Why is My Patient Anemic?

Locke J. Bryan, MD[a], Neil A. Zakai, MD, MSc[b,*]

KEYWORDS

• Anemia • Review • Clinical medicine • Blood cell count

Key Points

- Anemia is a common clinical question in consultative hematology.
- The causes and consequences of anemia have important implications for the care of patients.
- Anemia results from 1. red cell loss of sequestration, 2. red cell destruction, or 3. decreased red cell production.
- A systematic approach to the evaluation of anemia is needed to diagnose the cause of anemia.

Anemia is one of the most common clinical questions in consultative hematology.[1] The variety of causes and consequences of anemia reflects the key role of blood as a transport and messaging system for the entire body. Traditionally, anemia has been viewed as an innocent bystander, a marker of disease rather than a cause of disease, but emerging evidence suggests that anemia may affect quality of life, cardiovascular health, and mortality.[2]

Despite the association of anemia with a variety of diseases and with adverse clinical outcomes, the hemoglobin concentration that defines anemia is not established and variations within the normal range are associated with disease.[2,3] The World Health Organization (WHO) in 1958 defined sex-specific hemoglobin targets of 12 g/dL in women and 13 g/dL in men, but acknowledged that "[t]hese figures were chosen arbitrarily and it is still not possible to define normal precisely."[4] Most clinical laboratories define anemia as the bottom 2.5% of the distribution of hemoglobin values from a healthy population.[5]

The lack of a standard definition complicates the role of the clinician, because small changes in the definition of abnormal can change dramatically the number of people who are anemic. In certain situations, the patient serves as their own reference range

[a] University of Vermont College of Medicine, 111 Colchester Avenue, Smith Room 244, Burlington, VT 05401, USA
[b] Colchester Research Facility, University of Vermont College of Medicine, 208 South Park Drive, Colchester, VT 05446, USA
* Corresponding author.
E-mail address: Neil.Zakai@uvm.edu

Hematol Oncol Clin N Am 26 (2012) 205–230
doi:10.1016/j.hoc.2012.02.008 **hemonc.theclinics.com**
0889-8588/12/$ – see front matter

and changes in hemoglobin over time may provide an indicator of health or illness. In this review, the epidemiology and risk factors for anemia are discussed and a clinical guide to the evaluation of anemia is presented.

WHAT IS ANEMIA?

The Merriam-Webster dictionary defines anemia as "a condition in which the blood is deficient in red blood cells (RBC), in hemoglobin, or in total volume."[2] In clinical practice, anemia has multiple clinical definitions, usually based on RBC volume or hemoglobin concentration. **Table 1** presents multiple standards that are reported for healthy populations; however, many more are available. Most clinical laboratories define abnormal as the bottom 2.5% of the gender-specific population distribution, occasionally including age-specific ranges for adults, and thus reference ranges differ based on the local population.[5]

WHAT ARE THE DETERMINANTS OF HEMOGLOBIN CONCENTRATION?

Hemoglobin concentration is a complex phenotype, controlled by both genetic and environmental factors. Although the environmental risk factors for anemia have been studied for years, we are only just beginning to understand the genetics of hemoglobin concentration.

Genetics

Previous analyses of the genetics of hemoglobin concentration have focused on disease states involving hemoglobin variants (ie, thalassemias, hemoglobin C, S, or E disease), red cell structural and metabolism protein defects (ie, hereditary spherocytosis), and iron metabolism (ie, hemochromatosis). In a recent genome-wide association study of healthy individuals, variations in multiple genes, some known to affect hematopoiesis and others novel, were found that affect hemoglobin concentration (**Table 2**).[6] These variants explain only some of the observed variation in hemoglobin concentration in individuals with European ancestry, and further work is under way in non-European ancestry populations.

Age

Age is a known risk factor for anemia, although the physiology is often not completely understood.[7] As we age, a greater proportion of our bone marrow is replaced with fat, leaving fewer hematopoietic elements.[8] In an apparently healthy elderly cohort

Table 1
Hemoglobin reference ranges to define anemia in various populations

		Men (g/dL)		Women (g/dL)
WHO[4]		13		12
Williams hematology[1]		14.0		12.3
Beutler & Waalen[5]		Age 20–59 y	Age 60 y+	All ages
	White	13.7	13.2	12.2
	Black	12.9	12.7	11.5
Boston, MA[62]		13.5		12.0
Togo[63]		11.9		10.2
Tanzania[64]		13.7		11.1
Ethiopia[65]		13.9		12.2

Table 2
Genetic determinants of hemoglobin concentration from the CHARGE consortium

Protein Name	Chromosome	Function
Protein C kinase ε	2p21	Phosphorylates a wide variety of protein targets in cellular signaling
Transmembrane protein 163	2q21	Unknown
Aminomethyltransferase	3p21	Glycene cleavage system
v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	4q11	Transmembrane protein, target for stem cell factor; mutations are associated with various tumors
Hereditary hemochromatosis protein	6p21	Controls iron absorption by regulating interaction between transferrin and the transferrin receptor
HBS1L-like v-myb myeloblastosis viral oncogene homolog	6q23	Guanosine triphosphate elongation factor; regulates hematopoiesis; intragenic region associated with modification of severity of sickle cell anemia and thalassemia/ hemoglobin E disease
Erythropoietin	7q22	Regulates red cell production by promoting erythroid differentiation and initiating hemoglobin synthesis
Protein kinase, adenosine monophosphate-activated, γ2 noncatalytic subunit	7q36	Monitors cellular energy status and inactivates key enzymes involved in regulating synthesis of fatty acids and cholesterol
Hexokinase 1	10q22	Phosphorylates glucose to glucose-6-phosphate–energy metabolism
SH2B adaptor protein 3	12q24	Signaling protein believed to play a role in hematopoiesis
TSHZ3 teashirt zinc finger homeobox 3	19q12	Unknown
Transmembrane protease, serine 6	22q12	Hepatic protein, may be involved in regulating iron metabolism

Data from Ganesh SK, Zakai NA, van Rooij FJ, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nat Genet 2009;41(11):1191–8.

(age >64 years), the Cardiovascular Health Study, the prevalence of anemia (WHO criteria) at baseline was 8.5%.[3] In another study, for every 10-year increase in age, participants had a 30% increased odds of anemia using the WHO criteria.[9] Whether lower hemoglobin concentrations and increased anemia prevalence represents physiologic aging versus a condition of disease is poorly studied. Several studies in elderly individuals suggest hemoglobin decline is associated with increased mortality.[3,10,11]

Race

African Americans have lower hemoglobin levels and a nearly 3-fold increased odds of anemia than whites.[9,12] Some of the difference is associated with an increased prevalence of hemoglobin variants such as α-thalassemia.[12] When examining the distribution of hemoglobin between African Americans and whites (**Fig. 1**), the curves

are almost identical except for a shift to lower hemoglobin values in African Americans. Whether African Americans tolerate lower hemoglobin concentrations better is not known. In one analysis of elderly individuals, anemia by the WHO criteria was just as poorly tolerated in blacks as in whites.[3]

Gender

Women have lower hemoglobin concentrations than men. Most anemia criteria use sex-specific ranges and thereby the prevalence of anemia differs only slightly between men and women. In younger women, this difference may be partially explained by menstrual blood losses; however, this differences persists into older ages. Again, apart from a shift to lower hemoglobin values, there is no evidence of a skewed distribution in women versus men (see **Fig. 1**). Although there are no outcomes data to support using different hemoglobin criteria for men and women

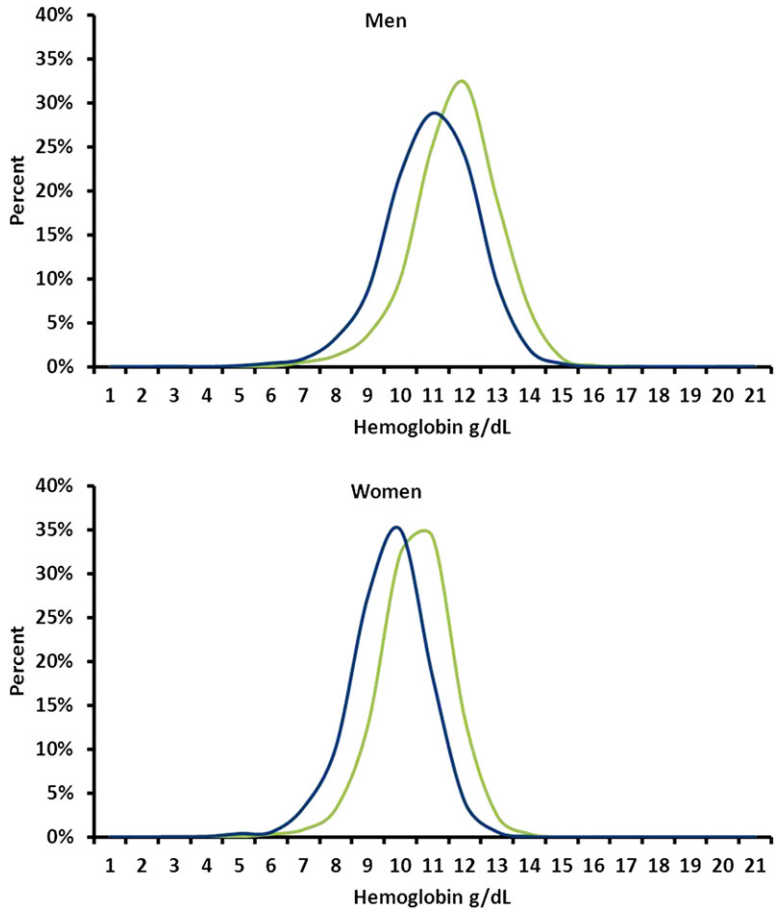

Fig. 1. Distribution of hemoglobin concentration for African American (*blue line*) and white (*green line*) men and women in the United States. (*Data from* Zakai NA, McClure LA, Prineas R, et al. Correlates of anemia in American blacks and whites: the REGARDS Renal Ancillary Study. Am J Epidemiol 2009;169(3):355–64.)

to define anemia, different ranges have been used for many years in clinical practice.

