Introduction

Maria A. Proytcheva

Pediatric hematopathology is a special and highly challenging field. Despite its importance, however, and despite the sea of publications that exist in the field of hematopathology, few texts focus on the diagnosis of benign and malignant hematologic disorders found in children. As a result, even though the uniqueness of developmental factors and pathology in children is well recognized and appreciated, the specifics are not widely known or understood, which poses great difficulties for the diagnosis of hematologic diseases in children.

Diagnostic Pediatric Hematopathology presents an accurate and up-to-date examination of such diseases in children – both non-neoplastic and neoplastic. One goal is to provide knowledge about how the hematopoietic and lymphoid systems develop and how this development affects what can be considered normal and abnormal findings in children at various ages. A second key goal is to focus on the morphologic, immunophenotypic, cytogenetic, and molecular genetic characteristics of most pediatric-specific hematologic diseases so as to provide a resource that can be helpful in reaching a proper diagnosis when evaluating pediatric peripheral blood, bone marrow, and lymph nodes. The text addresses these goals through a team of experienced pediatric hematopathologists and clinical scientists drawn from major academic children's hospitals in the United States, Canada, and Europe, and this text is a result of our collaborative efforts.

Several major differences between pediatric and adult hematopathology are especially important and create the need for a separate text such as this book.

First, the hematopoietic system is not fully developed at birth. Instead, it continues to evolve during childhood to reach its maturity during the teenage years. As a result, both the peripheral blood and the bone marrow findings will be related to the developmental stage, such that what should be considered normal will depend upon the age of the child. These differences – both between children of various ages and between children and adults – can be substantial and have great clinical relevance. They are explored in the chapters on hematologic values in the healthy fetus, neonate, and child and normal bone marrow.

Age, along with underlying genetic abnormalities, has been recognized as integral to the diagnosis of certain hematologic malignancies. The latest *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*,[1] for example, includes several disorders, such as juvenile myelomonocytic leukemia, childhood myelodysplastic syndrome, and myeloid proliferations related to Down syndrome, as conditions with unique morphologic features, underlying genetic mechanisms, and different treatment outcomes in children as compared to the adult counterparts. It also identifies disorders that are exclusively seen in children, such as systemic EBV+ T-cell lymphoproliferative diseases of childhood. The unique features of these hematologic malignancies are explored in the appropriate chapters.

Second, there are differences in the type and prevalence of hematologic diseases in children as compared to adults. For instance, acute leukemias, particularly lymphoblastic leukemias, are frequent in children, and lymphomas are rare. This is in contrast to adults, where mature B-cell lymphomas/leukemias are much more frequent, and acute leukemias are relatively rare. The pediatric leukemias have specific morphologic features and underlying genetic mechanisms that are explored in the chapters on precursor B- and T-lymphoblastic leukemias, acute myeloid leukemias, chromosomal abnormalities, and expression profiling in pediatric hematologic malignancies.

Third, the treatment for pediatric leukemia differs and the outcomes themselves are far superior than is the case for adults. The better outcomes are due in part to the unique pathogenetic mechanisms causing these diseases in children, but they are also due to the unusual speed with which advances have taken place in the treatment of the diseases in children. Those advances are based upon standardized protocols that have been developed for the treatment of children through randomized clinical trials. The trials have been conducted through the pediatric

[1] **Swerdlow SH**, **Campo E**, **Harris NL**, *et al.* (eds.). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (4th edn.). Lyon: IARC Press; 2008.

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cooperative groups, such as the Children's Oncology Group (COG) and St. Jude Children's Research Hospital in the United States, Berlin–Frankfurt–Münster ALL protocol in Germany, European Organization for Research and Treatment of Cancer – Children's Leukemia Group, and the Medical Research Council in the United Kingdom. In contrast with adults, most children with leukemia under the age of 15 in the United States, Canada, Europe, Australia, and New Zealand are currently treated on such protocols.

These protocols set specific requirements for a standardized approach to the diagnosis and follow-up evaluation of the bone marrow of children with leukemias. Accordingly, bone marrow studies are performed at specific time points to determine responses to therapy and detect minimal residual disease. However, the iatrogenic changes that occur following chemotherapy, along with the unique regeneration patterns of the bone marrow in children, may mimic residual disease, which makes the detection of minimal residual disease challenging. The dynamics of these changes are explored in the chapter covering the effects of therapy and hematopoietic stem cell transplants.

Information resulting from a child's particular response to therapy and the presence or absence of minimal residual leukemia is an important guide for the therapeutic management of children with leukemia. Given its importance, the hematopathologist is brought into the clinical oncology team to collaborate, not simply regarding initial diagnoses, but also for follow-up treatments. For that reason, the text includes an examination, coming from a clinician's perspective, of the advances in diagnosis, prognostication, and treatment of pediatric acute leukemia. The aim is to help broaden the pathologist's understanding of the likely clinical expectations of the oncology team during the management of such diseases.

As leukemias affect children and adults differently, the incidence of reactive lymphadenopathies and lymphomas differs as well. There is a higher prevalence of reactive lymphadenopathies early in life and a relative paucity of lymphomas. Most of the enlarged lymph nodes in children are reactive and frequently arise as part of the normal immunologic response to various antigens. However, the reaction can be so exuberant that it can resemble lymphoma, or children may present with constitutional and laboratory abnormalities suggesting malignancy in the settings of reactive lymphoid hyperplasia. Thus, it is crucial in making a proper diagnosis to avoid the considerable possibilities for misinterpreting lymph node changes of children. These types of issues, as well as the most common lymphadenopathies in children, are examined in the chapter on reactive lymphadenopathies. It also provides a comprehensive review of the development of lymph nodes and the normal immune reaction, which is important to appreciate in the morphologic examination of lymph nodes. A chapter on immunodeficiency associated with lymphoproliferative disorders is included as well, since many of these diagnostically challenging conditions occur mainly during childhood.

