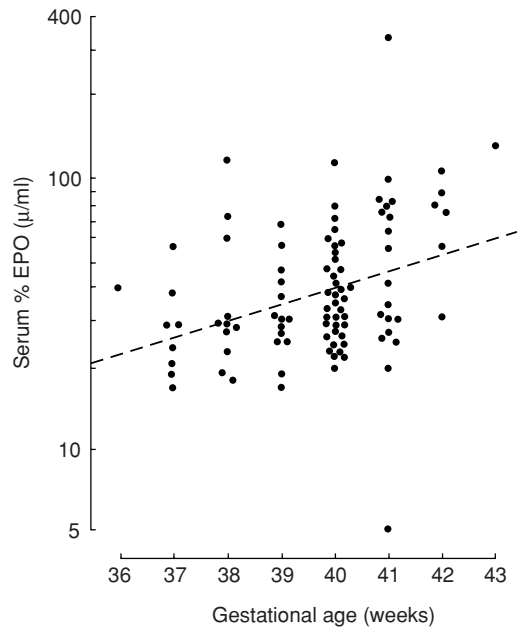


Fig. 17.3 Cord-blood erythropoietin (EPO) levels from infants of various gestational ages. Reproduced with permission. *Eur J Pediatr* Serum immunoreactive erythropoietin of children in health and disease. Eckardt, K. U., Hartmann, W., Vetter, U. *et al.* 149: 459–464, Fig. 3, 1990. © Springer–Verlag Gmbh & Co. [23]

Iron

Iron deficiency is quite rare in the newborn, and alternative diagnoses such as alpha thalassemia trait should be considered in the neonate with a low MCV. Measurements of iron sufficiency often are

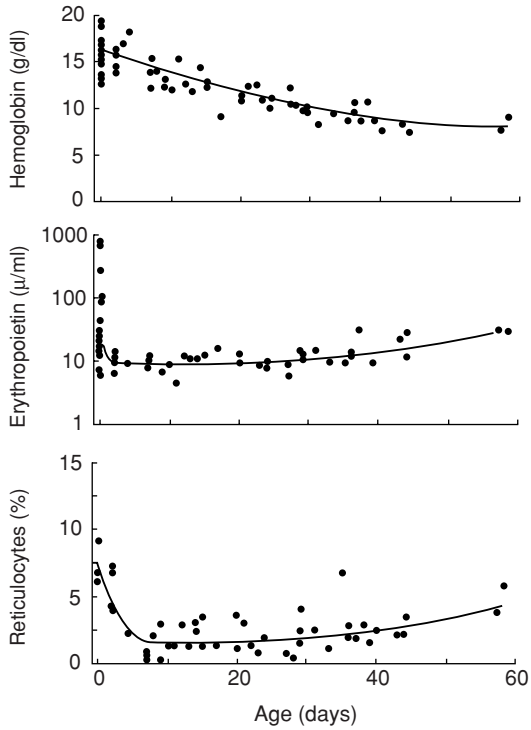


Fig. 17.4 Serum erythropoietin levels with hemoglobin and reticulocyte percentages in the first 60 days of life in preterm newborns. From Brown *et al.* [24]. Reproduced with permission. *J Pediatr* 1983; 103: 612–617.

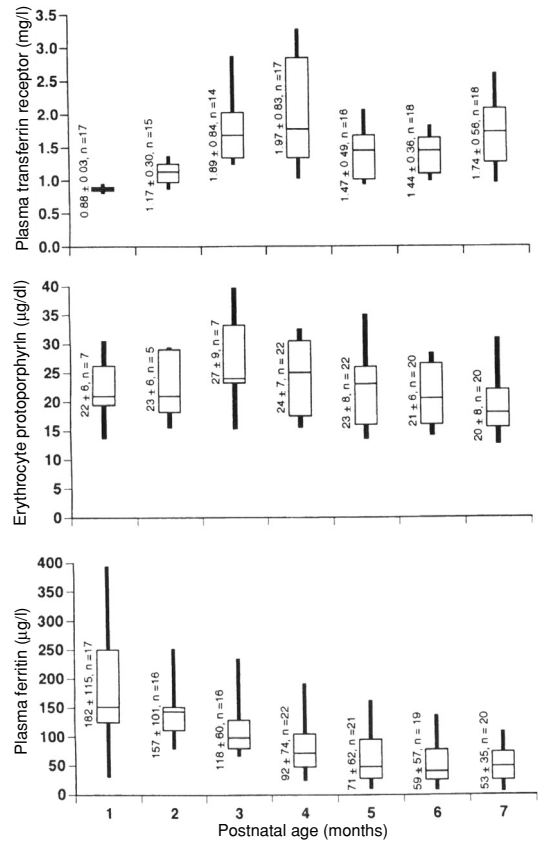


Fig. 17.5 Plasma transferrin receptor, erythrocyte protoporphyrin, and ferritin levels in infants aged one to seven months. Median values are indicated by the horizontal line within the rectangles, the upper and lower horizontal boundaries of the rectangles define the upper and lower quartiles, and the top and bottom of the thick vertical lines indicate the tenth and ninetieth percentiles. Reproduced with permission from Kling, P. J., Roberts, R. A. and Widness, J. A. Plasma transferrin receptor levels and indices of erythropoiesis and iron status in healthy term infants. *J Pediatr Hematol Oncol* 1988; 20: 309–314.

affected by other factors. Ferritin is an acute-phase reactant; hence, levels may increase with infection, inflammation, or other disorders. Therefore, while a low serum ferritin level indicates iron deficiency, a normal ferritin level does not necessarily confirm iron sufficiency. The ratio of the serum iron concentration to total iron-binding capacity (transferrin saturation ratio) reflects the iron status of the individual; however, serum iron concentrations fall with acute inflammation as well as with iron deficiency. Transferrin levels also may decrease with inflammation. The transferrin saturation ratio also can be affected by diurnal variation and acute iron ingestion. Newborns have relatively high levels of ferritin and transferrin saturation ratios in the first month of age, but these decline to reach trough levels in the fourth to fifth months (Table 17.5 and Fig. 17.5) [8]. Serum transferrin receptor levels, a relatively new method of measuring iron sufficiency, increase not only with iron deficiency but also with accelerated erythropoiesis. Serum transferrin receptor levels correlate with gestational age [25]. During infancy, plasma transferrin receptor values

Table 17.6 Serum cobalamin, serum folate, and whole-blood folate levels in term infants at birth and at six weeks of age

	Birth		6 weeks	
	Median	Interquartile range	Median	Interquartile range
Serum cobalamin (pmol/l)	299	238–429	230	158–287
Serum folate (nmol/l)	24.8	18.0–30.1	22.7	19.0–31.3
Whole-blood folate (nmol/l)	532	428–692	262	209–305

Adapted from Bjorke Monsen *et al.* [27]. Reproduced with permission from Bjorke Monsen *et al.* [27]. Copyright 2001.

are lowest in the first month of life and then increase (Fig. 17.5) [8]. The reticulocyte Hb concentration has been shown to be very sensitive to the diagnosis of iron deficiency in children [26]. Historically, the most reliable test was felt to be the presence or absence of stainable iron in the bone marrow, but marrow sampling is neither practical nor necessary in most instances.

Folate and vitamin B12

Both folate and vitamin B12 play key roles in erythropoiesis. Levels can be determined in serum or whole blood. Elevations in plasma methylmalonic acid and homocysteine also are seen in folate and vitamin B12 deficiency. Levels for both of these vitamins are increased in the newborn when compared with maternal levels and are shown in Table 17.6 [27].

Platelets

Platelet count

Platelet numbers can be quantified by automated cell counters or by manual platelet counting performed in counting chambers. Platelet activation at the site of phlebotomy can cause platelet aggregation and blood clotting, a common cause of pseudothrombocytopenia (spuriously low platelet counts). Typically, platelet counts from blood drawn into ethylene-diamine-tetraacetic acid (EDTA) are stable for up to five hours [28]. Automated cell counters typically identify platelets by size, identifying cells with volumes of roughly 2–30 fl as platelets. Red-blood-cell and leukocyte fragments also may fall into this size range and thus are interpreted by the instrument as platelets, resulting in an inappropriately high platelet count. Giant platelets may exceed this size and, hence can be excluded as platelets, resulting in a pseudothrombocytopenia. Automatic cell counters use additional analyses such as histogram evaluation and/or multiple samplings to help determine whether interference from nonplatelet sources is present [28]. The normal platelet counts for fetuses are shown in Table 17.7 [2], while values for term and preterm infants are given in Tables 17.8 and 17.9 [29–34].

