62 - 66 Anemia and Polycythemia

66 Anemia and Polycythemia

One last feature of the red cells to assess before moving to the white blood cells is the distribution of the red cells on the smear. In most individuals, the cells lie side by side in a single layer. Some patients have red cell clumping (called agglutination) in which the red cells pile upon one another; it is seen in certain paraproteinemias and autoimmune hemolytic anemias. Another abnormal distribution involves red cells lying in single cell rows on top of one another like stacks of coins. This is called rouleaux formation and reflects abnor mal serum protein levels. Finally, one examines the white blood cells. Three types of granu locytes are usually present: neutrophils, eosinophils, and basophils, in decreasing frequency. Neutrophils are generally the most abundant white cell. They are round, are 10–14 µm wide, and contain a lobulated nucleus with two to five lobes connected by a thin chromatin thread. Bands are immature neutrophils that have not completed nuclear condensation and have a U-shaped nucleus. Bands reflect a left shift in neutrophil maturation in an effort to make more cells more rap idly. Neutrophils can provide clues to a variety of conditions. Vacu olated neutrophils may be a sign of bacterial sepsis. The presence of 1- to 2-µm blue cytoplasmic inclusions, called Döhle bodies, can reflect infections, burns, or other inflammatory states. If the neutrophil gran ules are larger than normal and stain a darker blue, “toxic granulations” are said to be present, and they also suggest a systemic inflammation. The presence of neutrophils with more than five nuclear lobes suggests megaloblastic anemia. Large misshapen granules may reflect the inher ited Chédiak-Higashi syndrome. Eosinophils are slightly larger than neutrophils, have bilobed nuclei, and contain large red granules. Diseases of eosinophils are associated with too many of them rather than any morphologic or qualitative change. They normally total less than one-thirtieth the number of neu trophils. Basophils are even more rare than eosinophils in the blood. They have large dark blue granules and may be increased as part of chronic myeloid leukemia. Lymphocytes can be present in several morphologic forms. Most common in healthy individuals are small lymphocytes with a small dark nucleus and scarce cytoplasm. In the presence of viral infections, more of the lymphocytes are larger, about the size of neutrophils, with abundant cytoplasm and a less condensed nuclear chromatin. These cells are called reactive lymphocytes. About 1% of lymphocytes are larger and contain blue granules in a light blue cytoplasm; they are called large granular lymphocytes. In chronic lymphoid leukemia, the small lymphocytes are increased in number, and many of them are ruptured in making the blood smear, leaving a smudge of nuclear material without a surrounding cytoplasm or cell membrane; they are called smudge cells and are rare in the absence of chronic lymphoid leukemia. Monocytes are the largest white blood cells, ranging from 15 to 22 µm in diameter. The nucleus can take on a

variety of shapes but usually appears to be folded; the cytoplasm is gray. Abnormal cells may appear in the blood. Most often, the abnormal cells originate from neoplasms of bone marrow–derived cells, includ ing lymphoid cells, myeloid cells, and occasionally red cells. More rarely, other types of tumors can get access to the bloodstream, and rare epithelial malignant cells may be identified. The chances of seeing such abnormal cells are increased by examining blood smears made from buffy coats, the layer of cells that is visible on top of sedimenting red cells when blood is left in the test tube for an hour. Smears made from finger sticks may include rare endothelial cells. Acknowledgment Figures in this chapter were borrowed from Williams Hematology, 7th edition, M Lichtman et al (eds). New York, McGraw-Hill, 2005; Hematology in General Practice, 4th edition, RS Hillman, KA Ault. New York, McGraw-Hill, 2005.

Thomas G. DeLoughery, Dan L. Longo

Anemia and Polycythemia Anemia is one of the most common medical problems in the world, affecting almost 2 billion people, and it is a significant source of mor bidity and reduced quality of life. Many causes of anemia are treatable, and some forms of anemia can be a clue to underlying disorders. Anemia and Polycythemia CHAPTER 66 NORMAL RED CELL PRODUCTION Hematopoiesis is the process by which the formed elements of blood are produced. The process is regulated through a series of steps begin ning with the hematopoietic stem cell. Stem cells are capable of pro ducing red cells, all classes of granulocytes, monocytes, platelets, and the cells of the immune system. The precise molecular mechanism by which the stem cell becomes committed to a given lineage is not fully defined. However, experiments in mice suggest that erythroid cells come from a common erythroid/megakaryocytic progenitor that does not develop in the absence of expression of the GATA-1 and FOG-1 (friend of GATA-1) transcription factors (Chap. 101). Following lineage commitment, hematopoietic progenitor and precursor cells become increasingly under the regulatory influence of growth factors and hormones. For red cell production, erythropoietin (EPO) is the primary regulatory hormone. EPO is required for the maintenance of committed erythroid progenitor cells that, in the absence of the hormone, undergo programmed cell death (apoptosis). The regulated process of red cell production is erythropoiesis, and its key elements are illustrated in Fig. 66-1. In the bone marrow, the first morphologically recognizable ery throid precursor is the pronormoblast. This cell can undergo four to five cell divisions, which result in the production of 16–32 mature red cells. With increased EPO production, or the administration of EPO as a drug, early progenitor cell numbers are amplified and, in turn, give rise to increased numbers of erythrocytes. The regulation of EPO production is linked to tissue oxygenation. In mammals, oxygen is transported to tissues bound to the hemo globin contained within circulating red cells. The mature red cell is 8 μm in diameter, anucleate, discoid in shape, and extremely pliable in order to traverse the microcirculation successfully; its membrane integrity is maintained by the intracellular generation of ATP. The biconcave disk provides the greatest amount of surface area for a given volume; this maximizes oxygen delivery. Normal red cell production results in the daily replacement of 0.8–1% of all circulating red cells in the body, since the average red cell lives 100–120 days. The organ responsible for red cell production is called the erythron. The erythron is a dynamic organ made up of a rapidly proliferating pool of marrow erythroid precursor cells and a large mass of mature circulating red Iron folate B12 Erythroid marrow Red cell mass Red cell destruction Erythropoietin Plasma volume Hb Concentration Kidney tissue PO2 O2 Consumption Heart Lungs Vessels Atmospheric O2 levels FIGURE 66-1 The physiologic regulation of red cell production by tissue oxygen tension. Hb, hemoglobin.