Unlike adults, too, nearly all lymphomas in children are high grade, such as Burkitt lymphoma, diffuse large B-cell lymphoma, and ALK1+ anaplastic large cell lymphoma. The unique characteristics of the pediatric mature B- and T-cell lymphomas as well as Hodgkin lymphoma are covered in the respective chapters on them and, as with the rest of the text, the discussions are current with the latest WHO classification.

Small blue cell tumors metastatic to the bone marrow represent one more set of conditions for which the pathology is unique to children. These tumors have various propensities to metastasize to the marrow and can manifest with different patterns that may resemble leukemia. The characteristic morphologic patterns and how to avoid pitfalls in their diagnosis are discussed in the chapter on bone marrow metastasis.

Finally, the text also includes chapters on the disorders of erythrocyte production, hemoglobin synthesis, hemolytic anemia, bone marrow failure syndromes, and storage diseases that most frequently are seen in children, but where the differences between children and adults are not always so clear. In addition, some very infrequent diseases, such as cutaneous and subcutaneous lymphomas in children and histiocytic proliferations in childhood that are rarely systematically covered in the literature, are included here.

The *Diagnostic Pediatric Hematopathology* text focuses on the diagnostic aspects of benign and neoplastic hematologic diseases in children and discusses a wide range of key problems and issues that must be addressed in reaching a proper diagnosis. The text is intended as a helpful instrument in the everyday practice of pathologists, pediatric pathologists, and hematopathologists, along with fellows, pathology residents and medical students, and clinical scientists in the field. Since many pediatric hematologists/oncologists are involved in the diagnosis of childhood hematologic diseases as well, this book can be valuable for them, too.

Section 1 General and non-neoplastic hematopathology

Section 1
Chapter 1

General and non-neoplastic hematopathology

Hematologic values in the healthy fetus, neonate, and child

Maria A. Proytcheva

The hematopoietic system is not fully developed at birth, and the normal hematologic values of newborns and infants differ as compared to older children and adults. The differences are a manifestation of the unique characteristics of the embryonal and fetal development of the hematopoietic system that continues to evolve after birth. Furthermore, preanalytical and analytical factors unique for neonates and young children also contribute to these differences. This chapter will explore these factors and discuss how they define the normal hematologic values for different age groups.

Developmental hematopoiesis: a general view

The hematopoietic development, unlike any other organ system, occurs in successive anatomic sites where the hematopoietic stem cells (HSCs) are generated, maintained, and differentiate into various cell types [1]. The hematopoiesis begins in the yolk sac with the generation of angioblastic foci or "blood islands" that contain primitive erythroblasts. It then progresses further in several waves involving multiple anatomic sites: the aorta–gonadal–mesonephros (AGM) region, fetal liver, and bone marrow (BM) [2, 3]. Depending on the site of major hematopoietic activity, the hematopoiesis has been divided into three stages: the mesenchymal, hepatic, and myeloid stages with the yolk sac, liver, and bone marrow as major hematopoietic sites where hematopoietic cells with characteristic features are generated [4] (Fig. 1.1). There is a considerable temporal overlap between different stages. At birth and thereafter, the hematopoiesis is restricted to the bone marrow and continues to evolve in order to adapt to the new oxygen-rich environment and the needs of the growing organism.

It is currently accepted that the HSCs develop from hemangioblasts, which are mesodermal multipotent progenitors that give rise to hematopoietic as well as endothelial and vascular smooth muscle cells (Fig. 1.2A) [2, 5]. The first blood islands consisting of primitive erythroblasts surrounded by endothelial cells are formed in the *yolk sac* between days 16 and 19 of gestation [6]. During this stage, the hematopoiesis generates mostly primitive and only a few definitive erythroblasts, as well as a few megakaryocytes at the sixth and seventh weeks of gestation. The primitive erythroblasts differ from the definitive erythroblasts in several aspects (Table 1.1). They are macrocytic [mean corpuscular volume (MCV) of 250 fL/cell]. They differentiate within the vascular network and remain nucleated for their entire lifespan. These cells have an increased sensitivity to erythropoietin (EPO) and a shorter lifespan as compared to the later fetal, definitive erythroblasts and adult counterparts. The hallmark of the primitive erythroid cells is the expression of embryonic hemoglobins such as Gower 1 ($\zeta_2\varepsilon_2$), Gower 2 ($\alpha_2\varepsilon_2$), and Portland ($\zeta_2\gamma_2$). The yolk sac hematopoiesis declines after the eighth week of gestation.

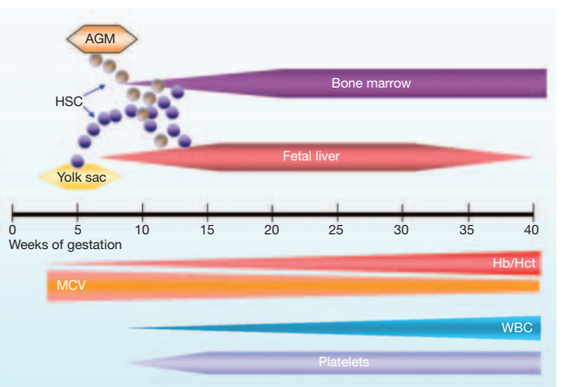

Fig. 1.1. Schematic representation of the developmental time windows for shifting sites of hematopoiesis in humans. The hematopoiesis occurs in successive anatomic sites – yolk sac, fetal liver, and bone marrow. It is currently believed that hematopoietic cells (HSCs) from extraembryonic (yolk sac) and intraembryonic (aorta, genital ridge, and mesonephros (AGM) region) sites seed the fetal liver and bone marrow where the HSCs are maintained and differentiate into various cell types. The blood generated during each phase has a different composition. With the advancing of the gestational age, the mean corpuscular volume (MCV) of the erythrocytes decreases and the hemoglobin, hematocrit, white blood cell, and platelet counts increase.

