Anemia

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Abnormalities in the routine blood count alert the physician to hematologic problems. The most common of these are red cell abnormalities as reflected in decreased levels of hemoglobin and hematocrit together with changes in mean corpuscular volume and red cell morphology. When the history and physical examination are not diagnostic, systematic use of laboratory tests can determine the category of anemia present. An approach for the laboratory workup of anemia is discussed. Particular attention is paid to the differential diagnosis of the most common anemias in which iron is the limiting factor in erythropoiesis.

As mild anemia of itself is usually asymptomatic, the physician often first becomes aware of anemia from a laboratory report. When symptoms do occur, however, they are usually cardiovascular in nature. These symptoms may not be marked, however, even in the patient with moderate anemia, particularly when it has developed slowly. There may be increased shortness of breath on exertion, palpitations, sweating, and pedal edema.[1] In the otherwise normal child, pallor and a systolic flow murmur may be the only indicators of anemia. The reduction of blood oxygen supply incurred by anemia in elderly patients with vascular disease may produce symptoms of local ischemia, which may mislead the physician. When anemia is secondary to another condition, the predominant presentation may relate to the primary illness.

DOES THE PATIENT HAVE ANEMIA?

Before embarking on an investigation, the physician must decide whether the hemoglobin or hematocrit level is appropriate for the patient. The hemoglobin level is preferable as a red cell measurement, because it is more directly related to the transport of oxygen. Hemoglobin may be converted to hematocrit by multiplying by three. From a physiologic standpoint, anemia may be defined as a reduced concentration of hemoglobin below the individual's normal value. In evaluating this description, it is necessary to consider several factors.

The normal reference value for hemoglobin used in the laboratory has been statistically derived from a "normal" population in which there is an overlap at the separation point between normal individuals and individuals shown to respond to iron therapy (Figure 1).[2] Because population norms may not relate to the physiologic norm of the individual, it is important, when possible, to compare the hemoglobin level with a previous level of the patient. In the absence of such a comparison, a single laboratory determination of hemoglobin below normal can only indicate a probability of anemia, with the probability increasing as the value of hemoglobin is decreased.[3]

The age and sex of the patient affect the mean normal values for hemoglobin.[4] In the preterm male infant, the maximum hemoglobin level, as measured in the cord blood, is achieved by the 32nd week of gestation, while the female fetus continues to increase her hemoglobin until the 40th week.[5] The newborn has an elevated hemoglobin level, which falls over the next four to eight weeks (this drop is more exaggerated and prolonged in the premature infant[6,7] and then increases gradually through childhood and adolescence to adulthood (Table 1). The mean hemoglobin level in the adult man is 15 g/dL; in the adult woman, it is 14 g/dL. In pregnancy, there is a fall of 1 g in the late second and third trimester. The woman maintains her hemoglobin level through old age, while the man drops his hemoglobin level slightly after the seventh decade.[8,9]

Altitude associated with arterial oxygen desaturation produces an increase in 1 g/dL of hemoglobin for each 3 to 4 percent decrease in arterial oxygen saturation.[10] Race may also be associated with different values. In the United States, average normal hemoglobin values for blacks are approximately 0.5 g/dL lower than those for whites.[11] In circumstances associated with plasma volume changes in the patient, reciprocal changes in hemoglobin are seen.

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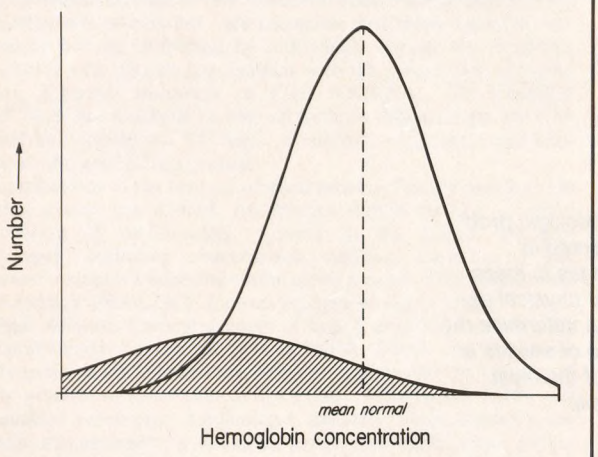

Figure 1. Anemia overlap. The symmetrical distribution of hemoglobin in normal individuals is shown with the mean indicated by a dotted line. Distribution of individuals with true iron-responsive anemia is indicated in the shaded area overlapping normal values