Geography

Environmental factors such as altitude have a profound effect on hemoglobin concentrations. At higher altitudes, decreased oxygen concentrations in the blood provoke a reactive polycythemia, potentially increasing hemoglobin concentrations by more than 2 g/dL in individuals acclimatized to sea level.[13] Polycythemia is seen in some populations native to high altitudes such as the Aymara in South America but not in others such as Tibetans in Asia, suggesting different genetic adaptations to altitude in different populations.[14] Recent studies indicate that there may be geographic differences in hemoglobin concentration independent of altitude, but the impact on health and anemia definitions have not been studied.[9] Apart from incorporating population-specific reference ranges in clinical laboratories serving discrete populations, no effort has been made to account for altitude and geography when establishing normal hemoglobin ranges.

Diseases and Medications

A variety of acute disease conditions affect hemoglobin concentration, but the role of chronic disease conditions is less recognized. Common medical conditions are independently associated with anemia, such as a history of stroke or myocardial infarction, diabetes mellitus, hypertension, and chronic kidney disease.[9] Some common medications such as angiotensin-converting enzyme inhibitors and antiandrogen medications are also associated with anemia.[15–18]

WHAT ARE THE CONSEQUENCES OF ANEMIA?

The effect of severe anemia on cardiovascular function is well recognized in clinical practice.[1] In contrast, less severe anemia has been considered a consequence of disease rather than a cause of disease.[2] A growing body of evidence suggests that anemia and even hemoglobin concentrations within the lower normal range are associated with increased morbidity and mortality in a variety of populations.[3,10] An alternate approach to defining anemia is to use the individual as their own reference range; a decreasing hemoglobin concentration, even within the normal range, has been shown to relate to future mortality in an elderly population.[11]

SUMMARY

A variety of factors both genetic and environmental can affect hemoglobin concentration and the prevalence of arbitrarily defined anemia. In most cases, a hemoglobin concentration less than 11 g/dL is abnormal. Hemoglobin concentrations between 11 g/dL and 14 g/dL may or may not be normal, depending on the clinical context. The role of the consulting hematologist is to help identify the cause of anemia and when appropriate recommend treatment options. Patients should be given transfusion support when experiencing severe anemia-related symptoms. However, there are no data to show that providing transfusion support for asymptomatic patients with the purpose of bringing hemoglobin concentrations into a specific normal range improves outcomes.

WHY IS MY PATIENT ANEMIC?

The reasons for anemia can be complex and multifactorial. To diagnose and manage anemia, the workup needs a systematic approach. Although there are innumerable

immediate causes of anemia, there are only a few overarching reasons why someone is anemic:

1. Blood loss or sequestration
2. Increased destruction of RBC
3. Decreased production of RBC.

Determining which of these processes is occurring in the patient helps determine the cause of the anemia. An initial clinical and laboratory assessment must focus on determining which of these processes are active. An initial workup consisting of a complete blood count, reticulocyte count, a clinical history, and a physical examination can help guide the anemia workup.

Clinical History

A careful history to establish whether or not the patient has an unstable volume is warranted in all anemias. A history of gastrointestinal (GI), urinary, or respiratory blood loss is essential, with confirmatory testing for the presence of blood. A dietary and medication/drug history determines whether the patient is at risk for nutritional deficiencies. The physical examination may help direct the workup for anemia:

- Head and neck: the presence of scleral icterus and mucosal jaundice can indicate increased RBC destruction and increase of indirect bilirubin in the serum. Thyromegaly can suggest anemia caused by thyroid disease.
- Lymph nodes: lymphadenopathy can denote an underlying infection or malignancy leading to anemia.
- Cardiac: the detection of murmurs associated with valvular heart disease can lead to suspicion for increased RBC destruction. In the appropriate clinical scenario, assess for signs of endocarditis, which can lead to both diminished RBC production and increased RBC destruction.
- Respiratory: blood loss can present as a hemothorax yielding diminished breath sounds and dullness to percussion. Basilar crackles can suggest congestive heart failure, which is commonly associated with anemia.
- Abdominal: detection of an enlarged spleen or liver can signify a malignancy, increased RBC destruction, or RBC sequestration. The presence of a palpable mass is suggestive of malignancy. A fluid wave or shifting dullness in the abdomen also can suggest a malignancy associated with ascites or portal hypertension and cirrhosis. Abdominal distention with tenderness and abdominal wall ecchymosis can denote a retroperitoneal hematoma associated with RBC loss.
- Extremities: thigh swelling or ecchymosis can point to a site of bleeding. Joint swelling or deformity can hint at an infectious or rheumatologic process leading to anemia.
- Skin: jaundice can be a sign of increased RBC destruction. Rashes can be associated with diseases such as systemic lupus erythematosus or vasculitis, which can lead to anemia.

Laboratory Studies

Several initial laboratory studies guide the workup, as can review of the peripheral smear:

- Peripheral smear blood
  - RBC size: the RBC is a biconcave disc that appears as round to slightly ovoid on review of the peripheral smear. Approximately one-third of the center appears

paler than the surrounding periphery. A normal RBC is typically the size of the nucleus of a nonreactive lymphocyte, approximately 7 mm (**Fig. 2**A). A change in the cell size or amount of central pallor constitutes an abnormal RBC. RBC greater than 8.5 μm are called macrocytes (see **Fig. 2**E). Macrocytic RBC occur with nutritional deficiencies (eg, vitamin B_{12} or folic acid), primary bone marrow disorders (eg, myelodysplastic syndromes, multiple myeloma), hypothyroidism, medications (eg, hydroxyurea), excess alcohol consumption and reticulocytosis in response to bleeding or hemolysis. Small RBC, microcytes, are seen with iron deficiency, lead poisoning, thalassemias, abnormal hemoglobins (eg, sickle disease), and sideroblastic anemia (see **Fig. 2**C). Spherocytes are small RBC less than 6.5 μm with absent central pallor (see **Fig. 2**D) seen in hereditary spherocytosis and autoimmune hemolytic anemias.

- RBC shape: when viewing the peripheral blood smear it is important to consider the shape of the RBC. Several distinct shapes can suggest a diagnosis. Schistocytes are small fragmented pieces of RBC caused by hemolysis (see **Fig. 2**B).

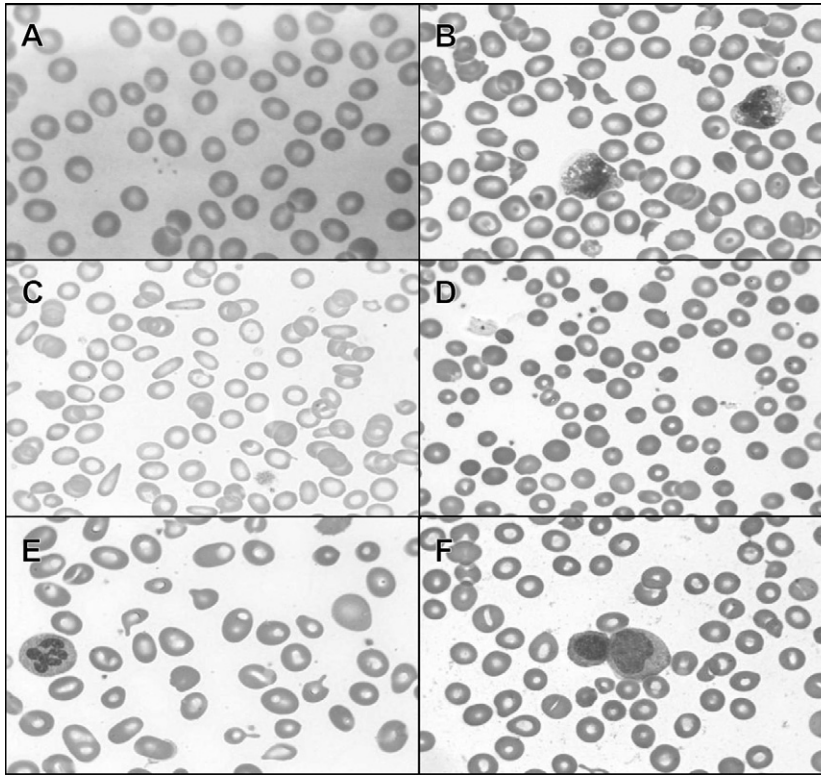

Fig. 2. Peripheral smears. (*A*) Normal RBC morphology. Note central pallor occupying approximately one-third of cell. (*B*) Schistocytes and burr cells consistent with microangiopathic hemolytic anemia. (*C*) Hypochromic microcytic RBC consistent with IDA. (*D*) Spherocytes as seen in hereditary spherocytosis or autoimmune hemolytic anemia. (*E*) Megaloblastic RBC with a hypersegmented neutrophil as seen in vitamin B_{12} deficiency. (*F*) Nucleated RBC and tear-drop cells consistent with leukoerythroblastic syndrome as seen in bone marrow infiltration. (*Courtesy of* John H. Lunde, Medical Director of the Hematology Lab.)

