

CHAPTER 57

Anemia and Polycythemia

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HEMATOPOIESIS AND THE PHYSIOLOGIC BASIS OF 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 beginning with the hematopoietic stem cell. Stem cells are capable of producing red cells, all classes of granulocytes, monocytes, platelets, and the cells of the immune system. The precise molecular mechanism—either intrinsic to the stem cell itself or through the action of extrinsic factors—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/megakaryocyte progenitor that does not develop in the absence of expression of the GATA-1 and FOG-1 (friend of GATA-1) transcription factors (Chap. 66). Following lineage commitment, hematopoietic progenitor and precursor cells come increasingly under the regulatory influence of growth factors and hormones. For red cell production, erythropoietin (EPO) is the 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. 57-1.

In the bone marrow, the first morphologically recognizable erythroid 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

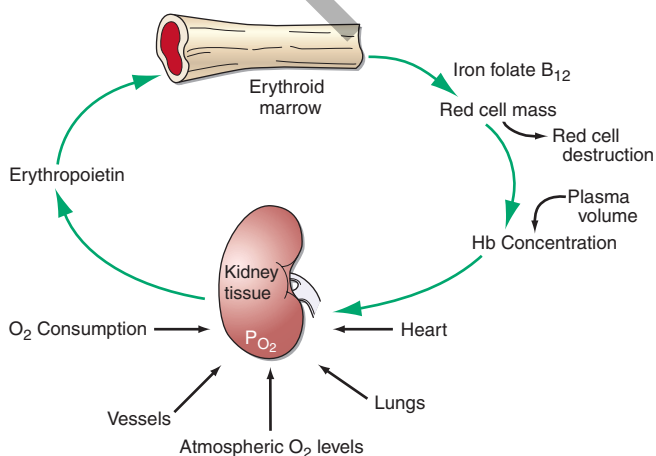


Figure 57-1 The physiologic regulation of red cell production by tissue oxygen tension. Hb, hemoglobin.

turn, give rise to increased numbers of erythrocytes. The regulation of EPO production itself is linked to tissue oxygenation.

In mammals, O_2 is transported to tissues bound to the hemoglobin 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. 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 blood cells. The size of the red cell mass reflects the balance of red cell production and destruction. The physiologic basis of red cell production 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 epithelial-like cells. A small amount of EPO is produced by hepatocytes. The fundamental stimulus for EPO production is the availability of O_2 for tissue metabolic needs. Key to EPO gene regulation is hypoxia-inducible factor (HIF)-1 α . In the presence of O_2 , HIF-1 α is hydroxylated at a key proline, allowing HIF-1 α to be ubiquitinated and degraded via the proteasome pathway. If O_2 becomes limiting, this critical hydroxylation step does not occur, allowing HIF-1 α to partner with other proteins, translocate to the nucleus, and upregulate the EPO gene, among others.

Impaired O_2 delivery to the kidney can result from a decreased red cell mass (*anemia*), impaired O_2 loading of the hemoglobin molecule or a high O_2 affinity mutant hemoglobin (*hypoxemia*), or, rarely, impaired blood flow to the kidney (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—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. 57-2). In circulation, EPO has a half-clearance time of 6–9 h. EPO acts by binding to specific receptors 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 components can lead to anemia. Generally, anemia is recognized in the laboratory when a patient's hemoglobin level or hematocrit is reduced below an expected value (the normal range). The likelihood and severity of anemia are defined based on the deviation of the patient's hemoglobin/hematocrit from values expected for age- and sex-matched normal subjects. The hemoglobin concentration in adults has a Gaussian distribution. The mean hematocrit value for adult males is 47% (\pm SD 7) and that for adult females is 42% (\pm 5). Any single hematocrit or hemoglobin value carries with it a likelihood of associated anemia. Thus, a hematocrit of \leq 39% in an adult male or \leq 35% in an adult female has only about a 25% chance of

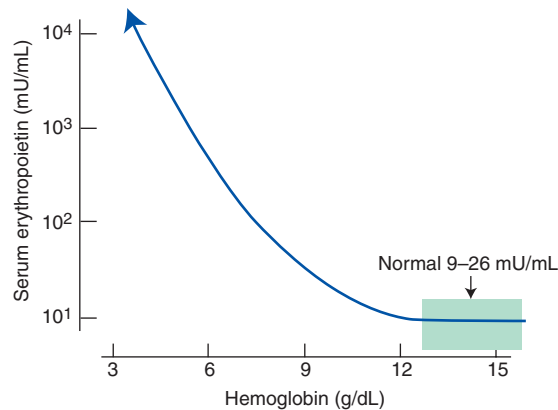


Figure 57-2 Erythropoietin (EPO) levels in response to anemia. When the hemoglobin level falls to 120 g/L (12 g/dL), plasma EPO levels increase logarithmically. In the presence of chronic kidney disease or chronic inflammation, EPO levels are typically lower than expected for the degree of anemia. As individuals age, the level of EPO needed to sustain normal hemoglobin levels appears to increase. (From Hillman *et al.*)

being normal. Suspected low hemoglobin or hematocrit values are more easily interpreted if previous values for the same patient are known for comparison. The World Health Organization (WHO) defines anemia as a hemoglobin level <130 g/L (13 g/dL) in men and <120 g/L (12 g/dL) in women.

The critical elements of erythropoiesis—EPO production, iron availability, the proliferative capacity of the bone marrow, and effective maturation of red cell precursors—are used for the initial classification of anemia (see below).

ANEMIA

CLINICAL PRESENTATION OF ANEMIA

Signs and symptoms

Anemia is most often recognized by abnormal screening laboratory tests. Patients less commonly present with advanced anemia and its attendant signs and symptoms. Acute anemia is due to blood loss or hemolysis. If blood loss is mild, enhanced O₂ delivery is achieved through changes in the O₂-hemoglobin dissociation curve mediated by a decreased pH or increased CO₂ (*Bohr effect*). With acute blood loss, hypovolemia dominates the clinical picture and the hematocrit and hemoglobin levels do not reflect the volume of blood lost. Signs of vascular instability appear with acute losses of 10–15% of the total blood volume. In such patients, the issue is not anemia but hypotension and decreased organ perfusion. When >30% of the blood volume is lost suddenly, patients are unable to compensate with the usual mechanisms of vascular contraction and changes in regional blood flow. The patient prefers to remain supine and will show postural hypotension and tachycardia. If the volume of blood lost is >40% (i.e., >2 L in the average-sized adult), signs of hypovolemic shock including confusion, dyspnea, diaphoresis, hypotension, and tachycardia appear (**Chap. 106**). Such patients have significant deficits in vital organ perfusion and require immediate volume replacement.

