Section I Peripheral Blood

Chapter 1

Peripheral Blood Smear Review

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Despite all the advances in laboratory medicine, microscopic review of the peripheral blood smear is still highly informative and clinically relevant and remains an indispensable diagnostic tool. In a pediatric hospital, a common and challenging request is the evaluation of a newborn blood smear. This chapter provides an overview of some of the characteristics of a newborn smear and common reactive conditions.

Blood smear evaluation is often performed for three essential reasons: first, to clarify a flagged result such as immature cells or a low platelet count to rule out pseudothrombocytopenia from platelet clumping; second, to evaluate the morphology of red blood cells, white blood cells, and platelets; and, third, to confirm morphologic findings identified by lab staff or an instrument [1]. The latter may be requested by a physician due to a clinical suspicion or by members of the laboratory staff for review of an abnormal finding. Common reasons for peripheral smear review include cytopenias or cytoses, abnormal cells, malignancy, and infections [2]. The criteria for when evaluation is performed also depend on national and institutional guidelines.

Examination of a well-prepared blood smear is essential in many clinical situations. Peripheral blood smear preparation should be performed by a laboratory professional and controlled for preanalytical variables in order to ensure optimal quality. Blood should be sampled correctly and is commonly drawn from a peripheral vein and placed in an anticoagulant tube. It is essential to have the blood-to-anticoagulant ratio in the correct proportion or this can affect the cytology. In the pediatric population, particularly in neonates, the lab routinely receives microcapillary containers from heel sticks. Ethylene diamine tetra-acetic acid (EDTA) is the most common anticoagulant used. Specimens should be sent to the laboratory as soon as possible and are best analyzed within 2 hours. This is not always possible, but delay in preparation can result in degeneration of the cells and pseudothrombocytopenia. Morphology of the cells is best viewed in the monolayer part of the smear. It is best to avoid the thickest part of the smear when reviewing cell morphology, but it may be useful when searching for parasites. The feathered edge of the slide is an ideal place to look for platelet clumps and large cells such as blasts.

Slide preparation should be performed by a trained laboratory medical technologist. The quality of the slide depends on proper smearing technique and the quality of the staining process so there is no over- or under-staining of the cells. These processes require good quality control and are essential for a quality blood smear review and differential count. Many labs now use automated analyzers that prepare stained slides.

Interpreting a peripheral blood smear requires a skilled approach and should be done by a trained medical technologist. Review of the peripheral blood smear should follow a systemic approach. It is essential to review all lineages and assess the size, shape, maturation, and morphology. Findings on the peripheral blood smear should be interpreted in the context of the patient's clinical history and other laboratory information. Anemia is a frequently encountered problem in the newborn nursery or intensive care unit, and the presence of other cellular abnormalities related to neutrophils and platelets is not uncommon, especially if the neonate is sick or has medical issues.

Once the decision is made to evaluate the smear, a well-mixed drop of blood of about 2–3 mm in diameter is placed about one quarter of an inch from one end of the slide. Then a "spreader" slide is placed with its edge in front of this drop of blood at an angle of approximately 35–45°. This slide is gently moved back to touch the blood and then, with one smooth motion, this "spreader" slide is pushed to the other end of the slide, maintaining the angle until a wedge is created (Figure 1.1). Hence, this method is often referred to as the wedge method. The slide is air dried and fixed with methanol or ethyl alcohol and stained. The thin portion of the blood smear 1–2 mm from the feathered edge is used to examine the morphology of the cells as they are likely

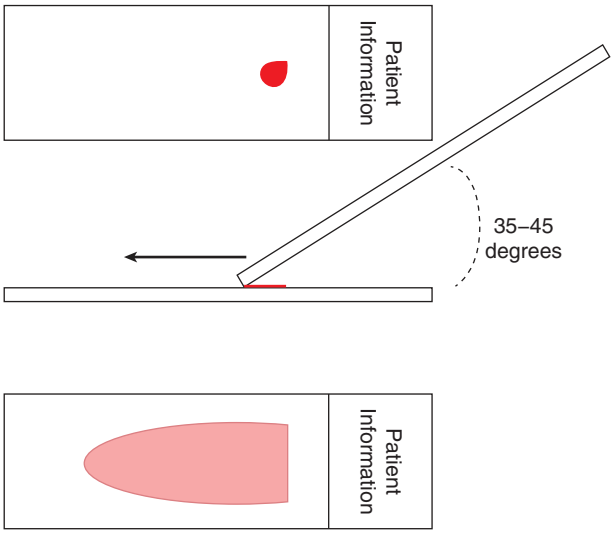


Figure 1.1 Diagram of the wedge method of peripheral blood smear preparation. The edge of the "spreader" slide is placed at an angle of approximately 35–45°. This slide is gently moved back to touch the blood and then, with one smooth motion, the "spreader" slide is pushed to the other end of the slide maintaining the angle.

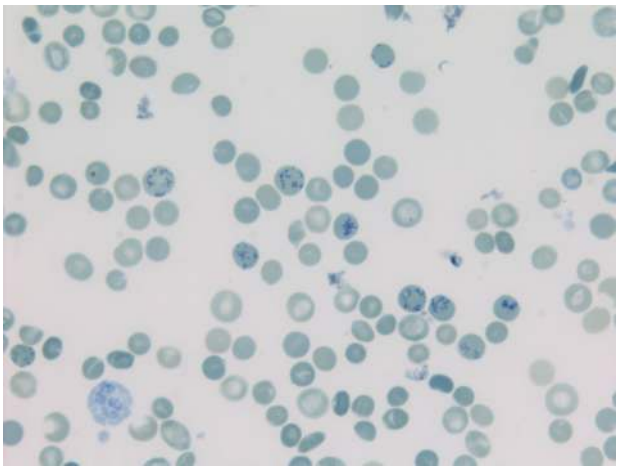


Figure 1.2 (Supravital stain, 50x) Immature RBCs show reticulofilamentous precipitates of ribosomes. The stain causes clumping and staining of residual nucleic acid present in immature cells. The stained cells represent reticulocytes and are counted as a percentage of total red blood cells.

separated from one another and not overlapping. A thick smear is used to evaluate for malarial parasites with low levels of viremia.