Because of the potential causes of pseudothrombocytopenia noted above, the blood film should be reviewed in cases of thrombocytopenia. The peripheral smear can be utilized to estimate platelet count as well as to look for the presence of microthrombocytes, megathrombocytes, and red-cell fragmentation. The edges of the smear should be evaluated for platelet clumping, indicative of pseudothrombocytopenia.

Platelet morphology and production

Electron microscopy of platelets has been used to identify ultrastructural abnormalities in many giant platelet syndromes [35]. Bone-marrow morphology also can be useful in the identification of disorders of platelet production. Megakaryocyte numbers and ploidy reflect the degree of platelet production and are discussed in detail in Chapter 10.

The mean platelet volume (MPV) and platelet distribution width (PDW) are analogous to these measurements in erythrocytes and are measured by the automated cell counters. Normal values for MPV and PDW in the newborn are shown in Table 17.10 [36].

Another measure of platelet production is the reticulated platelet count. Platelets with increased concentrations of RNA, hence released recently from

Table 17.7 Evolution of platelet counts in 163 normal fetuses during pregnancy

	Platelet count		
Weeks of gestation	*n*	Mean	Standard deviation
18–20	25	242 100	34 480
21–22	55	258 200	53 650
23–25	61	259 430	42 450
26–30	22	253 540	36 600

Adapted from Forestier *et al.* [2].

Table 17.8 Platelet count in full-term newborns

Ref.	Source	*n*	Platelet count
[30]	Heel puncture	105	190 000 ± 94 000
[31]	Umbilical cord	45	325 000 ± 50 000
[29]	Capillary blood	88	250 000 ± 125 000

Adapted from Ablin *et al.* [30], Sell *et al.* [31], and Aballi *et al.* [29].

Table 17.9 Platelet count in preterm newborns

		Age		Platelet count $\times 10^3$		
Ref.	*n*	Gestation (weeks)	Postnatal	Mean	SD	Range
[33]	73		0–28 days	212		156–302
[29]	273		0–2 days	220	68	55–518
	194		2 weeks	260	100	33–574
	112		4 weeks	309	125	85–682
[32]	60		Birth	203		80–356
	40		10 days	399		172–680
	40		28 days	384		212–625
[31][a]	8	27–30	<36 hours	280	110	
	29	31–33	<36 hours	295	115	
	24	34–36	<36 hours	305	90	
	45	37–41	<36 hours	295	114	
Hathaway[b]		27–31	Birth	275	60	
		32–36	Birth	290	70	

[a] Published reference and personal communication.

[b] Data from personal series combined with reported series of others.

SD, standard deviation.

Adapted with permission from Naiman [34].

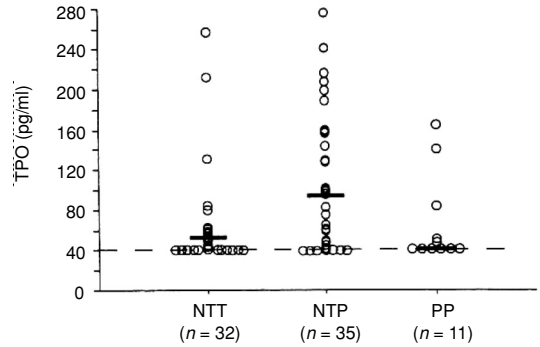


Fig. 17.6 Serum thrombopoietin (TPO) levels in nonthrombocytopenic term infants (NTT), nonthrombocytopenic preterm infants (NTP), and infants of mothers with pre-eclampsia (PP). From Albert *et al.* [39]. Reproduced with permission from *Pediatrics* 105: 1286–1291. Copyright 2000.

the megakaryocyte, are identified by flow-cytometric techniques. As shown in Table 17.11, reticulated platelet counts in infants 30–36 weeks' and >36 weeks' gestational age are similar, but are increased in infants <30 weeks' gestational age [37].

Thrombopoietin

Thrombopoietin (TPO) is a hematopoietic growth factor that regulates megakaryopoiesis. TPO levels have been measured by enzyme-linked immunosorbent assay (ELISA) in term and preterm infants. Sola and colleagues [38] found that cord-blood TPO levels were higher than in adult plasma, but these levels did not correlate with gestational age or platelet count at birth. Albert and colleagues [39] found higher TPO levels in cord blood from nonthrombocytopenic preterm infants when compared with nonthrombocytopenic term infants (Fig. 17.6).

Platelet-function analysis

Platelet function is more difficult to assess than platelet number, as screening tests may be unreliable and platelet-aggregation studies require relatively

Table 17.10 Mean platelet volume and platelet distribution of width in term and preterm infants

	Term	Preterm	*P*
Platelet count (/μl)	332 756 ± 80 400	293 107 ± 89 621	<0.05
Mean platelet volume (fl)	9.06 ± 0.85	8.51 ± 0.97	<0.001
Platelet distribution width	16.20 ± 0.68	17.21 ± 0.94	<0.001

Reproduced with permission from Patrick *et al.* [36].

Table 17.11 Reticulated platelet measurements in nonthrombocytopenic newborns in the first six hours of life

Gestational age (weeks)	*n*	Platelet count ($\times 10^3$/μl)	Reticulated platelet % (mean±SD)	Reticulated platelet % range	Absolute reticulated platelet count ($\times 10^3$/μl)
>36	39	246±65	4.0±2.4	2–12	10.5±8.7
30–36	25	255±48	4.6±1.7	2–7	11.9±5.0
<30	25	229±51	8.8±5.1	3–26	20.3±11.8

SD, standard deviation.
Reproduced with permission from Peterec *et al.* [37].

Table 17.12 Modified template method bleeding times in term and preterm infants

			Bleeding time (minutes)			
	n	Sphygmomanometer pressure (mm Hg)	Mean	SD	Range	Platelet count ($\times 10^3$/μl)
Term newborn	30	30	3.4	0.9	1.9–5.8	384±106
Preterm newborn	26		3.6	1.0	2.0–5.6	302±128
<1000 g	6	20	3.3	0.6	2.6–4.0	326±75
1000–2000 g	15	25	3.9	1.0	2.0–5.6	277±133
>2000 g	5	30	3.2	1.1	2.3–5.0	349±169
Child	17	30	3.4	1.3	1.0–5.5	356±115

SD, standard deviation.
From Feusner [41]. ©1980 American Society of Clinical Pathologists. Reprinted with permission.

large blood volumes. The bleeding time has been adapted to the newborn using a standardized incision made on the forearm with a blood-pressure cuff inflated to a specific pressure that varies with gestational age. While the bleeding time is well-standardized in the premature and full-term infant (Table 17.12) [40, 41] as well as in the older child and adult, it has a limited sensitivity and specificity for bleeding problems [42]. Furthermore, this test can be very technician-dependent and has the potential to leave a small scar at the site. The bleeding time is prolonged with thrombocytopenia. Several medications, including aspirin and nonsteroidal anti-inflammatory agents, can affect the bleeding time

Table 17.13 Platelet Function Analyzer (PFA-100®) closure times in healthy neonates and children

Subjects	*n*	Age (range) (years)	Hemoglobin (range) (g/dl)	Platelets (range) ($\times 10^9$/l)	Closure time (s)	
					Collagen/epinephrine (range)	Collagen/ADP (range)
Healthy children	57	11.3±3.9 (2.5–17)	13.2±1.0 (11.6–15.9)	263±51 (189–401)	117±23 (83–163)	91±13 (72–111)
Healthy neonates	17	>37 weeks	16.4±1.5 (14.7–20.8)	277±71 (175–466)	81±17 (61–108)	56±6 (48–65)
Healthy adults	31	34±5.8 (25–54)	14.2±1.1 (12.8–16.6)	232±53 (152–347)	106±21 (82–142)	85±16 (67–111)

Data expressed as mean ±1 SD.
ADP, adenosine diphosphate; SD, standard deviation.
From Carcao *et al.* [44]. Reproduced with permission.

as well as other tests of platelet function. In addition to platelet disorders, collagen defects such as those seen with Ehlers–Danlos and Marfan's syndrome may cause a prolonged bleeding time. The bleeding time also can be prolonged by anemia. When compared with adults, the bleeding time is shorter in the newborn than in older children and adults. This is likely due to the relatively high hematocrit and elevated von Willebrand factor levels of the newborn [40].