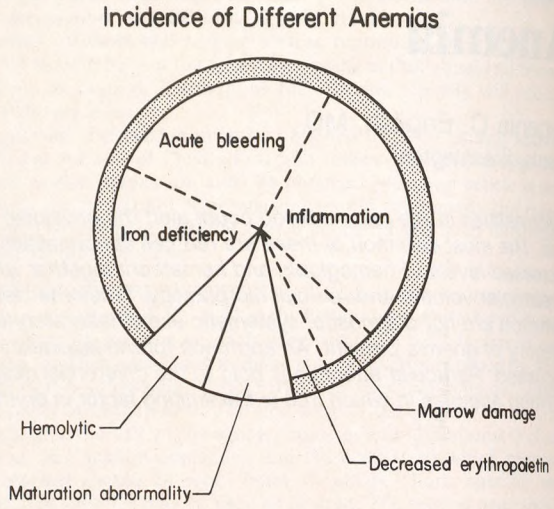

Figure 2. Diagrammatic representation of the incidence of different anemias. The stippled area indicates hypoproliferative anemia. Those anemias in which iron is the limiting factor include iron deficiency, inflammation, and bleeding

TABLE 1. MEAN NORMAL HEMOGLOBIN VALUES AT SEA LEVEL, ALL RACES

Age	Hemoglobin (g/dL)	
	Male	Female
32- to 33-week gestation	16	15
36- to 37-week gestation	16	16
newborn	17	17
4 to 8 weeks	14	14
1 to 2 years	12	12
5 to 10 years	13	13
11 to 14 years	14	13
Adult man	15	—
Menstruating woman	—	14
Pregnant woman (last trimester)	—	13
Over 70 years	14.5	14

Despite these complicating factors, it is necessary to have arbitrary values in mind in the detection of anemia. While hemoglobin levels of 9.5 to 11 g/dL have been used in 1-year-old infants to indicate anemia,[12–14] a value of less than 11.5 g/dL was found to be most useful in identifying those who would respond to iron.[15] Anemia in adults at sea level is considered likely with hemoglobin values of less than 13 g/dL in men, with values of less than 12 g/dL in nonpregnant women and less than 11 g/dL in pregnant women.[16] In practice, a more accurate assessment may be made if the usual hemoglobin concentration of the patient is known. A decrease of 1 g/dL from the patient's own known normal hemoglobin level is significant if plasma volume changes are excluded.

THE LOW MEAN CORPUSCULAR VOLUME

When anemia is believed to be present and its cause is not clear from the medical history and physical examination, simple laboratory tests can be used to define its nature. The most common cause of anemia is insufficient iron for hemoglobin formation (Figure 2). Lack of iron may be due to an inadequate iron supply (iron deficiency), with or without chronic blood loss, or an internal block in iron metabolism as seen in inflammation.[17] While about one half of such anemias are normocytic, a decreased mean corpuscular volume (MCV) in the other half provides a powerful means of identifying iron deficiency (Figure 3).[18] When the MCV is less than 84 μm^3 in the adult and less than 70 μm^3 in children, there is an impairment in hemoglobin formation that is due to either a deficient iron supply or a defect in globin formation (thalassemia). Hypochromia of red cells is a hallmark of deficient hemoglobin formation. In iron deficiency, in contrast to thalassemia, there is an associated increase in numbers of small platelets in the blood smear.

A determination of plasma transferrin saturation will identify an iron-deficient state (Figure 4). When the

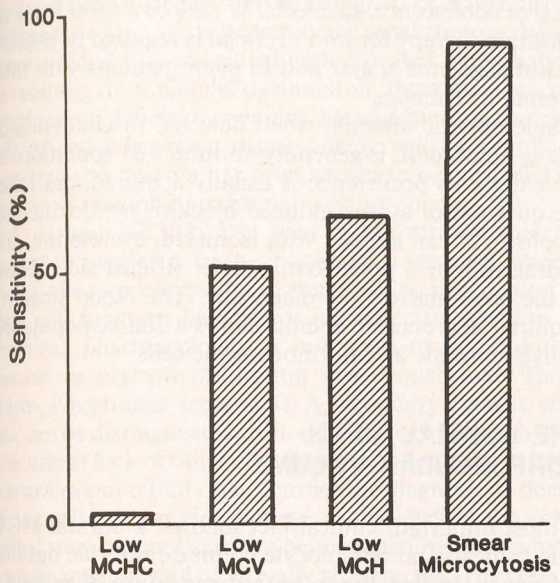

Figure 3. The sensitivity of red cell indices and smear microcytosis in the detection of iron deficiency (transferrin saturation less than 16 percent)