The frequently used stains are Wright or Wright-Giemsa stains; the latter ensures adequate staining of nuclear features and granular components. A Wright stain is a polychromatic stain consisting of a mixture of eosin and methylene blue. The Wright stain is methanol based; therefore, the slides do not need to be fixed. However, fixation helps reduce water artifact that can occur on humid days or with aged stain. Eosin Y is an acidic anionic dye and methylene blue is a basic cationic dye. Eosin stains the basic components such as hemoglobin and eosinophilic granules an orange to pink color. Methylene blue stains acidic cellular components such as nucleic acid and basophilic granules in varying shades of blue. The neutral components of the cells are stained by both components of the dye, producing variable colors. Another special stain used is a supravital stain. The supravital stain is performed to detect the reticulofilamentous pattern of ribosomal RNA in immature red blood cells (RBCs) using new methylene blue (Figure 1.2). The result provides information into bone marrow erythropoiesis. Another utility of a supravital stain is to detect Heinz bodies, which is not visible on a Wright-Giemsa stain. Heinz bodies are denatured hemoglobin seen in persons with glucose-6-phosphate dehydrogenase deficiency or unstable hemoglobin when their RBCs undergo oxidative damage. Reticulocyte counts are often automated but manual counts are still performed. The Reticulocyte Index (RI) is a calculated value in the diagnosis of anemia (see the following formula).

Reticulocyte Index (RI) = Retic percentage (%) × (Patient's hematocrit/Normal hematocrit)/2

Reticulocyte index interpretation:

RI should be between 0.5% and 2.5% for a healthy individual.

RI < 2% with anemia indicates maturation disorder – an inappropriate response to correct the anemia, such as iron deficiency anemia or myelodysplastic syndrome.

RI > 3% with anemia indicates an increased compensatory production of reticulocytes such as hemolytic anemia.

A common and challenging request is the evaluation of a newborn blood smear. Anemia is a frequently encountered problem in the newborn nursery or intensive care unit, and the presence of other cellular abnormalities related to neutrophils and platelets is not uncommon, especially if a neonate is sick or has medical issues. The next portion of this chapter provides an overview of the characteristics of a newborn smear.

Erythrocyte

As erythropoiesis switches from the fetal to the newborn period, the rate of hemoglobin synthesis and RBC production decreases drastically, secondary to the sudden increase in tissue oxygenation and marked decrease of erythropoietin. There is a period of physiologic anemia in the second week of life as RBC production reaches a nadir. Due to these changes, RBCs in the neonatal period have different indices compared to adult RBCs (Table 1.1). Neonatal RBCs demonstrate unique metabolic features due to enzymatic activities. Examination and familiarization of newborn smears are encouraged as they often exhibit variation in shapes including acanthocytes, echinocytes, schistocytes, stomatocytes, or target cells, which leads to decreased deformability in the first few weeks of life. Normal newborn RBC hemoglobin content is comprised of more than 70% fetal hemoglobin, and the remainder is adult hemoglobin. Additionally, RBC enzyme levels are lower in mature newborns. These characteristics of newborn RBCs make a diagnosis of an RBC membrane disorder very challenging. They also reduce the life span to 60 to 90 days compared to the adult RBC life span of 120 days. Premature RBCs tend to be more fragile and have an even shorter lifespan [3]. Nucleated RBCs are not an uncommon finding, as shown in Figure 1.3.

Table 1.1 Red blood cell and reticulocyte indices in neonatal and adult blood*

	Newborn (RBCs/ Reticulocytes)	Adult (RBCs/ Reticulocytes)
Red blood cells		
MCV (fL)	107.7/123	89.8/106
RDW (%)	22.1	11.6
CHCM (g/dL)	32.9/24.7	33.7/30.3
MCH (pg)	34.4/29.7	29.6/30.3
Life span	60–90 days	120 days
Reticulocytes		
	4.4%	1.2%

* Adapted from Nathan and Oski's *Hematology and Oncology of Infancy and Childhood*, 7th edition. The Neonatal Erythrocytes and Its Disorders, Chapter 2, 52–75.e8.

Lymphocytes and Neutrophils

In newborns, in the first 24 hours, higher numbers of neutrophils circulate when compared to adults, quickly reaching adult levels by 72 hours of life. Mature segmented neutrophils are often seen by 4–6 days after birth. Similarly, granule numbers and maturation are also variable in newborns [4]. Neutrophils mature throughout gestation and therefore are at risk for defects if this maturation process or the pregnancy is impaired or shortened. Neutrophils circulate as the first responders against infections in a newborn. Even after birth, a balance between neutrophil maturation in the bone marrow, release into the circulation, and subsequent passage into the tissues is critical. While interpreting neutrophil numbers in newborns, it is important to take into account and correlate with their gestational age, birth weight, and ongoing comorbidities such as maternal medical history, infection, and drugs. Lymphocytes in a newborn ("baby lymphocytes") have an immature blastoid appearance with fine chromatin and clefted nuclei (Figure 1.4) and can be diagnostically challenging. Recognizing these features would be helpful when a differential diagnosis of lymphoblastic leukemia arises.

