

# Diagnosis of Anemia

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The bone marrow contains pluripotential stem cells that have the capacity for self-renewal as well as differentiation into mature cells, including red blood cells. Erythropoietin stimulates primitive stem cells to undergo four rounds of cellular division—over six to seven days—and differentiation to form pronormoblasts and normoblasts. During the final step of differentiation the nucleus is extruded from the red blood cell precursor to form a reticulocyte, that still retains cytoplasmic RNA. Reticulocytes enter the peripheral circulation, appearing as faintly blue-gray polychromatophilic cells in Wright stained blood smears that persist for about 24 hours. Upon the loss of cytoplasmic RNA, a mature red blood cell is formed. A red blood cell remains in the peripheral circulation for 120 days before being removed via hemolysis by the spleen and reticuloendothelial system.

Usually red blood cell numbers remain relatively constant with removal from the peripheral circulation balanced by proliferation and differentiation within the marrow. However, many disease states will disrupt red blood cell homeostasis, leading to possible alterations in red blood cell number, appearance or hemoglobin concentration. The most common pathologic process associated with red blood cells is the decrease in overall red blood cell numbers. Morphologic characteristics and the complete blood count (CBC) performed by an automated hematology analyzer, will provide important information that will direct further laboratory testing to allow diagnosis and characterization of a red blood cell disorder.

## 1.1 Normal and Pathologic Red Blood Cells

The normal red blood cell is a biconcave disk, 6 to 9  $\mu\text{m}$  in diameter and 1.5 to 2.5  $\mu\text{m}$  thick. In the peripheral smear, red blood cells are anucleate and contain predominantly hemoglobin that is distributed to form a dense outer rim with a paler center that occupies approximately one third of the diameter of the cell. The hemoglobin imparts a uniform pink to orange-red color to the cytoplasm that is typically without inclusions. Normally all red blood cells are relatively uniform in size and shape. Numerous disease states affect the size, shape and hemoglobin content of red cells. Variation in size is referred to as “anisocytosis,” and variation in shape is termed “poikilocytosis.” Pathologic red cells may be larger or smaller than normal, may be abnormally shaped, or may contain inclusions. Knowledge of the disease states associated with specific appearances of red blood cells will provide important diagnostic clues that aid in the diagnosis of hematologic disorders. [t1.1](#) presents a summary of morphologic red blood cell abnormalities that may be seen in blood smears, including those associated with specific disease states.

## t1.1 Pathologic Red Blood Cells in Blood Smears

RBC Type	Description	Underlying Change	Disease States
Acanthocyte (spur cell)	Irregularly speckled cells with projections of varying length and dense center	Altered cell membrane lipids	Abetalipoproteinemia, parenchymal
Basophilic stippling	Punctate basophilic inclusions	Precipitated ribosomes (RNA)	Coarse stippling; lead intoxication, thalassemia; fine stippling; many anemias
Bite Cell (degmacyte)	Smooth semicircle taken from one edge	Heinz body "pitting" by spleen	G6PD deficiency, drug-induced oxidant hemolysis
Burr cell (echinocyte), or crenated cell	Cells with short, evenly spaced spicules and preserved central pallor	May be associated with altered membrane lipids	Usually artifactual; seen in uremia, bleeding ulcers, gastric carcinoma, artifact
Howell-Jolly bodies	Small, discrete basophilic dense inclusions; usually single	Nuclear remnant	Postsplenectomy, hemolytic anemia, megaloblastic anemia
Hypochromic cell	Prominent central pallor	Diminished hemoglobin synthesis	Iron deficiency anemia, thalassemia, sideroblastic anemia
Macrocyte	Cells larger than normal (>8.5 $\mu\text{m}$ ), well-filled hemoglobin	Reticulocytes, abnormal cell DNA maturation	Increased erythropoiesis, oval macrocytes in megaloblastic anemia, round macrocytes in liver disease
Microcyte	Cells smaller than normal (<7 $\mu\text{m}$ )	Abnormal hemoglobin production	Iron deficiency anemia, thalassemia, sideroblastic anemia
Ovalocyte (elliptocyte)	Elliptical-shaped cell	Abnormal cytoskeletal proteins	Hereditary elliptocytosis
Pappenheimer bodies	Small, dense basophilic granules	Iron-containing mitochondrial remnant or siderosome	Sideroblastic anemia, postsplenectomy
Polychromatophilia	Gray or blue hue frequently seen in reticulocytes	Ribosomal material	Reticulocytosis, premature marrow release of red blood cells
Rouleaux	Cell aggregates resembling stack of coins	Cell slumping by red cell interactions with paraprotein	Paraproteinemia, artifact
Schistocyte	Distorted, fragmented cell, two or three pointed ends	Mechanical destruction microvasculature by fibrin strands mechanical damage or prosthetic heart valve	Microangiopathic hemolytic anemia (DIC, TTP), prosthetic heart valves, severe burns
Sickle Cell (drepanocyte)	Bipolar, speckled forms, sickle-shaped, pointed at both ends	Molecular aggregation of hemoglobin S	Sickle cell disorders excludes S trait
Spherocyte	Spherical cell with dense hemoglobin and absent central pallor; usually decreased in diameter	Decreased membrane redundancy	Hereditary spherocytosis, immunohemolytic anemia, transfusion, artifact
Stomatocyte	Mouth- or cuplike deformity	Membrane defect with abnormal cation permeability	Hereditary stomatocytosis, immunohemolytic anemia
Target cell (codocyte)	Target-like appearance hypochromic with central hemoglobin	Increased redundancy of cell membrane	Liver disease, postsplenectomy, thalassemia, hemoglobin C disease, iron deficiency
Teardrop cell (dacrocyte)	Distorted, drop-shaped cell	Mechanical distortion of red cell	Myelofibrosis, myelophthisic anemia