Newer technologies are available to assess platelet function. The PFA-100® Platelet Function Analyzer uses blood aspirated through a stainless-steel capillary tube and then a 150 μl aperture in a membrane coated with collagen and either epinephrine or adenosine diphosphate (ADP). The platelet aggregates occlude the membrane, stopping blood flow (closure time). Factors affecting closure time include hematocrit, platelet count, leukocyte count, von Willebrand factor concentration, and prolonged time from collection (more than four to five hours) [43]. Neonatal normal values are shown in Table 17.13 [44]. As with the bleeding time, the shorter closure times in the neonate are likely related to their higher hematocrits and von Willebrand factor levels. The PFA-100® is more sensitive than the bleeding time to the diagnosis of von Willebrand disease and appears to be sensitive to the diagnoses of Glanzmann thrombasthenia, Bernard–Soulier syndrome, and somewhat sensitive to delta storage pool disorders. The collagen/epinephrine cartridge may be the more sensitive assay [43].

There are no readily available ex vivo techniques for measuring platelet adhesion. Platelet aggregation is measured by one of two methods. Optical platelet aggregation is performed in platelet-rich plasma. Platelets suspended in plasma form a relatively turbid solution. When agonists stimulate platelet aggregation, the platelets aggregate and fall out of solution, and light transmission increases [45]. The impedance method measures the resistance to transmission of an electronic charge between two electrodes immersed in a whole-blood solution. The agonist causes platelets to aggregate along the electrodes, increasing resistance [45]. The ability to measure platelet aggregation in whole blood allows testing using smaller volumes of blood. Results of impedance platelet aggregation testing in a series of newborns as compared with school-aged children and adults are shown in Table 17.14 [46]. In a small study of infants of various ages, the neonate exhibited decreased aggregation and release only in response to collagen [46].

Platelet release can be studied by measuring the amount of a platelet granule constituent released after platelet activation. Examples are serotonin uptake and release [47] and flow cytometry [48]. Lumiaggregometry is another method in which adenosine triphosphate (ATP) released from activated platelets causes firefly luciferin to luminesce.

Table 17.14 Whole-blood lumiaggregation and release in newborns, children, and adults

Agonist	Measured value	Infants/newborns	*n*	Schoolchildren	*n*	Adults	*n*
ADP	Rate	73.1±8.0	12	71.5±11.6	17	68.5±9.8	17
	Aggregation	22.7±7.3	12	23.5±9.1	17	18.2±5.6	17
	Release	1.30±0.56	12	1.64±1.1	17	1.02±0.48	17
Arachidonic acid	Rate	79.3±3.0	13	75.9±8.6	16	77.5±3.4	16
	Aggregation	23.9±5.4	13	21.7±8.5	16	21.3±3.0	16
	Release	0.92±0.37	13	0.79±0.47	16	0.80±0.26	16
Collagen	Rate	76.9±5.5	13	77.5±5.2	18	78.9±6.3	13
	Aggregation	25.0±6.6	13	33.4±7.5	18	33.0±9.7	13
	Release	0.39±0.18	13	0.91±0.46	18	0.86±0.24	13
Thrombin	Release	0.93±0.27	13	1.24±0.64	19	1.39±0.43	18
Ristocetin	Rate	84.0±4.2	12	77.4±14.4	19	82.5±3.8	8
	Aggregation	23.3±8.5	12	19.6±9.1	19	27.9±9.4	8

ADP, adenosine diphosphate.

From Knöfler *et al.* [46]. Platelet function tests in childhood: measuring aggregation and release reaction in whole blood. *Semin Thromb Hemost* 1998; 24: 513–521. Reproduced with permission.

The platelet release is then proportional to the amount of light measured (Table 17.14) [46].

Flow cytometry is used to identify the presence of surface receptors on the platelet. Defects in the platelet glycoprotein IIb/IIIa receptor are seen in Glanzmann thrombasthenia, while glycoprotein Ib receptor defects are found in Bernard–Soulier syndrome. Flow cytometry results may be falsely normal if the defect is caused by a dysfunctional but antigenically normal protein.

Coagulation

Normal reference ranges for the prothrombin time, activated partial thromboplastin time, thrombin time, coagulation factors II–XIII, and von Willebrand factor (vWF) for term and preterm infants are shown in Tables 17.15 and 17.16, respectively [49, 50]. Circulating anticoagulant proteins for term and preterm infants are shown in Table 17.17 [49, 50]. Fibrinolytic system values for term and preterm infants are shown in Table 17.18 [51].

The prothrombin time and activated partial thromboplastin time are commonly performed screening coagulation studies. The automated coagulation timers use either impedence or photo-optical techniques to identify fibrin, the final point of these assays, hence they are insensitive to factor XIII deficiency. The prothrombin time is sensitive to deficiencies of factors II, V, VII, and X and fibrinogen. The activated partial thromboplastin time is sensitive to deficiencies of all of the clotting factors, except for factors VII and XIII. Turbid, lipemic, or icteric plasma may interfere with photo-optical coagulation timers. The clotting times can easily be affected by the presence of heparin. Even low-dose heparin infusions can affect coagulation times, as can blood drawn from heparinized catheters [52]. Intermittent heparin flushes to anticoagulate arterial or venous catheters may infuse enough heparin into the infant to affect the activated partial thromboplastin time, even when it is drawn by peripheral venipuncture. As heparin has a short half-life, waiting a few hours after infusing such flushes should allow accurate measurement. An alternative is to perform heparin neutralization ex vivo [53].

Coagulation factor assays can be performed by clotting or chromogenic techniques. Clotting assays are prone to interference with agents that affect the coagulation times, including poor phlebotomy,

Table 17.15 Reference coagulation times and coagulant protein values for term infants

	Day 1 (range)	Day 5 (range)	Day 30 (range)	Day 90 (range)	Day 180 (range)	Adult (range)
PT (s)	13.0 (10.1–15.9)	12.4 (10.0–15.3)*	11.8 (10.0–14.3)*	11.9 (10.0–14.2)	12.3 (10.7–13.9)	12.4 (10.8–13.9)
APTT (s)	42.9 (31.3–54.5)	42.6 (25.4–59.8)	40.4 (25.6–55.2)	37.1 (24.1–50.1)	35.5 (28.1–42.9)	33.5 (26.6–40.3)
TCT (s)	23.5 (19.0–28.3)	23.1 (18.0–29.2)*	24.3 (19.4–29.2)	25.1 (20.5–29.7)	25.5 (19.8–31.2)	25.0 (19.7–30.3)
Fibrinogen (g/l)	2.83 (1.67–3.99)	3.12 (1.62–4.62)	2.70 (1.62–3.78)	2.43 (1.50–3.79)*	2.51 (1.50–3.87)*	2.78 (1.56–4.00)
Factor II (U/ml)	0.48 (0.26–0.70)	0.63 (0.33–0.93)	0.68 (0.34–1.02)	0.75 (0.45–1.05)	0.88 (0.60–1.16)	1.08 (0.70–1.46)
Factor V (U/ml)	0.72 (0.36–1.08)	0.95 (0.45–1.45)	0.98 (0.62–1.34)	0.90 (0.48–1.32)	0.91 (0.55–1.27)	1.06 (0.62–1.50)
Factor VII (U/ml)	0.66 (0.28–1.04)	0.89 (0.35–1.43)	0.90 (0.42–1.38)	0.91 (0.39–1.43)	0.87 (0.47–1.27)	1.05 (0.67–1.43)
Factor VIII (U/ml)	1.00 (0.5–1.78)*	0.88 (0.50–1.54)*	0.91 (0.50–1.57)*	0.79 (0.50–1.25)*	0.73 (0.50–1.09)*	0.99 (0.50–1.49)
vWF (u/ml)	1.53 (0.50–2.87)*	1.40 (0.50–2.54)*	1.28 (0.50–2.46)*	1.18 (0.50–2.06)*	1.07 (0.50–1.97)*	0.92 (0.50–1.58)*
Factor IX (U/ml)	0.53 (0.15–0.91)	0.53 (0.15–0.91)	0.51 (0.21–0.81)	0.67 (0.21–1.13)	0.86 (0.36–1.36)	1.09 (0.55–1.63)
Factor X (U/ml)	0.40 (0.12–0.68)	0.49 (0.19–0.79)	0.59 (0.31–0.87)	0.71 (0.35–1.07)	0.78 (0.38–1.18)	1.06 (0.60–1.52)
Factor XI (u/ml)	0.38 (0.10–0.66)	0.55 (0.23–0.87)	0.53 (0.27–0.79)	0.69 (0.41–0.97)	0.86 (0.38–1.34)	0.97 (0.67–1.27)
Factor XII (u/ml)	0.53 (0.13–0.93)	0.47 (0.11–0.83)	0.49 (0.17–0.81)	0.67 (0.25–1.09)	0.77 (0.39–1.15)	1.08 (0.52–1.64)
PK (U/ml)	0.37 (0.18–0.69)*	0.48 (0.20–0.76)	0.57 (0.23–0.91)	0.73 (0.41–1.05)	0.86 (0.56–1.16)	1.12 (0.62–1.62)
HMWK (u/ml)	0.54 (0.06–1.02)	0.74 (0.18–1.30)	0.77 (0.33–1.21)	0.82 (0.30–1.46)	0.82 (0.36–1.28)	0.92 (0.50–1.36)
Factor XIIIa (U/ml)	0.79 (0.27–1.31)	0.94 (0.44–1.44)	0.93 (0.39–1.47)	1.04 (0.36–1.72)	1.04 (0.46–1.62)	1.05 (0.55–1.55)
Factor XIIIb (U/ml)	0.76 (0.30–1.22)	1.06 (0.32–1.80)	1.11 (0.39–1.83)	1.16 (0.48–1.84)	1.10 (0.50–1.70)	0.97 (0.57–1.37)