Platelets

In the fetus and newborn, platelets are mostly produced in the liver and spleen compared to bone marrow in adults. Platelet count depends on gestational age, but usually reach the adult range of 150,000–450,000/μL by 22 weeks

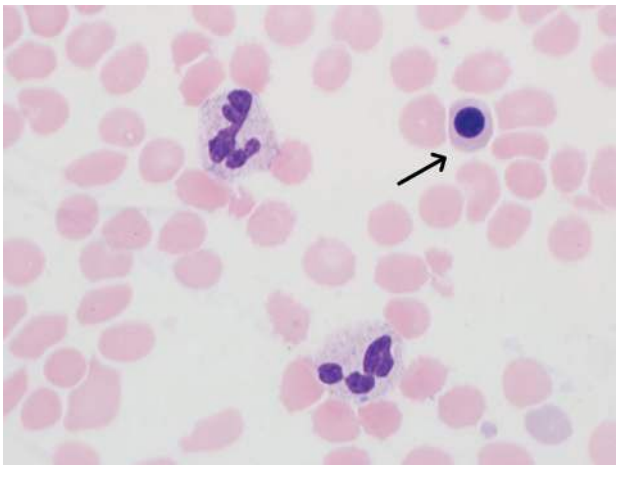


Figure 1.3 (Wright-Giemsa, 50x) Blood film from a healthy newborn showing a nucleated RBC (arrow).

of gestation. The platelet function is usually normal compared to adult platelets within a few days after a term birth. Several maternal and perinatal factors influence neonatal platelet dysfunction in at-risk infants, including maternal hypertension, medications, prematurity, birth weight, and infections, among others [5]. Many infections and systemic diseases can present with changes in the complete blood count and peripheral blood morphology. Next, we review general causes, and blood smears from infections and other causes are again discussed with pictures.

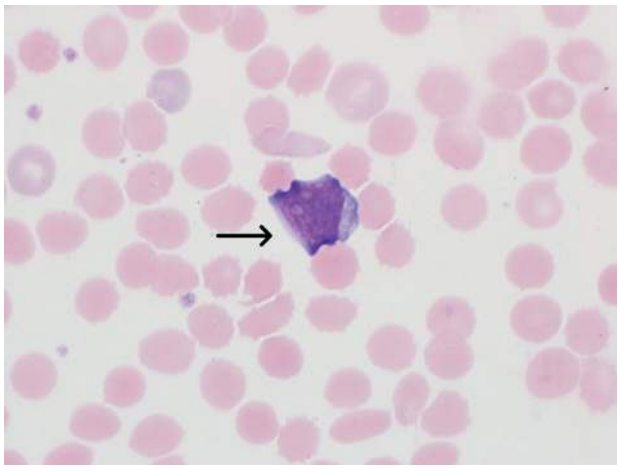


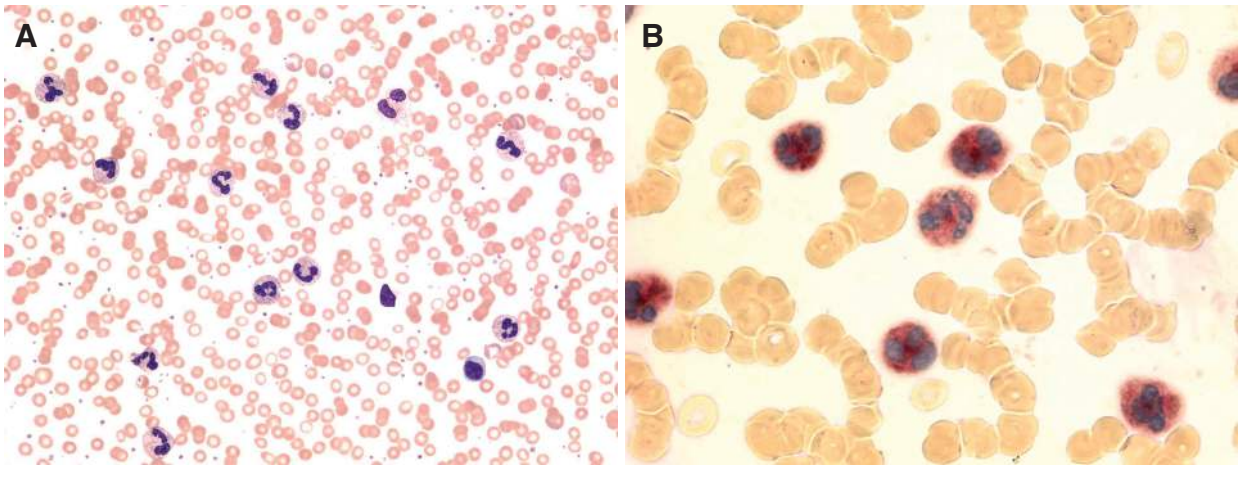
Figure 1.4 (Wright-Giemsa, 100x) Blast-like lymphocytes, often referred to as baby lymphocytes or pedi-lymphocytes, are seen in newborns.

Leukemoid Reaction

A leukemoid reaction is defined as a white blood cell (WBC) count greater than 50,000 cells/μL and is not secondary to a hematologic malignancy such as chronic myeloid leukemia. In most children, it is secondary to an acute infection, but other causes such as drugs – particularly steroids – solid organ cancers, and severe bleeding are possible. When a high WBC count is encountered, a thorough clinical history, physical examination, and blood smear review are essential for correct diagnosis. The blood smear in a leukemoid reaction is characterized by predominant neutrophilia and a left shift in maturation with many immature myeloid forms seen in the circulation (Figure 1.5a). Additionally, leukocyte alkaline phosphatase (LAP) can be markedly elevated (Figure 1.5b). LAP is predominantly found in neutrophils including immature forms, but not in lymphocytes or monocytes. In most cases, bone marrow examination is not required. When a bone marrow is performed, it is hypercellular with normal morphology and maturation of all cell lineages, along with normal immunophenotyping and cytogenetic studies [6].