The *ventral aspect of the aorta* is another site of erythropoietic activity in the human embryo from 20 to 40 days of gestation [6]. This region corresponds to the aorta, genital ridge,

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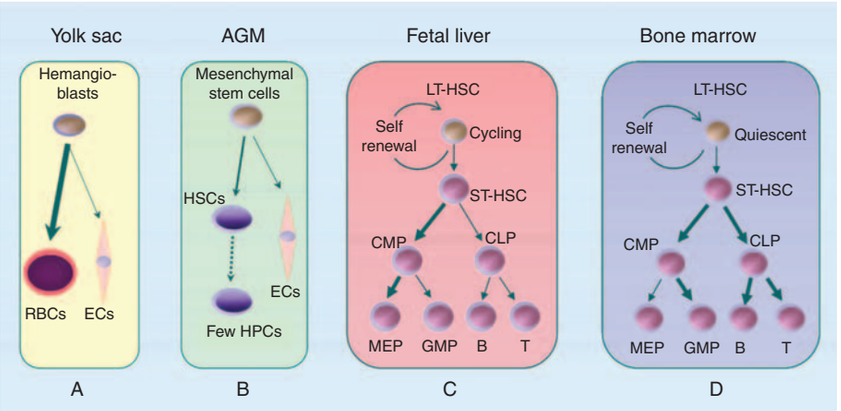

Fig. 1.2. Schematic representation of the hematopoiesis in each anatomic site with the production of specific blood lineages depending on each location. (A) The yolk sac favors the generation of primitive erythroblasts. (B) The AGM is mostly involved in the generation of hematopoietic progenitors which, along with the hematopoietic progenitors generated in the yolk sac, seed the fetal liver and bone marrow. (C) The fetal liver generates mostly definitive erythroid progenitors and to a lesser degree granulocytes, monocytes, and lymphocytes. (D) The fetal bone marrow generates multilineage hematopoiesis which in the early stages is mostly granulocytic. Abbreviations: AGM, aorta–gonadal–mesonephros region; EC, endothelial cell; RBC, red blood cell; HPC, hematopoietic progenitor cell; LT-HSC, long-term hematopoietic cell; ST-HSC, short-term hematopoietic cell; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte/erythroid progenitor; GMP, granulocyte/macrophage progenitor. (Redrawn with modifications from Orkin, Zon [4], with permission.)

Table 1.1. Characteristic features of the primitive and definitive erythropoiesis during ontogeny.

	Primitive hematopoiesis	Definitive hematopoiesis
Hematopoietic site	Yolk sac Vascular endothelium	Fetal liver and fetal bone marrow Hematopoietic niches
Type of hematopoiesis	Mostly erythroid	Multilineage hematopoiesis
RBC characteristics		
Nucleated	Remain nucleated during their entire lifespan	Enucleated RBCs
Cell size	Macrocytic (MCV 250 fL)	MCV decreases with gestational age
Sensitivity to EPO	Increased	Lower sensitivity
Lifespan	Short	Increases with gestation
Hemoglobins	Embryonic: Gower 1 ($\zeta_2\varepsilon_2$), Gower 2 ($\alpha_2\varepsilon_2$), and Portland ($\zeta_2\gamma_2$)	Fetal: Hb F ($\alpha_2\gamma_2$) and adult: Hb A ($\alpha_2\beta_2$)

Abbreviations: RBC, red blood cell; MCV, mean corpuscular volume; EPO, erythropoietin; Hb, hemoglobin.

and mesonephros (AGM) region in various vertebrate species. Most of the cells generated in this region are multipotent HSCs, and their number is highest around the 23rd day of gestation (Fig. 1.2B).

The HSCs from AGM (intraembryonic site) as well as cells from the yolk sac (extraembryonic site) colonize the fetal liver and bone marrow, where they are maintained, expanded, and differentiated into definitive blood cells [4, 6–8]. The definitive hematopoietic precursors develop in the hematopoietic niche, which provides a complex microenvironment generated by the stromal elements of the fetal liver, bone marrow, and the microvasculature endothelium; it plays a seminal role in the regulation of the hematopoiesis.

The *fetal liver* is the major site of hematopoiesis between 11 and 24 weeks of gestation (Fig. 1.2C). The hepatic hematopoiesis is mostly erythroid, and in the second trimester of pregnancy about half of the nucleated cells in the liver are erythroid progenitors. However, other cell lineages such as megakaryocytic, myeloid, and lymphoid progenitors are also generated in the fetal liver. Unlike the yolk sac erythropoiesis, the hepatic erythropoiesis occurs extravascularly within the complex cellular milieu of the fetal liver. The fetal erythropoiesis is definitive and resembles that found in postnatal life. It generates enucleated red blood cells that are seen in the circulation by eight weeks of gestation. The MCV of the definitive erythroblasts is lower than the MCV of the primitive erythroblasts. These cells contain predominantly Hb F ($\alpha_2\gamma_2$).

The *fetal bone marrow* hematopoiesis is initiated at about 6–8.5 weeks of gestation and occurs in several morphologically distinctive stages described in detail in Chapter 2 [9]. The process is completed by the 16th week of gestation, but the BM does not become a major site for hematopoiesis until the 25th week of gestation. The fetal BM hematopoiesis is multilineal and generates definitive enucleated RBCs containing Hb F and Hb A ($\alpha_2\beta_2$) as well as myeloid and lymphoid progenitors (Fig. 1.2D).