With acute hemolysis, the signs and symptoms depend on the mechanism that leads to red cell destruction. Intravascular hemolysis with release of free hemoglobin may be associated with acute back pain, free hemoglobin in the plasma and urine, and renal failure. Symptoms associated with more chronic or progressive anemia depend on the age of the patient and the adequacy of blood supply to critical organs. Symptoms associated with moderate anemia include fatigue, loss of stamina, breathlessness,

and tachycardia (particularly with physical exertion). However, because of the intrinsic compensatory mechanisms that govern the O₂-hemoglobin dissociation curve, the gradual onset of anemia—particularly in young patients—may not be associated with signs or symptoms until the anemia is severe [hemoglobin <70–80 g/L (7–8 g/dL)]. When anemia develops over a period of days or weeks, the total blood volume is normal to slightly increased, and changes in cardiac output and regional blood flow help compensate for the overall loss in O₂-carrying capacity. Changes in the position of the O₂-hemoglobin dissociation curve account for some of the compensatory response to anemia. With chronic anemia, intracellular levels of 2,3-bisphosphoglycerate rise, shifting the dissociation curve to the right and facilitating O₂ unloading. This compensatory mechanism can only maintain normal tissue O₂ delivery in the face of a 20–30 g/L (2–3 g/dL) deficit in hemoglobin concentration. Finally, further protection of O₂ delivery to vital organs is achieved by the shunting of blood away from organs that are relatively rich in blood supply, particularly the kidney, gut, and skin.

Certain disorders are commonly associated with anemia. Chronic inflammatory states (e.g., infection, rheumatoid arthritis, cancer) are associated with mild to moderate anemia, whereas lymphoproliferative disorders, such as chronic lymphocytic leukemia and certain other B cell neoplasms, may be associated with autoimmune hemolysis.

APPROACH TO THE PATIENT Anemia

The evaluation of the patient with anemia requires a careful history and physical examination. Nutritional history related to drugs or alcohol intake and family history of anemia should always be assessed. Certain geographic backgrounds and ethnic origins are associated with an increased likelihood of an inherited disorder of the hemoglobin molecule or intermediary metabolism. Glucose-6-phosphate dehydrogenase (G6PD) deficiency and certain hemoglobinopathies are seen more commonly in those of Middle Eastern or African origin, including African Americans who have a high frequency of G6PD deficiency. Other information that may be useful includes exposure to certain toxic agents or drugs and symptoms related to other disorders commonly associated with anemia. These include symptoms and signs such as bleeding, fatigue, malaise, fever, weight loss, night sweats, and other systemic symptoms. Clues to the mechanisms of anemia may be provided on physical examination by findings of infection, blood in the stool, lymphadenopathy, splenomegaly, or petechiae. Splenomegaly and lymphadenopathy suggest an underlying lymphoproliferative disease, while petechiae suggest platelet dysfunction. Past laboratory measurements are helpful to determine a time of onset.

In the anemic patient, physical examination may demonstrate a forceful heartbeat, strong peripheral pulses, and a systolic “flow” murmur. The skin and mucous membranes may be pale if the hemoglobin is <80–100 g/L (8–10 g/dL). This part of the physical examination should focus on areas where vessels are close to the surface such as the mucous membranes, nail beds, and palmar creases. If the palmar creases are lighter in color than the surrounding skin when the hand is hyperextended, the hemoglobin level is usually <80 g/L (8 g/dL).

LABORATORY EVALUATION Table 57-1 lists the tests used in the initial workup of anemia. A routine complete blood count (CBC) is required as part of the evaluation and includes the hemoglobin, hematocrit, and red cell indices: the mean cell volume (MCV) in femtoliters, mean cell hemoglobin (MCH) in picograms per cell, and mean concentration of hemoglobin per

TABLE 57-1 Laboratory Tests in Anemia Diagnosis

I. Complete blood count (CBC)	II. Iron supply studies
A. Red blood cell count	A. Serum iron
1. Hemoglobin	B. Total iron-binding capacity
2. Hematocrit	C. Serum ferritin
3. Reticulocyte count	III. Marrow examination
B. Red blood cell indices	A. Aspirate
1. Mean cell volume (MCV)	1. M/E ratio ^a
2. Mean cell hemoglobin (MCH)	2. Cell morphology
3. Mean cell hemoglobin concentration (MCHC)	3. Iron stain
4. Red cell distribution width (RDW)	B. Biopsy
C. White blood cell count	1. Cellularity
1. Cell differential	2. Morphology
2. Nuclear segmentation of neutrophils	
D. Platelet count	
E. Cell morphology	
1. Cell size	
2. Hemoglobin content	
3. Anisocytosis	
4. Poikilocytosis	
5. Polychromasia	

^aM/E ratio, ratio of myeloid to erythroid precursors.

volume of red cells (MCHC) in grams per liter (non-SI: grams per deciliter). The red cell indices are calculated as shown in [Table 57-2](#), and the normal variations in the hemoglobin and hematocrit with age are shown in [Table 57-3](#). A number of physiologic factors affect the CBC, including age, sex, pregnancy, smoking, and altitude. High-normal hemoglobin values may be seen in men and women who live at altitude or smoke heavily. Hemoglobin elevations due to smoking reflect normal compensation due to the displacement of O₂ by CO in hemoglobin binding. Other important information is provided by the reticulocyte count and measurements of iron supply including *serum iron*, *total iron-binding capacity* (TIBC; an indirect measure of the transferrin level), and *serum ferritin*. Marked alterations in the red cell indices usually reflect disorders of maturation or iron deficiency. A careful evaluation of the peripheral blood smear is important, and clinical laboratories often provide a description of both the red and white cells, a white cell differential count, and the platelet count. In patients with severe anemia

TABLE 57-2 Red Blood Cell Indices

Index	Normal Value
Mean cell volume (MCV) = (hematocrit × 10)/ (red cell count × 10 ⁶)	90 ± 8 fL
Mean cell hemoglobin (MCH) = (hemoglobin × 10)/ (red cell count × 10 ⁶)	30 ± 3 pg
Mean cell hemoglobin concentration = (hemoglobin × 10)/hematocrit, or MCH/MCV	33 ± 2%

TABLE 57-3 Changes in Normal Hemoglobin/Hematocrit Values With Age and Pregnancy

Age/Sex	Hemoglobin g/dL	Hematocrit %
At birth	17	52
Childhood	12	36
Adolescence	13	40
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 Hillman et al.

and abnormalities in red blood cell morphology and/or low reticulocyte counts, a bone marrow aspirate or biopsy can assist in the diagnosis. Other tests of value in the diagnosis of specific anemias are discussed in chapters on specific disease states.

The components of the CBC also help in the classification of anemia. *Microcytosis* is reflected by a lower than normal MCV (<80), whereas high values (>100) reflect *macrocytosis*. The MCH and MCHC reflect defects in hemoglobin synthesis (*hypochromia*). Automated cell counters describe the red cell volume distribution width (RDW). The MCV (representing the peak of the distribution curve) is insensitive to the appearance of small populations of macrocytes or microcytes. An experienced laboratory technician will be able to identify minor populations of large or small cells or hypochromic cells before the red cell indices change.

Peripheral Blood Smear The peripheral blood smear provides important information about defects in red cell production ([Chap. e17](#)). As a complement to the red cell indices, the blood smear also reveals variations in cell size (*anisocytosis*) and shape (*poikilocytosis*). The degree of anisocytosis usually correlates with increases in the 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 circulating 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 prematurely released 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 (fibrosis, infiltration of the marrow by malignant cells, etc.) that results in their disordered release from the marrow. The appearance of nucleated red cells, Howell-Jolly bodies, target cells, sickle cells, and others may provide clues to specific disorders ([Figs. 57-3 to 57-11](#)).