## 1.2 Automated Hematology

In both office and hospital settings, most patients' blood is evaluated with an automated electronic blood cell counter. Most instruments analyze individually and combine data to characterize the entire cell population. Analysis of cell characteristics is achieved by various methods including voltage pulse-impedance analysis and low- or high-angle light scatter from a coherent or laser light source. In an impedance counter, the passage of a particle through an orifice of standard size and volume displaces conductive electrolyte solution within the orifice. If an electric current is applied across the orifice, a change in resistance and conductivity of the electrolyte solution occurs as the particle passes through it. A detector notes a pulse when the particle passes through the orifice; this pulse is proportional to the volume of the electrolyte solution displaced by the particle. Thus, the counter counts and sizes particles simultaneously. In a light scatter counter, interruption of the light beam by a particle produces an electronic pulse. The angle of light scatter and the intensity of the light scattered at a particular angle delineates several physical properties of the cell, including cell size, volume, shape, and internal complexity. Many modern hematology analyzers utilize a combination of these two approaches to provide accurate data about red blood cells.

With the physical data collected, a hematology analyzer can generate a histogram of size distribution on the x-axis and relative number of particles on the y-axis. From these data, the red cell number (RBC) and mean corpuscular volume (MCV) can be determined, and other indices, such as mean corpuscular hemoglobin concentration (MCHC), can be calculated. Newer instruments also generate an index that provides the degree of dispersion of red blood cell sizes (anisocytosis) compared with a "normal" size distribution histogram, referred to as a "red cell distribution width" (RDW).

## 1.3 Evaluation of the Blood Smear

Examination of the blood smear by a physician who is aware of the patient's clinical condition is extremely useful in evaluating the patient with anemia, as some red cell disorders may have subtle changes that are easily overlooked, such as minimal hypersegmentation of neutrophils in patients with combined folate and iron deficiency (masked macrocytosis) or basophilic stippling in a patient with thalassemia and other complicating causes of anemia. Electronically derived red blood cell indices, although useful, are simply representations of the mean and overall degree of dispersion of the cellular population and provide little information about specific red blood cell shapes and the presence or absence of minor populations of abnormal red cells. Examination of the blood smear for specific shape variations, such as those listed in Table 1-1, can provide valuable information to aid in the diagnosis of a patient's underlying disease.

## 1.4 Anemia

The primary function of the red blood cell is to deliver oxygen to the tissues. Anemia is defined as a reduction in the total number of red blood cells, amount of hemoglobin in the circulation, or circulating red blood cell mass. This results in impaired oxygen delivery to tissues, giving rise to physiologic consequences of tissue hypoxia as well as compensatory mechanisms initiated by the organism to correct anoxia. Signs and symptoms of anemia include fatigue, syncope, dyspnea, or impairment of organ function due to decreased oxygen; pallor or postural hypotension due to decreased blood volume; and palpitations,

onset of heart murmurs, or congestive heart failure due to increased cardiac output. Anemia is not a diagnosis, but a sign of underlying disease. Hence, the evaluation of a patient with anemia is directed at elucidating the causes for the patient's decreased red blood cell mass. A thorough history and physical examination are crucial for an intelligent, directed approach to the differential diagnosis of anemia. **t1.2** and **t1.3** show important features in a patient's history and physical examination that can yield diagnostic clues as to the cause of anemia, and efficiently direct further laboratory testing.

### t1.2 Patient History in the Diagnosis of Anemia

Historical Information	Possible Causes of Anemia
Age of onset	Inherited or acquired disorder, continuous or recent onset.
Duration of illness	Results of previous examinations and blood counts
Prior therapy for anemia	Vitamin B <sub>12</sub> , iron supplementation, and how long ago.
Suddenness or severity of anemia	Symptoms of dyspnea, palpitations, dizziness, fatigue, postural hypotension.
Chronic blood loss	Menstrual and pregnancy history, gastrointestinal symptoms, black or bloody stools.
Hemolytic episodes	Episode of weakness with icterus and dark urine.
Toxic exposures	Drugs, hobbies, and occupational exposures.
Dietary history	Alcohol use, unusual diet, prolonged milk ingestion in infants.
Family history and racial background	Possible inherited disorder: family members with anemia, gallbladder disease, splenomegaly, splenectomy.
Underlying diseases	Uremia, chronic liver disease, hypothyroidism

### t1.3 Physical Signs in the Diagnosis of Anemia

Physical Sign	Associated Disease
<b>Skin and mucous membranes</b>	
Pallor	Any anemia
Scleral icterus	Hemolytic anemia
Smooth tongue	Pernicious anemia, severe iron deficiency
Petechiae	Thrombocytopenia and bone marrow replacement or aplastic anemia
Ulcers	Sickle cell disease
<b>Lymph nodes</b>	
Lymphadenopathy	Infectious mononucleosis, lymphoma, leukemia
<b>Heart</b>	
Cardiac dilatation, tachycardia, loud murmur	Severe anemia
Soft murmurs	Anemia, usually mild
<b>Abdomen</b>	
Splenomegaly,	Infectious mononucleosis, leukemia, lymphoma, hypersplenism
Massive splenomegaly	Chronic myelogenous leukemia, myelofibrosis
Hepatosplenomegaly with ascites	Liver disease
<b>Central nervous system</b>	
Subacute combined degeneration of spinal cord	Pernicious anemia (vitamin B <sub>12</sub> deficiency)
Delayed Achilles tendon reflex	Hypothyroidism