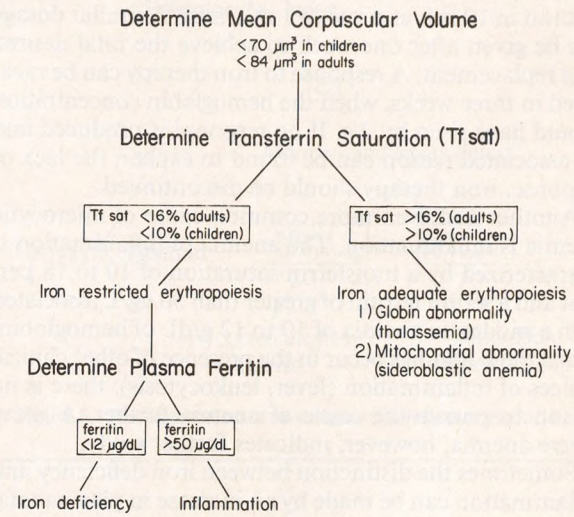

Figure 4. The separation of microcytic anemias

plasma transferrin saturation (plasma iron/total iron-binding capacity) is below 16 percent in the adult and below 10 percent in the child, there is insufficient iron to support normal red cell production. This condition may be more specifically identified as iron deficiency by a serum ferritin of less than 12 μg/dL, which indicates iron-depleted stores.[19] One may then proceed to determine the underlying cause of iron deficiency, which must be long-standing, because microcytosis takes several months to develop. Dietary iron lack in the infant and increased iron losses in the menstruating and pregnant woman are common causes of iron deficiency. In the adult man, however, dietary iron balance is usually favorable, so that iron deficiency must be assumed to be due to pathologic blood loss. Discovering the underlying lesion causing this blood loss is of primary importance.

The specific treatment for iron deficiency is oral iron.[20] In planning treatment, the amount of elemental iron rather than the amount of iron salt is the critical factor. Iron supplementation of infant formula is particularly useful for the low birth weight infant who has a greater risk of iron deficiency when the growth rate accelerates between 6 months and 2 years of age. Liquid iron preparations (ferrous sulfate solution USP containing 25 mg/mL of elemental iron, or ferrous sulfate syrup USP containing 8 mg/mL of elemental iron) are easily absorbed and well tolerated by children. The usual dosage of iron for infants and children is 5 mg/kg. When small children are present, it is essential that iron preparations be kept in tamper-proof containers as a precaution against the danger of iron poisoning. For adults, the usual therapeutic dosage is 2 to 3 mg/kg (120 to 200 mg elemental iron in three divided doses). In pregnancy, when iron is used prophylactically, only 10 to 20 mg of elemental iron a day is required to guard against the development of iron deficiency.

It is preferable to take iron apart from meals, because food reduces the availability of iron for absorption by one half or more.[21] In the patient who complains of intolerance, either a reduction in dose can be tried apart from meals, or the same dose can be taken with meals. After correction of anemia, therapy must be continued for those patients in whom blood loss is anticipated. If iron stores are to be reestablished, at least six months of therapy will be required in the nonbleeding patient. When malabsorption (sprue) exists, in the rare situation when an oral intolerance cannot be circumvented, or when iron stores need to be created, parenteral iron dextran may be used. As significant and even fatal reactions occur with parenteral iron, there should be a persuasive reason for such use. Intramuscular dextran injections produce prolonged discomfort and skin discoloration and can be associated with malignant change at the site of injection,[22] so that they are not advised. Intravenous dextran injections should be first tested for signs of anaphylactic reaction (0.1 mL intravenously with the patient observed for 10

minutes) before an infusion of 500 mg of iron as iron dextran in 10 mL at a rate of 1 mL/min. A similar dosage can be given after one week to achieve the total desired iron replacement. A response to iron therapy can be measured in three weeks, when the hemoglobin concentration should have risen by 2 g. If no response is produced and no associated reason can be found to explain the lack of response, iron therapy should be discontinued.

Another and even more common cause of microcytic anemia is inflammation. The anemia of inflammation is characterized by a transferrin saturation of 10 to 18 percent and a serum ferritin of greater than 50 μg/L associated with a moderate anemia of 10 to 12 g/dL of hemoglobin. When these findings occur in the presence of other clinical indices of inflammation (fever, leukocytosis), there is no reason to pursue the cause of anemia further. A more severe anemia, however, indicates further study.