Sepsis

Neutrophilia secondary to a bacterial infection is accompanied by toxic granulations in the neutrophils and precursors (Figure 1.6). Toxic granulations are blue-black or purplish granules distributed through the cytoplasm. Toxic granulation can also be seen in burns, drugs such as chemotherapy agents, and poisons. A higher proportion



Figures 1.5a and 1.5b (Wright-Giemsa, 20x) Figure 1.5a is from a toddler presenting with viral infection resulting in a leukemoid reaction that shows neutrophilia and a left shift in myeloid maturation with toxic granulations. Figure 1.5b shows an elevated LAP score. For this test, naphthyl AS-B1 phosphate is hydrolyzed by alkaline phosphatase to diazonium salt, forming a blue dye within the cytoplasm of the WBCs, as shown in this picture.

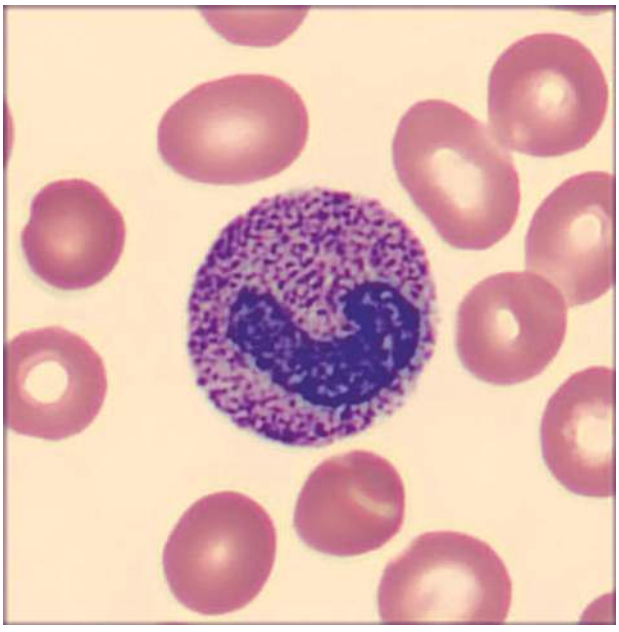


Figure 1.6 (Wright-Giemsa, 100x) Band with toxic granulations. Dark purple granules are in the cytoplasm of the band due to abnormal maturation of the primary granules.

of band forms (usually > 10%) is typically seen with sepsis. Alternatively, neutropenia can also be associated with infections, particularly viruses. It is important to note that there can be reduced number of erythrocytes with occasional clumping or even fragment cells, and platelet numbers can be either increased or decreased in severe cases [7].

Drugs

Drugs can induce quantitative and qualitative abnormalities of erythrocytes, platelets, and leukocytes to a variable degree. This can depend on the type of drug and the dose, and on the preexisting condition of the bone marrow. Review of clinical and medication history is crucial if findings on the blood smear are not within normal or expected range. Some of these conditions include drug-induced immune-hemolytic anemia, which may show schistocytes and thrombocytopenia (as shown in Figure 1.7 [8]), and drug-related eosinophilia (Figure 1.8). A common drug, recombinant granulocyte colony-stimulating factor, induces a marked leukocytosis with an increase in mature neutrophils, immature myeloid precursors, and monocytoid precursors [9].

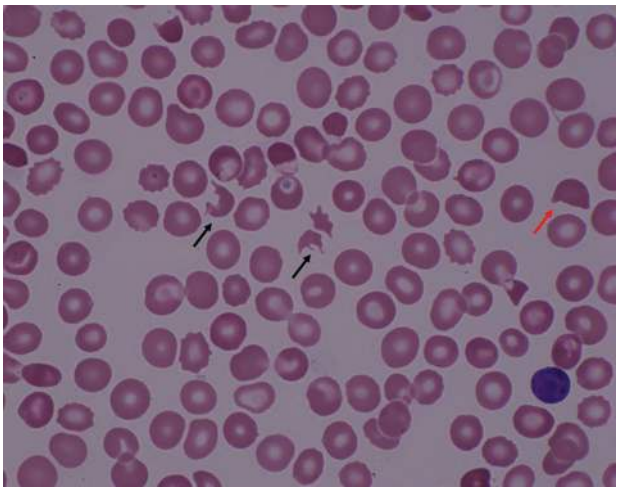


Figure 1.7 (Wright-Giemsa, 50x) Blood smear from a 2-year-old child presenting with drug-induced immune hemolytic anemia from ceftriaxone. The smear was taken a couple of days after presentation showing the presence of schistocytes (black arrows) and helmet cells (red arrows), along with paucity of platelets. This picture fits with drug-induced thrombotic microangiopathy.

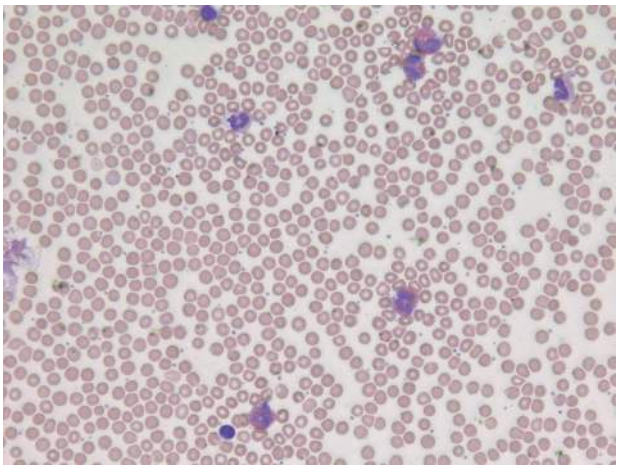


Figure 1.8 (Wright-Giemsa, 20x) Blood smear showing drug-related eosinophilia.