### 1.4.1 Examination of the Blood

Anemia has been classified by several different approaches, none of which is completely satisfactory. For practical purposes, an initial morphologic classification of anemia with integration of red blood cell indices and morphologic characteristics is probably most useful. With use of the MCV and the RDW or red cell morphologic index (RCMI), anemia may be classified into six categories **t1.4**. The anemia may be characterized by cell size as microcytic, normocytic, or macrocytic. The absence or presence of anisocytosis (as measured by RDW) further subdivides these three size categories. In general, anemia caused by nutritional deficiencies (such as iron, folate, or vitamin B<sub>12</sub>) tend to have a greater degree of anisocytosis than anemia caused by genetic defects or primary bone marrow disorders. However, difficulties arise in classification using this scheme, particularly with regard to anemia of chronic disease.

#### t1.4 Classification of Anemia Based on Red Blood Cell Size and Distribution Width

Cell Size	Normal RDW	High RDW
Microcytosis (MCV <70 $\mu\text{m}^3$ [70 fL])	Thalassemia minor, anemia of chronic disease, some hemoglobinopathy traits	Iron deficiency, hemoglobin H disease, some anemia of chronic disease, some thalassemia minor, fragmentation hemolysis
Normocytosis	Anemia of chronic disease, hereditary spherocytosis, some hemoglobinopathy traits, acute bleeding	Early or partially treated iron or vitamin deficiency, sickle cell disease
Macrocytosis (MCV >100 $\mu\text{m}^3$ [100 fL])	Aplastic anemia, some myelodysplasias	Vitamin B <sub>12</sub> or folate deficiency, autoimmune hemolytic anemia, cold agglutinin disease, some myelodysplasias, liver disease, thyroid disease, alcohol

*RDW = red cell distribution width; MCV = mean corpuscular volume*

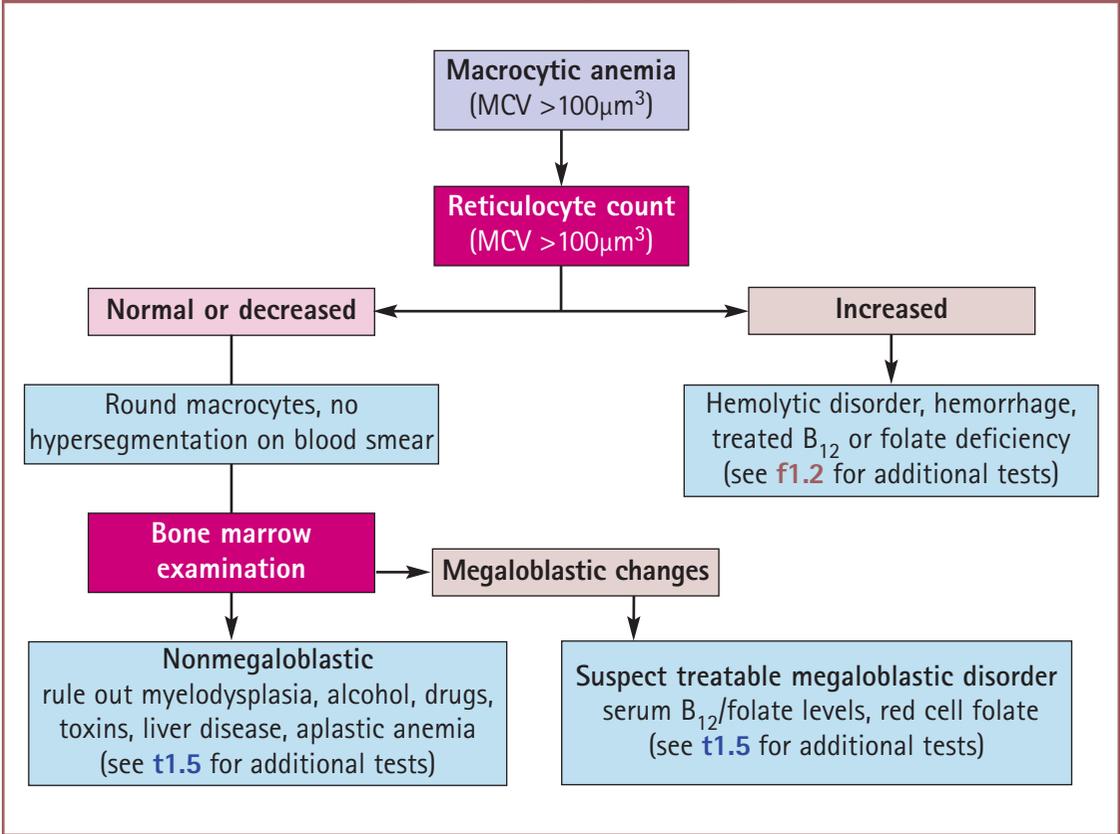
In addition to pure morphologic criteria, anemia also may be classified by the degree of bone marrow response or peripheral blood reticulocytosis as hyperproliferative, normoproliferative, or hypoproliferative. This often provides insights into the pathogenesis of the process. Thus, patients with defects in red blood cell proliferation or maturation tend to have little or no increase in reticulocytes, reflecting the inability of the bone marrow to increase red blood cell production in response to the anemia (hypoproliferative anemia). In contrast, patients with anemia caused by decreased survival of red blood cells with a normal bone marrow proliferative response often exhibit increased peripheral blood reticulocytes (normoproliferative or hyperproliferative anemia) (see **f1.1**). If the degree of reticulocytosis is adequate to replace the loss of red blood cells, the anemia is termed “compensated.” If the bone marrow response is inadequate, the anemia will progressively worsen.