Serum erythropoietin (mU/mL)

Normal 9–26 mU/mL PART 2 Cardinal Manifestations and Presentation of Diseases

Hemoglobin (g/dL) FIGURE 66-2 Erythropoietin (EPO) levels in response to anemia. When the hemoglobin level falls to 120 g/L (12 g/dL), plasma EPO levels increase logarithmically. In the presence of chronic kidney disease or chronic inflammation, EPO levels are typically lower than expected for the degree of anemia. As individuals age, the level of EPO needed to sustain normal hemoglobin levels appears to increase. (Reproduced with permission from RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) blood cells. The size of the red cell mass reflects the balance of red cell production and destruction. The physiologic basis of red cell produc tion and destruction provides an understanding of the mechanisms that can lead to anemia. The physiologic regulator of red cell production, the glycoprotein hormone EPO, is produced and released by peritubular capillary lining cells within the kidney. These cells are highly specialized epitheliallike cells. A small amount of EPO is produced by hepatocytes. The fundamental stimulus for EPO production is the availability of oxygen (O2) for tissue metabolic needs. Key to EPO gene regulation is hypoxia-

inducible factor (HIF)-1α. In the presence of O2, HIF-1α is hydroxylated at a key proline, allowing HIF-1α to be ubiquitinated and degraded via the proteasome pathway. If O2 becomes limiting, this critical hydrox ylation step does not occur, allowing HIF-1α to partner with other proteins, translocate to the nucleus, and upregulate the expression of the EPO gene, among others. Impaired O2 delivery to the kidney can result from a decreased red cell mass (anemia), impaired O2 loading of the hemoglobin molecule, a high O2 affinity mutant hemoglobin (hypoxemia), or, rarely, impaired blood flow to the kidney (e.g., renal artery stenosis). EPO governs the day-to-day production of red cells, and ambient levels of the hormone can be measured in the plasma by sensitive immunoassays with the normal level being 10–25 U/L. When the hemoglobin concentration falls below 100–120 g/L (10–12 g/dL), plasma EPO levels increase in proportion to the severity of the anemia (Fig. 66-2). In circulation, EPO has half-life time of 6–9 h. EPO acts by binding to specific recep tors on the surface of marrow erythroid precursors, inducing them to proliferate and to mature. With EPO stimulation, red cell production can increase four- to fivefold within a 1- to 2-week period, but only in the presence of adequate nutrients, especially iron. The functional capacity of the erythron, therefore, requires normal renal production of EPO, a functioning erythroid marrow, and an adequate supply of substrates for hemoglobin synthesis. A defect in any of these key com ponents can lead to anemia. APPROACH TO THE DIAGNOSIS OF ANEMIA There are four initial steps in the diagnosis of anemia. The first step is a good history; one should ask about previous episodes of anemia, any previous therapy such as iron pills or transfusions, family history of anemia, and being a blood donor. Attention should also be paid to symptoms of disease that can lead to anemia. For example, diarrhea can be a sign of celiac disease or inflammatory bowel disease. In people who menstruate, a good menstrual history should be taken, including

duration of periods, number of pads/tampons used, and passing large clots. The physical exam should be focused on the consequences of ane mia such as a cardiac flow murmur and clues to the cause of the anemia such as the presence of splenomegaly or blood in the stool. A review of the blood smear is a crucial part of any evaluation for anemia as changes in red cell morphology can

point to specific causes of anemia. Measuring the number of new red cells—the reticulocyte count—assesses the function of the bone marrow. Both of these tests are discussed in more detail below. ■ ■SIGNS AND SYMPTOMS OF ANEMIA Patients who gradually develop anemia over months can tolerate amaz ingly low hemoglobin levels due to compensatory mechanisms. The overall health status of an individual will also determine their response to anemia. Since blood delivers oxygen, many of the clinical signs are related to lack of oxygen delivery, such as tiredness and shortness of breath. On exam, this is manifested by paleness of the mucosa/con junctiva and resting tachycardia. If patients have atherosclerosis, they may suffer ischemic symptoms such as angina or transient ischemic attacks/strokes. Also, in cases of nutritional deficiency, there may be symptoms related to that such as pica in iron deficiency or neuropathy in B12 deficiency. In general, the signs and symptoms of anemia are very unreliable in predicting the patient's hematocrit. ■ ■COMPENSATION FOR ANEMIA The body has a tremendous ability to compensate for anemia. This compensation improves over time, so inherited anemias or those that occur gradually are better tolerated than acute anemias. There are three physiologic compensatory mechanisms for anemia, which are described below. The first compensatory mechanism is an increase in cardiac out put. Oxygen delivery to tissues is a function of cardiac output times hemoglobin, so if the hemoglobin is lower, the cardiac output rises to compensate. Therefore, patients with a limited cardiac reserve will have symptoms of anemia at a higher hematocrit than patients with normal cardiac function. This compensatory mechanism can occur within minutes. The second compensatory mechanism is via increased levels of 2,3-disphosphoglyceric acid (2,3-DPG). 2,3-DPG decreases oxygen affinity for hemoglobin by increasing the stability of deoxygenated hemoglobin. While this may seem counterintuitive, this decreased affinity leads to more oxygen delivery in the tissues. The high ambient oxygen tension in the alveoli results in full oxygenation of hemoglobin despite this decreased oxygen affinity. This mechanism takes place over hours to days. Finally, over time, plasma volume increases which preserves cardiac output and maintains blood pressure and in theory may lower blood viscosity. In some cases, this increased plasma volume can overwhelm the heart, leading to edema and other signs of heart failure. This com pensation for anemia occurs over weeks. ■ ■LABORATORY TESTING Because most anemia develops slowly, anemia is most often detected by finding low hemoglobin and hematocrit levels on a complete blood count. Current testing of the blood count is performed by electronic cell counters that can directly measure red cell size (mean corpuscular volume [MCV]), number of red cells, and hemoglobin levels with a variety of measurements derived from these values. For example: × Hematocrit = Red cell number/liter MCV

The classic red cell indices reflect red cell size and hemoglobin con centration (Table 66-1). The index most commonly used is the MCV because the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration will trend with the MCV. Normal variations in hemoglobin and hematocrit with age are shown in Table 66-2. A normal peripheral blood smear is shown in Fig. 66-3.