Target cells, codocytes, are small RBC with a central area of pigmentation. Target cells are present in the hemoglobinopathies and may also appear in iron-deficiency anemia (IDA). Dacrocytes, tear-drop cells, appear in the presence of myelophthisis or leukoerythroblastosis when the bone marrow is infiltrated by another process (see **Fig. 2**F). Sickle cells are present in the sickle hemoglobinopathies. Rouleaux formation or the appearance of RBC as a coin stack can be present in Waldenstrom macroglobulinemia, multiple myeloma, and inflammatory states. Clumping of RBC suggest cold antibodies.

- Abnormal circulating cells: the peripheral smear can reveal crucial information beyond direct visualization of the RBC. Review of the circulating white blood cells (WBC) and platelets is essential. Hypersegmented neutrophils, neutrophils with greater than 6 nuclear lobes, may be present in vitamin B_{12} deficiency (see **Fig. 2**C). Immature lymphocytes or numerous lymphocytes can reveal the diagnosis of acute or chronic leukemias, respectively. Abnormal platelets can raise suspicion for the myelodysplastic or myeloproliferative disorders.

- Red cell distribution width (RDW): the RDW is a measure in the variation of the size of RBC, with an abnormally high RDW indicating increased RBC variation. An increased RDW is the first sign of an iron-depleted state and when noted in the setting of microcytosis is consistent with IDA. An increased RDW with macrocytosis can indicate a mixed nutritional deficiency (eg, combined iron and vitamin B_{12} or folic acid deficiency), or reticulocytosis in response to either RBC loss, RBC destruction, or correction of a nutritional deficiency.
- Reticulocyte count: a reticulocyte count helps differentiate anemias of increased RBC loss/destruction from anemias of decreased RBC production. Reticulocytes are young RBC forced into the circulation in response to increased RBC loss or destruction. A low reticulocyte count with anemia suggests decreased RBC production or inadequate time for RBC production to increase. Reticulocytes can be reported as either a percentage of RBC or as an absolute count. If the reticulocytes are presented as a percentage of RBC, a correction factor $\left(\text{corrected reticuocyte count} = \%\ \text{reticulocytes} \times \left(\frac{\text{Patient Hct}}{\text{Normal Hct}}\right)\right)$ should be applied to adjust for the severity of anemia.[19]
- Other initial laboratory tests: increases in lactate dehydrogenase (LDH) and indirect bilirubin levels with a decrease in haptoglobin level are signs of increased RBC destruction. A positive direct antibody test (DAT) is suggestive of immune-mediated RBC destruction.

RED BLOOD CELL (RBC) LOSS OR DILUTION ANEMIA

There are many potential sources of RBC loss. These sources include occult GI bleeding, urologic bleeding, menstruation, pulmonary bleeding, and phlebotomies (eg, blood donation, laboratory testing). Blood loss must be addressed as a potential cause for all anemias because identification of an early-stage malignancy such as colon cancer can be potentially life saving. Indolent blood loss over a period of time often results in iron deficiency and loss of the ability of the bone marrow to properly increase RBC production. Anemia secondary to blood loss and IDA should prompt a complete workup in men, regardless of age, and in postmenopausal women for a source of bleeding. In menstruating women, an initial trial of iron is warranted, but with further evaluation if there is no response to therapy. Occasionally, anemia is secondary to an increased plasma volume rather than a decrease in RBC mass. The only common clinical situations in which this situation occurs are pregnancy

and iatrogenic fluid overload from intravenous fluids.[1] Hemoglobin concentration in pregnancy is typically reduced to 11 to 12 g/dL.

Clinical History

Careful questioning about blood loss is important. Unless there is evidence of an overdose, blood loss on anticoagulant and antithrombotic medications is not physiologic and the source must be identified rather than attributed to anticoagulation.

GI blood losses are one of the most common sources of blood loss. Past history of medications that can cause GI ulceration or inflammation such as nonsteroidal antiinflammatory agents, aspirin, and bisphosphonates may help the practitioner narrow down potential sources. A history of gastric reflux symptoms or use of gastric reflux medications may indicate the potential for GI blood loss secondary to chronic gastritis. Patients may report hematemesis with either bright red blood or emesis described as like coffee grounds in appearance. Questioning about bright red blood with bowel movements, dark tarry stools or melena, known diverticular disease, or previous diverticular bleeding are important to identify possible GI losses. Occasionally, swallowed blood from the upper respiratory tract can present as apparent GI blood loss. Confirmation of colorectal cancer screening is crucial.

In women, a careful menstrual history is mandatory, including the amount of blood loss, the frequency of periods, and the duration of menstruation. The prevalence of IDA in menstruating women is reported to be between 25% and 47.5%.[20,21] Women with decreases in iron intake or even small increases in blood loss as seen in menorrhagia or metrorrhagia can further deplete iron stores, contributing to worsened anemia.

Urinary blood loss is often underappreciated. Changes in urine color, with variations of a pink tint to deep red, can point to a urinary source. Microscopic hematuria may represent underlying urinary disease, including benign prostatic hypertrophy, renal calculi, polycystic kidney disease, or more concerning causes such as bladder, prostate, or kidney cancer. Intrinsic renal disease represents another potential for microscopic hematuria that can result in IDA.[22]

Pulmonary and upper airway bleeding often present as varying degrees of hemoptysis. Patients may ascribe to a chronic cough with hemoptysis described as either obvious blood-colored or rust-colored sputum, raising suspicion of a pulmonary lesion. Patients may not recognize hemoptysis because most sputum is swallowed and never visualized.

There are several potential spaces within the body that can sequester large amounts of blood and be relatively asymptomatic: the pleural space, the retroperitoneal space, and the thigh. Trauma, even minor, especially in the setting of antithrombotic or anticoagulant medications can result in significant blood loss. Intracranial, retinal, or joint space hemorrhages are small in volume and are unlikely to present as anemia. Hip or flank pain, vague abdominal complaints, and lower extremity paresis can be symptoms associated with retroperitoneal hemorrage.[23]

A careful history of blood donation, frequency of phlebotomy for laboratory examinations, and potential surreptitious phlebotomy should be obtained. Iron deficiency from blood donation is a reported cause of anemia.[24] Repetitive phlebotomy during hospitalization can result in a meaningful decline in hemoglobin.[25] Rarely, Munchausen syndrome has been associated with anemia in patients seeking unneeded therapy by bloodletting or intentional diet modifications.[26]

Physical Examination

The focus of the physical examination depends largely on the acuity of the blood loss. Orthostatic vital signs are important in acute blood loss because a patient at rest can

seem hemodynamically stable and become profoundly hypotensive with standing. GI blood loss may be evident on nasogastric lavage or digital rectal examination. Inspection of the urine color may reveal hematuria. Suspicion of gynecologic bleeding requires a complete pelvic examination. Trauma, lacerations, or venous puncture sites may be visible. A complete musculoskeletal examination may reveal focal pain or swelling, suggesting a hematoma or internal blood loss.

Laboratory Findings

The severity of anemia varies depending on the amount and chronicity of blood loss. Typically, anemia of acute blood loss remains normocytic and as iron stores are depleted the RDW increases and the mean corpuscular volume (MCV) decreases. Initial screening tests include stool for occult blood, urinalysis, or sputum analysis, as directed by the clinical history. Localized complaints of pain or swelling may represent collections of blood and can direct imaging studies. If a patient history and workup are consistent with blood loss, a negative screen for bleeding does not eliminate the need for direct imaging of likely sites by procedures such as colonoscopy, esophagogastroduodenoscopy, or visualization of the upper aerodigestive tract by endoscopy.

Red Blood Cell (RBC) Destruction

RBC destruction is a challenging cause of anemia because of the large number of conditions in the differential diagnosis. The normal erythrocyte has a life span of approximately 100 to 140 days.[1] The processes through which the body identifies older or damaged RBC for clearance are not fully understood; hypotheses include decreased deformability or alterations in the RBC membrane. There are two main sites for the body to destroy RBC: within the blood vessels (intravascular hemolysis) and ingestion of RBC by macrophages in the spleen and liver (extravascular hemolysis).[1,27]

Intravascular

Under normal circumstances, only some RBC are destroyed in the vascular space. Circulating haptoglobin binds to free hemoglobin, and haptoglobin-hemoglobin complexes are cleared by the liver, with the hemoglobin converted to iron and biliverdin (further converted to bilirubin).[1,19]

Extravascular

Macrophages in the spleen and liver engulf RBC and heme is cleaved into iron and biliverdin, which is later converted into bilirubin. This is the main route for normal RBC catabolism. Haptoglobin is decreased in extravascular hemolysis as well from hemoglobin spilling from macrophages.[1,19]

It is possible to divide hemolytic anemias into intravascular and extravascular processes; however, the cause often overlaps between these 2 processes. Further, chronic RBC destruction can lead to nutritional deficiencies such as iron deficiency from urinary losses and folate deficiency from increased nucleic acid turnover.[1] A reasonable clinical approach is to divide hemolysis into mechanical, immune-mediated, and intrinsic RBC abnormalities and recognize that the destruction can occur at multiple sites.