Finally, anemia caused by decreased red blood cell survival are often subdivided by pathogenetic mechanism into those caused by intrinsic or inherited defects and those that are acquired or caused by extrinsic factors. This classification is often useful in understanding the underlying disease processes and may facilitate the evaluation and diagnosis of anemia that arises secondary to extrinsic processes.

### 1.4.2 Differential Diagnosis of Anemia

Anemia may be either relative (due to increased plasma volume with a normal red blood cell mass) or absolute (due to a decreased red blood cell mass). It is important to rule out causes of relative anemia, such as pregnancy, excessive hydration or macroglobulinemia, as they represent disturbances in plasma volume rather than a true decrease in red blood cell mass. Similarly,

**f1.1 Classification of macrocytic anemia by reticulocyte count**



decreased plasma volume, caused by dehydration, may mask a real decrease in circulating red blood cell mass.

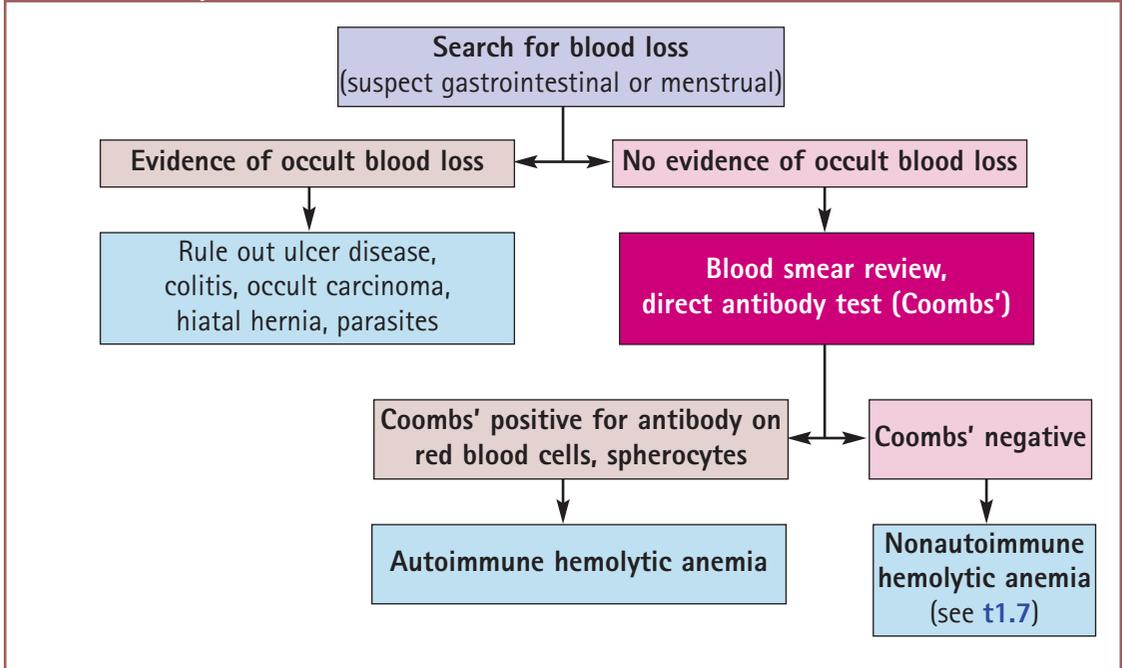
Use of a morphologic classification scheme in combination with red blood cell indices and the reticulocyte count allows for practical classification of anemia into broad groups. This will facilitate selection of additional laboratory tests to determine the underlying cause of the anemia.

**1.4.3 Macrocytic Anemia**

Macrocytic anemia (MCV >100 µm<sup>3</sup> [>100 fL]) are less common than normocytic or microcytic anemia. Macrocytic anemia may be subdivided into those with a normal RDW (principally those caused by bone marrow failure, such as aplastic anemia and myelodysplasia), and those with a high RDW (caused by either deficiencies of vitamin B<sub>12</sub> or folic acid; by autoimmune hemolysis or cold agglutinins). However, many exceptions to this general classification scheme exist. For example, a mild degree of macrocytosis (MCV between 10<sup>2</sup> and 10<sup>5</sup> µm<sup>3</sup> [10<sup>2</sup> and 10<sup>5</sup> fL]) with a normal RDW is relatively common as a direct toxic effect of alcohol. Similarly, some cases of myelodysplasia may have a high RDW.