TABLE 66-1 Red Cell Indices NORMAL VALUES COMMENT INDEX FORMULA Mean corpuscular volume (MCV) Hct/RBC

count × 10 85–95 fL RBC size Mean corpuscular hemoglobin concentration Hgb/Hct × 100 33.8–34.2 g/dL Changes very little in most cases of anemia; therefore, of limited value Mean corpuscular hemoglobin (MCH) Hgb/RBC

count × 10 28.5–32.3 pg Varies linearly with MCV; therefore, of limited additional value Abbreviations: Hct, hematocrit; Hgb, hemoglobin; RBC, red blood cell. ■ ■BLOOD SMEAR Blood is one of the few tissues of the body that allows direct visual ization. The morphology of the red cell on the blood smear can be a diagnostic clue to a variety of anemias. As a complement to the red cell indices, the blood smear also reveals variations in cell size (aniso cytosis) and shape (poikilocytosis). The degree of anisocytosis usually correlates with increases in the red cell distribution width (RDW) or the range of cell sizes. Poikilocytosis suggests a defect in the maturation of red cell precursors in the bone marrow or fragmentation of circu lating red cells. The blood smear may also reveal polychromasia—red cells that are slightly larger than normal and grayish blue in color on the Wright-Giemsa stain. These cells are reticulocytes that have been released prematurely from the bone marrow, and their color represents residual amounts of ribosomal RNA. These cells appear in circulation in response to EPO stimulation or to architectural damage of the bone marrow (e.g., fibrosis, infiltration of the marrow by malignant cells) that results in their disordered release from the marrow. The appear ance of nucleated red cells, Howell-Jolly bodies, target cells, sickle cells, and other red cell morphology changes may provide clues to specific disorders (Figs. 66-4 to 66-12). (See also Table 66-3 and the Atlas of Hematology, Chap. A6.) ■ ■BURR CELLS Also called echinocytes, these cells have multiple small projections. This can be a laboratory artifact but is most often seen with liver dis ease or uremia. ■ ■ELLIPTOCYTES These elongated red cells may be seen most commonly in heredity elliptocytosis or with severe iron deficiency. ■ ■HOWELL-JOLLY BODIES These are small remnants of the red cell nucleus. Usually these are rap idly cleared by the spleen, but if the spleen is missing or not function ing, these red cell inclusions may be seen. ■ ■HYPOCHROMIA/MICROCYTOSIS These are cells that have larger areas of central pallor than normal. This is a sign of inadequate hemoglobinization of the red cell. This can be seen in iron deficiency, thalassemia, or in the rare sideroblastic anemia. TABLE 66-2 Changes in Normal Hemoglobin/Hematocrit Values with Age, Sex, and Pregnancy AGE/SEX HEMOGLOBIN, g/dL HEMATOCRIT, % At birth

Childhood

Adolescence

Adult man 16 (±2) 47 (±6) Adult woman (menstruating) 13 (±2) 40 (±6) Adult woman (postmenopausal) 14 (±2) 42 (±6) During pregnancy 12 (±2) 37 (±6) Source: From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.

Anemia and Polycythemia CHAPTER 66 FIGURE 66-3 Normal blood smear (Wright stain). High-power field showing normal red cells, a neutrophil, and a few platelets. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) FIGURE 66-4 Severe iron-deficiency anemia. Microcytic and hypochromic red cells smaller than the nucleus of a lymphocyte associated with marked variation in size (anisocytosis) and shape (poikilocytosis). (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) FIGURE 66-5 Macrocytosis. Red cells are larger than a small lymphocyte and well hemoglobinized. Often macrocytes are oval shaped (macro-ovalocytes). (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.)

PART 2 Cardinal Manifestations and Presentation of Diseases FIGURE 66-6 Howell-Jolly bodies. In the absence of a functional spleen, nuclear remnants are not culled from the red cells and remain as small homogeneously staining blue inclusions on Wright stain. (From M Lichtman et al (eds): Williams Hematology, 7th ed. New York, McGraw-Hill, 2005; RS Hillman, KA Ault: Hematology in General Practice, 4th ed. New York, McGraw-Hill, 2005.) FIGURE 66-7 Red cell changes in myelofibrosis. The left panel shows a teardropshaped cell. The right panel shows a nucleated red cell. These forms can be seen in myelofibrosis. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) FIGURE 66-8 Target cells. Target cells have a bull's-eye appearance and are seen in thalassemia and in liver disease. (From M Lichtman et al (eds): Williams Hematology, 7th ed. New York, McGraw-Hill, 2005; RS Hillman, KA Ault: Hematology in General Practice, 4th ed. New York, McGraw-Hill, 2005.)

FIGURE 66-9 Red cell fragmentation. Red cells may become fragmented in the presence of foreign bodies in the circulation, such as mechanical heart valves, or in the setting of thermal injury. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) FIGURE 66-10 Uremia. The red cells in uremia may acquire numerous regularly spaced, small, spiny projections. Such cells, called burr cells or echinocytes, are readily distinguishable from irregularly spiculated acanthocytes shown in Fig. 66-11. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGrawHill, 2010.) FIGURE 66-11 Spur cells. Spur cells are recognized as distorted red cells containing several irregularly distributed thorn-like projections. Cells with this morphologic abnormality are also called acanthocytes. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.)