Reticulocyte Count An accurate reticulocyte count is key to the initial classification of anemia. Normally, reticulocytes are red cells that have been recently released from the bone marrow. They are identified by staining with a supravital dye that precipitates the ribosomal RNA ([Fig. 57-12](#)). These precipitates appear as blue or black punctate spots. This residual RNA is metabolized over the first 24–36 h of the reticulocyte's life span in circulation. Normally, the reticulocyte count ranges from 1 to

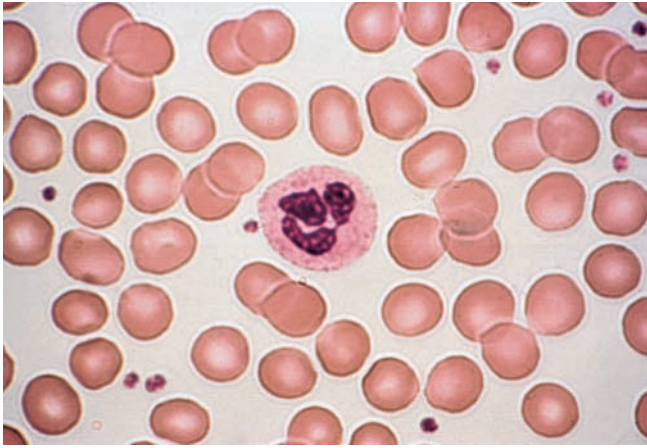


Figure 57-3 Normal blood smear (Wright stain). High-power field showing normal red cells, a neutrophil, and a few platelets. (From Hillman et al.)

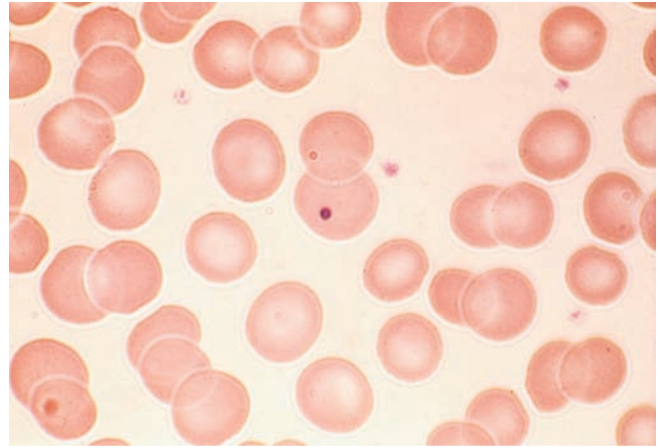


Figure 57-6 Howell-Jolly bodies. In the absence of a functional spleen, nuclear remnants are not culled from the red cells and remain as small homogeneously staining blue inclusions on Wright stain. (From Hillman et al.)

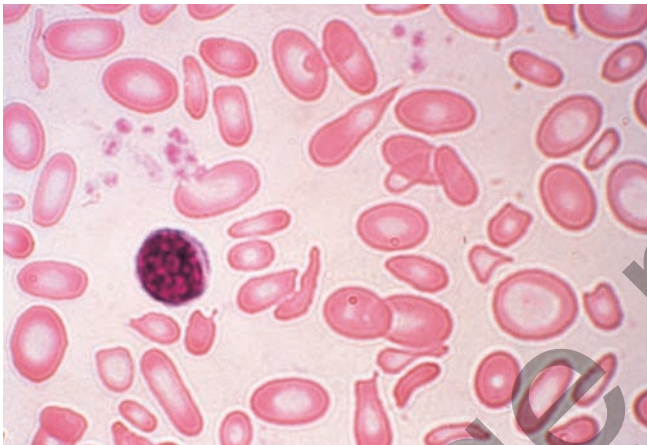


Figure 57-4 Severe iron-deficiency anemia. Microcytic and hypochromic red cells smaller than the nucleus of a lymphocyte associated with marked variation in size (anisocytosis) and shape (poikilocytosis). (From Hillman et al.)

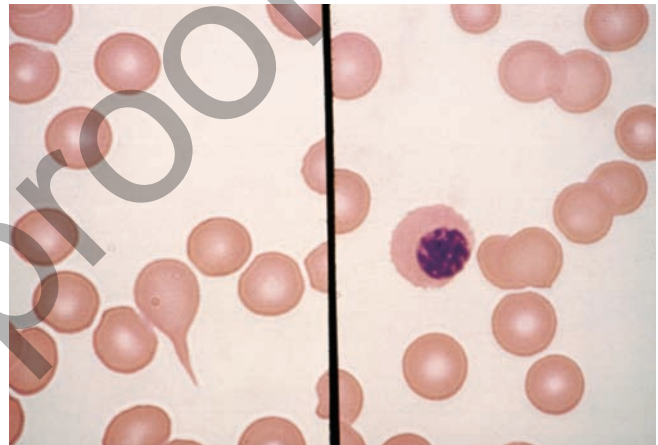


Figure 57-7 Red cell changes in myelofibrosis. The left panel shows a teardrop-shaped cell. The right panel shows a nucleated red cell. These forms are seen in myelofibrosis.

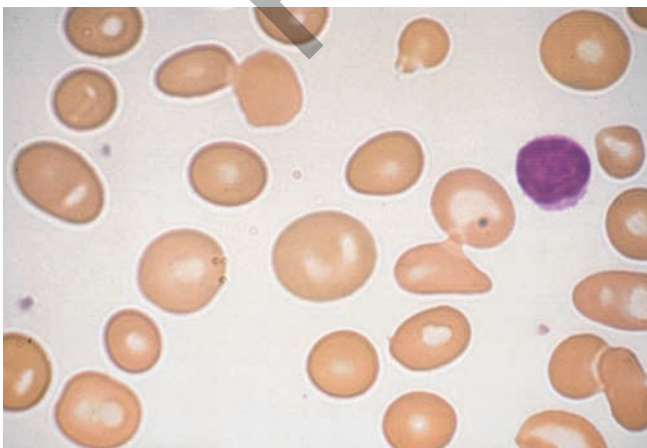


Figure 57-5 Macrocytosis. Red cells are larger than a small lymphocyte and well hemoglobinized. Often macrocytes are oval shaped (macro-ovalocytes).

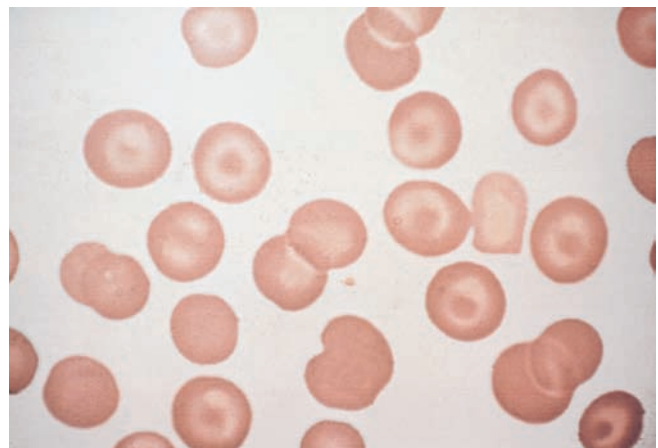


Figure 57-8 Target cells. Target cells have a bull's-eye appearance and are seen in thalassemia and in liver disease. (From Hillman et al.)