Further classification of a macrocytic anemia based on the presence or absence of a reticulocyte response is also helpful (see f1.1). Hemolytic anemia, blood loss, and partially treated vitamin B<sub>12</sub> or folic acid deficiencies will demonstrate an increased reticulocyte count. Normal to increased reticulocyte counts are more likely to be associated with autoimmune hemolysis, disorders of membrane structural proteins (eg, elliptocytosis or spherocytosis), paroxysmal nocturnal hemoglobinuria, and fragmentation hemolysis f1.2. For those patients with a normal or decreased corrected reticulocyte count, disorders associated with decreased bone marrow function—including untreated vitamin deficiency, drugs, toxins, liver and thyroid disease, or primary bone marrow

## f1.2 Classification of normocytic or megaloblastic anemias with elevated reticulocyte counts



failure—should be suspected. Blood smears that show morphologic features compatible with megaloblastic anemia (oval macrocytes and hypersegmented neutrophils) may warrant further evaluation with vitamin assays but not bone marrow examination. When megaloblastic changes are present without signs of vitamin B<sub>12</sub> or folate deficiency, bone marrow examination and additional testing **t1.5** may be needed. A more extensive discussion of the diagnosis and evaluation of macrocytic anemia is found in Chapter 5.

### t1.5 Ancillary Tests for Macrocytic Anemia Without Increased Reticulocyte Response

#### Megaloblastic bone marrow changes present only in erythroid line:

Thyroid function tests

Assess iron stores—serum iron, iron-binding capacity, ferritin

Cytogenetic analysis—evaluate for myelodysplasias

#### Megaloblastic bone marrow changes present in more than one cell line:

Dietary and drug history

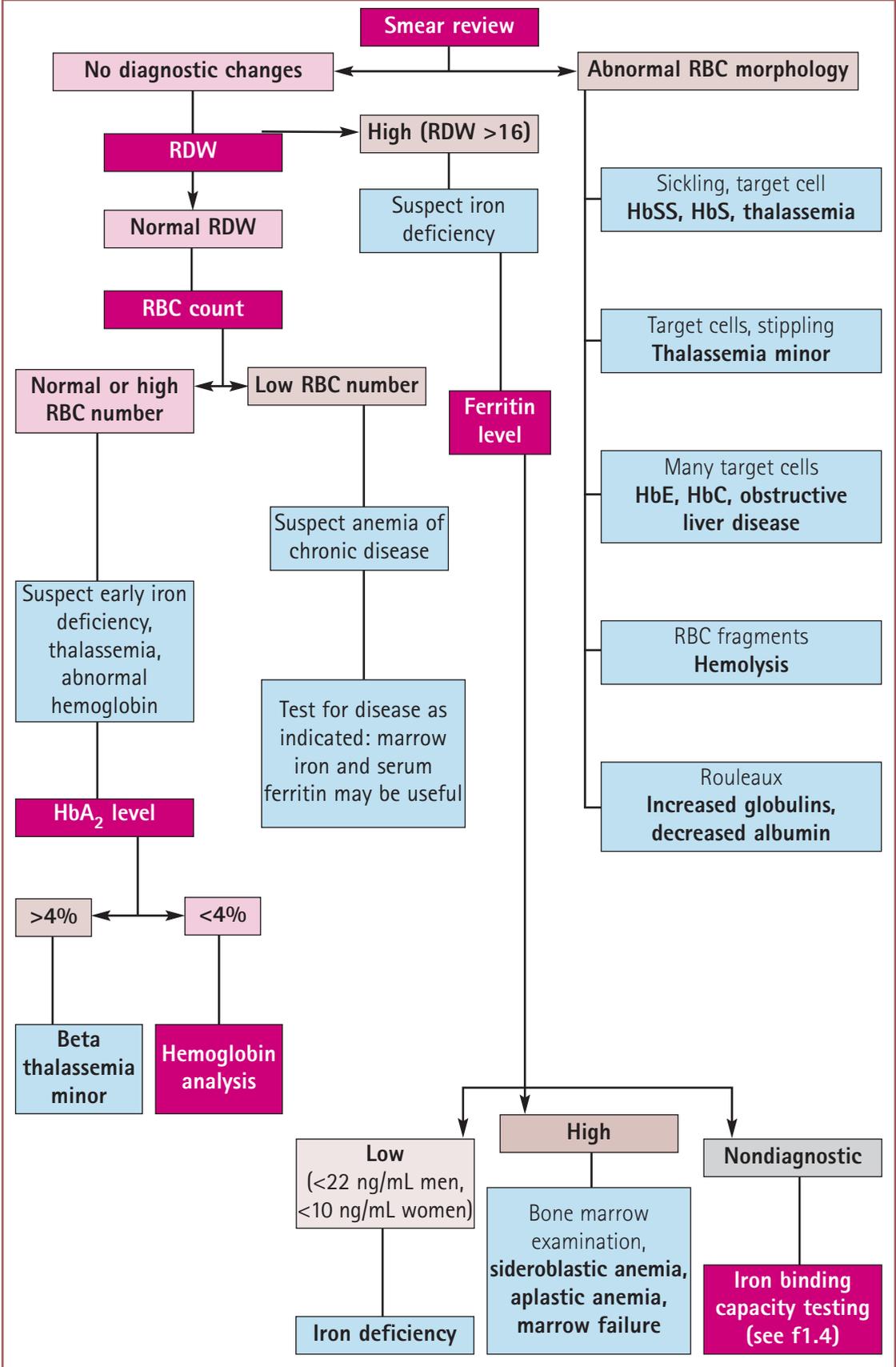
Malabsorption studies

Schilling test if vitamin B<sub>12</sub> deficiency

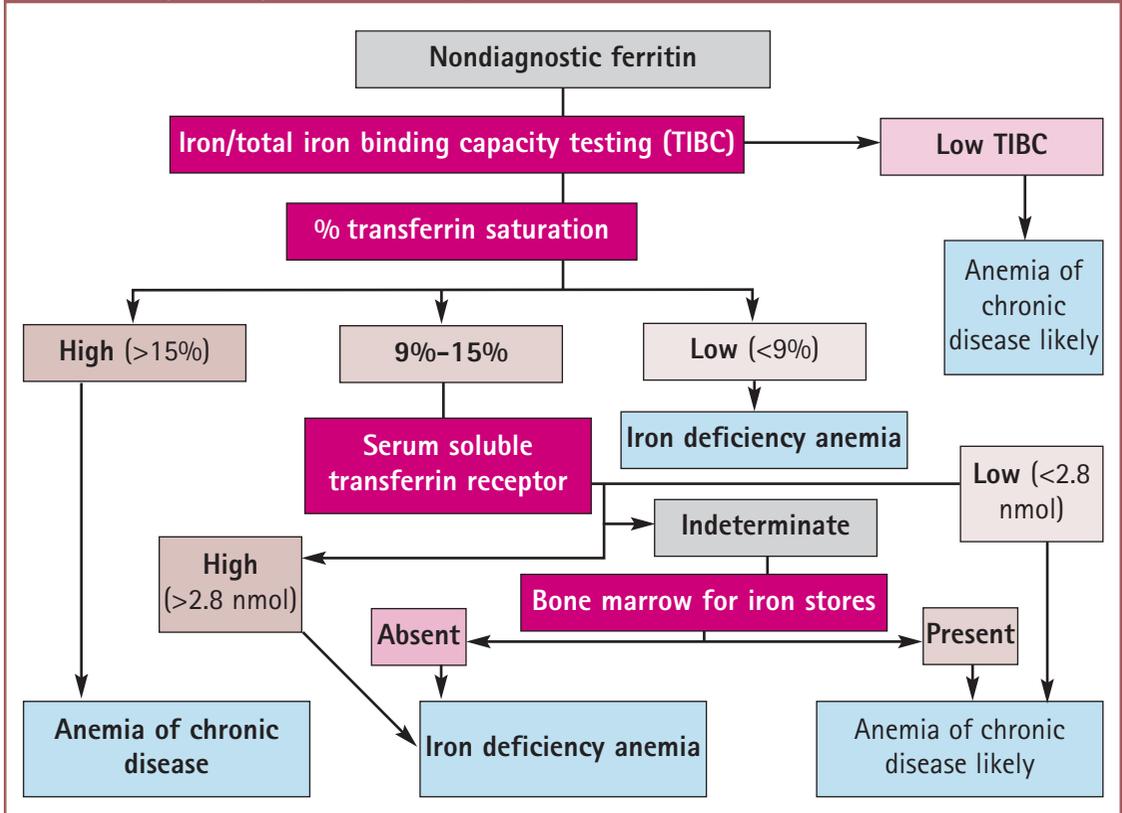
### 1.4.4 Microcytic Anemia

The three most common causes of microcytic anemia (MCV <75  $\mu\text{m}^3$  [ $<75$  fL]) are iron deficiency, thalassemia minor, and anemia of chronic disease **f1.3**. The RDW is useful in distinguishing thalassemia, which generally (but not invariably) produces elevated red blood cell counts and a lower RDW than would be expected for the degree of anemia. Iron deficiency is almost always associated with a high RDW. The values seen in anemia of chronic disease are extremely variable with some being normocytic, while others are microcytic (particularly in patients with renal disease). By using additional laboratory testing to determine body iron stores, such as serum iron and