Mechanical

Within blood vessels, RBC can be destroyed by physical trauma such as from prosthetic cardiac valves and by repetitive trauma of vascular beds, as is seen in march hemoglobinuria. Pathologic processes such as systemic infections, thrombotic thrombocytopenic purpura (TTP), and vasculitis sheer RBC passing through obstructed

small vessels. Injury from toxins and heat as well as injury from parasites such as malaria and *Babesia* and bacteria such as *Clostridium* species are other possible means of RBC destruction.

Immune-mediated

Immune-mediated destruction or RBC can be from autoreactive antibodies or alloreactive antibodies. Autologous antibodies can result from neoplastic, infectious, drug-associated, and idiopathic processes, whereas alloreactive antibodies are not apparent unless there is sensitization and exposure to allergenic RBC. Depending on the antibody, immune-mediated hemolysis can occur in the intravascular space, extravascular space, or both.

Hereditary syndromes

Hereditary syndromes can present as chronic hemolysis throughout the lifetime of the individual or as paroxysms of hemolysis with an environmental trigger. A full discussion of hemoglobin variants (ie, sickle cell anemia, thalassemias) is beyond the scope of this review but should be considered in the appropriate clinical setting. Carriers of hemoglobin S, C, and E rarely have obvious hematologic abnormalities, but carriers of thalassemia minor (both α and β) may be microcytic and mildly anemic. An important group of patients are those with RBC enzyme deficiencies because these can present in adulthood as specific episodes of hemolysis caused by an environmental trigger.

Clinical History and Physical Examination

A clinical history suggestive of RBC destruction includes the normal signs and symptoms of anemia (eg, fatigue, pallor). Hemolysis should be strongly suspected with a history of jaundice and changes in urine color. A careful history of recent illnesses and medications is important in the diagnosis of immune-mediated hemolysis or drug-induced hemolysis. Review of travel history, sick contacts, and sexual history may be clues of underlying infections. Hemolysis can be associated with a hypercoagulable state and patients may present with venous thromboembolic disease.

The physical examination is useful in assessing the stability of the patient. Most patients with hemolysis have jaundice. If there is an underlying infectious or neoplastic disorder, lymphadenopathy or splenomegaly may be apparent. Patients with vasculitis may present with neurologic complaints and skin lesions. Patients with TTP or hemolytic uremic syndrome (HUS) may have accompanying symptoms, including fevers and neurologic complaints. Occasionally, patients present with cholelithisis or cholecystitis from pigmented gallstones.

Laboratory Findings

The degree of anemia is variable in hemolytic anemias and can range from mild to severe. Markers of hemolysis such as LDH, indirect bilirubin, and urinary urobilinogen are increased, and haptoglobin is suppressed. A reticulocyte count is usually increased except in acute hemolysis in which the bone marrow has not had a chance to increase RBC production or in chronic hemolysis if individuals have developed concurrent nutritional deficiencies.

Review of the peripheral blood smear is important. In nonimmune-mediated hemolysis, the peripheral blood smear may reveal characteristic burr cells, schistocytes, helmet cells, and microspherocytes from physical destruction of the RBC (see **Fig. 2**B). In immune-mediated hemolysis, the peripheral smear may reveal spherocytes, fragmented red cells, acanthocytes, bite or blister cells, or RBC clumping (see **Fig. 2**D).

Mechanical hemolysis

Mechanical breakdown of RBC is the result of physical trauma, microangiopathic hemolysis, infections associated with destruction, and several other miscellaneous processes.

- Physical trauma: repetitive trauma to vascular beds can cause destruction of erythrocytes, as seen in runners or in soldiers forced to march long distances, resulting in march hemoglobinuria. Individuals present with discrete episodes of hemolysis, which may include dark urine or muscle aches/pains. Laboratory and physical examination findings are usually negative but occasionally jaundice and hepatosplenomegaly are present. Malfunctioning prosthetic cardiac valves or abnormalities of native valves can cause shearing of RBC and hemolysis. Hemolysis is seen in many prosthetic valves, with a higher risk in mitral versus aortic valve replacement and mechanical versus bioprosthetic valves.[28] Review of the peripheral smear shows red cell fragments (see **Fig. 2**B).
- Microangiopathic hemolytic anemia: the underlying process is destruction or shearing of the RBC in the microcirculation and leads to the classic finding of schistocytes or helmet cells on review of the peripheral blood smear (see **Fig. 2**B). Schistocytes are seen in most individuals; however, in pathologic conditions, the percentage of schistocytes usually exceeds 1%.[29] Signs and symptoms of anemia are often secondary to signs and symptoms of the underlying disorder. The clinical setting varies, but markers of hemolysis (increased LDH, decreased haptoglobin) are almost universally present. Disorders that can cause a microangiopathic hemolytic anemia include TTP, HUS, systemic infections (with or without diffuse intravascular coagulation), systemic malignancy, malignant hypertension, and preeclampsia/HELLP syndrome in pregnant women. In general, coagulation studies are normal and the diagnosis is made with clinical criteria in the setting of a microangiopathic hemolytic anemia on peripheral blood smear. The diagnosis and treatment of microangiopathic hemolytic anemias is complex and an accurate diagnosis is important. Classic TTP can be diagnosed with low levels of the ADAMTS13 protease and an excess of high-molecular-weight Von Willebrand multimers.[30] The diagnosis of other microangiopathic hemolytic anemias has recently been reviewed.[1,30]
- Infections: malaria and *Babesia* are parasites that reside within the RBC. Intravascular hemolysis occurs as a result of reproduction of the parasites within the RBC. Severe malarial infections can mimic the signs and symptoms of TTP; however, review of the peripheral smear reveals parasites within RBC.[31,32] Toxins produced by infections such as *E coli* O157:H7 and *Clostridium* species result in direct RBC destruction; diagnosis is made by the clinical history and isolation of bacterial toxins from stool or bacteria from the blood (**Table 3**).[1]
- Miscellaneous: snake, spider, and insect toxins can cause hemolysis. The clinical picture is usually dominated by the particular toxin to which the patient is exposed. Accidental infusion of hypotonic solutions can cause rupture of RBC. A careful review of all clinical events surrounding hemolysis and review of all infusions can help make the diagnosis.

Immune-mediated hemolysis

Autoimmune hemolytic anemias are divided into primary and secondary processes.[1,27] Patients have variable degrees of anemia and typical laboratory findings of hemolysis (increased LDH, depressed haptoglobin, increased indirect bilirubin) and anemia. The diagnosis of autoimmune hemolytic anemia is made by showing binding of antibodies

Table 3
Infections associated with anemia

Type	Organism	Pathophysiology
Bacterial	*Clostridium perfringens*	Toxin-mediated direct RBC lysis
	E coli (O157:H7)	Toxin-mediated direct RBC lysis
	Helicobacter pylori	B_{12} deficiency secondary to gastritis
	Mycoplasma pneumoniae	Cold autoimmune hemolysis
	Streptococcus pneumoniae	Mechanical hemolysis with bacterial endocarditis or DIC
Fungal	*Histoplasma capsulatum*	Bone marrow infiltration typically associated with HIV
	Mycobacterium tuberculosis and *M avium*	Bone marrow infiltration typically associated with HIV
Parasitic	*Babesia microti* and *B divergens*	Direct RBC lysis and RBC membrane fragility
	Plasmodium falciparum, *P malariae*, and *P vivax*	Direct RBC lysis and RBC membrane fragility
Viral	Cytomegalovirus	Cold autoimmune hemolysis
	Epstein-Barr virus	Cold autoimmune hemolysis
	Hepatitis B and C	Bone marrow suppression
	HIV	Bone marrow suppression or infiltration, chronic inflammation
	Parvovirus B19	Bone marrow suppression or failure (pure red cell aplasia)

Abbreviation: DIC, disseminated intravascular coagulation.

or complement to RBC as assessed with the direct antiglobulin (DAT or Coombs) test. The indirect antiglobulin test measures the ability of patients' sera to bind to allogenic RBC. In general, the indirect antiglobulin test is less helpful than the DAT, except for the rare occasions when the patient has a low-affinity antigen. Autoimmune hemolytic anemia is divided into warm and cold autoimmune hemolytic anemia depending on the reactivity of the antibody at body temperature.