FIGURE 66-12 Reticulocytes. Methylene blue stain demonstrates residual RNA in newly made red cells. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) ■ ■MACRO-OVALOCYTES These are red cells that are larger in size and slightly oval-shaped. This is caused by disruption of DNA synthesis in the developing red cell from B12/folate deficiency, certain drugs, or bone marrow disorders. ■ ■NUCLEATED RED BLOOD CELLS The nucleus of the red cell is cleared by the marrow before being released; any remaining are cleared by the spleen. The presence of nucleated red cells can be seen with asplenia, severe marrow stress such as massive bleeding or hemolysis, and many bone marrow disorders, especially those that disrupt the marrow architecture such as marrow fibrosis. ■ ■SCHISTOCYTES These are fragments of red cells that have been physically disrupted by blockage in the blood vessels by platelets or fibrin strands or by external forces. They can be seen in a wide variety of processes such as thrombotic thrombocytopenic purpura or march hemoglobinuria. ■ ■SICKLE CELLS As the name implies, the blood cells are sickle-shaped. This can be seen in hemoglobin defects, most commonly in sickle cell anemia. TABLE 66-3 Red Cell Morphology PATHOPHYSIOLOGY DISEASE STATES Macro-ovalocytes Hemoglobin excess (nuclear-cytoplasmic dyssynchrony) B12 and/or folate deficiencies Myelodysplasia Spherocytes Loss of membrane Hereditary spherocytosis Immune hemolytic anemia Hypochromia Hemoglobin deficiency (corresponds to low mean corpuscular hemoglobin concentration) Iron deficiency Thalassemia Sideroblastic anemia Anemia of chronic disease Schistocyte Red blood cell fragmentation Microangiopathic hemolysis Heart-valve hemolysis Sickle cell Hemoglobin polymerization Sickle cell disease Target cell Relative membrane excess Liver disease Thalassemia Hemoglobinopathy Polychromatophilia Persistence of polyribosomes (corresponds to high reticulocyte count) Hemolytic anemia Basophilic stippling Pathologic precipitation of polyribosomes Thalassemia Lead poisoning

■ ■SPHEROCYTES These are cells that on the blood smear lack central pallor as they are spherical and not biconcave disks. This can be caused by any process that leads to the loss of red cell membrane. This membrane loss turns the biconcave disk into a sphere as this is the shape with the least amount of surface area for a given volume. Spherocytes are most commonly seen in autoimmune hemolytic anemia and hereditary spherocytosis.

■ ■SPUR CELLS As opposed to burr cells, spur cells have fewer longer projections from the red cell and are most commonly seen in severe liver disease. They result from cholesterol crystal formation in the red cell membrane due to the abnormal lipid metabolism in liver disease. These crystals get caught in narrow passages of the spleen, resulting in these projections. They can also be seen in asplenic patients and those with McLeod blood group. Anemia and Polycythemia CHAPTER 66 ■ ■TARGET CELLS These can be seen where there is a redundant red cell membrane. In thalassemia, there is less hemoglobin filling the red cell, and in liver disease, there is excess red cell membrane. ■ ■TEARDROP CELLS These are cells that look like a teardrop and are most often seen with disruption of marrow architecture or severe iron deficiency. ■ ■THE RETICULOCYTE COUNT Red cells still contain mRNA for about 24 h after being released by the marrow. This mRNA can be detected by staining, and these cells are called “reticulocytes.” The number of reticulocytes present is a measure of red cell production and is helpful in separating increased destruction from anemias due to impaired red cell production. The oldest method of determining reticulocyte counts is to stain the blood smear with new methylene blue and determine the percentage of red cells that take up the stain; this is the reticulocyte count. However, this needs to be adjusted for the hematocrit as the reticulocyte percent age will appear to increase with decreasing blood counts when the abso lute count has not actually increased. For example, a reticulocyte count of 1% will increase to 2% with a hematocrit of 23% (Fig. 66-13). There fore, the reticulocyte count needs to be corrected for the hematocrit: Corrected reticulocyte count = Measured reticulocyte count × (Patient hematocrit/45%) (Normal hematocrit) Many newer complete blood count machines can directly quantitate the reticulocytes present in a given volume of blood, and this is the absolute reticulocyte count. Since this is per volume, no adjustment for anemia is required. Absolute reticulocyte number = Reticulocyte count/100 × Red blood cell number ■ ■BONE MARROW EXAM The bone marrow may be easily sampled in a bedside procedure and can be helpful in the diagnosis of anemia or other hematologic pro cesses. With bone marrow aspiration, the marrow is removed from the posterior iliac spine and stained with Wright’s or Wright-Giemsa stains (photomicrographs of normal marrow [Fig. 66-14], marrow showing 2% reticulocytes 10% reticulocytes 1% reticulocytes FIGURE 66-13 Interpretation of reticulocyte counts. If the absolute reticulocyte count is not provided, the percent reticulocytes must be adjusted based on the hematocrit.