### f1.3 Classification of microcytic anemia



### f1.4 Distinguishing iron deficiency anemia from anemia of chronic disease



total iron binding studies, iron deficiency anemia and anemia of chronic disease may usually be distinguished without a bone marrow examination **f1.4**. A more detailed consideration of the evaluation and diagnosis of microcytic anemia is found in Chapter 2.

#### 1.4.5 Normocytic, Normochromic Anemia

Patients with normal or hypoproliferative reticulocyte counts and normocytic, normochromic anemia generally require bone marrow evaluation. A peripheral blood smear may provide valuable clues for the differential diagnosis **t1.6**. Patients with normocytic anemia and an elevated reticulocyte count should undergo the same general evaluation as patients with macrocytosis and an elevated reticulocyte count (see **f1.2**). Normocytic, normochromic anemia with an elevated reticulocyte count can be divided into those with positive direct antiglobulin test results (DAT or Coombs' test) and those lacking evidence of red blood cell-bound

### t1.6 Normochromic, Normocytic Anemia Without High Reticulocyte Response

#### Findings on Peripheral Blood Smear

Leukoerythroblastosis

Abnormal white blood cells  
Rouleaux

No abnormal cells

#### Further Workup

**Suspect myelophthitic process**—bone marrow examination for space-occupying lesion (metastatic tumor, lymphoma, myelofibrosis) in children suspect infection

**Suspect leukemia, lymphoma**—bone marrow examination

**Suspect myeloma**—serum and urine electrophoresis, radiographs to look for lytic lesions, bone marrow examination

**Suspect anemia of chronic disease or sideroblastic anemia**—bone marrow examination; rule out chronic disease processes, ferritin, total iron-binding capacity, and percent transferrin saturation as indicated

antibodies. Coombs'-negative hemolytic anemia is a heterogeneous group of disorders. The blood smear and patient history often suggest possible causes for the anemia **t1.7**. A more detailed discussion of non-hemolytic normocytic anemia may be found in Chapter 3, and discussion of different causes of hemolytic anemia are presented in Chapters 6 through 15.