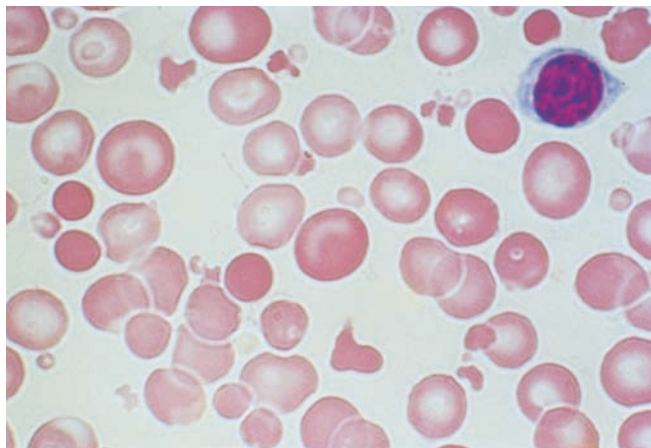


Figure 57-9 Red cell fragmentation. Red cells may become fragmented in the presence of foreign bodies in the circulation, such as mechanical heart valves, or in the setting of thermal injury. (From Hillman et al.)

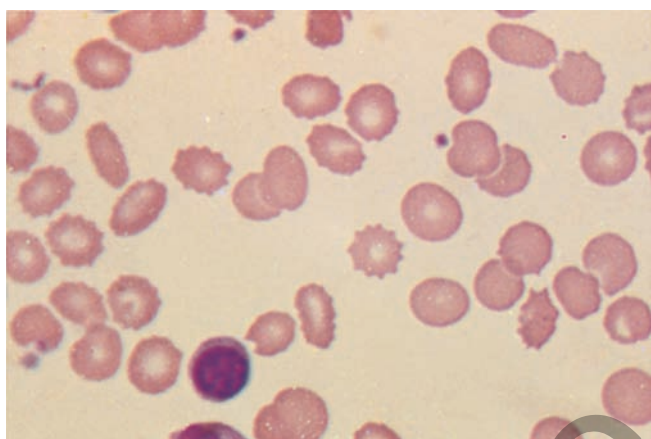


Figure 57-10 Uremia. The red cells in uremia may acquire numerous regularly spaced, small, spiny projections. Such cells, called burr cells or echinocytes, are readily distinguishable from irregularly spiculated acanthocytes shown in Fig. 57-11.

2% and reflects the daily replacement of 0.8–1.0% of the circulating red cell population. A corrected reticulocyte count provides a reliable measure of red cell production.

In the initial classification of anemia, the patient's reticulocyte count is compared with the expected reticulocyte response. In general, if the EPO and erythroid marrow responses to moderate anemia [hemoglobin < 100 g/L (10 g/dL)] are intact, the red cell production rate increases to two to three times normal within 10 days following the onset of anemia. In the face of established anemia, a reticulocyte response less than two to three times normal indicates an inadequate marrow response.

In order to use the reticulocyte count to estimate marrow response, two corrections are necessary. The first correction adjusts the reticulocyte count based on the reduced number of circulating red cells. With anemia, the percentage of reticulocytes may be increased while the absolute number is unchanged. To correct for this effect, the reticulocyte percentage is multiplied by the ratio of the patient's hemoglobin or hematocrit to the expected hemoglobin/hematocrit for the age and gender of the patient (Table 57-4). This provides an estimate of the reticulocyte count corrected for anemia. In order to convert the corrected reticulocyte count to an index of marrow production, a further correction is required, depending on whether some

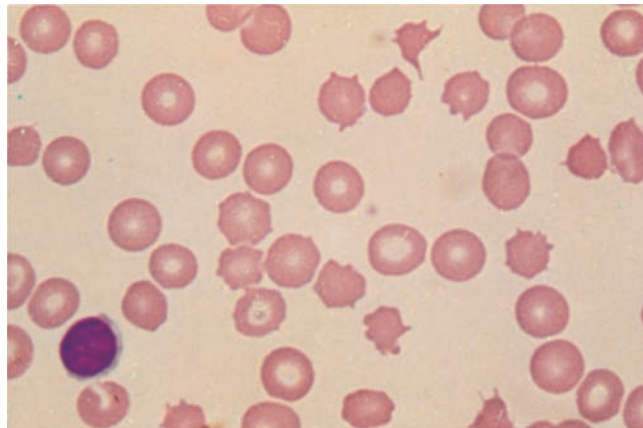


Figure 57-11 Spur cells. Spur cells are recognized as distorted red cells containing several irregularly distributed thornlike projections. Cells with this morphologic abnormality are also called acanthocytes. (From Hillman et al.)

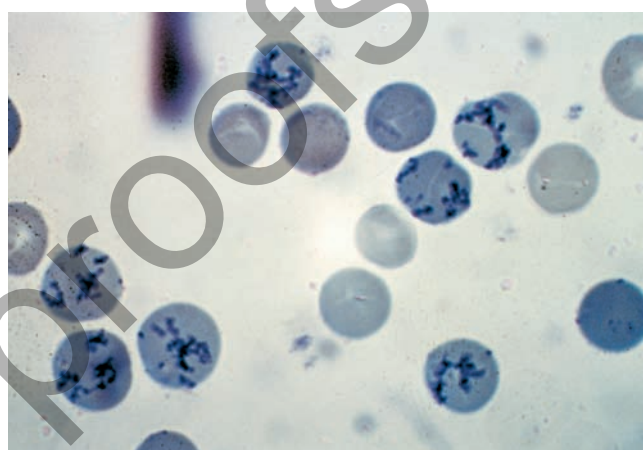


Figure 57-12 Reticulocytes. Methylene blue stain demonstrates residual RNA in newly made red cells. (From Hillman et al.)

of the reticulocytes in circulation have been released from the marrow prematurely. For this second correction, the peripheral blood smear is examined to see if there are polychromatophilic macrocytes present.