- Warm autoimmune hemolytic anemias: in general, warm autoimmune hemolytic anemias are caused by immunoglobulins of IgG subclasses and react to RBC at body temperature (37°C). The DAT are positive for IgG with or without C3d. Most IgG subclasses fix complement poorly and the predominant site of RBC destruction is the spleen. Review of the peripheral smear reveals an increase in the number of reticulocytes, spherocytes, and some bite cells (see **Fig. 2**D). Secondary causes of warm autoimmune hemolytic anemia include viral infections (usually in children), autoimmune and connective tissues disorders, lymphomas and lymphoproliferative disorders, and drugs.[27,33]
- Cold autoimmune hemolytic anemias: cold autoimmune hemolytic anemias are a group of disorders in which the red cells are bound by antibodies with a thermal amplitude less than body temperature, but greater than temperatures in the peripheral circulation (approximately 30°C).[1,27] Most cold autoimmune hemolytic anemias are secondary to another process. The IgM multimers (pentamers and hexamers) are large enough to bind more than 1 RBC, causing agglutination of RBC. Exposure to cold in the periphery can result in agglutination of RBC in small vessels and acrocyanosis. Review of the peripheral blood smear may reveal RBC clumps. The IgM antibodies fix complement on the RBC membrane and can

cause direct lysis of the RBC as well as macrophage-mediated RBC ingestion in the spleen and liver. The DAT is characterized by binding of C3d to RBC. Occasionally, IgM is seen on the RBC surface if the thermal amplitude is sufficiently high. The titer of the cold antibody is reported as the minimum concentration at which the patient's serum agglutinates allogenic RBC. The thermal amplitude is the maximum temperature at which the antibody causes RBC agglutination. The degree of hemolysis is related to the amount and reactivity of the antibody and the ability of the antibody to bind complement. In some individuals, hemolysis is minimal or absent and the only presenting symptom is acrocyanosis.

There are several naming conventions for cold autoimmune hemolytic anemia, but understanding the pathophysiology helps overcome this limitation. Antibody specificity is occasionally helpful in determining the cause of the hemolytic anemia; anti-I specificity is associated with primary cold autoimmune hemolytic anemia, as well as *Mycoplasma pneumoniae* and lymphoma. Anti-i is associated with hemolysis caused by mononucleosis or lymphoma.

- Primary cold agglutinin disease: patients have a chronic hemolysis and usually have a monoclonal IgM autoantibody detected on serum electrophoresis. Malignancies such as lymphoma and Waldenstrom macroglobulinemia should be ruled out with imaging and bone marrow examination in most cases. Antibody specificity is usually anti-I.
- Secondary cold agglutinin disease: secondary cold agglutinin disease is caused by hematologic malignancies (chronic lymphocytic leukemia, lymphomas), nonhematologic malignancies, and infections. Infections commonly associated with cold autoimmune hemolytic anemia are mononucleosis and *Mycoplasma pneumoniae.* Hemolysis occurs several weeks after the infection and usually lasts 2 to 4 weeks. Antibody specificity is Anti-i. Neoplastic processes can have anti-I or anti-i; however, finding an anti-i in the absence of an infectious cause is suspicious for lymphoma.

- Drug-mediated: drugs can induce immune-mediated RBC injury through several mechanisms (**Table 4**).
  - Hapten: the combination of drug bound to RBC membrane proteins elicits an immune response. Classically, this response is associated with high-dose penicillin. Hemolysis usually begins 7 to 10 days after exposure, and resolves days to weeks after the drug is withdrawn.[1]
  - Ternary complex: drug-antibody complexes form and bind weakly to RBC membranes and fix complement, causing destruction of RBC. Hemolysis can occur quickly after exposure to drug.
  - Autoantibody: in the presence of drug, nondrug-dependent autoantibodies form; α-methyldopa is the classic representation of this effect.
- Alloimmune: transfused RBC invoke an immune response. Reactions are either immediate or delayed. Immediate hemolytic transfusion reactions are usually related to ABO incompatibility or with preformed alloantibodies (with previous exposure either through blood products or pregnancy). Delayed transfusion reactions usually require formation of alloantibodies. Diagnosis is based on an indirect antiglobulin test (antibody screen) and discovery of an antibody reactive to allogenic RBC only.

Table 4
Medications associated with anemia

Category	Class/Medication	Pathophysiology
Autoimmune hemolysis	α-Methyldopa	Autoimmune RBC binding with splenic sequestration
	Penicillins	Hapten/drug absorption with splenic sequestration
	Sulfonamides	Neoantigen formation with autoimmune RBC destruction
	Quinidine	Ternary complex formation with direct RBC lysis
Bone marrow suppression	ACE inhibitors	Suppress erythropoietin
	Alkylating agents	Direct marrow toxicity
	Anthracyclines	Direct marrow toxicity
	Antiandrogens	Decreased testosterone
	Antimetabolites	Direct marrow toxicity
	Chloramphenicol	Direct toxicity to cell mitochondria
	Colchicine	Direct marrow toxicity
	Zidovudine (AZT)	Megaloblastic anemia by inhibition of nucleic acid synthesis
Decreased RBC production	Anticonvulsants	Megaloblastosis associated with folic acid deficiency
	Hydroxyurea	Megaloblastic anemia by inhibition of nucleic acid synthesis
	Metformin	Decreased folic acid intestinal absorption
	Methotrexate	Dihydrofolate reductase inhibitor causing folic acid deficiency
	Proton pump inhibitors	Decreases vitamin B_{12} levels with long-term use

Abbreviation: ACE, angiotensin-converting enzyme.

Enzyme deficiencies, abnormal hemoglobins, and red blood cell (RBC) membrane abnormalities

Abnormalities in RBC metabolism, structural proteins, or hemoglobin result in fragile RBC predisposed to destruction. The number of abnormalities is vast and can be either acquired or hereditary.

- Acquired
  - Medications and toxins: a variety of drugs and toxins can bind and damage RBC membranes. Toxins include arsenic hydride, lead, and copper. There are case reports of many medications causing RBC membrane abnormalities.[1] Evaluation is based on exposure (see **Table 4**).
  - Paroxysmal nocturnal hemoglobinuria (PNH): PNH is an acquired clonal disorder in which mutations in the PIG-A gene cause defects in glycosylphosphatidylinositol (GPI) anchor synthesis and absence of many proteins from the surface of circulating RBC. Laboratory and clinical workup reveals hemolysis, no evidence of immune-mediated hemolysis, and perhaps a history of aplastic anemia or thrombosis. Diagnosis is made by measuring the presence of GPI-anchored proteins on circulating cells.
- Inherited: the number of inherited RBC disorders resulting in increased RBC destruction and anemia is vast, reflecting the complex nature of the RBC. Disorders range from defects in hemoglobin (ie, thalassemias and sickle cell anemias),

RBC membrane proteins (hereditary spherocytosis), and RBC enzyme deficiencies (eg, glucose-6-phosphate dehydrogenase [G6PD] deficiency). Many of these defects are detected at birth, although thalassemia minor/trait may not be diagnosed until adulthood. A full discussion of these defects is beyond the scope of this review, but should be suspected if abnormally shaped RBC are present on peripheral smear without evidence for another cause. G6PD deficiency and other RBC enzyme deficiencies are relatively common and affect RBC survival, especially after exposure to certain foods and drugs. Hemoglobin electrophoresis and specific enzyme assays can make these diagnoses in the nonacute setting. Bite cells (degmacytes) are seen with G6PD deficiency, and a special test for Heinz bodies (denatured hemoglobin clumps in RBCs) may be helpful in making a diagnosis.

RED BLOOD CELL (RBC) PRODUCTION

The ability to maintain a stable hemoglobin concentration depends on the ability of the bone marrow to produce RBC to balance losses from bleeding, natural aging of RBC, and RBC destruction. Failure of the bone marrow to produce RBC results in anemia. RBC have a life span of 100 to 140 days in the circulation under normal circumstances.[1] Abrupt cessation of hematopoiesis seldom results in abrupt decreases in hemoglobin concentration in the absence of increased blood loss or decreased life span of the RBC. Nutrient deficiencies, organ dysfunction, bone marrow dysfunction from primary marrow processes, and hemoglobin synthesis errors can result in decreased RBC production.

Clinical History

The symptoms of anemia are often less apparent than the primary pathologic process such as thyroid dysfunction, malignant processes, or infection. A careful nutritional history is essential because various dietary restrictions predispose individuals to deficiencies in iron, vitamin B_{12}, and folic acid. History of bowel resection or bariatric procedures may prevent proper nutrient absorption. A drug and alcohol history is also important. More obscure are nonfood substances such as lead, which may result from use of improperly manufactured cookware or work in various occupations.[34]

Physical Examination

Decreased RBC production is usually not discernable on physical examination; however, a physical examination may help guide the diagnosis by focusing on potential sites of organ dysfunction or malignancy.

Laboratory Findings

The size of the RBC is varied in anemia associated with decreased production. In general, the reticulocyte count is inappropriately low for the hemoglobin, and other blood cell lines may be suppressed. Review of the peripheral blood smear is important, with a variety of morphologic findings possible depending on the underlying cause.

Causes for Alterations in RBC Production

The causes of decreased RBC production are best divided into 3 main categories: nutrient deficiencies, organ dysfunction, and bone marrow dysfunction.

Nutrient deficiencies

IDA is the most common cause of anemia worldwide and accounts for more than half of cases of anemia. Other common nutrient deficiencies are vitamin B_{12} (cobalamin) and

vitamin B_9 (folic acid). In general, vitamin B_{12}, folic acid, and iron status should be evaluated in anyone with an anemia associated with decreased RBC production because these conditions are not rare and multiple deficiencies can present in the same patient.