### t1.7 Workup of Coombs'-Negative Anemia

Feature of Anemia	Possible Process	Tests
Episodic anemia	Enzyme deficiency Paroxysmal nocturnal hemoglobinuria	G6PD, other RBC enzymes Sucrose hemolysis, Ham's test, flow cytometry
RBC fragmentation	DIC, TTP, HUS	Coagulation tests, serum haptoglobin levels
Abnormal RBC stippling	Lead poisoning Thalassemia	Lead levels Hemoglobin electrophoresis
Abnormal shapes or increased target cells	Hemoglobinopathy, thalassemia	Hemoglobin analysis
Spherocytes	Hereditary spherocytosis	Osmotic fragility test

*G6PD = glucose-6-phosphate dehydrogenase; DIC = disseminated intravascular coagulation; TTP = thrombotic thrombocytopenic purpura; HUS = hemolytic-uremic syndrome*

The differential diagnosis of anemia is often tempered or modified by knowledge of other patient data. All algorithmic classification schemes should be qualified by the pragmatic knowledge of the physician in considering the probable causes of anemia in an individual patient or patient population. For example, as 98% or more of anemia in children under the age of 4 years is caused by iron deficiency, many pediatricians simply treat all children with this type of anemia with iron supplementation and perform workups only for those who fail to respond to this therapy. In many situations, clinical knowledge can suggest several possible causes of anemia. Thus, the provided algorithms are suggested pathways for physicians to use for determining test utilization and should not be considered required clinical work-ups.

## 1.5 Laboratory Tests

### Test 1.5.1 Manual Reticulocyte Count

**Purpose.** This test enumerates the number of reticulocytes, indicating bone marrow production of new red blood cells.

**Principle.** Residual RNA in immature red blood cells is precipitated and stained with a supravital dye.

**Specimen.** Venous or capillary blood may be used for this test.

**Procedure.** A blood smear is made, and the red blood cells are stained with brilliant cresyl blue or methylene blue. Cells containing stained reticular material are enumerated per 1000 red blood cells and expressed as percent reticulocytes (absolute number per 100 red cells). Many automated hematology analyzers now analyze reticulocyte counts based on staining and light-scatter properties as an optional function of the complete blood count.

**Interpretation.** Reticulocytes are immature red blood cells that contain at least two dots of stainable reticulin material in their cytoplasm. More immature forms have multiple dots and small networks of skeins of bluish-staining material. Intra-observer variation and uneven distribution of reticulocytes introduce a high analytic variation in manual reticulocyte counting, with interlaboratory coefficients of variation often in the range of 20%. Duplicate reticulocyte counts or 3-day average values may help to reduce the imprecision of the raw reticulocyte count. Automated reticulocyte counts (see **Test 1.2**), owing to larger sample analysis and mechanically defined criteria, tend to be more reproducible.

Effective red blood cell production is a dynamic process, and the number of reticulocytes should be compared with the expected number to be released in a patient without anemia. This is calculated as 1% of  $5 \times 10^6/\text{mm}^3$  ( $5 \times 10^{12}/\text{L}$ ) red cells daily for an absolute reticulocyte production of  $50 \times 10^3/\text{mm}^3$  ( $50 \times 10^9/\text{L}$ ). The corrected reticulocyte count takes into account normal red blood cell proliferation for a specific hematocrit and may be calculated with the following formula:

$$\text{Corrected Reticulocyte Count} = (\% \text{ Observed Reticulocytes} \times \text{Hematocrit}) \div 45$$

Another complicating factor in reticulocyte count correction is that patients with anemia may release reticulocytes prematurely into the circulation. Reticulocytes are usually present in the blood for 24 hours before they extrude the residual RNA and become erythrocytes. If they are released early from the bone marrow, immature reticulocytes may persist in peripheral blood for 2 or 3 days. This is most likely to occur when severe anemia causes a marked acceleration in erythropoiesis and release. Some authors have advocated correction of the reticulocyte count for immature reticulocytes (thought to be the best reflection of bone marrow response to anemia), called the "reticulocyte production index" (RPI):

$$\text{RPI} = [(\% \text{ Reticulocyte} \times \text{Hematocrit Value}) \div 45] \times [1 \div \text{Correction Factor}]$$

The correction factor calculation is shown in **t1.8**.

### t1.8 Correction Factor Calculation

Patient's Hematocrit Value, %	Correction Factor
40-45	1.0
35-39	1.5
25-34	2.0
15-24	2.5
<15	3.0

In cases of low erythropoietin (often seen in patients with renal or hepatic disease), application of an RPI correction may mask a failure of bone marrow response, because the shift does not take place fully or at all. In general, RPI values less than 2 indicate failure of bone marrow red blood cell production or a hypoproliferative anemia. Reticulocyte production indexes of 3 or greater indicate marrow hyperproliferation or appropriate response to anemia.

### Test 1.5.2 Automated Reticulocyte Count

**Purpose.** Determination of reticulocyte numbers provides insight into the underlying pathophysiology of an anemia. Use of automated staining and determination of reticulocytes in a hematology analyzer provides accurate reticulocyte enumeration by allowing evaluation of many more red blood cells than can be studied with manual supravital staining and also can provide information as to time since release from the bone marrow (reticulocyte maturity stage) by the staining patterns.