PART 2 Cardinal Manifestations and Presentation of Diseases FIGURE 66-14 Normal bone marrow. This is a low-power view of a section of a normal bone marrow biopsy stained with hematoxylin and eosin (H&E). Note that the nucleated cellular elements account for ~40–50% and the fat (clear areas) accounts for ~50–60% of the area. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) erythroid hyperplasia [Fig. 66-15], and marrow showing myeloid hyperplasia [Fig. 66-16] are shown). The biopsy disturbs the marrow architecture but allows for examination of individual cell morphology, determination of a differential cell count, and determination of the myeloid-to-erythroid ratio. This ratio is normally 2.5:1 (range 2:1–5:1). Bone marrow iron stores can be examined by use of an appropriate stain. Samples can also be drawn for

specialized testing such as flow cytometry, genetic testing, and/or microbiological testing. For a marrow biopsy, a “core” sample of marrow is removed intact from the iliac spine and then decalcified, sectioned, and stained with hematoxylin and eosin. This technique reveals the undisturbed mar row architecture and is useful for the determination of cellularity and the presence of abnormal marrow infiltrates or fibrosis. The normal bone marrow is ~50% cellular (i.e., half hematopoietic cells and half fat cells). In general, the marrow fat percentage is roughly equivalent to the patient’s age. A bone marrow examination is a necessary diagnostic study in situ ations where the blood and clinical findings suggest the possibility of marrow infiltration by abnormal or nonhematopoietic elements or the likelihood of generalized marrow dysfunction (e.g., aplastic anemia or myelodysplasia). In most situations, it is necessary to perform both an aspiration and a biopsy. The biopsy should precede the aspiration. If the aspiration is done first, the subsequent biopsy tends to be distorted by the bleeding induced by the aspiration. FIGURE 66-15 Erythroid hyperplasia. This marrow shows an increase in the fraction of cells in the erythroid lineage as might be seen when a normal marrow compensates for acute blood loss or hemolysis. The myeloid/erythroid (M/E) ratio is about 1:1. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.)

FIGURE 66-16 Myeloid hyperplasia. This marrow shows an increase in the fraction of cells in the myeloid or granulocytic lineage as might be seen in a normal marrow responding to infection. The myeloid/erythroid (M/E) ratio is >3:1. (From RS Hillman et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010.) General indications for bone marrow examination are:

1. Circulating immature cells (e.g., blasts)
2. Severe pancytopenia
3. Very low reticulocyte counts (<0.1%)
4. Circulating nucleated red blood cells
5. Evidence of marrow infiltration (teardrop red cells, nucleated red blood cells)
6. Staging of certain malignancies (e.g., lymphoma)
7. Unexplained severe anemia ■ ■ANEMIA DEFINITION AND CLASSIFICATION Anemia is simply defined as blood counts below normal for a given population. There are two general ways of classifying anemia (Table 66-4). A time-honored and still practical way is by the size of the red cell, as this can help guide diagnostic workup. Another method is by mechanism of anemia. Anemia classification by red cell size was pioneered by the hema tologist Max Wintrobe. In this classification, anemia is grouped by MCV, with those with smaller MCV called microcytic; normal MCV, normocytic; and larger MCV, macrocytic. Microcytic anemia is due to any process that interferes with hemo globin production; less hemoglobin leads to smaller red cells. This would include: • Thalassemia: defects of hemoglobin protein synthesis • Iron deficiency: unable to make heme • Anemia of chronic disease: lack of iron delivery to red cell (note: the anemia of chronic disease is often associated with normal red cell volume; the mechanism remains the inhibition of iron reutilization based on cytokine inhibition) • Sideroblastic anemias: defects of heme synthesis Macrocytic anemia may be due to defects in DNA synthesis and has two subdivisions: round macrocytes and oval macrocytes. Oval macrocytes are due to defects in DNA synthesis, while round macro cytes are caused by membrane defects. Unlike the limited differential of microcytosis, many processes can lead to macrocytosis. The classic macrocytic anemias are due to B12 and folate deficiency, but these account for a minority

of macrocytic anemias. Normocytic anemia includes all other causes of anemia; the broad differential for this classification of anemia is a drawback of this ane mia classification scheme. An alternative method of classifying anemias is by mechanism. The absolute reticulocyte count is an important first indicator of mechanism. If reticulocytes are elevated, the mechanism of anemia is increased loss or destruction of red cells. If reticulocytes are low or

TABLE 66-4 Classification of Anemia By Size of Red Cell Microcytic Anemia of inflammation Iron deficiency Sideroblastic Thalassemia Macrocytic Oval Macrocytes Vitamin B12 deficiency Folate deficiency Medications (chemotherapy, some antiseizure medications) Myelodysplasia Round Macrocytes Alcohol use Dysproteinemia Hypothyroidism Hypoxia Liver disease Reticulocytosis Smoking Normocytic Aplastic anemia Endocrinopathies Marrow invasion Myeloma Pure red cell aplasia Renal disease By Mechanism Hyperproduction Bleeding Hemolysis Acquired Autoimmune Mechanical Congenital Hemibrain defect Hemoglobinopathies Enzyme defects Underproduction Nutritional Deficiency Vitamin B12 Copper Folate Iron Vitamin C Absence of Red Cell Precursors Aplastic anemia Pure red cell aplasia Lack of Erythropoietin Anemia of inflammation Renal disease Anemia of aging Marrow Replacement Granulomatous disease Infection Neoplasm Stem Cell Defects Acute leukemia Chronic leukemia Myelodysplasia

inappropriately low for the level of anemia, the mechanism is impaired production of red cells. Impaired production includes:

• Nutritional: deficiencies such as iron or B12 • Marrow replacement by infection, cancer, granulomas • Absence of red cell precursors: aplastic anemia, pure red cell aplasia • Stem cell defects: myelodysplasia • Lack of EPO: renal disease, anemia of inflammation, anemia of aging ■ ■HYPERPRODUCTION: HEMOLYSIS/BLEEDING In general, the finding of an elevated reticulocyte count should raise the suspicion of hemolysis. Hemolysis is where red cell breakdown is accelerated. This can be due to extrinsic causes such as autoimmune hemolytic anemia or processes intrinsic to the red cell such as enzyme defects. Laboratories will show evidence of red cell breakdown includ ing high lactate dehydrogenase (LDH; an enzyme found in abundance in red cells), low haptoglobin (a serum protein that salvages free hemoglobin), and increased blood/urine free hemoglobin. Often the blood smear will show evidence of hemolysis with abnormal cells such as schistocytes or spherocytes. Testing is then focused on finding the particular cause of hemolysis. For example, autoimmune hemolytic anemia will present with spherocytes, high LDH, and low haptoglobin; testing will show the presence of autoantibodies on red cells. Anemia and Polycythemia CHAPTER 66 ■ ■UNDERPRODUCTION At least 75% of all cases of anemia are hypoproliferative in nature. The most common cause is mild to moderate iron deficiency or inflammation. ■ ■NUTRITIONAL Production of red cells is dependent on a consistent supply of nutrients. The most vital nutrient is iron; four atoms are required for every hemo globin molecule, with about 1 billion iron atoms in every red cell. Iron deficiency is the most common nutritional deficiency worldwide and is very common in premenopausal women due to obligate menstrual losses. The laboratory measurements that reflect the availability of iron for hemoglobin synthesis include the serum iron, the total ironbinding capacity (TIBC), and the percent transferrin saturation. The percent transferrin saturation is derived by dividing the serum iron level (× 100) by the TIBC. The normal serum iron ranges from 9 to