APTT, activated partial thromboplastin time; HMWK, high molecular weight kininogen; PK, prekallekrein; PT, prothrombin time; TCT, Thrombin clotting time; vWF, von Willebrand factor.

*All factors except fibrinogen are expressed as units per milliliter where pooled plasma contains 1.0 u/ml. Values expressed as mean ±2SD. Values that are skewed due to a disproportionate number of high values. For these, the lower limit that excludes the lower 2.5th percentile is given.

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Table 17.16 Reference coagulation times and coagulant protein values for preterm infants

	Day 1 (range)	Day 5 (range)	Day 30 (range)	Day 90 (range)	Day 180 (range)	Adult (range)
PT (s)	13.0 (10.6–16.2)	12.5 (10.0–15.3)*	11.8 (10.0–13.6)	12.3 (10.0–14.6)	12.5 (10.0–15.0)	12.4 (10.8–13.9)
APTT (s)	53.6 (27.5–79.4)	50.5 (26.9–74.1)	44.7 (26.9–62.5)	39.5 (28.3–50.7)	37.5 (21.7–53.3)	33.5 (26.6–40.3)
TCT (s)	24.8 (19.2–30.4)	24.1 (18.8–29.4)	24.4 (18.8–29.9)	25.1 (19.4–30.8)	25.2 (18.9–31.5)	25.0 (19.7–30.3)
Fibrinogen (g/l)	2.43 (1.50–3.73)*	2.80 (1.60–4.18)*	2.54 (1.50–4.14)*	2.46 (1.50–3.52)*	2.28 (1.50–3.60)*	2.78 (1.56–4.00)
Factor II (U/ml)	0.45 (0.20–0.77)*	0.57 (0.29–0.85)	0.57 (0.36–0.95)*	0.68 (0.30–1.06)	0.87 (0.51–1.23)	1.08 (0.70–1.46)
Factor V (U/ml)	0.88 (0.41–1.44)*	1.00 (0.46–1.54)	1.02 (0.48–1.56)	0.99 (0.59–1.39)	1.02 (0.58–1.46)	1.06 (0.62–1.50)
Factor VII (U/ml)	0.67 (0.21–1.13)	0.84 (0.30–1.38)	0.83 (0.21–1.45)	0.87 (0.31–1.43)	0.99 (0.47–1.51)	1.05 (0.67–1.43)
Factor VIII (U/ml)	1.11 (0.50–2.13)*	1.15 (0.53–2.05)*	1.11 (0.50–1.99)*	1.06 (0.58–1.88)*	0.99 (0.50–1.87)*	0.99 (0.50–1.49)
vWF (u/ml)	1.36 (0.78–2.10)*	1.33 (0.72–2.19)*	1.36 (0.66–2.16)*	1.12 (0.75–1.84)*	0.98 (0.54–1.58)*	0.92 (0.50–1.58)
Factor IX (U/ml)	0.35 (0.19–0.65)*	0.42 (0.14–0.74)*	0.44 (0.13–0.80)*	0.59 (0.25–0.93)	0.81 (0.50–1.20)*	1.09 (0.55–1.63)
Factor X (U/ml)	0.41 (0.11–0.71)	0.51 (0.19–0.83)	0.56 (0.20–0.92)	0.67 (0.35–0.99)	0.77 (0.35–1.19)	1.06 (0.70–1.52)
Factor XI (u/ml)	0.30 (0.08–0.52)*	0.41 (0.13–0.69)	0.43 (0.15–0.71)	0.59 (0.25–0.93)	0.78 (0.46–1.10)	0.97 (0.67–1.27)
Factor XII (u/ml)	0.38 (0.10–0.66)	0.39 (0.09–0.69)	0.43 (0.11–0.75)	0.61 (0.15–1.07)	0.82 (0.22–1.42)	1.08 (0.52–1.64)
PK (U/ml)	0.33 (0.09–0.57)	0.45 (0.26–0.75)*	0.59 (0.31–0.87)	0.79 (0.37–1.21)	0.78 (0.40–1.16)	1.12 (0.62–1.62)
HMWK (u/ml)	0.49 (0.09–0.89)	0.62 (0.24–1.00)	0.64 (0.16–1.12)	0.78 (0.32–1.24)	0.83 (0.41–1.25)	0.92 (0.50–1.36)
Factor XIIIa (U/ml)	0.70 (0.32–1.08)	1.01 (0.57–1.45)	0.99 (0.51–1.47)	1.13 (0.71–1.55)	1.13 (0.65–1.61)	1.05 (0.55–1.55)
Factor XIIIb (U/ml)	0.81 (0.35–1.27)	1.10 (0.68–1.58)	1.07 (0.57–1.57)	1.21 (0.75–1.67)	1.15 (0.67–1.63)	0.97 (0.57–1.37)

APTT, activated partial thromboplastin time; HMWK, high molecular weight kininogen; PK, prekallekrein; PT, prothrombin time; TCT, Thrombin clotting time; vWF, von Willebrand factor. All factors except fibrinogen are expressed as units per milliliter where pooled plasma contains 1.0 u/ml. Values expressed as mean followed by lower and upper boundary encompassing 95% of the population (boundary).

*Values that are skewed due to a disproportionate number of high values. For these, the lower limit that excludes the lower 2.5th percentile is given.

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Table 17.17 Reference circulating anticoagulant values for term and preterm infants