Stress Response

Bone marrow stress is a collective term used to identify the response of bone marrow or the general hematopoietic response to infection, inflammation, drugs, stem cell transplant, and so forth. This results in circulating nucleated red cells and polychromasia (reticulocytes) into the peripheral circulation prior to maturation. These enucleated

polychromatic erythrocytes are larger and have higher levels of erythropoietin. Similarly, biological stress can result in leukocytosis and thrombocytosis.

SARS-CoV-2

COVID-19 caused by SARS-CoV-2 has brought about a global pandemic. The symptoms of COVID-19 range from mild flu-like symptoms to severe respiratory illness that can be fatal. Laboratory medicine plays an important role in diagnosis and prognosis in these patients. Recent studies have described hematology parameters such as lymphopenia, lymphocytosis, neutrophilia, and atypical coagulation. The peripheral blood smear findings seen in COVID-19–infected patients have been published and data continue to emerge. The morphology on peripheral blood smears seen in COVID-19 patients includes a spectrum of findings ranging from neutrophils with pseudo-Pelger-Huet anomalies (Figure 1.9), abnormal lobations (Figure 1.10), toxic granulations, atypical lymphocytes (Figure 1.11), plasmacytoid lymphocytes, vacuolated monocytes (Figure 1.12), platelet clumping, and RBCs with schistocytes and basophilic stippling. Pediatric patients who presented with multisystem inflammatory syndrome in children (MIS-C) showed RBC abnormalities significant for burr cells and increased schistocytes (Figures 1.13a and 1.13b) [10, 11, 12, 13].

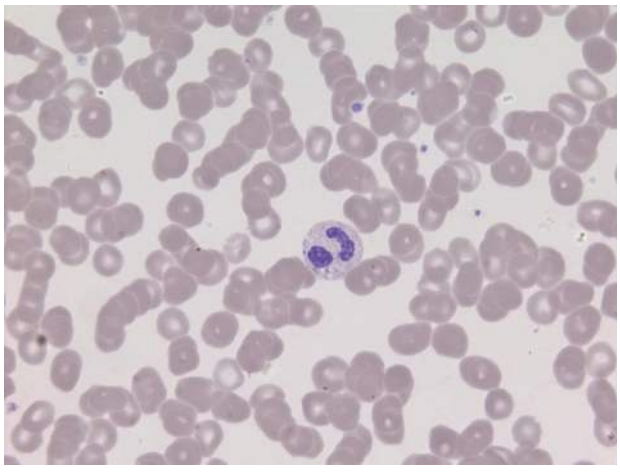


Figure 1.9 (Wright-Giemsa, 50x) Blood smear from a 16-year-old COVID-19 patient showing pseudo-Pelger-Huet anomaly.

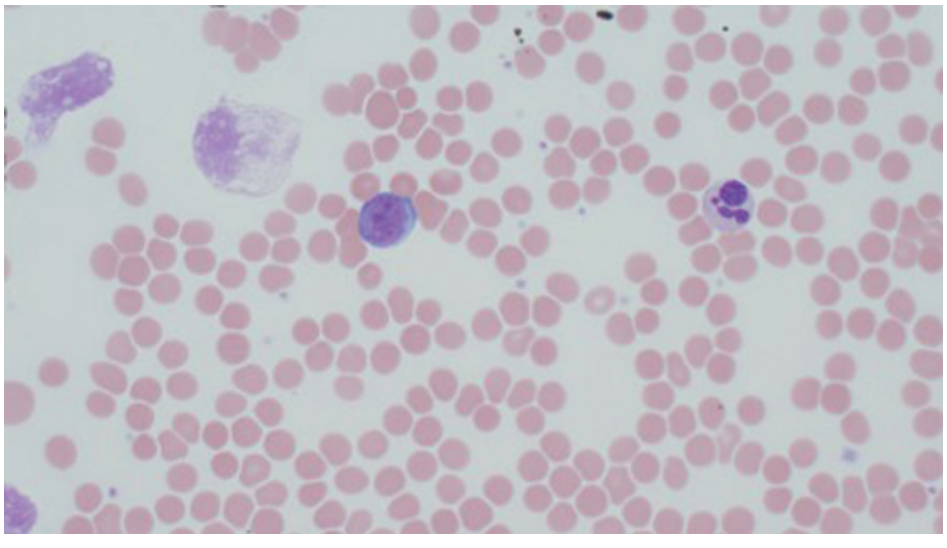


Figure 1.10 (Wright-Giemsa, 600x) Peripheral blood from a 10-year-old COVID-19 patient with neutrophil with abnormal lobation and an atypical lymphocyte.

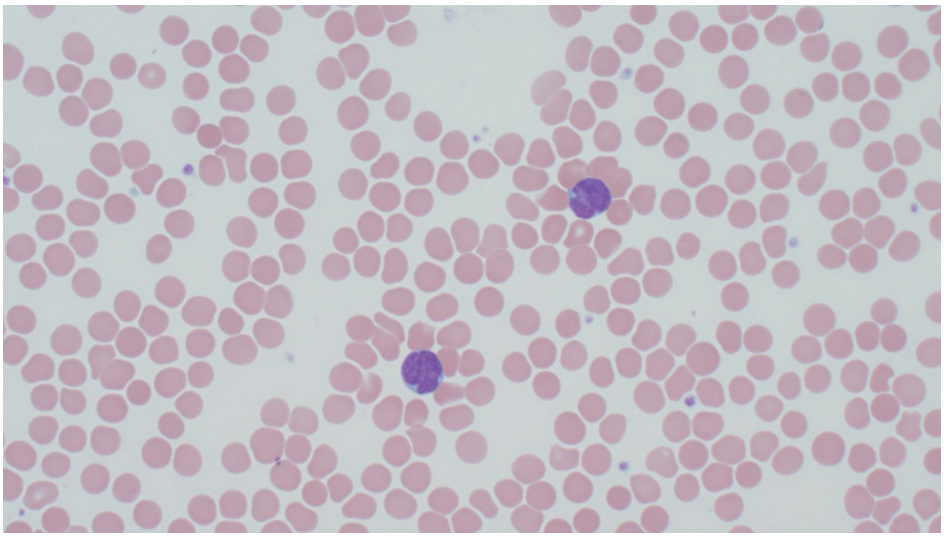


Figure 1.11 (Wright-Giemsa, 600x) Peripheral blood from a 12-year-old COVID-19 patient with atypical lymphocytes.