Between the 14th and 24th week of gestation, both the fetal liver and bone marrow are hematopoietic organs concomitantly, yet each supports a somewhat distinctive set of hematopoietic lineages [10]. The liver is the major site for erythropoiesis (Fig. 1.3) whereas, in the BM, the hematopoiesis is shifted to granulopoiesis and lymphopoiesis and the erythropoiesis is only a minor component (Fig. 1.4) [11]. These differences are a result of different stimulatory and/or inhibitory

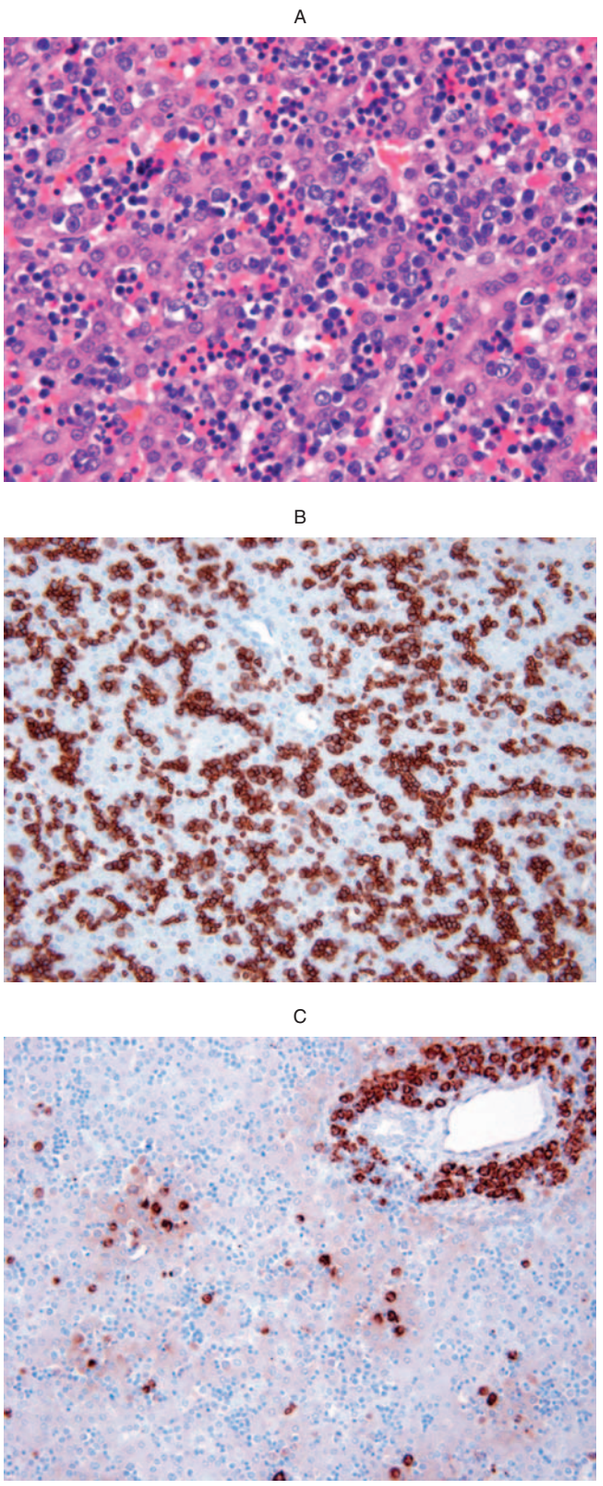

Fig. 1.3. Fetal liver, 21 weeks of gestation. (A) Numerous hematopoietic progenitors and hepatocytes [hematoxylin and eosin (H&E) stain]. (B) Predominance of glycophorin A-positive erythroid progenitors (immunoperoxidase stain). (C) Paucity of myeloid progenitors in the fetal liver parenchyma and higher number of myeloid precursors in the periportal spaces (immunoperoxidase stain, myeloperoxidase). (Courtesy of Dr. Linda Ernst.)