	Day 1 (range)	Day 5 (range)	Day 30 (range)	Day 90 (range)	Day 180 (range)	Adult (range)
Term						
Antithrombin (U/ml)	0.63 (0.39–0.87)	0.67 (0.41–0.93)	0.78 (0.48–1.08)	0.97 (0.73–1.21)	1.04 (0.84–1.24)	1.05 (0.79–1.31)
$Alpha_2$ macroglobulin (U/ml)	1.39 (0.95–1.83)	1.48 (0.98–1.98)	1.50 (1.06–1.94)	1.76 (1.26–2.26)	1.91 (1.49–2.33)	0.86 (0.52–1.20)
C1 esterase inhibitor (U/ml)	0.72 (0.36–1.08)	0.90 (0.60–1.20)	0.89 (0.47–1.31)	1.15 (0.71–1.59)	1.41 (0.89–1.93)	1.01 (0.71–1.31)
$Alpha_1$ antitrypsin (U/ml)	0.93 (0.49–1.37)	0.89 (0.49–1.29)	0.62 (0.36–0.88)	0.72 (0.42–1.02)	0.77 (0.47–1.07)	0.93 (0.55–1.31)
Heparin cofactor II (U/ml)	0.43 (0.00–0.93)	0.48 (0.00–0.96)	0.47 (0.07–0.87)	0.72 (0.00–1.46)	1.20 (0.50–1.90)	0.96 (0.66–1.26)
Protein C (U/ml)	0.35 (0.17–0.53)	0.42 (0.20–0.64)	0.43 (0.21–0.65)	0.54 (0.28–0.80)	0.59 (0.37–0.81)	0.96 (0.64–1.28)
Protein S (U/ml)	0.36 (0.12–0.60)	0.50 (0.22–0.78)	0.63 (0.33–0.93)	0.86 (0.54–1.18)	0.87 (0.55–1.19)	0.92 (0.60–1.24)
Preterm						
Antithrombin (U/ml)	0.38 (0.14–0.62)	0.56 (0.30–0.82)	0.59 (0.37–0.81)	0.83 (0.45–1.21)	0.90 (0.52–1.28)	1.05 (0.79–1.31)
$Alpha_2$ macroglobulin (U/ml)	1.10 (0.56–1.82)*	1.25 (0.71–1.77)	1.38 (0.72–2.04)	1.80 (1.20–2.66)*	2.09 (1.10–3.21)*	0.86 (0.52–1.20)
C1 esterase inhibitor (U/ml)	0.65 (0.31–0.99)	0.83 (0.45–1.21)	0.74 (0.40–1.24)*	1.14 (0.60–1.68)	1.40 (0.96–2.04)*	1.01 (0.71–1.31)
$Alpha_1$ antitrypsin (U/ml)	0.90 (0.36–1.44)	0.94 (0.42–1.46)	0.76 (0.38–1.12)	0.81 (0.49–1.13)	0.82 (0.48–1.16)	0.93 (0.55–1.31)
Heparin cofactor II (U/ml)	0.32 (0.00–0.60)	0.34 (0.00–0.69)	0.43 (0.15–0.71)	0.61 (0.20–1.11)*	0.89 (0.45–1.40)*	0.96 (0.66–1.26)
Protein C (U/ml)	0.28 (0.12–0.44)	0.31 (0.11–0.51)	0.37 (0.15–0.59)	0.45 (0.23–0.67)	0.57 (0.31–0.83)	0.96 (0.64–1.28)
Protein S (U/ml)	0.26 (0.14–0.38)	0.37 (0.13–0.61)	0.56 (0.22–0.90)	0.76 (0.40–1.12)	0.82 (0.44–1.20)	0.92 (0.60–1.24)

Adapted from Andrew *et al.* [49, 50]. All values are expressed in U/ml where pooled plasma contains 1.0 u/ml. Values expressed as mean with (± 2SD) for term infants and mean followed by lower and upper boundary encompassing 95% of the population (boundary) for preterm infants.

*Values that are skewed due to a disproportionate number of high values. For these, the lower limit that excludes the lower 2.5th percentile is given.

Table 17.18 Reference values for components of the fibrinolytic system in term and preterm infants

	Day 1 (range)	Day 5 (range)	Day 30 (range)	Day 90 (range)	Day 180 (range)	Adult (range)
Term						
Plasminogen (U/ml)	1.95 (1.25–2.65)	2.17 (1.41–2.93)	1.98 (1.26–2.70)	2.48 (1.74–3.22)	3.01 (2.21–3.81)	3.36 (2.48–4.24)
t-PA (ng/ml)	9.6 (5.0–18.9)	5.6 (4.0–10.0)	4.1 (1.0–6.0)	2.1 (1.0–5.0)	2.8 (1.0–6.0)	4.9 (1.4–8.4)
$Alpha_2$ antiplasmin (U/ml)	0.85 (0.55–1.15)	1.00 (0.70–1.30)	1.00 (0.76–1.24)	1.08 (0.76–1.40)	1.11 (0.83–1.39)	1.02 (0.68–1.36)
Plasminogen activator inhibitor (U/ml)	6.4 (2.0–15.1)	2.3 (0.0–8.1)	3.4 (0.0–8.8)	7.2 (1.0–15.3)	8.1 (6.0–13.0)	3.6 (0.0–11.0)
Preterm						
Plasminogen (U/ml)	1.70 (1.12–2.48)*	1.91 (1.21–2.61)	1.81 (1.09–2.53)	2.38 (1.58–3.18)	2.75 (1.91–3.59)	3.36 (2.48–4.24)
t-PA (ng/ml)	8.48 (3.00–16.70)	3.97 (2.00–6.93)	4.13 (2.00–7.79)	3.31 (2.00–5.07)	3.48 (2.00–5.85)	4.96 (1.46–8.46)
$Alpha_2$ antiplasmin (U/ml)	0.78 (0.40–1.16)	0.81 (0.49–1.13)	0.89 (0.55–1.23)	1.06 (0.64–1.48)	1.15 (0.77–1.53)	1.02 (0.68–1.36)
Plasminogen activator inhibitor (U/ml)	5.4 (0.0–12.2)	2.5 (0.0–7.1)	4.3 (0.0–10.9)	4.8 (1.0–11.8)	4.9 (1.0–10.2)	3.6 (0.0–11.0)

t-PA, tissue plasminogen activator. Reproduced with permission from Andrew *et al.* [51].
Values given as a mean followed by upper and lower boundary encompassing 95% of the population (boundary).
*Values that are skewed due to a disproportionate number of high values. For these, the lower limit that excludes the lower 2.5th percentile is given.

heparin, and circulating inhibitors of coagulation. Chromogenic assays may be less sensitive than clotting assays to defects in the coagulation factor that lie outside of its active area, such as those that occur with warfarin therapy or vitamin K deficiency. Immunologic factor assays measure the quantity of specific clotting factor protein but do not measure function. The ratio of the functional to antigenic concentration of a factor is useful in order to identify dysfunctional proteins. Immunologic measurements also may be useful in the prenatal diagnosis of known familial clotting factor deficiencies, such as factor VIII deficiency. As factor XIII is not measured by automated timers, many institutions screen for this deficiency by assessing the stability of the clot upon exposure to urea (urea solubility assay). While small amounts of factor XIII are enough to result in a normal urea solubility test, this reflects the physiologic situation where only small amounts are necessary for adequate hemostasis.

Von Willebrand disease is the most common inherited bleeding disorder. vWF is an acute-phase reactant protein; levels in the newborn are elevated when compared with adult values (Tables 17.15 and 17.16). vWF activity most frequently is measured by the ristocetin cofactor assay. Another functional vWF assay is the collagen-binding assay. The quantity of von Willebrand protein (vWF: Ag) is measured by a number of immunologic methods, such as the Laurell rocket and ELISA techniques. The multimeric composition of vWF is assessed by agarose-gel electrophoresis. Improper specimen handling can cause proteolysis of vWF, leading to an appearance of decreased amounts of the more functional high-molecular-weight form. Platelet-aggregation studies using ristocetin are useful in identifying individuals with "gain-of-function" variants whose platelets aggregate excessively in response to low concentrations of ristocetin [54]. Finally, gene analysis can be performed to identify known or new gene defects [55].

Circulating anticoagulants are measured by clot-based, chromogenic, or immunologic methods. The clot-based or chromogenic assays measure activity, while the immunologic assays measure antigen. The clot-based methods of measurement used for protein C and protein S can be affected by heparin and the presence of the lupus anticoagulant. The neonatal levels for circulating anticoagulants are noted in Table 17.17 [49, 50]. It is important to note that while the levels of many anticoagulants in the newborn are decreased compared with levels in adults, there is a wide range. The levels of proteins C and S decrease acutely with thrombosis and disseminated intravascular coagulation (DIC). They are also vitamin K-dependent proteins. Parental studies may be necessary to diagnose heterozygous congenital protein C or protein S deficiency in the infant. Protein S circulates in the plasma bound to C4 binding protein, but levels of this protein are very low in the neonate. Antithrombin antigen can be measured immunologically by a number of techniques, while levels of antithrombin function can be measured by clot-based or chromogenic assays. Both can be affected by the presence of heparin. Functional assays for antithrombin can be influenced by high levels of heparin cofactor II. Congenital prothrombotic conditions are discussed in Chapter 13.