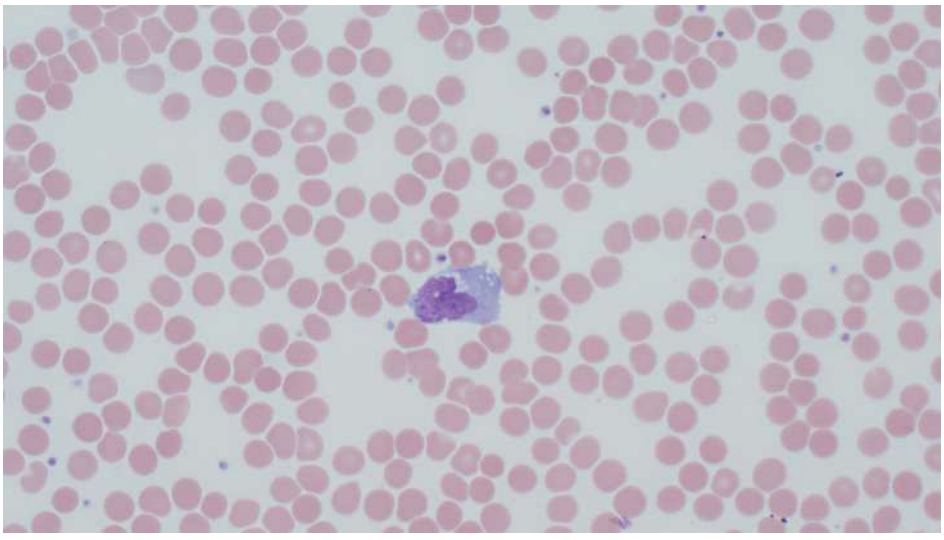
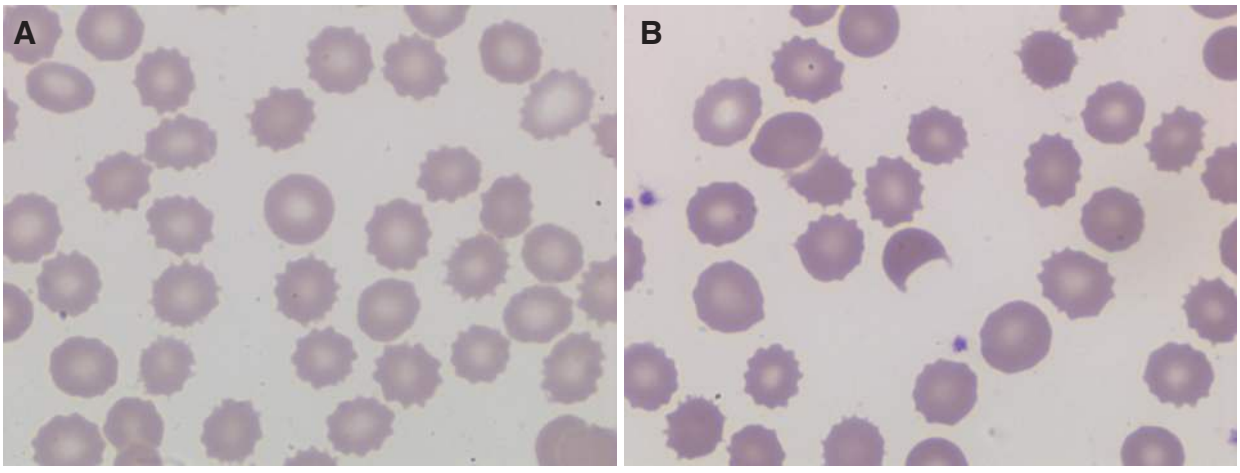


Figure 1.12 (Wright-Giemsa, 600x) Peripheral blood from a 8-year-old COVID-19 patient with vacuolated monocyte.



Figures 1.13a and 13b (Wright-Giemsa, 50x) Blood smear from a 10-year-old girl with COVID-19 and MIS-C with increased burr cells and schistocytes.