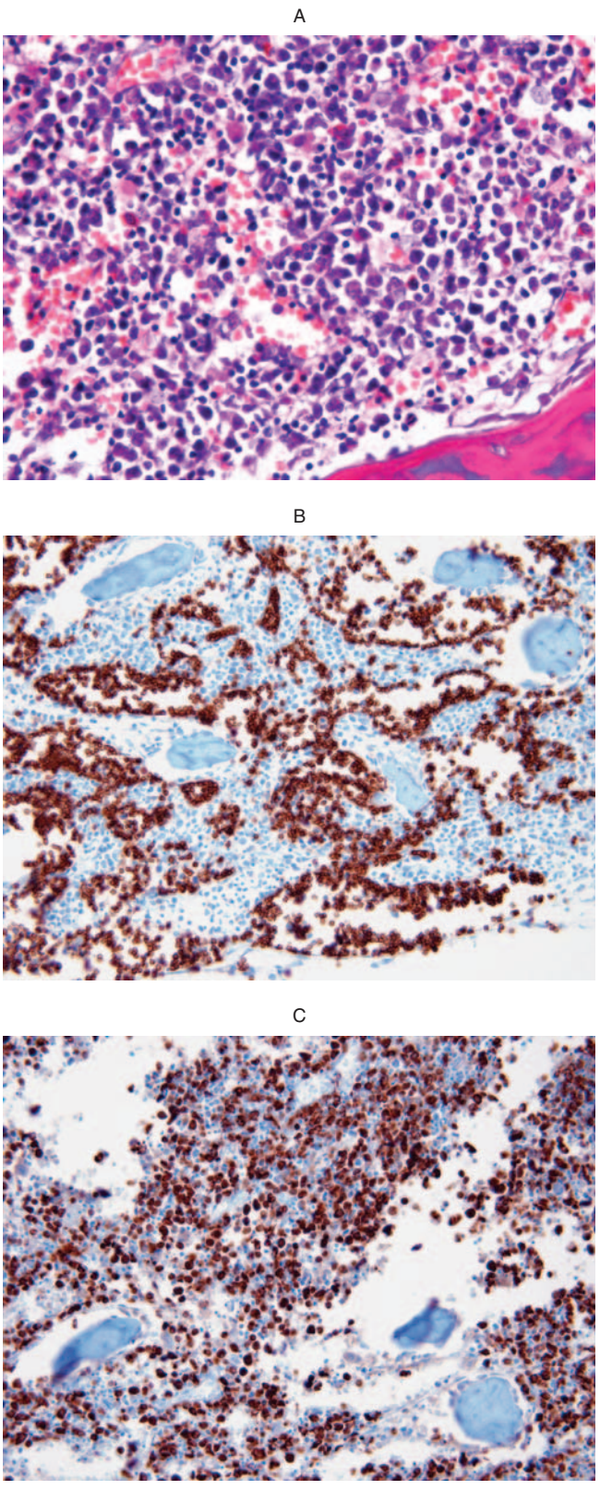

Fig. 1.4. Fetal bone marrow, 21 weeks of gestation. (A) Cellular bone marrow with numerous hematopoietic progenitors (H&E stain). (B) There is a paucity of erythroid progenitors; most of the positive cells are erythrocytes filling the dilated sinuses (immunoperoxidase stain for glycophorin A). (C) Predominance of myeloid progenitors in the fetal bone marrow (immunoperoxidase stain for myeloperoxidase). (Courtesy of Dr. Linda Ernst.)

signals from the microenvironment of the liver and BM that determine the fate of the HSC and their differentiation into erythroid or myeloid pathways. A quantitative analysis of the leukocyte content along with the percentage of CD34+ cells, lymphocytes, granulocytes, and monocytes in fetal liver, BM, and spleen, revealed a comparable absolute number of CD34+ cells in the liver and marrow throughout the second trimester of gestation but different proportions of maturing hematopoietic cells [12]. While the liver in the second trimester appears to be responsible for sustaining erythropoiesis, and to a lesser degree for the myelopoiesis and megakaryopoiesis, the BM is mainly involved in granulopoiesis and B-cell lymphogenesis. The *fetal spleen* in mid gestation, however, does not normally function as an active erythropoietic organ, and along with the thymus is involved in the B-cell and T-cell lineage development [12–14].

There are significant differences between the fetal hematopoietic stem cells and adult bone marrow progenitors. The fetal hematopoietic progenitors are notable for a rapid cycling rate resulting in constant expansion of the pool size, higher sensitivity to EPO, and a low sensitivity to granulocyte/monocyte colony stimulating factor (GM-CSF). As a result, during fetal development the hematopoietic cells mature mostly along the erythroid pathway. *In vitro* studies show that the fetal hematopoietic progenitors form threefold more burst-forming units – erythrocytes (BFU-Es) at mid trimester than at birth [15].

The EPO plays a central role and is the most important cytokine regulator of mammalian erythropoiesis. Targeted disruption of either EPO or its receptor leads to almost complete block of fetal liver erythropoiesis, resulting in fetal death. During mid and late gestation, EPO mRNA has been detected in the liver and to a lesser degree in the spleen, bone marrow, and kidney [16]. This is in contrast to adults where the kidney is the major site for EPO synthesis. The precise EPO signaling of prenatal erythropoiesis is still not completely understood. It is known that between 13 and 23 weeks of gestation the erythroid progenitors are more sensitive to growth factors: colony forming units – erythrocytes (CFU-Es), EPO, BFU-Es [17]. In adults, the EPO provides both a proliferative signal to early erythroid progenitors and a differentiation signal to late erythroid progenitors such as to prevent apoptosis of early progenitors.

Growth factors and cytokines, such as a granulocyte colony stimulating factor (G-CSF), interleukin (IL)-6, IL-1, IL-4, IL-9, and insulin growth factor-1, also regulate the rate of the proliferation and differentiation of the hematopoietic precursors [4, 18]. During the second trimester, the hematopoietic stem cells are less sensitive to GM-CSF and G-CSF. The total white blood cell (WBC) count is very low and gradually increases prior to birth. The monocytes are the first and the neutrophils are the last white blood cells to appear in the fetal blood. The neutrophil count is very low and gradually increases from the 20th to the 30th week of gestation. During the second and third trimester, the lymphocytes comprise the largest white blood cell population.

Composition of fetal blood

Blood samples obtained fetoscopically from live fetuses early in the second and third trimester of pregnancy have demonstrated the marked changes that occur in the composition of fetal blood during this stage of development [15, 19–21]. The results from two major studies, involving almost 3000 fetal blood samples, are presented in Table 1.2. They point out that, with the advance of gestation, the mean hemoglobin (Hb) progressively increases from 10.9 ± 0.7 g/dL at age 10 weeks to 16.6 ± 4 g/dL at age 39 weeks, while the MCV, mean corpuscular hemoglobin (MCH), and reticulocyte counts gradually decrease (Fig. 1.5). In addition to these quantitative characteristics, fetal erythrocytes have different membrane properties, unique metabolic profiles, and contain fetal hemoglobin (Hb F).

The *white blood cell count* is very low and gradually increases from 1.6 ± 0.7 at the 15th week of gestation to reach 6.40 ± 2.99 at the 30th week of gestation [15, 19]. Immunophenotyping of fetal blood in the second and third trimester by flow cytometry reveals that the lymphocytes are the major white blood cell population of the fetal blood and that their absolute numbers are similar to those in adults [20, 22, 23]. The majority of lymphocytes in fetal blood are naïve and express CD45RA, a marker of "virgin" cells. These fetal lymphocytes remain almost entirely unprimed prior to birth, and only a minority express CD45RO, a marker of primed lymphocytes [22].

Most of the fetal lymphocytes express T- or B-cell surface differentiation antigens. During the second and third trimester, the percentage and absolute number of T-cells is lower as compared to adults [20, 24] (Table 1.3). The CD4+ T-cell subset predominates and the proportion of CD8+ T-cells is low. In addition, γδ T-cell and NK-cell counts are lower in fetal blood than in neonates and adults. In contrast, the proportion of B-cells in fetal blood is higher than in adults.