- IDA: IDA is the most common cause of anemia worldwide, affecting as many as 20% of the world's population.[35] Iron absorption occurs mostly in the jejunum, and altered uptake, nutrition, digestion, and poor absorption can result in iron deficiency. Microcytosis is a marker of iron-deficient hematopoiesis but usually occurs when the hemoglobin decreases below normal. An increased RDW is often an early marker of iron deficiency, indicating developing anisocytosis. A serum ferritin level is a simple, inexpensive test. A serum ferritin level of 30 ng/mL or lower is diagnostic for IDA. Ferritin is an acute-phase reactant, thus making levels difficult to interpret in the setting of acute inflammation. Previously, a serum ferritin level of 70 ng/mL or lower in the setting of acute inflammation was considered diagnostic of IDA; however, what constitutes acute inflammation is unclear.[36] Given changes associated with acute inflammation, the serum ferritin level that effectively excludes IDA in the setting of inflammation is not established. Review of the peripheral blood smear reveals numerous hypochromic RBC with central pallor in patients with severe IDA (see **Fig. 2**C). **Table 5** reviews the laboratory findings in IDA. A therapeutic trial of iron can also aid in the diagnosis. The provider must determine the cause of iron deficiency because malignancy is not uncommon. The gold standard for the diagnosis of IDA is a bone marrow biopsy, revealing absence of iron stores.[1]
- Cobalamin (vitamin B_{12}) deficiency: the gastric parietal cells produce intrinsic factor, which binds to dietary vitamin B_{12}, allowing absorption in the ileum. Severe vitamin B_{12} deficiency results in megaloblastic macrocytosis, although only if there is inadequate folic acid. In addition to large RBC, the peripheral blood smear may reveal hypersegmented neutrophils (see **Fig. 2**E). In severe cases, total white cell count and platelet count are decreased. Vitamin B_{12} deficiency can result from poor oral intake or poor absorption. A vitamin B_{12} level greater than 300 pg/mL effectively excludes vitamin B_{12} deficiency and a vitamin B_{12}

Table 5
Diagnosis of IDA

	Normal	Iron Deficiency Without Anemia	Mild IDA	Severe IDA
Hemoglobin (g/dL)	Normal	Normal	9–12	6–8
Ferritin (ng/mL)	40–200	<40	<20	<10
Serum iron (μg/dL)	60–180	60–180	<60	<40
TIBC (μg/dL)	250–450	>450	>450	>450
Transferrin saturation (%)	20–50	30	<15	<10
Transferrin (μg/dL)	300–360	300–390	350–400	>410
MCHC	27–31	Normal	<27	<27
MCV	82–96	Normal	Normal	<82
RDW	11.5–14.5	Normal	Normal	>14.5
Red cell morphology	Normal	Normal	Slightly hypochromic	Hypochromic and microcytosis

Abbreviations: MCHC, mean corpuscular hemoglobin concentration; TIBC, total iron-binding capacity.

Table 6
Diagnosis of vitamin B_{12} (cobalamin) and vitamin B_9 (folic acid) deficiency

	Deficiency Excluded	Vitamin B_{12} Deficiency	Deficiency Excluded	Vitamin B_{12} Deficiency ± Folate Deficiency	Folic Acid Deficiency
Vitamin B_{12} (pg/mL)	>300	<140	≤300	≤300	≤300
Serum folic acid (ng/mL)	>4	>4	≤4	≤4	≤4
Erythrocyte folate (μg/L)	>140	NA	NA	NA	<140
Homocysteine (mmol/L)	NA	NA	5–14	5–14; >14	>14
MMA[a] (nmol/L)	NA	NA	70–270	>270	70–270

Abbreviation: NA, not applicable.
[a] MMA can be increased in kidney failure.

level less than 140 pg/mL is diagnostic of vitamin B_{12} deficiency. For vitamin B_{12} levels between 140 and 300 pg/mL, increased methylmalonic acid (MMA) and homocysteine levels support the diagnosis (**Table 6**). A diagnosis of pernicious anemia resulting from autoimmune destruction of the gastric parietal cells was historically made using a Schilling test, but presence of intrinsic factor or antiparietal cell antibodies are now most often used.

- Folic acid (vitamin B_9) deficiency: folic acid deficiency results in a macrocytic anemia. Causes of folic acid deficiency include poor nutritional intake and malabsorption. In addition, folic acid deficiency can result from several medications used to treat seizure disorders (phenytoin), autoimmune diseases (methotrexate), infection (pentamidine, trimethoprim), and malignancy (hydroxyurea, methotrexate). Metformin and cholestyramine can decrease folate absorption, and patients may require supplementation. Disorders of increased red cell turnover (such as hemolysis) may result in increased folate requirements and folate deficiency. Serum folate levels fluctuate daily depending on diet so measurements are not useful. Red cell folate levels should be ordered if folic acid deficiency is suspected. A normal MMA level and an increased homocysteine level strongly support the diagnosis of folic acid deficiency (see **Table 6**).
- Other nutritional deficiencies: deficiencies in vitamin A, vitamin B_6, copper, and dietary protein are rare causes of anemia.[1]

Organ dysfunction

- Thyroid: the cause of anemia from hypothyroidism is unclear but may be from decreased stimulation of erythropoiesis by thyroid hormone.[37] Red cell size is variable and workup depends on other signs and symptoms of hypothyroidism.[38] Evaluation should include testing serum thyroid-stimulating hormone and free T4 level.
- Liver: liver dysfunction from any cause is associated with anemia.[39] Physical examination may reveal jaundice, ascites, caput medusa, spider angiomas, or hepatomegaly, prompting an evaluation of underlying liver disease.

- Kidney: chronic kidney disease causes normocytic anemia secondary to a decrease in erythropoietin production.[40] Erythropoietin is a humoral factor produced by the kidney that regulates RBC production. Anemia varies in severity and most patients present with mild to moderate reductions in hemoglobin. Even mild decreases in the glomerular filtration rate are associated with increased rates of anemia.[9,41–43]
- Hypogonadism: testosterone increases hemoglobin in men beginning at the time of puberty and is likely the cause of increased hemoglobin levels in men compared with women.[15] A decreased level of testosterone in both elderly men and women has been shown to be a risk factor for anemia.[16] Gonadotropin-releasing hormone analogues such as leuprolide used in treatment of prostate cancer decrease erythropoiesis by suppression of testosterone production.[17] Symptoms associated with hypogonadism include diminished libido, decreased body hair, truncal obesity, decreased energy, and generalized weakness. The anemia is usually mild.
- Heart failure: anemia is common in individuals with heart failure. Causes may be varied and include decreased renal perfusion, hepatic congestion, or decreased absorption of vital nutrients from the gut.[44]

Bone marrow dysfunction

Bone marrow disorders can be divided into primary marrow disorders and secondary marrow disorders. In primary marrow disorders, abnormalities within the bone marrow result in the inability to produce RBC. In secondary bone marrow disorders, a systemic process from outside the marrow space invades the bone marrow or suppresses production of RBC.

- Primary bone marrow disorders: these disorders reflect processes that are intrinsic to the bone marrow. Review of the peripheral blood smear and bone marrow biopsy are important in identifying these processes.
  - Myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD): MDS/MPD occasionally result in isolated marrow dysfunction of the erythroid cell lines and present with anemia and decreased reticulocyte count.[19] Patients may have additional cell lineages involved with abnormal numbers of WBC or platelets. Diagnosis is typically made with bone marrow biopsy with aspiration. The evaluation of peripheral blood for the JAK V617F can help with the diagnosis MPD.
  - Leukemia: acute leukemias often present abruptly with obvious manifestations and circulating immature WBC. Rarely, acute leukemia may present with isolated anemia. Chronic leukemias can be more subtle in presentation. Review of the peripheral blood smear and bone marrow examination are crucial in diagnosis. Flow cytometry of peripheral blood may assist in the diagnosis of chronic lymphocytic leukemia and evaluation of the peripheral blood for the BCR/ABL transcript may assist in the diagnosis of chronic myeloid leukemia.
  - Multiple myeloma: multiple myeloma is on the differential for decreased red cell production, especially in the setting of concurrent renal failure, hypercalcemia, or pathologic bone fractures. Serum protein electrophoresis, urine protein electrophoresis, evaluation of serum free light chain ratio, skeletal survey, and a bone marrow biopsy are helpful diagnostic tests.
  - Bone marrow failure syndromes: failure of marrow progenitor cells results in varying conditions. Pure red cell aplasia results in normocytic anemia with absent reticulocytosis. It is associated with T-cell–mediated attack of erythroid progenitors.[45] Aplastic anemia typically presents as pancytopenia from T-cell–

mediated attack. Bone marrow cellularity is markedly reduced on bone marrow biopsy.[46] PNH, although more commonly thought of as an increased RBC destructive process, lies on a continuum with aplastic anemia and also can have diminished erythropoeisis.[47]