27 µmol/L (50–150 µg/dL), whereas the normal TIBC is 54–64 µmol/L (300–360 µg/dL); the normal transferrin saturation ranges from 25 to 50%. A diurnal variation in the serum iron leads to a variation in the percent transferrin saturation. The serum ferritin is used to evaluate total-body iron stores. Adult males have serum ferritin levels that aver age ~100 µg/L, corresponding to iron stores of ~1 g. Adult premeno pausal females have lower serum ferritin levels averaging 30 µg/L, reflecting lower iron stores (~300 mg). A serum ferritin less than 30 µg/L indicates depletion of body iron stores. However, ferritin is also an acute-phase reactant and, in the presence of acute or chronic inflammation, may rise several-fold above baseline levels. As a rule, a serum ferritin >200 µg/L means there is at least some iron in tissue stores. The classic finding of iron deficiency in the blood is a microcytic anemia, but this can be absent in early iron deficiency or when other confounding issues are present like liver disease. Laboratory findings show a low serum ferritin and may show a low iron saturation. Vitamin B12 is another crucial nutrient. The red cell requires vitamin B12 in order to synthesize DNA, so deficiency results in impaired DNA synthesis. This leads to the classic hematologic finding of B12 deficiency—macrocytosis. In addition, the neutrophils will show hypersegmentation and the marrow will be remarkably hypercellular with large abnormal red cell precursors. Folate is also required for red cell DNA synthesis; deficiencies pres ent similar to B12 deficiency, and these two are grouped together as “megaloblastic anemia.” In countries that supplement flour with folate, deficiencies are rare but may still be seen in those with a very poor diet or severe malabsorption. Other nutrient deficiencies lead to anemia, but these tend to be more unusual. Copper deficiency is often associated with neurologic

disease and neutropenia. Vitamin C deficiency can be associated with severe anemia as well as the classic clinical findings of scurvy.

■ ■MARROW REPLACEMENT Several processes, most notably infections and neoplasms, can invade the marrow and crowd out hematopoietic elements. Many infections can occupy the marrow including infections such as histoplasmosis or tuberculosis. Metastasis to the marrow from any tumor can also lead to anemia. For example, patients with prostate cancer may have their marrow replaced by tumor, leading to severe anemia. More commonly in older patients, multiple myeloma can present with anemia. In many cases of marrow replacement, the blood smear can show a myelophthisic picture with the presence of nucleated red cells, tear drop cells, and immature white cells. These findings mandate marrow examination. PART 2 Cardinal Manifestations and Presentation of Diseases ■ ■LACK OF ERYTHROPOIETIN As noted earlier, EPO is essential for red cell production. Since the kidney is the primary source of this hormone, renal disease is often associated with anemia. While anemia is almost always present with a glomerular filtration rate of <30 mL/min/1.73 m2, some patients will be anemic with higher levels of renal function. Certain medications, most notably angiotensin-converting enzyme inhibitors, can also suppress EPO production. Inflammatory cytokines such as tumor necrosis factor can decrease production of EPO. One of the hallmarks of the anemia of inflamma tion is a low EPO level for any given degree of anemia. In addition, inflammation will increase levels of hepcidin, which then blocks iron absorption and its release from stores. Anemia is common in people over age 65 years; 11% of community-

dwelling and up to 40% of nursing home residents over age 65 are ane mic. Anemia is associated with increased risk of death, hospitalization, and frailty. EPO levels tend to be lower than expected for the degree of anemia, and administration of EPO can increase the hemoglobin level, but it is unclear whether the adverse consequences of anemia are reduced by treatment. ■ ■ABSENCE OF

RED CELL PRECURSORS Aplastic anemia is a disease where the marrow is very hypocellular. This can be caused by autoimmune processes, reactions to certain medications, or as a result of toxins/radiation. The bone marrow shows markedly reduced levels of all precursors, and other blood elements such as platelets and neutrophils will be reduced. Pure red cell aplasia is defined as only the red cell precursors in the marrow being reduced. Autoimmune processes that target only the red cell can lead to this, as well as infections with parvovirus B19. The patient will present with a very low reticulocyte count, and the bone marrow will show absent or markedly reduced erythroid cells. ■ ■STEM CELL DEFECTS Finally, processes intrinsic to the marrow can lead to anemia. Primary neoplasms of the marrow such as acute myelogenous leukemia or chronic myelogenous leukemia will often have a component of severe anemia. Patients most often will have elevated white cell counts with immature cells present; rare patients will present only with anemia. Myelodysplastic syndromes are caused by stem cell defects that lead to impaired marrow function. Patients can present with only a mac rocytic anemia but can also present with pancytopenia. The natural history can vary; some patients' courses are measured in years while others rapidly evolve into acute leukemia. Diagnosis of leukemia and myelodysplasia is most often made by bone marrow testing. Currently, the pathologic examination is augmented by molecular testing for mutations that are diagnostic of leukemia or myelodysplasia. ■ ■EVALUATION OF ANEMIA The results of the complete blood count and reticulocyte count together can guide further testing (Fig. 66-17). If the reticulocyte count is high and bleeding has been ruled out, then specific testing for hemolysis can be performed, including LDH, haptoglobin, and direct