Leukocytes

Specimens used for leukocyte counts are first placed in diluting fluids that lyze the red blood cells. The leukocyte number can then be determined either manually using a hematocytometer or, more frequently, using an automated cell counter. The technology used for cell counters has been reviewed [1]. Nucleated RBCs are included in the total leukocyte count, and a corrected white blood cell count should be used if there are nucleated red blood cells in the differential blood count. Heparin contamination may be a cause of falsely elevated leukocyte counts [1]. Recently, automated cell counters have been developed that can perform an automated differential blood count. These instruments use a combination of impedence, light scatter, and cytochemical techniques to separate neutrophils, immature neutrophils, lymphocytes, monocytes, eosinophils, and basophils. They are designed to identify or "flag" for review samples that have a high probability of

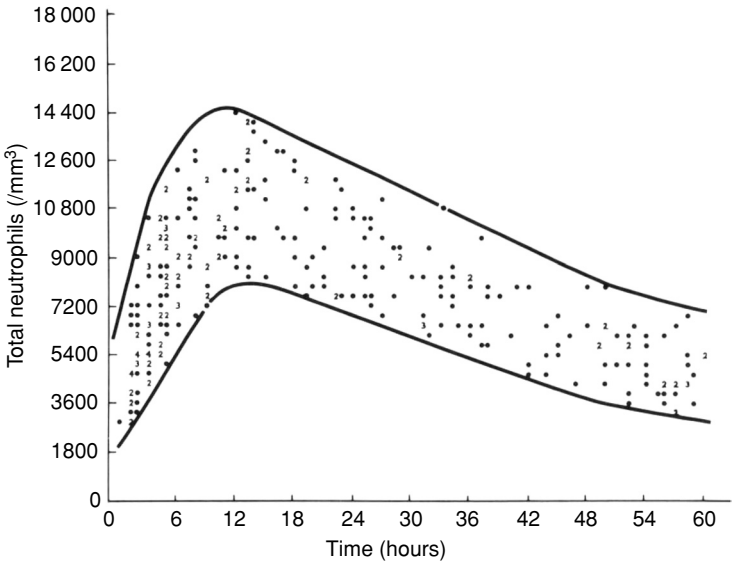

Fig. 17.7 Absolute neutrophil counts from term and preterm infants over the first 60 hours of age. From Manroe *et al.* [59]. Reproduced with permission from *J Pediatr* 1979; 95: 89–98.

abnormal cells. When studied in the newborn, a large percentage of samples were so identified, and the majority of specimens required manual review [56]. In addition, the automated differential differs from the manual differential in a greater percentage of newborn cases than noted for adults. This difference is greater for lymphocytes [57] than for total neutrophils, and can be improved by diluting the sample 1 : 1 in normal saline before analysis [58].

Manroe and colleagues published normal ranges for neutrophil counts during the first 28 days of life derived from a large number of subjects, including both term and preterm infants (Figs. 17.7 and 17.8) [59]. Others have shown that absolute neutrophil counts in preterm newborns are lower than for term newborns [60, 61]. Mouzinho and colleagues found that 67% of infants with birthweights under 1500 g had absolute neutrophil counts outside of the normal range defined by Manroe and colleagues, and 95% of these neutrophil counts were low. Ninety percent reference ranges for total neutrophil counts for these infants are shown in Fig. 17.9 [61]. Total neutrophil counts were reported to be lower and lymphocyte counts were higher in Nigerian newborns than in European and North American infants (Table 17.19) [62]. Stressful activities, and even mild exercise such as that induced by chest physiotherapy, may be sufficient to increase the total neutrophil count [63].

Site of sampling can affect total neutrophil counts. Capillary samples generally have higher total neutrophil counts than venous samples [12, 63, 64]. This does not appear to be affected by gestational age and does not appear to affect the immature/mature neutrophil ratio [64]. Arterial samples have lower neutrophil counts than do venous samples [63].

Gessler and colleagues [65] found that granulocyte colony-stimulating factor (G-CSF) levels, measured by an ELISA assay, peak in the first seven hours of age and correlated with gestational age. Laver and colleagues [66] also showed that cord-blood levels of G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF), using a biologic assay, are higher than values from adults.

Eosinophils

Eosinophil counts vary widely in neonates. Xanthou described a normal range of 100–2500/μl in the first

Table 17.19 Leukocyte values in Nigerian infants in the first month of life

	Day 1		Day 7		Day 28	
Total white blood cell count	Mean	SD	Mean	SD	Mean	SD
Preterm	12 120	8000	9750	4030	9880	1880
Full-term	12 230	5490	8230	2270	8160	1690
Post-term	13 400	4200	9400	2500		
Absolute differential count[a]	Mean	Range	Mean	Range	Mean	Range
Neutrophils	5670	980–12 900	2010	570–6500	1670	650–3200
Band forms	1160	160–2300	550	0–1500	360	0–390
Eosinophils	210	0–900	160	0–1900	420	160–960
Lymphocytes	5100	1400–8000	5630	2200–15 500	6550	3200–9900
Monocytes	40	0–340	80	0–1210	30	0–120

[a]Differential counts are derived from data from pooled preterm, term, and post-term infants. No significant differences were noted between the groups.

Data from Scott-Emuakpor *et al.* [62].

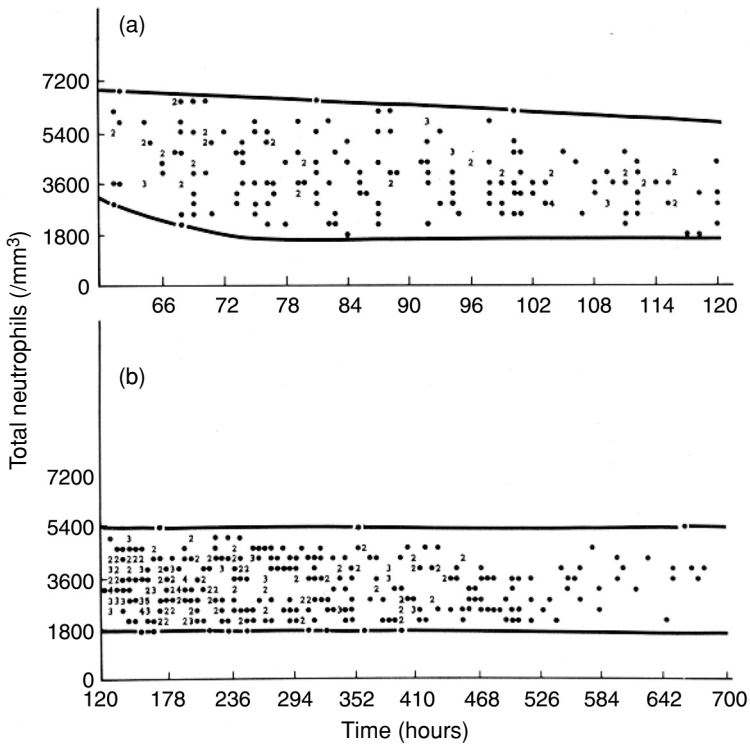

Fig. 17.8 Neutrophil counts from term and preterm infants for hours 60–120 of life (a) and for days 5–28 of life (b). From Manroe *et al.* [59]. Reproduced with permission from *J Pediatr* 1979; 95: 89–98.

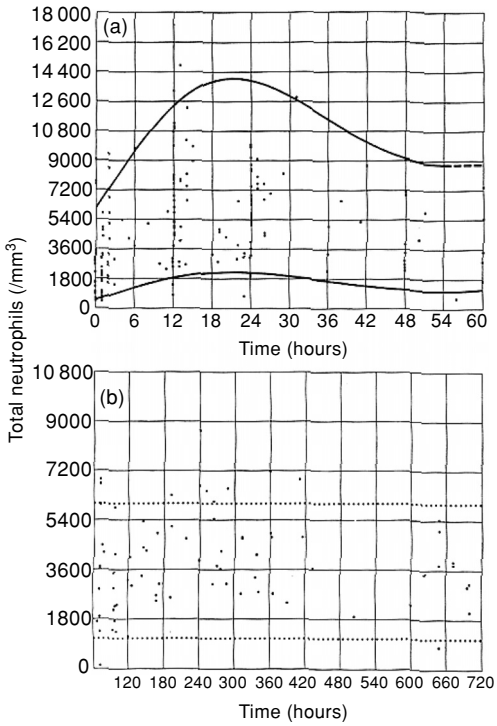

Fig. 17.9 Neutrophil counts for preterm infants for hours 0–60 of life (a) and for days 2.5–28 of life (b). From Mouzinho *et al.* [61]. Reproduced with permission from *Pediatrics* 94: 76–82. Copyright 1994.

week of life [67]. In a mixed population of healthy premature and term infants, Weinberg and colleagues [68] noted the eosinophil count to be a median of 136/μl (range 0–843/μl) in the first 2.5 days of life, rising to 176/μl (range 0–808/μl) on days 2.5–5 and 235/μl (range 0–839/μl) on days 5–30. Several studies have documented that in the premature infant, the eosinophil count increases after the first week of life and the majority reach absolute eosinophil counts in excess of 700/μl. Bhat and Scanlon [69] showed that in the smaller premature infant (gestational age less than 30 weeks), the absolute eosinophil count exceeded 700/μl in over 90% and that these infants had higher peak eosinophil counts that lasted for a longer time than infants over 30 weeks' gestational age. Gibson and colleagues [70] reported a correlation between the onset of eosinophilia in premature infants and improved nutritional status. While there may not be a lower limit of normal for absolute eosinophil count, several observations have documented a fall in this count in the presence of neonatal sepsis [68–70].