There are also functional differences between the fetal and postnatal lymphocytes. While fetal lymphocytes have been shown to proliferate spontaneously *in vitro*, they fail to respond to mitogen phytohemagglutinin (PHA) or allogeneic stimulations *in vitro*. Fetal mononuclear cells are unable to produce IL-2, IL-4, and IFN-γ (interferon gamma) *in vitro* but do secrete IL-10, IL-6, and TNF-α (tumor necrosis factor alpha) *in vitro* [24].

The first morphologically recognizable *platelets* appear in the fetal circulation at 8 to 9 weeks of gestation, and the number of platelets reaches adult levels before 18 weeks of gestation. Intrauterine thrombocytopenia can be reliably diagnosed through fetal blood sampling after 18 weeks of gestation.

Hematologic values in term neonates and infants

After birth, the marked improvement in tissue oxygenation results in a dramatic drop in EPO levels, leading to a significant decline in the red cell production by a factor of 2 to 3 during the first few days of life, and by a factor of about

Table 1.2. Hematologic values of normal fetuses between 15 and >30 weeks of gestation.

Weeks of gestation (Number of subjects)	RBC count (×10^{12}/L)	Hb (g/dL)	Hct (%)	MCV (fL)	WBC and NRBC[a] count (×10^9/L)	WBC corrected[b] count (×10^9/L)	PLT count (×10^9/L)
Data from Millar DS, Davis LR, Rodeck CH, *et al.* Normal blood cell values in the early mid-trimester fetus. *Prenatal Diagnosis.* 1985;5:367–373							
15 (*N* = 6)	2.43 ±0.26	10.9 ±0.7	34.6 ±3.6	143 ±8	–	1.6 ±0.7	190 ±31
16 (*N* = 5)	2.68 ±0.21	12.5 ±0.8	38.1 ±2.1	143 ±12	–	2.4 ±1.7	208 ±57
17 (*N* = 16)	2.74 ±0.23	12.4 ±0.9	37.4 ±2.8	137 ±8	–	2.0 ±0.8	202 ±25
18 (*N* = 18)	2.77 ±0.33	12.4 ±1.2	37.3 ±4.2	135 ±9	–	2.4 ±0.9	192 ±45
19 (*N* = 29)	2.92 ±0.27	12.3 ±1.2	37.5 ±3.1	129 ±6	–	2.5 ±0.8	211 ±48
20 (*N* = 12)	3.12 ±0.36	13.0 ±1.1	39.3 ±4.1	126 ±6	–	2.6 ±1.2	170 ±60
21 (*N* = 13)	3.07 ±0.42	12.30 ±0.8	37.3 ±3.5	123 ±8	–	2.7 ±0.7	223 ±61
Data from Forestier F, Daffos F, Catherine N, *et al.* Developmental hematopoiesis in normal human fetal blood. *Blood.* 1991;77:2360–2363							
18–21 (*N* = 760)	2.8 ±0.42	11.69 ±1.27	37.3 ±4.32	131.1 ±10.97	4.68 ±2.96	2.57 ±0.42	234 ±57
22–25 (*N* = 1 200)	3.09 ±0.34	12.20 ±1.60	38.59 ±3.94	125.1 ±7.84	4.72 ±2.82	3.73 ±2.17	247 ±59
26–29 (*N* = 460)	3.46 ±0.41	12.91 ±1.38	40.88 ±4.40	118.5 ±7.96	5.16 ±2.53	4.08 ±0.84	242 ±69
>30 (*N* = 440)	3.82 ±0.64	13.64 ±2.21	43.55 ±7.20	114.4 ±9.34	7.71 ±4.99	6.40 ±2.99	232 ±87

[a] Total nucleated blood cell count includes WBCs and nucleated red blood cells.
[b] WBC count after correction for the nucleated red blood cells.
Values are given as mean ± standard deviation (SD). The blood samples were analyzed with *Coulter "S" Plus* and *Coulter "S" Plus II* (Coulter, Hialeah, FL) instruments. Abbreviations: RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; WBC, white blood cell; NRBC, nucleated red blood cell; PLT, platelet.

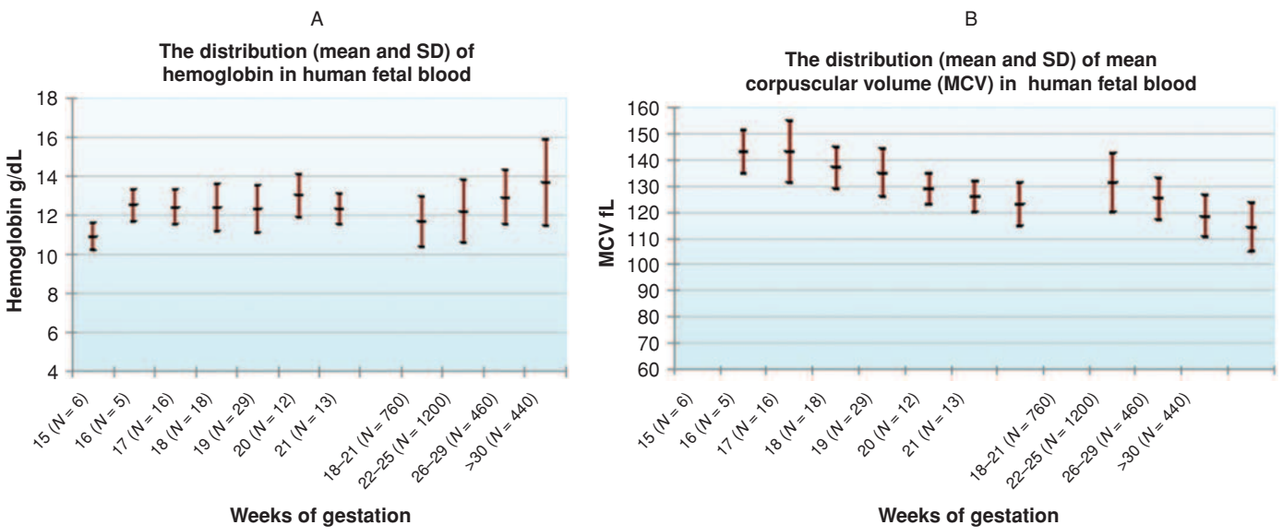

Fig. 1.5. Dynamics of red cell indices with advancing of gestational age. (A) Hemoglobin concentration. (B) Mean corpuscular volume (MCV). (Data reanalyzed from Millar *et al.* [19] and Forestier *et al.* [15].)