antibody testing. If there are clues present on the blood smear such as sickle cells, then focused testing can be done. If the reticulocyte count is not elevated, workup is guided by the MCV. If the MCV is low, a ferritin should be performed, and if normal, then the patient should be assessed for thalassemia. Another clue to distinguishing iron deficiency from thalassemia is the RDW. Iron defi ciency is characterized by a high RDW because of anisocytosis. The red cells in thalassemia are more homogeneous in volume and have a low RDW. Anemia of inflammation is a diagnosis of exclusion and usually occurs in the presence of an inflammatory disorder such as cancer or infection. The workup of macrocytosis is guided by the blood smear. If signs of megaloblastic anemia are seen (hypersegmented neutrophils and macro-ovalocytes), then B12 and folate levels should be assessed. Some experts distinguish large round red cells from large oval red cells on smear. If macro-ovalocytes are seen and nutritional tests are normal, this raises the concern for myelodysplasia, and bone marrow testing is the next step. With round macrocytosis, often the history can provide clues, such as presence of liver disease or alcoholism, for example. Workup of normocytic anemia involves assessment of renal func tion, EPO levels, and other disease processes that can lead to anemia and consideration of marrow exam if no other cause is apparent. Additional laboratory tests may be of value in confirming specific diagnoses. For details of these tests and how they are applied in spe cific disorders, see Chaps. 102 to 106. TREATMENT Anemia While definitive treatment of anemia requires knowing the cause, some patients may need treatment with transfusions to support them during the diagnostic workup. Clear indications for blood transfusion are hypotension or signs of cardiac compromise such as angina. For some patients such as those with bone marrow failure, transfusion may be needed to support them while definitive therapy is being performed. Often, the cause of the anemia is multifactorial. For example, a patient with severe rheumatoid arthritis who has been taking anti-inflammatory drugs may have a hypoproliferative anemia associated with chronic inflammation as well as chronic blood loss associated with intermittent gastrointestinal bleeding. In every circumstance, it is important to

evaluate the patient’s iron status fully before and during the treatment of any anemia. Transfusion is discussed in Chap. 118; iron therapy is discussed in Chap. 102; treatment of megaloblastic anemia is discussed in Chap. 104; treatment of other entities is discussed in their respective chap ters (sickle cell anemia, Chap. 103; hemolytic anemias, Chap. 105; aplastic anemia and myelodysplasia, Chap. 107). Therapeutic options for the treatment of anemias have expanded dramatically during the past 30 years. Blood component therapy is available and safe. Recombinant EPO as an adjunct to anemia man agement has transformed the lives of patients with chronic renal failure on dialysis and reduced transfusion needs of anemic can cer patients receiving chemotherapy. Transforming growth factor β inhibitors (anemia associated with myelodysplastic syndrome), complement inhibitors (associated with paroxysmal nocturnal hemoglobinuria), and other therapies are making a difference in the quality of life in selected types of anemia. Eventually, patients with inherited disorders of globin synthesis or mutations in the globin gene, such as sickle cell disease, may benefit from the successful introduction of targeted genetic therapy (Chap. 483). POLYCYTHEMIA Polycythemia is defined as an increase in the hemoglobin above nor mal. This increase may be real or only apparent because of a decrease in plasma volume (spurious or relative polycythemia). The term erythro cytosis may be used interchangeably with polycythemia, but some draw a distinction between them: erythrocytosis implies documentation of

Low absolute reticulocyte count High absolute reticulocyte count Red cell morphology MCV Normal hypoproliferative Elevated nuclear defects Low cytoplasmic defects Ferritin Normal or elevated Low Anemia of inflammation* Sideroblastic anemia Thalassemia Iron deficiency Look for marrow damage • Infiltration/fibrosis • Aplasia OR stimulation ↓ • Inflammation: ↑ hepcidin, ↓ iron reutilization • Metabolic defect • Renal disease • Aging (↓ EPO) EPO level • BUN/creatinine • SPEP/light chains • Bone marrow exam *MCV may be normal B12/folate deficiency Myelodysplasia Medication effect FIGURE 66-17 The physiologic classification of anemia. BUN, blood urea nitrogen; CBC, complete blood count; EPO, erythropoietin level; MCV, mean corpuscular volume; SPEP, serum protein electrophoresis. increased red cell mass, whereas polycythemia refers to any increase in red cells. Often patients with polycythemia are detected through an incidental finding of elevated hemoglobin or hematocrit levels. Concern that the hemoglobin level may be abnormally high is usu ally triggered at 17 g/dL (170 g/L) for men and 15 g/dL (150 g/L) for women. Hematocrit levels of >50% in men or >45% in women may be abnormal. Hematocrit levels of >60% in men and >55% in women are almost invariably associated with an increased red cell mass. Given that the machine that quantitates red cell parameters actually measures hemoglobin concentrations and calculates hematocrits, hemoglobin levels may be a better index. Features of the clinical history that are useful in the differential diagnosis include smoking, currently living at high altitude, a history of diuretic use, congenital heart disease, sleep apnea, or chronic lung dis ease. It is also useful to inquire about the use of testosterone, EPO, and SGLT2 (sodium-glucose transport protein 2) inhibitors (gliflozins). Patients with polycythemia may be asymptomatic or experience symptoms related to the increased red cell mass or the underlying disease process that leads to the increased red cell mass. The dominant symptoms from an increased red cell mass are related to hyperviscosity and thrombosis (both venous and arterial), because the blood viscosity increases logarithmically at hematocrit levels of >55%. Manifestations include neurologic symptoms such as vertigo, tinnitus, headache,

Anemia CBC, reticulocyte count Anemia and Polycythemia CHAPTER 66 Hemolysis/ hemorrhage Blood loss Intravascular hemolysis Metabolic defect Membrane abnormality Smear