Monocytes

Weinberg and colleagues [68] found the median value for absolute monocyte count to be 600/μl in the first 60 hours of life, with 0–1912/μl encompassing the 5–95 percentiles. From days 2.5 to 5, and from days 5 to 30, the 5–95 percentiles are 0–1740/μl and 0–1717/μl, respectively. Xanthou found that the absolute monocyte count peaks during the first postnatal day, declines slightly in the first week, and then returns to a median level of approximately 1000/μl for the first month of life [71].

Lymphocytes

Total lymphocytes are enumerated either as a percentage of the nucleated cells seen on the peripheral blood smear or, more accurately, by flow-cytometric techniques. It is generally more appropriate to refer to the absolute numbers of lymphocytes and their subsets rather than the relative percentages. The absolute lymphocyte count falls slightly in the first week of life [67, 68]. Table 17.20 shows the size of the lymphocyte populations in the newborn and their progression over the first year of life [72].

While immunoglobulin M (IgM), immunoglobulin A (IgA) and immunoglobulin G (IgG) can be made by the fetus in utero, IgG also is transmitted transplacentally to the fetus. IgG levels in the fetus increase in response to this maternal transfer over the third trimester. Humoral immunity in the neonate is discussed in Chapter 12. Quantitative immunoglobulin levels for the term neonate over the first year are shown in Table 17.21 [73], while those for preterm infants of gestational ages 25–28 weeks and 29–32

Table 17.20 Lymphocyte subpopulations in blood (absolute counts ($\times 10^9$/l): median and 5–95 percentiles

Lymphocyte subpopulations	Neonatal	1 week–2 months	2–5 months	5–9 months	9–15 months	15–24 months	Adult
Lymphocytes	4.8 (0.7–7.3)	6.7 (3.5–13.1)	5.9 (3.7–9.6)	6 (3.8–9.9)	5.5 (2.6–10.4)	5.6 (2.7–11.9)	1.8 (1–2.8)
CD19+ B-lymphocytes	0.6 (0.04–1.1)	1.0 (0.6–1.9)	1.3 (0.6–3)	1.3 (0.7–2.5)	1.4 (0.6–2.7)	1.3 (0.6–3.1)	0.2 (0.1–0.5)
CD3+ T-lymphocytes	2.8 (0.6–5)	4.6 (2.3–7)	3.6 (2.3–6.5)	3.8 (2.4–6.9)	3.4 (1.6–6.7)	3.5 (1.4–8)	1.2 (0.7–2.1)
CD3+/CD4+ T-lymphocytes	1.9 (0.4–3.5)	3.5 (1.7–5.3)	2.5 (1.5–5)	2.8 (1.4–5.1)	2.3 (1–4.6)	2.2 (0.9–5.5)	0.7 (0.3–1.4)
CD3+/CD8+ T-lymphocytes	1.1 (0.2–1.9)	1.0 (0.4–1.7)	1.0 (0.5–1.6)	1.1 (0.6–2.2)	1.1 (0.4–2.1)	1.2 (0.4–2.3)	0.4 (0.2–0.9)
CD3+/HLA-DR+ T-lymphocytes	0.09 (0.03–0.4)	0.3 (0.03–3.4)	0.2 (0.07–0.5)	0.2 (0.07–0.5)	0.2 (0.1–0.6)	0.3 (0.1–0.7)	0.09 (0.03–0.2)
CD3-/CD16–56+NK cells	1.0 (0.1–1.9)	0.5 (0.2–1.4)	0.3 (0.1–1.3)	0.3 (0.1–1)	0.4 (0.2–1.2)	0.4 (0.1–1.4)	0.3 (0.09–0.6)

NK, natural killer.

From Comans-Bitter *et al.* [72]. Reproduced with permission.

Table 17.21 Normal values for immunoglobulins for term infants to age three years

Age	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Newborn	598–1672	0–5	5–15
1–3 months	218–610	20–53	11–51
4–6 months	228–636	27–72	25–60
7–9 months	292–816	27–73	12–124
10–18 months	383–1070	27–169	28–113
2 years	423–1184	35–222	32–131
3 years	477–1334	40–251	28–113

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

From Buckley *et al.* [73]. Reproduced with permission. © Copyright 1968.

weeks are shown in Tables 17.22 and 17.23, respectively [74].

Progenitor cell assays

In 1966, Bradley and Metcalf developed an in vitro assay to grow colonies derived from mouse bone-marrow cells [75]. By changing the growth-stimulating factor layer to include erythropoietin, Stephenson and colleagues expanded this assay to form erythroid colonies [76] and Metcalf and colleagues demonstrated the growth of megakaryocyte colonies using a variant of the assay [77]. Colony assays have evolved, but the basic principle still remains. The assays utilize a semisolid medium to immobilize the cells of interest and stimulate them with the appropriate cytokine/cytokines to induce the growth of specific colonies. The growth factors are now specific cytokine mixtures that stimulate specific lineage growth. Serum has been replaced by chemical additives such as transferrin and insulin. However, the role of these assays in clinical medicine is not yet defined.

Erythroid colonies are distinguished easily because of their red color. Megakaryocytic colonies are more difficult to distinguish. Agarose colony assays can be stained with peroxidase-linked anti-CD61 or -CD41a antibodies, making the recognition of megakaryocytic colonies much easier. The early singleton colonies are easy to count. The burst-like colonies (less mature colonies) can be more difficult to count, since one colony can merge with another. This problem is greater with plasma clot colony assays. Like many biological assays, there is great variability in the results from these assays, and internal controls are always advisable. Both the

Table 17.22 Plasma immunoglobulin concentrations in premature infants of 25–28 weeks' gestation

Age	IgG[a] (mg/dl)	IgM[a] (mg/dl)	IgA[a] (mg/dl)
1 week	251 (114–552)[b]	7.6 (1.3–43.3)	1.2 (0.07–20.8)
2 weeks	202 (91–446)	14.1 (3.5–56.1)	3.1 (0.09–10.7)
1 month	158 (57–437)	12.7 (3.0–53.3)	4.5 (0.65–30.9)
1.5 months	134 (59–307)	16.2 (4.4–59.2)	4.3 (0.9–20.9)
2 months	89 (58–136)	16.0 (5.3–48.9)	4.1 (1.5–11.1)
3 months	60 (23–156)	13.8 (5.3–36.1)	3.0 (0.6–15.6)
4 months	82 (32–210)	22.2 (11.2–43.9)	6.8 (1.0–47.8)
6 months	159 (56–455)	41.3 (8.3–205)	9.7 (3.0–31.2)
8–10 months	273 (94–794)	41.8 (31.1–56.1)	9.5 (0.9–98.6)

[a]Geometric mean.

[b]Normal ranges determined by taking antilog of (mean logarithm ± 2 SD of the logarithms).

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; SD, standard deviation.

From Ballow *et al.* [74], with permission.

Table 17.23 Plasma immunoglobulin concentrations in premature infants of 29–32 weeks' gestation

Age	IgG (mg/dl)[a]	IgM (mg/dl)[a]	IgA (mg/dl)[a]
1 week	368 (186–728)[b]	9.1 (2.1–39.4)	0.6 (0.04–1.0)
2 weeks	275 (119–637)	13.9 (4.7–41)	0.9 (0.01–7.5)
1 month	209 (97–452)	14.4 (6.3–33)	1.9 (0.3–12.0)
1.5 months	156 (69–352)	15.4 (5.5–43.2)	2.2 (0.7–6.5)
2 months	123 (64–237)	15.2 (4.9–46.7)	3.0 (1.1–8.3)
3 months	104 (41–268)	16.3 (7.1–37.2)	3.6 (0.8–15.4)
4 months	128 (39–425)	26.5 (7.7–91.2)	9.8 (2.5–39.3)
6 months	179 (51–634)	29.3 (10.5–81.5)	12.3 (2.7–57.1)
8–10 months	280 (140–561)	34.7 (17–70.8)	20.9 (8.3–53)

[a]Geometric mean.