Sometimes the distinction between iron deficiency and inflammation can be made by an increase in plasma total iron-binding capacity in the former and a decrease in the latter. A more reliable distinction can be made by a plasma ferritin determination. While a plasma ferritin value of 12 μg/dL or less is diagnostic of iron deficiency, a plasma ferritin value of greater than 50 μg/dL, when associated with an iron transferrin saturation below 16 percent, indicates the presence of inflammation.

A microcytic anemia with a plasma transferrin saturation above 16 percent raises other possibilities, such as the thalassemias (globin formation defects) and the sideroblastic anemias (mitochondrial function defects). While family studies are frequently helpful in demonstrating the genetic nature of thalassemia, specific diagnosis depends on hemoglobin electrophoresis (cellulose acetate) and other special studies for the measurements of fetal hemoglobin, hemoglobin A_2, and identification of abnormal hemoglobins. A hemoglobin A_2 level above 3.5 percent is diagnostic of the majority of β-thalassemia traits, but there is no clinical laboratory method for detecting α-thalassemia trait.[23,24] It may be possible, however, to obtain presumptive evidence of the thalassemia trait from red cell volume distribution curves[25] and discriminant functions based on red cell indices generated by electronic cell counters.[26,27] Measurement of the degree of anisocytosis as expressed by the red cell distribution width (RDW) can be helpful in differentiating thalassemia trait (low RDW) from iron deficiency (high RDW), but requires ultimate confirmation by a serum iron determination (high in thalassemia trait and low in iron deficiency).[28,29]

Management of the patient with thalassemia includes genetic and family counseling. Obviously iron is not indicated when transferrin saturation is normal and can be harmful in those patients who are already assimilating excess iron. In patients with severe anemia, supportive red cell transfusions may be required, and in later childhood or adolescence, splenectomy may be a consideration. Chelation therapy for iron overload is required in patients with thalassemia major and in some patients with thalassemia intermedia.

Sideroblastic anemia, when detected in childhood or young adulthood, is generally an inherited condition. In the elderly its occurrence is usually acquired and raises the question of a drug-induced block in pyridoxine metabolism, as can happen with isoniazid, cycloserine, and hydralazine or a preleukemic state. Ringed sideroblasts in the bone marrow are diagnostic. The blood smear in acquired sideroblastic anemia shows a double population of hypochromic and normochromic cells.

THE NORMAL MEAN CORPUSCULAR VOLUME

A most important clinical fact follows: a normal MCV, blood smear, and reticulocyte count do not rule out iron deficiency. In fact, the most frequent cause of anemia is still that due to a lack of iron, either from iron deficiency or inflammation. The MCV is normal because the duration of anemia is insufficient to have allowed microcytosis to develop. As discussed above, the deficient iron supply can be identified by a transferrin saturation of less than 16 percent.

Other causes of normocytic anemia can be differentiated by a determination of the reticulocyte index.[17] When the reticulocyte index is less than 3 in the presence of anemia, there is a defect in red cell production (Figure 5). Production defects are due to either faulty red cell formation with consequent ineffective erythropoiesis, which is shown by an increased erythroid to myeloid ratio in the bone marrow, or reduced red cell production in which there is a basal or decreased erythroid to myeloid ratio. The latter is designated as hypoproliferative anemia.

The hypoproliferative anemias result from either a failure in erythropoietin production (as in chronic renal disease) or stem cell dysfunction (marrow hypoplasia). Lack of the usual erythropoietin response occurs with endocrine (thyroid, pituitary, adrenal, or gonadal) insufficiency or protein deprivation. The clinical presentation of such primary conditions is often readily identifiable.

A mild normocytic anemia with a hemoglobin level stabilizing between 10 to 12 g/dL suggests an endocrine or protein insufficiency. Identification of the specific deficiency and treatment with replacement therapy usually corrects the anemia. In the therapy of hypothyroidism, an initial worsening of the anemia is due to rapid reexpansion of the plasma volume, which has been decreased. In the hypothyroid woman, metromenorrhagia can result in a concurrent iron deficiency.

A more severe anemia is seen with chronic renal disease. A blood urea nitrogen above 50 mg/dL or a serum creatinine over 3 mg/dL will identify the renal etiology. When there is blood loss associated with repeated hemodialysis or resulting from platelet dysfunction, there may be a coexisting iron deficiency anemia. Management of the anemia of end-stage renal disease likely will be simplified now that this anemia has been shown to be corrected by the use of recombinant human erythropoietin.[30,31]