References

1. Gulati G, Song J, Florea AD, Gong J. Purpose and criteria for blood smear scan, blood smear examination, and blood smear review. Ann Lab Med. 2013; **33**(1): 1–7.
2. Bain BJ. Diagnosis from the blood smear. N Engl J Med. 2005; **353**: 498–507.
3. Steiner LA, Gallagher PG. Erythrocyte disorders in the perinatal period. Semin Perinatol. 2007; **31**(4): 254–61.
4. Lawrence SM, Corriden R, Nizet V. Age-appropriate functions and dysfunctions of the neonatal neutrophil. Front Pediatr. 2017; **5**: 23.
5. Kühne T, Imbach P. Neonatal platelet physiology and pathophysiology. Eur J Pediatr. 1998; **157**(2): 87–94.
6. Sakka V, Tsiodras S, Giamarellos-Bourboulis EJ, Giamarellou H. An update on the etiology and diagnostic evaluation of a leukemoid reaction. Eur J Intern Med. 2006; **17**(6): 394–8.
7. Aird WC. The hematologic system as a marker of organ dysfunction in sepsis. Mayo Clin Proc. 2003; **78**(7): 869–81.
8. Chonat S, Graciaa S, Shin HS, Newton JG, Quarmyne MO, Boudreaux J, et al. Eculizumab for complement mediated thrombotic microangiopathy in sickle cell disease. Haematologica. 2020. Dec 1; **105**(12): 2887–91.
9. Reykdal S, Sham R, Phatak P, Kouides P. Pseudoleukemia following the use of G-CSF. Am J Hematol. 1995; **49**(3): 258–9.
10. Nazarullah A, Liang C, Villarreal A, Higgins RA, Mais DD. Peripheral blood examination findings in SARS-Co-V-2 infection. AM J Clin Pathol. 2020; **154**(3): 319–29.
11. Berber I, Cagascar O, Sarici A, Berber NK, Aydogdu I, Ulutas O, et al. Peripheral blood smear findings of COVID-19 patients provide information about the severity of the disease and the duration of the hospital stay. Mediterr J Hematol Infect Dis. 2021; **13**(1): e2021009.
12. Diorio C, Henrickson SE, Vella LA, McNerney KO, Chase J, Burudpakdee C, et al. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. J Clin Invest. 2020; **130**(11): 5967–75.
13. Merino A, Vlagea A, Molina A, Egri N, Laguna J, Barrera K, et al. Atypical lymphoid cells circulating in blood in COVID-19 infection: Morphology, immunophenotype and prognosis value. J Clin Pathol. 2020; **75**(2): 104–11.

Chapter 2

Red Blood Cell Disorders

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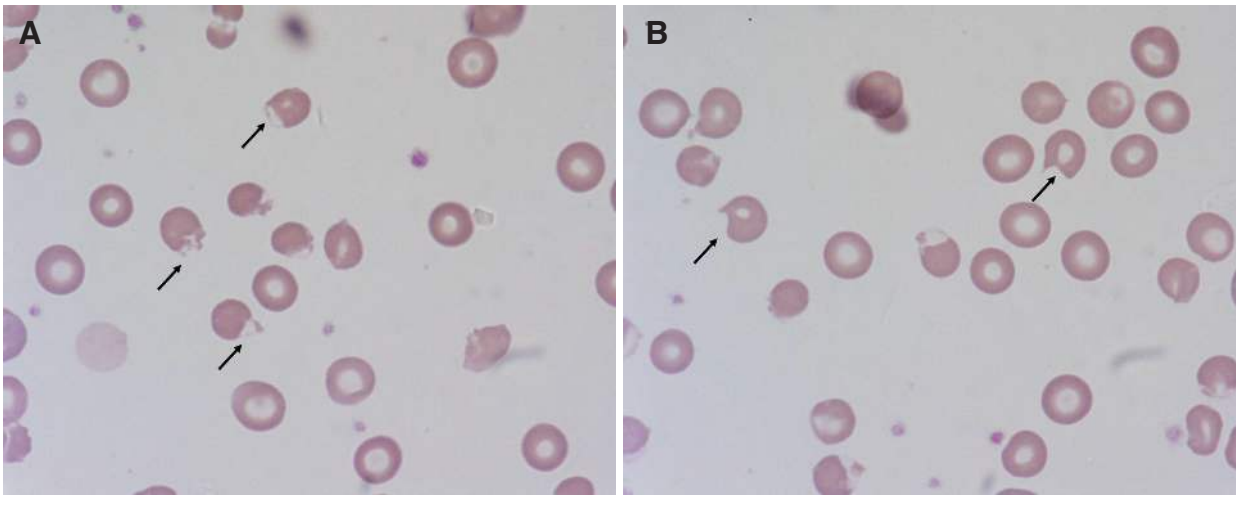
Congenital nonimmune hemolytic anemias are disorders of the red blood cells (RBCs) that occur infrequently in children and adults. These disorders can be divided into three categories: disorders affecting RBC metabolism, the RBC membrane, and hemoglobin synthesis. In this chapter, we briefly describe these conditions and the blood smear morphology. Increasing use of ektacytometry, flow cytometry, or the less sensitive osmotic fragility test can help in analyzing RBC membrane disorders. In addition to morphology of RBCs, RBC enzyme and/or genetic testing can be confirmatory for diagnosis.

Enzyme Deficiencies

Glucose-6-phosphate dehydrogenase (G6PD) deficiency: This is the most common RBC enzyme disorder worldwide. It is a genetic disorder characterized by a defect or deficiency in G6PD, which is involved in the hexose monophosphate pathway. It is required for the production of nicotinamide adenine dinucleotide phosphate (NADPH), which protects RBCs from oxidative damage. Absence of the G6PD enzyme in RBCs results in the buildup of reactive oxidants, which causes hemoglobin to denature and precipitate, which in turn damages the structure and function of the RBC membrane [1]. Heinz bodies – particles of denatured hemoglobin – may be seen in supravital stained preparations. Figures 2.1a and 2.1b are examples of blister and bite cells that can be noted in patients with G6PD presenting with hemolytic crisis.

Pyruvate kinase (PK) deficiency: PK deficiency is the second most common RBC enzymopathy after G6PD deficiency, with an estimated incidence of 1 in 20,000 whites [2]. It is an autosomal recessive disorder characterized by a deficiency or complete absence of PK, which plays a vital role in the glycolytic pathway to produce pyruvate and adenosine triphosphate (ATP). Decreased ATP release shortens RBCs' life span due to membrane instability and results in a nonspherocytic hemolytic anemia, which can range from mild to a severe transfusion-dependent hemolytic anemia. Blood



Figures 2.1a and 2.1b These blood smears are from a child presenting with acute severe anemia and jaundice after ingestion of large quantities of fava beans. The arrows in Figure 2.1a point to the blister cells with a thin rim of cytoplasm, including some cells that are in the process of rupturing. The black arrows in Figure 2.1b are the bite cells that form after the rupture of blister cells.

smear can show occasional echinocytes, which are increased after splenectomy (Figure 2.2).