TABLE 57-4 Calculation of Reticulocyte Production Index

Correction #1 for Anemia:

This correction produces the corrected reticulocyte count
In a person whose reticulocyte count is 9%, hemoglobin 7.5 g/dL, hematocrit 23%, the absolute reticulocyte count = $9 \times (7.5/15)$
[or $\times (23/45)$] = 4.5%

Correction #2 for Longer Life of Prematurely Released Reticulocytes in the Blood:

This correction produces the reticulocyte production index
In a person whose reticulocyte count is 9%, hemoglobin 7.5 gm/dL, hematocrit 23%, the reticulocyte production index

$$= 9 \times \frac{(7.5/15)(\text{hemoglobin correction})}{2(\text{maturation time correction})} = 2.25$$

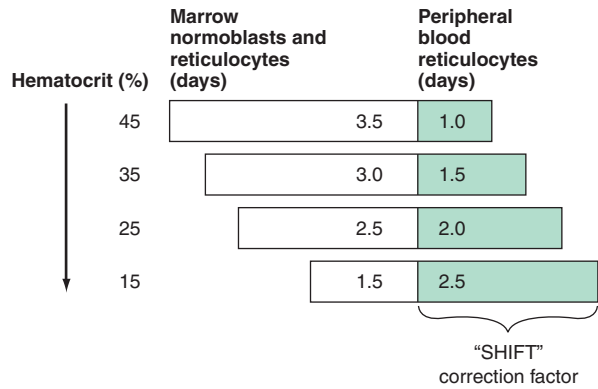


Figure 57-13 Correction of the reticulocyte count. In order to use the reticulocyte count as an indicator of effective red cell production, the reticulocyte percentage must be corrected based on the level of anemia and the circulating life span of the reticulocytes. Erythroid cells take ~4.5 days to mature. At a normal hemoglobin, reticulocytes are released to the circulation with ~1 day left as reticulocytes. However, with different levels of anemia, reticulocytes (and even earlier erythroid cells) may be released from the marrow prematurely. Most patients come to clinical attention with hematocrits in the mid-20s, and thus a correction factor of 2 is commonly used because the observed reticulocytes will live for 2 days in the circulation before losing their RNA.

These cells, representing prematurely released reticulocytes, are referred to as “shift” cells, and the relationship between the degree of shift and the necessary shift correction factor is shown in Fig. 57-13. The correction is necessary because these prematurely released cells survive as reticulocytes in circulation for >1 day, thereby providing a falsely high estimate of daily red cell production. If polychromasia is increased, the reticulocyte count, already corrected for anemia, should be divided again by 2 to account for the prolonged reticulocyte maturation time. The second correction factor varies from 1 to 3 depending on the severity of anemia. In general, a correction of 2 is commonly used. An appropriate correction is shown in Table 57-4. If polychromatophilic cells are not seen on the blood smear, the second correction is not required. The now doubly corrected reticulocyte count is the *reticulocyte production index*, and it provides an estimate of marrow production relative to normal.

Premature release of reticulocytes is normally due to increased EPO stimulation. However, if the integrity of the bone marrow release process is lost through tumor infiltration, fibrosis, or other disorders, the appearance of nucleated red cells or polychromatophilic macrocytes should still invoke the second reticulocyte correction. The shift correction should always be applied to a patient with anemia and a very high reticulocyte count to provide a true index of effective red cell production. Patients with severe chronic hemolytic anemia may increase red cell production as much as six- to sevenfold. This measure alone, therefore, confirms the fact that the patient has an appropriate EPO response, a normally functioning bone marrow, and sufficient iron available to meet the demands for new red cell formation. Table 57-5 demonstrates the normal marrow response to anemia. If the reticulocyte production index is <2 in the face of established anemia, a defect in erythroid marrow proliferation or maturation must be present.

TABLE 57-5 Normal Marrow Response to Anemia

Hematocrit	Production Index	Reticulocytes (incl corrections)	Marrow M/E Ratio
45	1	1	3:1
35	2.0–3.0	4.8%/3.8/2.5	2:1–1:1
25	3.0–5.0	14%/8/4.0	1:1–1:2
15	3.0–5.0	30%/10/4.0	1:1–1:2

Tests of Iron Supply and Storage The laboratory measurements that reflect the availability of iron for hemoglobin synthesis include the serum iron, the TIBC, and the percent transferrin saturation. The percent transferrin saturation is derived by dividing the serum iron level ($\times 100$) by the TIBC. The normal serum iron ranges from 9 to 27 $\mu\text{mol/L}$ (50–150 $\mu\text{g/dL}$), while the normal TIBC is 54–64 $\mu\text{mol/L}$ (300–360 $\mu\text{g/dL}$); 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 average ~100 $\mu\text{g/L}$, corresponding to iron stores of ~1 g. Adult females have lower serum ferritin levels averaging 30 $\mu\text{g/L}$, reflecting lower iron stores (~300 mg). A serum ferritin level of 10–15 $\mu\text{g/L}$ represents 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 $\mu\text{g/L}$ means there is at least some iron in tissue stores.

Bone Marrow Examination A bone marrow aspirate and smear or a needle biopsy can be useful in the evaluation of some patients with anemia. In patients with hypoproliferative anemia and normal iron status, a bone marrow is indicated. Marrow examination can diagnose primary marrow disorders such as myelofibrosis, a red cell maturation defect, or an infiltrative disease (Figs. 57-14 to 57-16). The increase or decrease of one cell lineage (myeloid vs. erythroid) compared to another is obtained by a differential count of nucleated cells in a bone

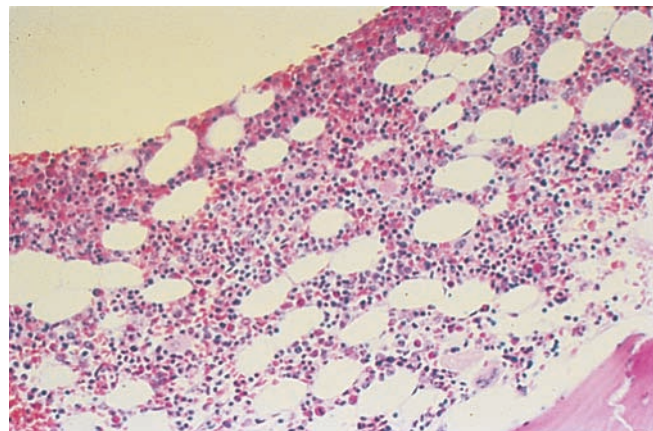


Figure 57-14 Normal bone marrow. This is a low-power view of a section of a normal bone marrow biopsy stained with hematoxylin and eosin (H&E). Note that the nucleated cellular elements account for ~40–50% and the fat (clear areas) accounts for ~50–60% of the area. (From Hillman et al.)

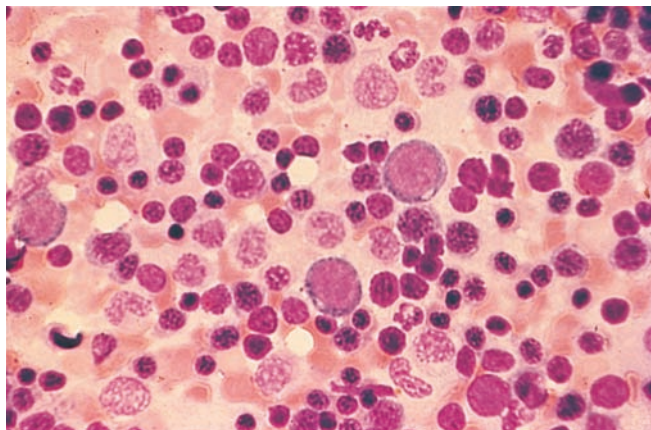


Figure 57-15 Erythroid hyperplasia. This marrow shows an increase in the fraction of cells in the erythroid lineage as might be seen when a normal marrow compensates for acute blood loss or hemolysis. The M/E ratio is about 1:1. M/E, myeloid/erythroid. (From Hillman et al.)

marrow smear [the myeloid/erythroid (M/E) ratio]. A patient with a hypoproliferative anemia (see below) and a reticulocyte production index <2 will demonstrate an M/E ratio of 2 or 3:1. In contrast, patients with hemolytic disease and a production index >3 will have an M/E ratio of at least 1:1. Maturation disorders are identified from the discrepancy between the M/E ratio and the reticulocyte production index (see below). Either the marrow smear or biopsy can be stained for the presence of iron stores or iron in developing red cells. The storage iron is in the form of ferritin or *hemosiderin*. On carefully prepared bone marrow smears, small ferritin granules can normally be seen under oil immersion in 20–40% of developing erythroblasts. Such cells are called *sideroblasts*.