- Secondary bone marrow dysfunction: several causes of infiltrative processes disrupt the ability of the bone marrow to produce RBC, resulting in anemia. Leukemia, lymphoma, fibrosis, infectious agents, and metastatic solid tumors to the bone marrow can result in a normocytic anemia with pancytopenia.[19] Infiltrative processes can result in displacement of the hemopoietic bone marrow tissue to the peripheral blood, resulting in myelophthisis or leukoerythroblastic reaction. The peripheral blood smear reveals tear-drop cells (dacrocytes) and nucleated RBC, and occasionally immature WBC (see **Fig. 2**F). Often, patients are older and present with signs and symptoms consistent with the underlying malignancy. Splenomegaly, hepatomegaly, and adenopathy may be present on physical examination. Diagnosis is confirmed with a bone marrow biopsy.
  - Infiltration by malignancies: most malignancies have the potential to invade the bone marrow, the more common malignancies to metastasize to the bone marrow include solid tumors including lung, breast, GI, and prostate.[48]
  - Infiltration by infectious processes: *Mycobacterium avium* complex, *Mycobacterium tuberculosis*, and *Histoplasma capsulatum* infections can result in anemia by bone marrow infiltration and are more often seen in patients with AIDS (see **Table 3**).[49]
  - Infiltration by other disorders: the bone marrow can rarely be infiltrated by other systemic processes such as sarcoidosis or amyloidosis.[50,51]
- Bone marrow suppression: the bone marrow can be suppressed, resulting in an inability to produce RBC. The broad categories of bone marrow suppression include inflammation, infection, autoimmune, and toxicity. Anemia of inflammation and autoimmune causes are discussed in a separate section.
  - Infection: parvovirus B19, human immunodeficiency virus (HIV), and viral hepatitis are known to have immune-mediated effects on bone marrow with the potential to cause isolated anemia to pancytopenia. Parvovirus B19, a single-stranded DNA virus, can cause anemia by direct RBC destruction but also arrest of erythroid maturation in the bone marrow.[52] Parvovirus B19 polymerase chain reaction assays are the most sensitive test for diagnosis. Most patients with HIV have anemia at some point in their disease course; anemia is associated with increased mortality independent of the severity of their infection.[49] The anemia associated with HIV has a complex pathogenesis and is related to direct marrow suppression by the virus, invasion of the marrow by malignancies, and increased destruction of RBC.[53]
  - Medications: toxicity from drugs, chemotherapy, and irradiation destroys the pluripotent stem cells of the bone marrow and can result in anemia or pancytopenia. The most commonly associated drugs and chemotherapeutic agents associated with bone marrow suppression include alkylating agents, topoisomerase inhibitors, and antimetabolites (see **Table 4**). Anemia associated with the treatment of malignancy is an often-anticipated side effect. Most medications have been linked to bone marrow suppression. **Table 4** lists a few common medications. Antiandrogens such as spironolactone and bicalutamide can result in decreased erythropoiesis by decreasing testosterone activity.
  - Alcohol: alcohol is a commonly overlooked bone marrow suppressant. Chronic alcohol consumption suppresses bone marrow function and has a direct effect

on heme synthesis.[54] Alcoholism is the most common cause of macrocytic anemia (which may not be related to B_{12} or folate deficiencies) and accounts for 15% to 65% of cases depending on the population.[55] Testing γ-glutamyltransferase levels can confirm suspicion of alcohol consumption in patients who report abstinence. Once a patient is abstinent from alcohol, the macrocytic anemia corrects over 1 to 2 months. Patients with alcoholism are at risk of underlying vitamin B_{12} or folate deficiencies.

- Environmental toxins: some chemical exposures can cause bone marrow suppression. Benzene, used in industrial dyes, detergents, explosives, pesticides, synthetic rubber, plastics, and pharmaceuticals, can have toxic effects on the bone marrow. Radiation exposure also has toxic effects on bone marrow. Occupational history may reveal a potential environmental exposure.
- Heavy metal toxicity: patients exposed to toxic levels of heavy metals can develop anemia. Potential heavy metal toxins include lead and zinc. Toxic levels of lead result in alterations of hemoglobin synthesis, altering RBC production. Sources of heavy metal exposure in adults include drinking water, faulty cookware, and metal working. The Agency for Toxic Substances and Disease Registry, an agency within the US Department of Health and Human Services, releases a detailed report on lead toxicity that lists all potential exposures.[34] Associated symptoms include abdominal pain and behavioral changes. Patients with a potential exposure history and microcytic anemia should have their serum lead level checked. If the result is positive, the entire household should be screened. A peripheral blood smear may reveal basophilic stippling.

ANEMIA OF INFLAMMATION

Anemia of inflammation (or anemia of chronic disease) is a well-known but poorly defined entity. The pathophysiology is complex and involves both decreased RBC production and increased RBC destruction. At times, the source of the inflammation is evident such as inflammatory bowel disease, a rheumatologic disease, cancer, or chronic infection such as osteomyelitis. At other times, the source of inflammation is not apparent. Anemia of inflammation is the second most common cause of anemia worldwide. In older adults, anemia of inflammation represents the cause of nearly one-fifth of anemias.[56]

Pathophysiology

Initially a diagnosis of exclusion, anemia of inflammation was believed to represent a broad process of inflammation affecting erythropoiesis. Research has revealed an increasingly complicated pathogenesis that results in changes of iron homeostasis, altered proliferation of erythoid progenitor cells, decreased production of erythropoietin, and shortened life span of the RBC.[57] Iron metabolism is altered by diversion of iron to within cells of the reticuloendothelial system. Removal of circulating iron restricts the ability to produce RBC, creating a pseudoiron-deficient state. Sequestration of iron in inflammatory states is believed to be an adaptive process because previous evidence has shown that iron increases microbial growth and increases proliferation of malignant cells.[58] Alteration in proliferation of erythroid progenitor cells is not clearly understood but seems most associated with increases of interferon-γ.[59] In vitro studies of erythropoietin have shown decreased production in inflammatory states associated with increases in interleukin-1 and tumor-necrosing factor α.[60] In addition, inflammatory states increase circulating cytokines, which damage RBC membranes, and induce free radicals, resulting in increased erythrophagocytosis.[57]

Together these processes inhibit normal RBC production and turnover, resulting in refractory anemia.

Multiple causes of anemia of inflammation have been implicated, including autoimmune diseases, chronic kidney disease, chronic transplant rejection, infections, and malignancy. Autoimmune diseases most often associated with anemia of inflammation include rheumatoid arthritis, systemic lupus erythematosus, vasculitis, inflammatory bowel disease, and sarcoidosis. Multiple infectious processes have been implicated in anemia of inflammation, but evaluation of HIV represents a chronic and often indolent process that must be considered.[57]

Patient History

The inflammatory illness can be obvious or occult. Review of the past medical history and any presenting symptoms may provide some clues. Common illnesses include infections, rheumatologic diseases, inflammatory bowel disease, and malignancies. Often a careful history and physical examination can guide the workup. Sometimes no cause is evident, or the inflammation is transient and the findings resolve. Following the patient over time may provide an answer.

Physical Examination

Examination of a patient should focus on potential findings to suggest an underlying inflammatory disease. The examination should be directed toward identifying the inflammatory illness. A lymph node examination, abdominal examination for masses, cardiac examination for a new murmur, and examination of the joints and the skin may reveal the underlying condition.

Laboratory Findings

Anemia varies in severity, and most patients present with mild to moderate reductions in hemoglobin. Laboratory workup typically reveals a normochromic normocytic anemia, although hypochromic microcytic anemia is occasionally seen.[61] Reticulocyte count is low, consistent with suppression of RBC production. In anemia of inflammation, serum iron and total iron-binding capacity is low, whereas ferritin levels remain normal or increased. It is important to evaluate for IDA, although both conditions can exist concurrently. Increases of the C-reactive protein level and the erythrocyte sedimentation rate and low albumin are nonspecific markers of inflammation that can be helpful in supporting the diagnosis of anemia of inflammation. The soluble transferin receptor serum test is not increased in the anemia of inflammation but is increased in IDA, but the clinical utility is not established.

SUMMARY

Anemia is a common clinical condition. The workup and evaluation are difficult because there is not a precise definition of what is normal and abnormal. A careful clinical assessment with initial laboratory studies helps determine the mechanism of anemia in most cases as being from increased RBC loss, increased RBC destruction, or from decreased RBC production. The evaluation and assessment of anemia requires a detailed knowledge of hematopoiesis as well as an overall understanding of the interconnections within the human body.

This review does not discuss the treatment of anemia. Treatment must be tailored for each individual. There is little to lose and much to gain in identifying and stopping a bleeding source, supplementing a nutritional deficiency, or improving organ function. The difficulty arises as to whether we should treat anemia as a disease. Does

a mild anemia associated with diabetes and mild chronic kidney disease require any other treatment than to maximize diabetes treatment and prevent further damage to the kidneys?

Regardless of whether an anemia is treated or not, it is important to determine why the patient is anemic. This strategy often leads to insights into the patient's overall health or catches diseases at early or curable stages. A systematic and logical approach, often over many visits, may be required to diagnose and treat anemia.