**Principle.** Reticulocytes are immature red blood cells in the final stage of differentiation that have been recently released from the bone marrow and still retain intracellular protein and RNA. They may be stained with RNA avid dyes that can be detected by fluorescence, light scatter properties or absorbance characteristics. Specific, proprietary dyes vary between types of hematology analyzers, but show similar reticulocyte staining patterns. The maturity level of the reticulocytes can be determined by the amount and intensity of staining, with the most immature reticulocyte fraction having the highest staining (highest RNA levels). Usually reticulocytes are fractionated into two to three different populations (immature, intermediate and mature), with the immature reticulocyte fraction being the most accurate reflection of erythropoietic activity that provides insight into the bone marrow proliferative response to an anemia.

**Specimen.** Anti-coagulated whole blood, usually in EDTA.

**Procedure.** Whole blood is stained with the RNA avid dye and analyzed in the hematology analyzer using the reticulocyte enumeration program. Data is provided as a percentage of red blood cell reticulocytes. The instrument will also fractionate the reticulocytes into an immature and mature fraction, with some instruments providing an intermediate reticulocyte fraction based on levels of staining.

**Problems and Pitfalls.** Automated reticulocyte counts may vary widely dependent on the methodology and instrumentation used, and monitoring should be done using the same methodology over time. Methods using fluorescence and argon laser detection may be more sensitive at detecting low numbers of reticulocytes. There is significant imprecision at very low reticulocyte numbers, reflecting limitations of analytic sensitivity in the method. Samples with significant numbers of reticulocytes are usually reproducible on the same instrument over time, both in determination of total numbers of reticulocytes and the immature fraction.

### Test 1.5.3 Bone Marrow Examination

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**Purpose.** Bone marrow examination allows assessment of the cellularity, maturation, and composition of the hematopoietic elements in the bone marrow, as well as evaluation of iron stores. Some infections also may be cultured from the bone marrow.

**Principle.** The cortical bone is penetrated and a sample of the bone marrow is aspirated. In most cases, a small biopsy specimen of the medullary bone and marrow is obtained. The most common sites for the procedure are the posterior or anterior iliac crest and the sternum.

**Specimen.** Bone marrow aspiration and biopsy samples.

**Procedure.** Bone marrow aspiration and biopsy are innocuous procedures when performed by experts. Several sites in the skeleton have been used for bone marrow sampling. Because active hematopoiesis occurs in the long bones of the arms and legs in infants under the age of 8 months, aspiration from the anterior aspect of the tibial tuberosity is useful. For adults, the posterior iliac crest is the recommended site. Patients who are unable to lie on their stomachs may be approached through the anterior iliac crest or sternum. The sternum is aspirated relatively easily, but its structure does not allow biopsy. In elderly patients, sternal bone marrow may be most representative of the patient's hematopoietic status and superior to that of the relatively acellular iliac crest. Sternal aspiration also may be most appropriate for patients who have lesions in the sternum or ribs.

**Notes and Precautions.** Processing and interpretation have significant technical variables and require experienced personnel. Bone marrow examination should be limited to situations in which non-invasive procedures do not yield clear answers. **t1.9** gives the most common indications for bone marrow examination.

## t1.9 Indications for Bone Marrow Examination in Anemia

### Abnormalities in blood counts and/or peripheral blood smear

Unexplained cytopenias  
 Unexplained leukocytosis or abnormal white blood cells  
 Teardrop cells or leukoerythroblastosis  
 Rouleaux  
 No or low reticulocyte response to anemia

### Evaluation of systemic disease

Unexplained splenomegaly, hepatomegaly, lymphadenopathy  
 Tumor staging: solid tumors, lymphomas  
 Monitoring of chemotherapy effect  
 Fever of unknown origin (with bone marrow cultures)  
 Evaluation of trabecular bone in metabolic disease (use undecalcified bone)

**Interpretation.** When both a Wright-stained aspirate preparation and a histologic core needle biopsy are available, optimal evaluation may be performed. The false-negative rate for metastatic carcinoma using aspiration alone is about 25%; for lymphomas it seems to be somewhat higher—30% to 40%—depending on the cell type. Because of the small nature of the biopsy specimen, sampling errors still may be a problem, causing false-negative results. Additional testing, such as iron stains to evaluate iron stores, immunohistochemical staining, flow cytometric analysis, and cytogenetic analysis, may be performed on aspirated bone marrow specimens to provide additional information about the disease process.

## 1.5 Treatment

Treatment of anemia is usually aimed at correcting the underlying abnormality. This may involve identification of a source of blood loss, iron or vitamin supplementation, or discontinuation of a drug that predisposes a patient to hemolysis. Acquired anemia associated with hematopoietic abnormalities (such as myelodysplasia or aplastic anemia) or inherited anemia (such as red blood cell membrane defects, enzymopathies or hemoglobinopathies) may require transfusions when symptoms arise due to decreased oxygen delivery to the tissues. The benefit of transfusion therapy must be balanced carefully against the risks of disease transmission and iron overload. Usually transfusions are not required unless the hemoglobin concentration falls below 7 g/dL (70 g/L), unless significant cardiac or pulmonary disease is present and hypoxia would be exacerbated by even modest decreases in oxygen delivery. Long-term transfusion therapy may cause iron overload, leading to subsequent organ iron deposition and failure of function as patients are unable to appropriately decrease gut mucosal iron absorption when iron loading occurs via transfusion.

## 1.6 References

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