OTHER LABORATORY MEASUREMENTS Additional laboratory tests may be of value in confirming specific diagnoses. For details of these tests and how they are applied in individual disorders, see Chaps. 103 to 107.

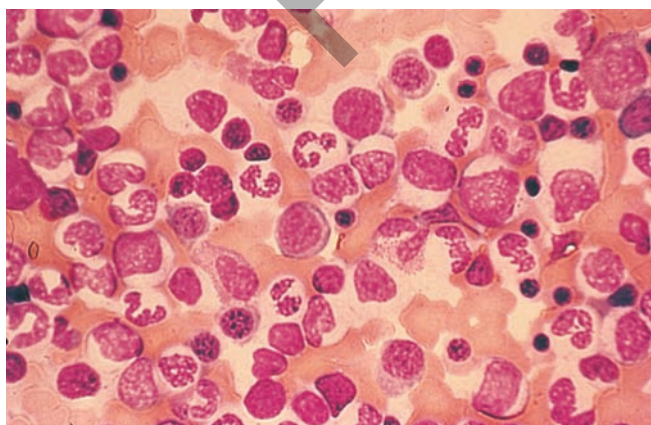


Figure 57-16 Myeloid hyperplasia. This marrow shows an increase in the fraction of cells in the myeloid or granulocytic lineage as might be seen in a normal marrow responding to infection. The M/E ratio is $>3:1$. M/E, myeloid/erythroid. (From Hillman et al.)

DEFINITION AND CLASSIFICATION OF ANEMIA

Initial classification of anemia

The functional classification of anemia has three major categories. These are (1) marrow production defects (*hypoproliferation*), (2) red cell maturation defects (*ineffective erythropoiesis*), and (3) decreased red cell survival (*blood loss/hemolysis*). The classification is shown in Fig. 57-17. A hypoproliferative anemia is typically seen with a low reticulocyte production index together with little or no change in red cell morphology (a normocytic, normochromic anemia) (Chap. 103). Maturation disorders typically have a slight to moderately elevated reticulocyte production index that is accompanied by either macrocytic (Chap. 105) or microcytic (Chaps. 103, 104) red cell indices. Increased red blood cell destruction secondary to hemolysis results in an increase in the reticulocyte production index to at least three times normal (Chap. 106), provided sufficient iron is available. Hemorrhagic anemia does not typically result in production indices of more than 2.0–2.5 times normal because of the limitations placed on expansion of the erythroid marrow by iron availability.

In the first branch point of the classification of anemia, a reticulocyte production index >2.5 indicates that hemolysis is most likely. A reticulocyte production index <2 indicates either a hypoproliferative anemia or maturation disorder. The latter two possibilities can often be distinguished by the red cell indices, by examination of the peripheral blood smear, or by a marrow examination. If the red cell indices are normal, the anemia is almost certainly hypoproliferative in nature. Maturation disorders are characterized by ineffective red cell production and a low reticulocyte production index. Bizarre red cell shapes—macrocytes or hypochromic microcytes—are seen on the peripheral blood smear. With a hypoproliferative anemia, no erythroid hyperplasia is noted in the marrow, whereas patients with ineffective red cell production have erythroid hyperplasia and an M/E ratio $<1:1$.

ALGORITHM OF THE PHYSIOLOGIC CLASSIFICATION OF ANEMIA

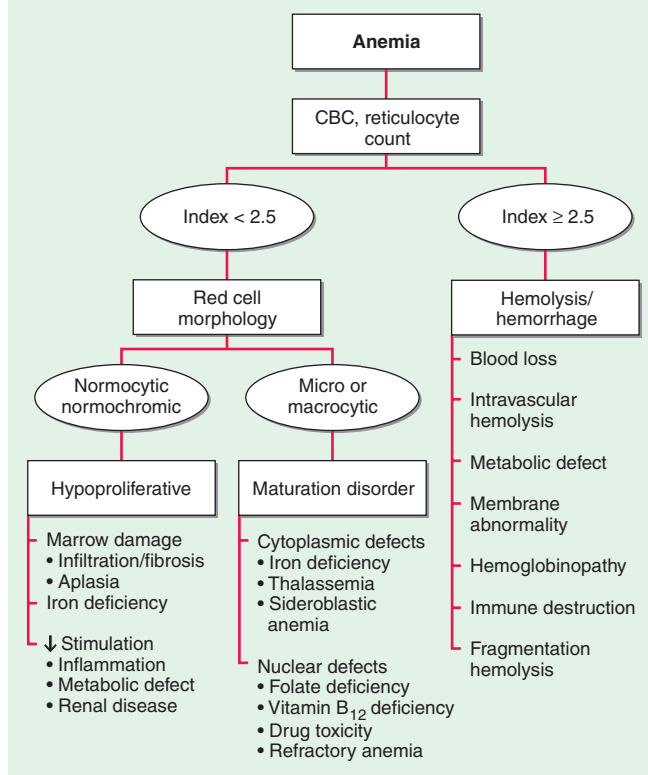


Figure 57-17 The physiologic classification of anemia. CBC, complete blood count.

Hypoproliferative anemias

At least 75% of all cases of anemia are hypoproliferative in nature. A hypoproliferative anemia reflects absolute or relative marrow failure in which the erythroid marrow has not proliferated appropriately for the degree of anemia. The majority of hypoproliferative anemias are due to mild to moderate iron deficiency or inflammation. A hypoproliferative anemia can result from marrow damage, iron deficiency, or inadequate EPO stimulation. The last may reflect impaired renal function, suppression of EPO production by inflammatory cytokines such as interleukin 1, or reduced tissue needs for O₂ from metabolic disease such as hypothyroidism. Only occasionally is the marrow unable to produce red cells at a normal rate, and this is most prevalent in patients with renal failure. With diabetes mellitus or myeloma, the EPO deficiency may be more marked than would be predicted by the degree of renal insufficiency. In general, hypoproliferative anemias are characterized by normocytic, normochromic red cells, although microcytic, hypochromic cells may be observed with mild iron deficiency or long-standing chronic inflammatory disease. The key laboratory tests in distinguishing between the various forms of hypoproliferative anemia include the serum iron and iron-binding capacity, evaluation of renal and thyroid function, a marrow biopsy or aspirate to detect marrow damage or infiltrative disease, and serum ferritin to assess iron stores. An iron stain of the marrow will determine the pattern of iron distribution. Patients with the anemia of acute or chronic inflammation show a distinctive pattern of serum iron (low), TIBC (normal or low), percent transferrin saturation (low), and serum ferritin (normal or high). These changes in iron values are brought about by hepcidin, the iron regulatory hormone that is increased in inflammation (Chap. 103). A distinct pattern of results is noted in mild to moderate iron deficiency (low serum iron, high TIBC, low percent transferrin saturation, low serum ferritin) (Chap. 103). Marrow damage by drugs, infiltrative disease such as leukemia or lymphoma, or marrow aplasia are diagnosed from the peripheral blood and bone marrow morphology. With infiltrative disease or fibrosis, a marrow biopsy is required.