Table 1.3. Lymphocyte subsets in fetal blood as compared to full-term neonates and adults.

	Week 16–20 fetus (*N* = 10)	Week 20–27 fetus (*N* = 7)	Full-term neonate (*N* = 15)	Adult (*N* = 10)
Absolute lymphocyte count[a] ($\times 10^9$/L)	1.9 ± 0.7	2.6 ± 0.7	5.6 ± 1.0	2.1 ± 0.7
CD3+ [b] T-lymphocytes	68.2 ± 10.3	71.1 ± 8.7	73.2 ± 6.4	76.9 ± 5.6
CD4+ T-lymphocytes	47.7 ± 6.0	50.2 ± 7.2	51.3 ± 4.8	46.1 ± 4.2
CD8+ T-lymphocytes	18.2 ± 6.9	23.1 ± 7.6	24.3 ± 4.5	27.8 ± 4.8
CD4 : CD8 ratio	2.95 ± 1.1	2.41 ± 0.9	2.17 ± 0.5	1.65 ± 0.35
CD19+ B-lymphocytes	15.6 ± 8.7	22 ± 8.1	163 ± 7.9	9.4 ± 5.5
Leu7+ NK-cells	0.3 ± 0.6	1.4 ± 2.2	0.6 ± 0.7	6.4 ± 4.2

[a] Blood samples were obtained by ultrasound-guided aspiration from the umbilical vein at the placental cord insertion.
[b] The lymphocyte subsets were determined by immunogold-silver staining with OKT3 (CD3), OKT4 (CD4), OKT8 (CD8), Leu12 (CD19), and Leu7 (an NK-cell marker) antibodies.
Data from De Waele *et al.* [20].

Table 1.4. Postnatal changes in some red cell parameters of capillary blood in the first 12 weeks of life in healthy full-term infants according to Matoth *et al.* [25].

Age	No. of cases	RBC count ($\times 10^{12}$/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCHC (%)	Retic count (%)
Days							
1	19	5.14 ±0.7	19.3 ± 2.2	61 ± 7.4	119 ± 9.4	31.6 ± 1.9	3.2 ± 1.4
2	19	5.15 ± 0.8	19.0 ± 1.9	60 ±6.4	115 ± 7.0	31.6 ± 1.4	3.2 ± 1.3
3	19	5.11 ± 0.7	18.8 ± 2.0	62 ± 9.3	116 ± 5.3	31.1 ± 2.8	2.8 ± 1.7
4	10	5.00 ± 0.6	18.6 ± 2.1	57 ± 8.1	114 ± 7.5	32.6 ± 1.5	1.8 ± 1.1
5	12	4.97 ± 0.4	17.6 ± 1.1	57 ± 7.3	114 ± 8.9	30.9 ± 2.2	1.2 ± 0.2
6	15	5.00 ± 0.7	17.4 ± 2.2	54 ± 7.2	113 ± 10.0	32.2 ± 1.6	0.6 ± 0.2
7	12	4.86 ± 0.6	17.9 ± 2.5	56 ± 9.4	118 ± 11.2	32.0 ± 1.6	0.5 ± 0.4
Weeks							
1–2	32	4.80 ± 0.8	17.3 ± 2.3	54 ± 8.3	112 ± 19.0	32.1 ± 2.9	0.5 ± 0.3
2–3	11	4.20 ± 0.6	15.6 ± 2.6	54 ± 8.3	111 ± 8.2	33.9 ± 1.9	0.8 ± 0.6
3–4	17	4.00 ± 0.6	14.2 ± 2.1	43 ± 5.7	105 ± 7.5	33.5 ± 1.6	0.6 ± 0.3
4–5	15	3.60 ± 0.4	12.7 ± 1.6	36 ± 4.8	101 ± 8.1	34.9 ± 1.6	0.9 ± 0.8
5–6	10	3.55 ± 0.2	11.9 ± 1.5	36 ± 6.2	102 ± 10.2	34.1 ± 2.9	1.0 ± 0.7
6–7	10	3.40 ± 0.4	12.0 ± 1.5	36 ± 4.8	105 ± 12.0	33.8 ± 2.3	1.2 ± 0.7
7–8	17	3.40 ± 0.4	11.1 ± 1.1	33 ± 3.7	100 ± 13.0	33.7 ± 2.6	1.5 ± 0.7
8–9	13	3.40 ± 0.5	10.7 ± 0.9	31 ± 2.5	93 ± 12.0	34.1 ± 2.2	1.8 ± 1.0
9–10	12	3.60 ± 0.3	11.2 ± 0.9	32 ± 2.7	91 ± 9.3	34.3 ± 2.9	1.2 ± 0.6
10–11	11	3.70 ± 0.4	11.4 ± 0.9	34 ± 2.1	91 ± 7.7	33.2 ± 2.4	1.2 ± 0.7
11–12	13	3.70 ± 0.3	11.3 ± 0.9	33 ± 3.3	88 ± 7.9	34.8 ± 2.2	0.7 ± 0.3

Abbreviations: RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Retic, reticulocyte.