REFERENCES

1. Lichtman MA, Beutler E, Kipps TJ, et al, editors. Williams hematology. 7th edition. New York (NY): The McGraw-Hill Companies, Inc.; 2006.
2. Nissenson AR, Goodnough LT, Dubois RW. Anemia: not just an innocent bystander? Arch Intern Med 2003;163(12):1400–4.
3. Zakai NA, Katz R, Hirsch C, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. Arch Intern Med 2005;165(19):2214–20.
4. Nutritional anaemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser 1968;405:5–37.
5. Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood 2006;107(5):1747–50.
6. Ganesh SK, Zakai NA, van Rooij FJ, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nat Genet 2009;41(11):1191–8.
7. den Elzen WP, Gussekloo J. Anaemia in older persons. Neth J Med 2011;69(6): 260–7.
8. Kuk JL, Saunders TJ, Davidson LE, et al. Age-related changes in total and regional fat distribution. Ageing Res Rev 2009;8(4):339–48.
9. Zakai NA, McClure LA, Prineas R, et al. Correlates of anemia in American blacks and whites: the REGARDS Renal Ancillary Study. Am J Epidemiol 2009;169(3):355–64.
10. Culleton BF, Manns BJ, Zhang J, et al. Impact of anemia on hospitalization and mortality in older adults. Blood 2006;107(10):3841–6.
11. Zakai NA, French B, Arnold A, et al. Hemoglobin decline and health outcomes in the elderly: the cardiovascular health study. ASH Annual Meeting Abstracts 2008; 112(11):3448.
12. Beutler E, West C. Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. Blood 2005;106(2):740–5.
13. Dill DB, Terman JW, Hall FG. Hemoglobin at high altitude as related to age. Clin Chem 1963;12:710–6.
14. Beall CM, Brittenham GM, Strohl KP, et al. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. Am J Phys Anthropol 1998;106(3): 385–400.
15. Shahidi NT. Androgens and erythropoiesis. N Engl J Med 1973;289(2):72–80.
16. Ferrucci L, Maggio M, Bandinelli S, et al. Low testosterone levels and the risk of anemia in older men and women. Arch Intern Med 2006;166(13):1380–8.
17. Curtis KK, Adam TJ, Chen SC, et al. Anaemia following initiation of androgen deprivation therapy for metastatic prostate cancer: a retrospective chart review. Aging Male 2008;11(4):157–61.
18. Pratt MC, Lewis-Barned NJ, Walker RJ, et al. Effect of angiotensin converting enzyme inhibitors on erythropoietin concentrations in healthy volunteers. Br J Clin Pharmacol 1992;34(4):363–5.

19. Adamson J, Longo D. Anemia and Polycythemia. In: Fauci A, Braunwald E, Kasper D, et al, editors. Harrison's Principles of Internal Medicine. 17th edition. New York: McGraw-Hill; 2008. p. 337. Chapter 57.
20. Hallberg L, Hulthén L, Bengtsson C, et al. Iron balance in menstruating women. Eur J Clin Nutr 1995;49(3):200–7.
21. Bermejo B, Olona M, Serra M, et al. Prevalence of iron deficiency in the female working population in the reproductive age. Rev Clin Esp 1996;196(7):446–50 [in Spanish].
22. Yun EJ, Meng MV, Carroll PR. Evaluation of the patient with hematuria. Med Clin North Am 2004;88(2):329–43.
23. González C, Penado S, Llata L, et al. The clinical spectrum of retroperitoneal hematoma in anticoagulated patients. Medicine (Baltimore) 2003;82(4):257–62.
24. Skikne B, Lynch S, Borek D, et al. Iron and blood donation. Clin Haematol 1984; 13(1):271–87.
25. Salisbury AC, Reid KJ, Alexander KP, et al. Diagnostic blood loss from phlebotomy and hospital-acquired anemia during acute myocardial infarction. Arch Intern Med 2011;171(18):1646–53.
26. Zahner J, Schneider W. Munchausen syndrome in hematology: case reports of three variants and review of the literature. Ann Hematol 1994;68(6):303–6.
27. Lechner K, Jager U. How I treat autoimmune hemolytic anemias in adults. Blood 2010;116(11):1831–8.
28. Mecozzi G, Milano AD, Carlo MD, et al. Intravascular hemolysis in patients with new-generation prosthetic heart valves: a prospective study. J Thorac Cardiovasc Surg 2002;123(3):550–6.
29. Burns ER, Lou Y, Pathak A. Morphologic diagnosis of thrombotic thrombocytopenic purpura. Am J Hematol 2004;75(1):18–21.
30. George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. Blood 2010;116(20):4060–9.
31. White NJ. The treatment of malaria. N Engl J Med 1996;335(11):800–6.
32. Hatcher JC, Greenberg PD, Antique J, et al. Severe babesiosis in Long Island: review of 34 cases and their complications. Clin Infect Dis 2001;32(8): 1117–25.
33. Crowther M, Chan YLT, Garbett IK, et al. Evidence-based focused review of the treatment of idiopathic warm immune hemolytic anemia in adults. Blood 2011; 118(15):4036–40.
34. Toxicological profile for lead. Atlanta (GA): US Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry; 2007. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=96&tid=22. Accessed November 01, 2011.
35. Stoltzfus R. Defining iron-deficiency anemia in public health terms: a time for reflection. J Nutr 2001;131(2S-2):565S–7S.
36. Cook JD. Diagnosis and management of iron-deficiency anaemia. Best Pract Res Clin Haematol 2005;18(2):319–32.
37. Das KC, Mukherjee M, Sarkar TK, et al. Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. J Clin Endocrinol Metab 1975;40(2):211–20.
38. Colon-Otero G, Menke D, Hook CC. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. Med Clin North Am 1992;76(3):581–97.
39. Qamar AA, Grace ND. Abnormal hematological indices in cirrhosis. Can J Gastroenterol 2009;23(6):441–5.

40. Humphries JE. Anemia of renal failure. Use of erythropoietin. Med Clin North Am 1992;76(3):711–25.
41. Peralta CA, Shlipak MG, Judd S, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. JAMA 2011;305(15): 1545–52.
42. Astor BC, Muntner P, Levin A, et al. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med 2002;162(12):1401–8.
43. Ble A, Fink JC, Woodman RC, et al. Renal function, erythropoietin, and anemia of older persons: the InCHIANTI study. Arch Intern Med 2005;165(19): 2222–7.
44. Kosiborod M, Curtis JP, Wang Y, et al. Anemia and outcomes in patients with heart failure: a study from the National Heart Care Project. Arch Intern Med 2005;165(19):2237–44.
45. Fisch P, Handgretinger R, Schaefer HE. Pure red cell aplasia. Br J Haematol 2000;111(4):1010–22.
46. Young NS. Acquired aplastic anemia. Ann Intern Med 2002;136(7):534–46.
47. Bacigalupo A, Passweg J. Diagnosis and treatment of acquired aplastic anemia. Hematol Oncol Clin North Am 2009;23(2):159–70.
48. Makoni SN, Laber DA. Clinical spectrum of myelophthisis in cancer patients. Am J Hematol 2004;76(1):92–3.
49. Volberding PA, Baker KR, Levine AM. Human immunodeficiency virus hematology. Hematology Am Soc Hematol Educ Program 2003;294–313. Available at: http://asheducationbook.hematologylibrary.org/content/2003/1.toc. Accessed February 21, 2012.
50. Browne PM, Sharma OP, Salkin D. Bone marrow sarcoidosis. JAMA 1978; 240(24):2654–5.
51. Licci S. Extensive bone marrow amyloidosis. Ann Hematol 2011.
52. Leguit RJ, van den Tweel JG. The pathology of bone marrow failure. Histopathology 2010;57(5):655–70.
53. Semba RD, Martin BK, Kempen JH, et al. The impact of anemia on energy and physical functioning in individuals with AIDS. Arch Intern Med 2005;165(19): 2229–36.
54. Hourihane DO, Weir DG. Suppression of erythropoiesis by alcohol. Br Med J 1970;1(5688):86–9.
55. Kaferle J, Strzoda CE. Evaluation of macrocytosis. Am Fam Physician 2009;79(3): 203–8.
56. Patel KV. Epidemiology of anemia in older adults. Semin Hematol 2008;45(4): 210–7.
57. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352(10): 1011–23.
58. Zarychanski R, Houston DS. Anemia of chronic disease: a harmful disorder or an adaptive, beneficial response? CMAJ 2008;179(4):333–7.
59. Wang CQ, Udupa KB, Lipschitz DA. Interferon-gamma exerts its negative regulatory effect primarily on the earliest stages of murine erythroid progenitor cell development. J Cell Physiol 1995;162(1):134–8.
60. Jelkmann W. Proinflammatory cytokines lowering erythropoietin production. J Interferon Cytokine Res 1998;18(8):555–9.
61. Sears DA. Anemia of chronic disease. Med Clin North Am 1992;76(3):567–79.

62. Kratz A, Ferraro M, Sluss PM, et al. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. N Engl J Med 2004;351(15):1548–63.
63. Kueviakoe I, Segbena AY, Jouault H, et al. Hematological reference values for health adults in Togo. ISRN Hematol 2011;2011:1–5.
64. Saathoff E, Schneider P, Kleinfeldt V, et al. Laboratory reference values for healthy adults from southern Tanzania. Trop Med Int Health 2008;13(5):612–25.
65. Tsegaye A, Messele T, Tilahun T, et al. Immunohematological reference ranges for adult Ethiopians. Clin Diagn Lab Immunol 1999;6(3):410–4.