Maturation disorders

The presence of anemia with an inappropriately low reticulocyte production index, macro- or microcytosis on smear, and abnormal red cell indices suggests a maturation disorder. Maturation disorders are divided into two categories: nuclear maturation defects, associated with macrocytosis, and cytoplasmic maturation defects, associated with microcytosis and hypochromia usually from defects in hemoglobin synthesis. The inappropriately low reticulocyte production index is a reflection of the ineffective erythropoiesis that results from the destruction within the marrow of developing erythroblasts. Bone marrow examination shows erythroid hyperplasia.

Nuclear maturation defects result from vitamin B₁₂ or folic acid deficiency, drug damage, or myelodysplasia. Drugs that interfere with cellular DNA synthesis, such as methotrexate or alkylating agents, can produce a nuclear maturation defect. Alcohol, alone, is also capable of producing macrocytosis and a variable degree of anemia, but this is usually associated with folic acid deficiency. Measurements of folic acid and vitamin B₁₂ are critical not only in identifying the specific vitamin deficiency but also because they reflect different pathogenetic mechanisms.

Cytoplasmic maturation defects result from severe iron deficiency or abnormalities in globin or heme synthesis. Iron deficiency occupies an unusual position in the classification of anemia. If the iron-deficiency anemia is mild to moderate, erythroid marrow proliferation is blunted and the anemia is classified as hypoproliferative. However, if the anemia is severe and prolonged, the erythroid

marrow will become hyperplastic despite the inadequate iron supply, and the anemia will be classified as ineffective erythropoiesis with a cytoplasmic maturation defect. In either case, an inappropriately low reticulocyte production index, microcytosis, and a classic pattern of iron values make the diagnosis clear and easily distinguish iron deficiency from other cytoplasmic maturation defects such as the thalassemias. Defects in heme synthesis, in contrast to globin synthesis, are less common and may be acquired or inherited (Chap. 358). Acquired abnormalities are usually associated with myelodysplasia, may lead to either a macro- or microcytic anemia, and are frequently associated with mitochondrial iron loading. In these cases, iron is taken up by the mitochondria of the developing erythroid cell but not incorporated into heme. The iron-encrusted mitochondria surround the nucleus of the erythroid cell, forming a ring. Based on the distinctive finding of so-called ringed sideroblasts on the marrow iron stain, patients are diagnosed as having a sideroblastic anemia—almost always reflecting myelodysplasia. Again, studies of iron parameters are helpful in the differential diagnosis of these patients.

Blood loss/hemolytic anemia

In contrast to anemias associated with an inappropriately low reticulocyte production index, hemolysis is associated with red cell production indices ≥ 2.5 times normal. The stimulated erythropoiesis is reflected in the blood smear by the appearance of increased numbers of polychromatophilic macrocytes. A marrow examination is rarely indicated if the reticulocyte production index is increased appropriately. The red cell indices are typically normocytic or slightly macrocytic, reflecting the increased number of reticulocytes. Acute blood loss is not associated with an increased reticulocyte production index because of the time required to increase EPO production and, subsequently, marrow proliferation. Subacute blood loss may be associated with modest reticulocytosis. Anemia from chronic blood loss presents more often as iron deficiency than with the picture of increased red cell production.

The evaluation of blood loss anemia is usually not difficult. Most problems arise when a patient presents with an increased red cell production index from an episode of acute blood loss that went unrecognized. The cause of the anemia and increased red cell production may not be obvious. The confirmation of a recovering state may require observations over a period of 2–3 weeks, during which the hemoglobin concentration will be seen to rise and the reticulocyte production index fall.

Hemolytic disease, while dramatic, is among the least common forms of anemia. The ability to sustain a high reticulocyte production index reflects the ability of the erythroid marrow to compensate for hemolysis and, in the case of extravascular hemolysis, the efficient recycling of iron from the destroyed red cells to support red cell production. With intravascular hemolysis, such as paroxysmal nocturnal hemoglobinuria, the loss of iron may limit the marrow response. The level of response depends on the severity of the anemia and the nature of the underlying disease process.

Hemoglobinopathies, such as sickle cell disease and the thalassemias, present a mixed picture. The reticulocyte index may be high but is inappropriately low for the degree of marrow erythroid hyperplasia (Chap. 104).

Hemolytic anemias present in different ways. Some appear suddenly as an acute, self-limited episode of intravascular or extravascular hemolysis, a presentation pattern often seen in patients with autoimmune hemolysis or with inherited defects of the Embden-Meyerhof pathway or the glutathione reductase pathway. Patients with inherited disorders of the hemoglobin molecule or red cell membrane generally have a lifelong clinical history typical of the disease process. Those with chronic hemolytic disease, such as

hereditary spherocytosis, may actually present not with anemia but with a complication stemming from the prolonged increase in red cell destruction such as symptomatic bilirubin gallstones or splenomegaly. Patients with chronic hemolysis are also susceptible to aplastic crises if an infectious process interrupts red cell production.

The differential diagnosis of an acute or chronic hemolytic event requires the careful integration of family history, the pattern of clinical presentation and—whether the disease is congenital or acquired—by a careful examination of the peripheral blood smear. Precise diagnosis may require more specialized laboratory tests, such as hemoglobin electrophoresis or a screen for red cell enzymes. Acquired defects in red cell survival are often immunologically mediated and require a direct or indirect antiglobulin test or a cold agglutinin titer to detect the presence of hemolytic antibodies or complement-mediated red cell destruction.

TREATMENT Anemia

An overriding principle is to initiate treatment of mild to moderate anemia only when a specific diagnosis is made. Rarely, in the acute setting, anemia may be so severe that red cell transfusions are required before a specific diagnosis is made. Whether the anemia is of acute or gradual onset, the selection of the appropriate treatment is determined by the documented cause(s) of the anemia. 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. 113](#); [iron therapy is discussed in Chap. 103](#); [treatment of megaloblastic anemia is discussed in Chap. 105](#); [treatment of other entities is discussed in their respective chapters \(sickle cell anemia, Chap. 104 hemolytic anemias, Chap. 106; aplastic anemia and myelodysplasia, Chap. 107\)](#).

Therapeutic options for the treatment of anemias have expanded dramatically during the past 25 years. Blood component therapy is available and safe. Recombinant EPO as an adjunct to anemia management has transformed the lives of patients with chronic renal failure on dialysis and reduced transfusion needs of anemic cancer patients receiving chemotherapy. 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. 68](#)).