10 during the first week of life [17]. This results in dramatic changes in the blood composition after birth. The blood of newborns and neonates has been subject to detailed studies for several decades [25–28]. At birth, there is polycythemia and macrocytosis followed by a gradual decrease in the red blood cell (RBC) count, Hb concentration, and the MCV in the first several months of life (Table 1.4). The mean RBC count drops steadily, reaching its lowest point in the 7th week, but the hemoglobin concentration lags and reaches its lowest level during the 9th week. This delay in the drop of hemoglobin is explained by the relatively high MCV that gradually decreases and reaches adult levels by the 11th week. While the RBC counts, Hb, and MCV levels are higher in newborns, the mean corpuscular hemoglobin concentration (MCHC) is relatively

low by adult standards. The MCHC increases significantly in the first five to six weeks and remains constant thereafter. Thus, while the neonatal erythrocytes are bigger and contain more hemoglobin relative to their increased size, the hemoglobin within the cells is neither more nor less concentrated than for adults. The red cell distribution width (RDW) in newborns and neonates is increased as compared to adults and reflects a significant variability in the size of the neonatal red cells. The reticulocyte count is relatively high immediately after birth and through the first several days, indicating the persistence of considerable erythropoietic activity. After the initial drop at the end of the first week, the reticulocyte count thereafter remains low: in the 0.5–1% range. It slowly increases and reaches its peak during the ninth week.

There is considerable morphologic heterogeneity in the erythrocytes of the newborn. Newborns have a higher number of irregularly shaped erythrocytes as compared to adults. Using scanning electron microscopy, Zipursky *et al.* found a markedly increased number of stomatocytes (bowl-shaped red blood cells) as compared to adults' erythrocytes (40% vs. 18%) [29]. In neonates, the number of normal, disc-shaped red cells (discocytes) was significantly lower than in adults (43% vs. 78%). Interference-contrast microscopy reveals a significantly higher number of erythrocytes with pits or craters in term neonates as compared to adults (24.3% vs. 2.6%) [30]. In this study, the mean pit count reached near-adult levels at two months of age. The significance of pitted erythrocytes is still unknown; it is suspected that the pits are cytoplasmic vacuoles and represent hypofunction of the spleen and/or other reticuloendothelial cell-rich sites in trapping and eliminating pitted erythrocytes from the circulation. Pits like this have been observed with such high frequency, so far, only in asplenic patients.

Nucleated red blood cells (NRBCs) are present in the blood of healthy newborns in the first day of life. Finding 0–10 NRBCs/100 WBCs is typical, although these values are highly dependent on the total WBC count. In a term neonate, NRBCs are rapidly cleared from the circulation after birth, and in a healthy neonate virtually no NRBCs are found after the third or fourth day of life, although they may persist in small numbers up to one week in preterm newborns [31].

In full-term neonates, 50–80% of the hemoglobin is Hb F and 15–50% is Hb A. After birth, the majority of the newly produced red blood cells contain Hb A, which has a lower affinity for oxygen as compared to Hb F. As a consequence, there is an improvement in tissue oxygenation after birth, along with a resultant decline of EPO production. After birth, the EPO synthesis shifts from the liver and bone marrow to the kidney [17].

Newborns have a high WBC count and a high percentage of neutrophils at birth. Two major studies performed almost 40 years apart demonstrate that the total WBC and neutrophil counts are dynamic and change in the first five days of life [27, 28]. The high neutrophil count at birth continues to rise in the first 8–12 hours, when it reaches a peak and then gradually decreases to its nadir observed around 72 hours after birth. After that time, the neutrophil count gradually increases again to reach a stable level by day five, and remains unchanged to the end of the neonatal period.

The cause of the leukocytosis and neutrophilia at birth and shortly thereafter is still not entirely understood. Most likely, the increased neutrophil count is a result of bone marrow mobilization of the preexisting neutrophil pool due to stress during labor, and less likely it is due to an increase in white blood cell production [17]. Multiple factors play a role. Epinephrine stimulation of the neutrophil demargination may be one such factor. However, the level of epinephrine and its relatively short action cannot explain the slow return to normal, taking several days, which suggests other mechanisms. Perinatal factors, such as the mode of delivery, maternal hypertension, maternal fever prior to delivery, hemolytic disease, and hemorrhage, have also been suggested [28]. In a recent study, gender was found to be a statistically significant factor in blood neutrophil concentration, with females averaging concentrations 2.0×10^9/L higher than males [27].

At birth, along with the neutrophilia, there is a granulocytic shift to immaturity with an increased number of metamyelocytes, myelocytes, and even circulating blasts [32]. In the early neonatal period, rare circulating micromegakaryocytes may also be present, and such a finding should not be considered pathologic.

While at birth the lymphocytes comprise a smaller population relative to the granulocytes, they become the major white blood cell population shortly after birth. The absolute number of CD19+ B-cells increases twofold after birth, remains stable until two years of age, and then subsequently gradually decreases sixfold from two years to adult age [33]. CD3+ T-cells increase 1.5-fold immediately after birth and decrease threefold from two years to adult age. The absolute CD3+/CD4+ pattern follows the T-cells. CD3+/CD8+ cells remain stable until two years of age and then decrease three- to fourfold until adulthood. NK-cells decrease two- to threefold after birth and remain stable thereafter (Table 1.5).

A detailed longitudinal analysis of the changes in lymphocyte subpopulations in 11 healthy infants followed from birth to one year of age defines these trends more specifically, though the sample is a small one [34]. It shows that the total lymphocyte count and T-lymphocytes increase at one week of age, the B-lymphocytes increase at six weeks of age, and the number of natural killer (NK) cells is highest at birth and sharply declines thereafter. In addition, it shows that most of the T-cells express T-cell receptor (TCR) αβ, and most of the T-lymphocytes are CD45RA+ naïve T-cells. In contrast to adults, the relative frequency of CD45RO+ memory T-cells is lower in infants. The CD4+ helper T-cells follow the pattern of total T-lymphocyte count with an increase at one week of age. The CD4 : CD8 ratio during the first year of life is higher than in adults.

With respect to the normal B-cell progenitors, also known as hematogones, hardly any such early B-cells are present in the blood of infants and adults. Moreover, there is no indication that in infants these cells leave the bone marrow at an earlier stage