Hemoglobinopathy Round macrocytes Oval macrocytes Immune destruction Alcohol excess Liver disease Hypersegmentation? Fragmentation hemolysis Yes No and visual disturbances. Hypertension is often present. Patients with polycythemia vera may have aquagenic pruritus, symptoms related to hepatosplenomegaly, easy bruising, epistaxis, or bleeding from the gas trointestinal tract. Peptic ulcer disease is common. Such patients also may present with digital ischemia, Budd-Chiari syndrome, or hepatic or splenic/mesenteric vein thrombosis. Patients with hypoxemia may develop cyanosis on minimal exertion or have headache, impaired mental acuity, and fatigue. The physical examination usually reveals a ruddy complexion. Splenomegaly favors polycythemia vera as the diagnosis (Chap. 108). The presence of cyanosis or evidence of a right-to-left shunt suggests congenital heart disease presenting in the adult, particularly tetralogy of Fallot or Eisenmenger's syndrome (Chap. 280). Increased blood viscosity raises pulmonary artery pressure; hypoxemia can lead to increased pulmonary vascular resistance. Together, these factors can produce cor pulmonale. Polycythemia can be spurious (related to a decrease in plasma volume; Gaisbock's syndrome), primary, or secondary in origin. The secondary causes are nearly all mediated by EPO: either a physiologi cally adapted appropriate level based on tissue hypoxia (lung disease, high altitude, carbon monoxide [CO] poisoning, high-affinity hemo globinopathy) or an abnormal overproduction (renal cysts, renal artery stenosis, tumors with ectopic EPO production). A rare familial form of

Elevated red cell count, hemoglobin, or hematocrit Obtain prior blood counts If elevated, exclude hypoxia (O2 saturation <93% at rest or exercise) If no prior elevation and asymptomatic, repeat the studies in 1 month If persistent or symptomatic or if elevation is substantial and O2 saturation is normal PART 2 Cardinal Manifestations and Presentation of Diseases Obtain a serum erythropoietin level Normal or low Elevated JAK2, LNK mutation assays (VAF) Renal disease Tumors Chuvash polycythemia (VHL) EGLN1 (PHD2) (HIF-1 alpha) EPAS1 (HIF-2 alpha) High O2 affinity hemoglobin Positive (VAF ≥ 5%) Negative or VAF ≤ 5% Polycythemia vera Polycythemia vera Renal disease Tumors Erythropoietin receptor mutation High O2 affinity hemoglobin FIGURE 66-18 An approach to the differential diagnosis of patients with an elevated hemoglobin (possible polycythemia). VAF, variant allele frequency; VHL, von HippelLindau syndrome. (Reproduced with permission from Jerry L. Spivak.) polycythemia is associated with normal EPO levels but hyperrespon sive EPO receptors due to mutations. Rare hemochromatosis (HFE mutations) may have elevated hemoglobin levels. APPROACH TO THE PATIENT Polycythemia As shown in Fig. 66-18, the first step is to search for earlier blood counts that might suggest the chronicity of the increase. Ideally one would document the presence of an increased red cell mass; however, the dependence of this technique on radiolabeled red cells has led to its abandonment. If the red cell mass is normal (<36 mL/kg in men, <32 mL/kg in women), the patient has spurious or relative polycythemia. If the red cell mass is increased (>36 mL/kg

in men, >32 mL/kg in women), serum EPO levels should be measured. It must be acknowledged that measurement of red cell mass is a physiologic approach to distinguishing polycythemia, and because of the use of radionuclide-labeled red cells, it is rarely performed. It is more common to measure EPO levels in a person with an elevated hemoglobin level or hematocrit once it has been documented that the patient is not hypoxic (i.e., O2 saturation is

> 93%). If EPO levels are low or unmeasurable, the patient most likely has polycythemia vera. A mutation in JAK2 (Val617Phe), a key member of the cytokine intracellular signaling pathway, can be found in 90–95% of patients with polycythemia vera. Many of those without this particular JAK2 mutation have mutations in exon

12. If EPO levels are low, check for JAK2 mutation(s), and perform an abdominal ultrasound to assess spleen size. Tests that support the diagnosis of polycythemia vera include elevated white blood cell count, increased absolute basophil count, and thrombocytosis. In practice, many physicians order EPO levels and assessment for JAK2 mutations at the same time.

If serum EPO levels are elevated, one needs to distinguish whether the elevation is a physiologic response to hypoxia or related to autonomous EPO production. Patients with low arte rial O2 saturation (<92%) should be further evaluated for the presence of heart or lung disease, if they are not living at high altitude. Patients with normal O2 saturation who are smokers may have elevated EPO levels because of CO displacement of O2. If carboxyhemoglobin (COHb) levels are high, the diagnosis is “smoker’s polycythemia.” Such patients should be urged to stop smoking. Those who cannot stop smoking require phlebotomy to control their polycythemia. Patients with normal O2 saturation who do not smoke either have an abnormal hemoglobin that does not deliver O2 to the tissues (evaluated by finding elevated O2–hemo globin affinity) or have a source of EPO production that is not responding to the normal feedback inhibition. Further workup is dictated by the differential diagnosis of EPO-producing neo plasms. Hepatoma, uterine leiomyoma, and renal cancer or cysts are all detectable with abdominopelvic computed tomography scans. Cerebellar hemangiomas may produce EPO, but they pres ent with localizing neurologic signs and symptoms rather than polycythemia-related symptoms. ■ ■FURTHER READING Camaschella C: Iron deficiency. Blood 133:30, 2019. Hillman RS et al: Hematology in Clinical Practice, 5th ed. New York, McGraw-Hill, 2010. McMullin MF et al: Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. Br J Haematol 130:174, 2005. Sankaran VG, Weiss MJ: Anemia: Progress in molecular mechanisms and therapies. Nat Med 21:221, 2015. Spivak JL: How I manage polycythemia vera. Blood 134:341, 2019.

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