[b]Normal ranges determined by taking antilog of (mean logarithm ± 2 SD of the logarithms).

IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; SD, standard deviation.

From Ballow *et al.* [74], with permission.

nature of the media and the cytokines added can affect the number of progenitors demonstrated in the culture system.

Progenitor cell colony assays are now commercially available and standardized. This can improve the variability of the assays. Table 17.24 and Fig. 17.10 detail the numbers of progenitor cells in cord blood and bone marrow from fetuses and from full-term and premature newborns [78–82]. Fig. 17.11 illustrates the numbers of

Table 17.24 Frequency of progenitor cells of different lineages found in cord blood (CB), peripheral blood, and bone marrow (BM) from fetuses, premature newborns, full-term newborns, and adults. Each column comes from a single laboratory. Numbers of progenitors are reported as progenitors per 10^5 ([a]) cells or per ml of blood ([b]).

	CB LTC-IC[a]	CB CFU-GEMM[b]	CB CFU-GEMM[a]	CB CFU-GM[b]	CB CFU-GM[a]	CB BFU-E[b]	CB BFU-E[a]	CB BFU-E[a]	BM BFU-E[a]	BFU-Mk[b]	CFU-Mk[b]
Fetal											
Early		1050±130	76±142	330±40	438±554	540±80	1292±1012				
Mid		3250±1570	33±30	3140±1200	137±110	1970±1260	1130±1195	160±107			
Late		560±70	13.2±1.2	970±570	130±55	1110±210	380±100				
Preterm	2.6±1.2			1937±348		3972±528				414±61	2444±337
Term	6.7±2.9							48±19		151±18	869±64
Adult								3.6±3	16±9		

BFU-E, burst forming unit – erythroid; BFU-MK, burst forming unit – megakaryocyte; CFU-GEMM, colony forming units – granulocyte, erythroid, megakaryocyte and macrophage; CFU-GM, colony forming units – granulocyte-macrophage.
Table adapted from data from Gasparoni *et al.* [78], Murray *et al.* [79], Wyrsch *et al.* [80], and Campagnoli *et al.* [81]

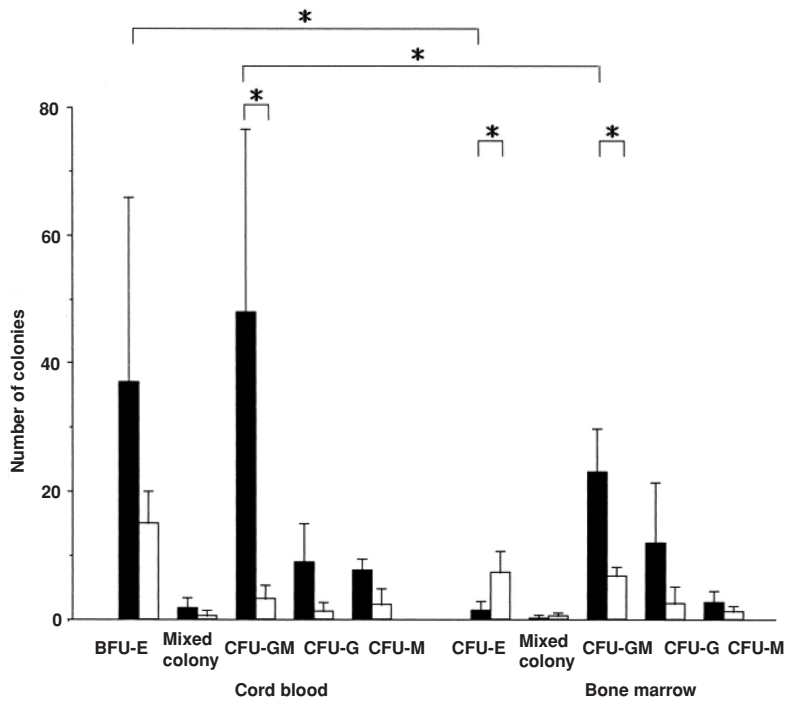

Fig. 17.10 In vitro colony-forming assays from preterm cord-blood and bone-marrow cells. Number of colonies are reported as per 10^5 cells planted. Reproduced with permission from Jin *et al.* [82].
BFU-E, burst forming unit – erythroid; CFU-E, colony forming unit – erythrocyte; CFU-G, colony forming unit – granulocyte; CFU-GM, colony forming units – granulocyte-macrophage; CFU-M, colony forming unit – macrophage.

Table 17.25 Percentages of cell types (mean ± standard deviation) from tibial bone-marrow aspirate samples from healthy infants aged 0–12 months (n = number of infants studied at each point)

Age (months)	0	1	2	3	4	5	6	9	12
n	57	71	48	24	19	22	22	16	18
Small lymphocytes	14.42±5.54	47.05±9.24	42.68±7.90	43.63±11.83	47.06±8.77	47.19±9.93	47.55±7.88	48.76±8.11	47.11±11.32
Transitional cells	1.18±1.13	1.95±0.94	2.38±1.35	2.17±1.64	1.64±1.01	1.83±0.89	2.31±1.16	1.92±1.39	2.32±1.90
Total erythroblasts	14.48±7.24	8.04±5.00	14.70±3.86	11.90±3.52	7.62±2.56	8.70±2.69	8.25±4.31	8.72±3.34	7.81±4.26
Reticulocytes	4.18±1.46	1.06±1.13	3.39±1.22	2.90±0.91	1.65±0.73	1.38±0.65	1.74±0.80	1.67±0.52	1.79±0.79
Total neutrophils and neutrophil precursors	60.37±8.66	32.35±7.68	30.31±7.27	34.01±11.95	34.21±8.61	33.12±8.34	32.75±7.03	32.33±7.75	33.79±8.76
Total eosinophils	2.70±1.27	2.61±1.40	2.50±1.22	2.54±1.46	2.37±1.13	1.98±0.86	2.08±1.16	1.74±1.08	1.92±1.09
Total basophils	0.12±0.20	0.07±0.16	0.08±0.10	0.09±0.09	0.11±0.14	0.09±0.13	0.10±0.13	0.11±0.13	0.13±0.15
Monocytes	0.88±0.85	1.01±0.89	0.91±0.83	0.68±0.56	0.75±0.75	1.29±1.06	1.21±1.01	1.17±0.97	1.46±1.52
Other[a]	6.38±2.84	6.39±2.63	5.94±1.94	5.63±2.36	5.66±2.30	5.66±1.41	5.78±1.16	5.55±1.74	5.90±2.03

[a]Other cells = megakaryocytes, plasma cells, unknown blasts, unknown cells, and damaged cells.
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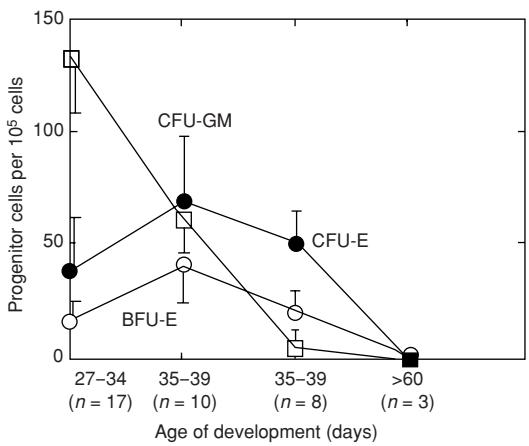

Fig. 17.11 Number of progenitors in the yolk sac as a function of the age of development. From Dommergues *et al.*, with permission. For abbreviations, see Fig. 17.10 [83].

progenitors in the yolk sac at increasing gestational age [83].

Bone marrow

There are relatively few studies of bone-marrow aspirates in normal infants. A technique for performing tibial marrow sampling has been described [84]. Rosse and colleagues [85] evaluated bone-marrow aspirate samples in addition to blood counts and physical examinations from 88 otherwise healthy infants in the first four days of life, monthly for the first six months of life, and every three months until age 18 months. Sixty-four of the infants continued throughout the first year and their data are shown in Table 17.25. Several trends were noted. Myeloid precursors are the predominant cell type at birth but fall precipitously in the first month, due mostly to a decrease in bands and neutrophils. Myeloid cells then account for 30–35% of the nucleated cells in the marrow. Erythroid precursors also decline significantly in the first month, increase transiently in the second and third months, and then account for 7–9% of the marrow cells. Small lymphocytes increase from 14.4% at birth to 47% in the first month and continue to account for more than 40% of the marrow cells past the first year of life.

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