POLYCYTHEMIA

Polycythemia is defined as an increase in the hemoglobin above normal. This increase may be real or only apparent because of a decrease in plasma volume (spurious or relative polycythemia). The term *erythrocytosis* may be used interchangeably with polycythemia, but some draw a distinction between them: erythrocytosis implies documentation of 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 usually triggered at 170 g/L (17 g/dL) for men and 150 g/L (15 g/dL) for women. Hematocrit levels >50% in men or >45% in women may be abnormal.

Hematocrits >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 history; current living at high altitude; or a history of congenital heart disease, sleep apnea, or chronic lung disease.

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 hematocrits >55%. Manifestations range from digital ischemia to Budd-Chiari syndrome with hepatic vein thrombosis. Abdominal vessel thromboses are particularly common. Neurologic symptoms such as vertigo, tinnitus, headache, and visual disturbances may occur. Hypertension is often present. Patients with *polycythemia vera* may have aquagenic pruritus and symptoms related to hepatosplenomegaly. Patients may have easy bruising, epistaxis, or bleeding from the gastrointestinal tract. Peptic ulcer disease is common. 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 syndrome ([Chap. 236](#)). 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 all associated with increases in EPO levels: either a physiologically adapted appropriate elevation based on tissue hypoxia (lung disease, high altitude, CO poisoning, high-affinity hemoglobinopathy) or an abnormal overproduction (renal cysts, renal artery stenosis, tumors with ectopic EPO production). A rare familial form of polycythemia is associated with normal EPO levels but hyperresponsive EPO receptors due to mutations.

APPROACH TO THE PATIENT Polycythemia

As shown in [Fig. 57-18](#), the first step is to document the presence of an increased red cell mass using the principle of isotope dilution by administering ^{51}Cr -labeled autologous red blood cells to the patient and sampling blood radioactivity over a 2-h period. 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. If EPO levels are low or unmeasurable, the patient most likely has polycythemia vera. Tests that support this diagnosis include elevated white blood cell count, increased absolute basophil count, and thrombocytosis. A mutation in *JAK-2* (Val617Phe), a key member of the cytokine intracellular signaling pathway, can be found in 70–95% of patients with polycythemia vera.

If serum EPO levels are elevated, one needs to distinguish whether the elevation is a physiologic response to hypoxia or is related to autonomous EPO production. Patients with low

AN APPROACH TO DIAGNOSING PATIENTS WITH POLYCYTHEMIA

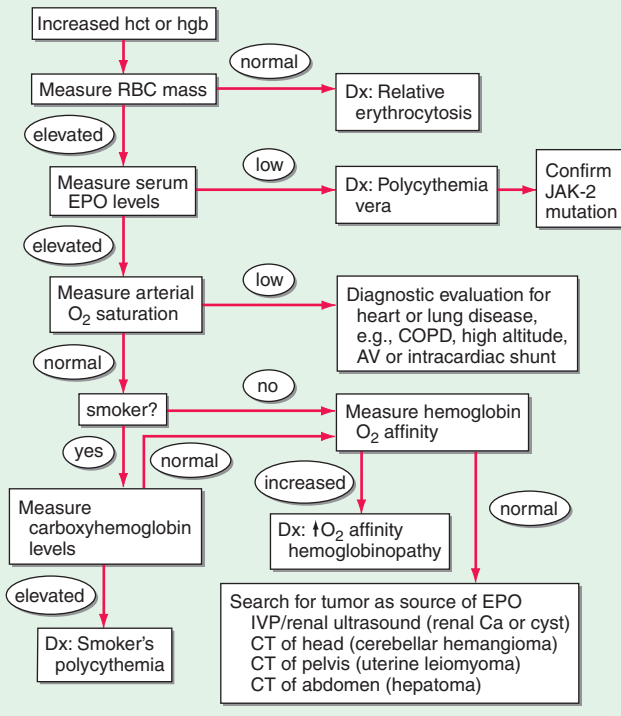


Figure 57-18 An approach to the differential diagnosis of patients with an elevated hemoglobin (possible polycythemia). RBC, red blood cell; EPO, erythropoietin; COPD, chronic obstructive pulmonary disease; AV, atrioventricular; IVP, intravenous pyelogram; hct, hematocrit.

arterial 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-hemoglobin 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 neoplasms. Hepatoma, uterine leiomyoma, and renal cancer or cysts are all detectable with abdominopelvic CT scans. Cerebellar hemangiomas may produce EPO, but they present with localizing neurologic signs and symptoms rather than polycythemia-related symptoms.

FURTHER READINGS

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CHAPTER 58

Bleeding and Thrombosis

Barbara Konkle

The human hemostatic system provides a natural balance between procoagulant and anticoagulant forces. The procoagulant forces include platelet adhesion and aggregation and fibrin clot formation; anticoagulant forces include the natural inhibitors of coagulation and fibrinolysis. Under normal circumstances, hemostasis is regulated to promote blood flow; however, it is also prepared to clot blood rapidly to arrest blood flow and prevent exsanguination. After bleeding is successfully halted, the system remodels the damaged vessel to restore normal blood flow. The major components of the hemostatic system, which function in concert, are (1) platelets and other formed elements of blood, such as monocytes and red cells; (2) plasma proteins (the coagulation and fibrinolytic factors and inhibitors); and (3) the vessel wall.

STEPS OF NORMAL HEMOSTASIS

PLATELET PLUG FORMATION

On vascular injury, platelets adhere to the site of injury, usually the denuded vascular intimal surface. Platelet adhesion is mediated primarily by von Willebrand factor (VWF), a large multimeric

protein present in both plasma and the extracellular matrix of the subendothelial vessel wall, which serves as the primary “molecular glue,” providing sufficient strength to withstand the high levels of shear stress that would tend to detach them with the flow of blood. Platelet adhesion is also facilitated by direct binding to subendothelial collagen through specific platelet membrane collagen receptors.

Platelet adhesion results in subsequent platelet activation and aggregation. This process is enhanced and amplified by humoral mediators in plasma (e.g., epinephrine, thrombin); mediators released from activated platelets (e.g., adenosine diphosphate, serotonin); and vessel wall extracellular matrix constituents that come in contact with adherent platelets (e.g., collagen, VWF). Activated platelets undergo the release reaction, during which they secrete contents that further promote aggregation and inhibit the naturally anticoagulant endothelial cell factors. During platelet aggregation (platelet-platelet interaction), additional platelets are recruited from the circulation to the site of vascular injury, leading to the formation of an occlusive platelet thrombus. The platelet plug is anchored and stabilized by the developing fibrin mesh.

The platelet glycoprotein (Gp) IIb/IIIa ($\alpha_{IIb}\beta_3$) complex is the most abundant receptor on the platelet surface. Platelet activation converts the normally inactive Gp IIb/IIIa receptor into an active receptor, enabling binding to fibrinogen and VWF. Because the surface of each platelet has about 50,000 Gp IIb/IIIa-binding sites, numerous activated platelets recruited to the site of vascular injury can rapidly form an occlusive aggregate by means of a dense network of intercellular fibrinogen bridges. Since this receptor is the key mediator of platelet aggregation, it has become an effective target for antiplatelet therapy.