Hexokinase (HK) deficiency: HK is required for the initial step of glycolysis and the pentose shunt. HK deficiency is rare with most patients belonging to Northern European ancestry. This is a heterogeneous disease characterized by mild bone marrow aplasia, and macrocytosis on blood smear. Notably, the deficiency also leads to decreased levels of 2,3-bisphosphoglyceric acid (BPG) and a consequent leftward shift of the oxyhemoglobin dissociation curve.

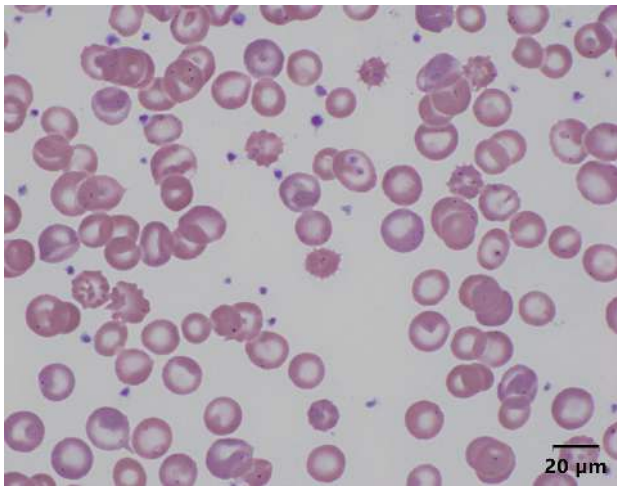


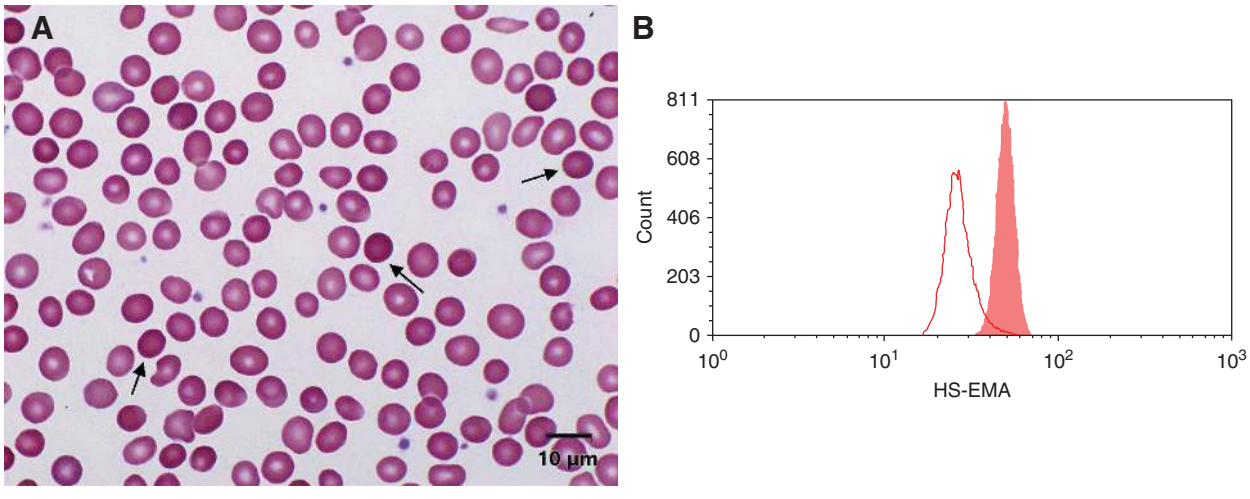
Figure 2.2 Blood smear displaying some degree of anisopoikilocytosis, characteristic echinocytes (densely staining spiculated cells with spicules of uniform size), among other polychromasia suggestive of reticulocytosis.

Triosephosphate isomerase (TPI) deficiency: TPI deficiency is a rare glycolytic enzyme defect associated with congenital hemolytic anemia and progressive neurological dysfunction. It is an autosomal recessive multisystem disorder, affecting cardiac, neuromuscular, and immune function. Reduced TPI catalytic activity in erythrocytes leads to elevated levels of dihydroxyacetone phosphate (DHAP), which decomposes to methylglyoxal. Methylglyoxal is highly reactive and produces advanced glycation end products and consequential oxidative stress. Moreover, it is neurotoxic and may contribute to the associated neurodegeneration seen in TPI deficiency [3].

Glucose 6-phosphate isomerase (GPI) deficiency: GPI deficiency is an autosomal recessive disorder characterized by non-spherocytic hemolytic anemia. GPI deficiency primarily affects erythrocytes as it interferes with the second step of the Embden-Meyerhof glycolytic pathway and ATP production. It results in varying degrees of anemia and genotype does not easily dictate phenotype.

Red Blood Cell Membrane Disorder

Hereditary spherocytosis (HS): This is the most common RBC membranopathy. It is a genetic disorder caused by alteration in the vertical associations between the RBC lipid bilayer and inner membrane skeleton. The most commonly implicated proteins are α-spectrin, β-spectrin, ankyrin, band



Figures 2.3a and 2.3b A. Peripheral blood film from a 12-year-old Caucasian female who was diagnosed with ankyrin deficiency-related hereditary spherocytosis (HS) at 3 weeks of life with no transfusion requirements. She presented with anemia and reticulocytosis of mild severity. Many of these RBCs have a spherical shape (spherocytes, black arrows). B. The eosin-5′-maleimide (EMA) binding assay is a flow-based assay measuring the amount of EMA binding to band 3 membrane protein. The mean fluorescence intensity of EMA-stained RBCs in this HS patient (shown by a thin orange line) is left shifted (lower) when compared with control RBCs (orange shaded), suggestive of reduced band 3 proteins seen in some HS patients.