A normal MCV is also seen in the rare anemias of marrow hypoplasia. In these anemias a stem cell abnormality affects proliferation of stem cells or suppression of stem cell function because of idiosyncratic reaction to chemical, pharmacologic, or infectious toxins; immune disease; or marrow infiltration and displacement (neoplasm, lymphoma, leukemia). A secondary aplastic anemia can be distinguished from pure red cell aplasia by the concurrent lack of other circulating cell elements. A bone marrow examination often clarifies the diagnosis. A defect of one cell line implies damage at the level of the committed stem cell and carries a better prognosis than a marrow specimen devoid of virtually all elements. The clinical evaluation includes a search for the possible cause, particularly when exposure to toxins or chemotherapeutic drugs is in question. Therapy is symptomatic with supportive red cell platelet transfusions, when necessary, to maintain a hemoglobin level of about 7 to 8 g/dL. Therapeutic considerations for the more severely affected patient include antithymocyte globulin or marrow transplantation when a compatible donor is available.

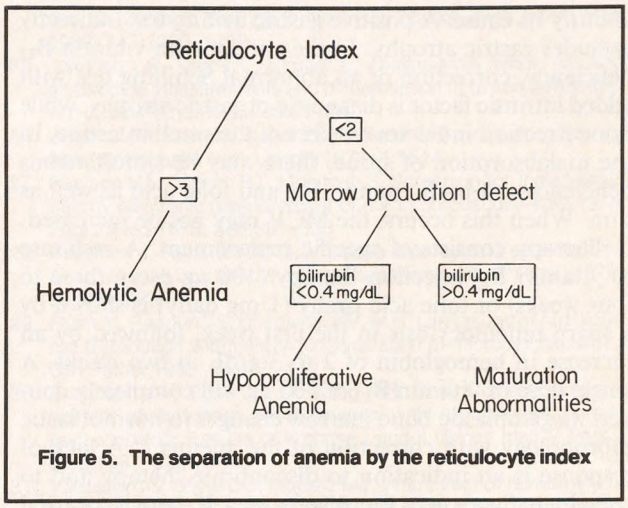

Figure 5. The separation of anemia by the reticulocyte index

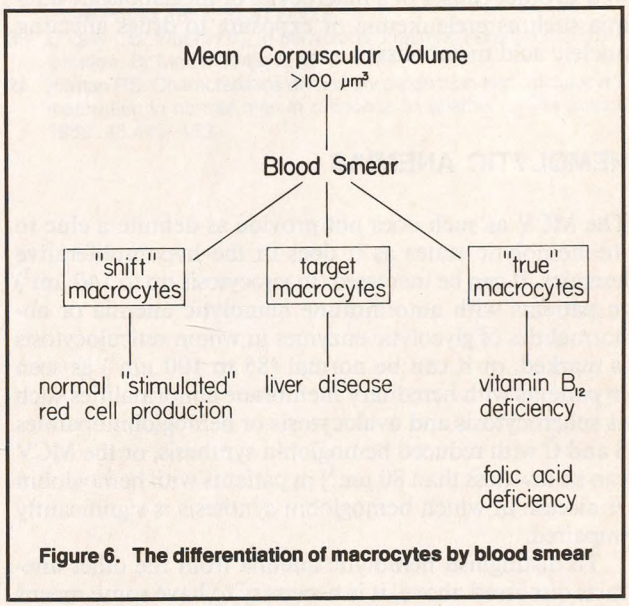

Figure 6. The differentiation of macrocytes by blood smear

THE HIGH MEAN CORPUSCULAR VOLUME

An elevated MCV (100 to 140 μm^3) is most often found when the erythroid marrow is stimulated in the presence of adequate iron, but increased corpuscular volumes are also characteristic of the anemias in which there is a nuclear maturation defect of the red cell (megaloblastosis). The blood smear differentiates between these two conditions (Figure 6). Polychromatic macrocytes ("shift" reticulocytes) indicate a response of marrow stimulated by erythropoietin,[32] while in the presence of true macrocytes and hypersegmentation of polymorphonuclear leukocytes indicates a nuclear maturation defect. A third form of macrocytosis is associated with liver disease, distinguished by targeting on blood smear. Other complicating features, however, are often present with liver disease including folate deficiency, inflammation, and hemolysis.

Of these macrocytic anemias, it is important to recognize the megaloblastic anemias, as they are readily treatable. Vitamin B_{12} deficiency is caused by disease of the stomach (where intrinsic factor is made) or of the ileum (where vitamin B_{12} is absorbed). Folate deficiency results from a dietary lack, from drugs that affect folate absorption or mobilization, or from increased physiologic requirements that occur during pregnancy, infection, neoplasmas, or prolonged increased erythropoiesis (chronic hemolytic states). The folate deficiency of alcoholism results from a dietary insufficiency as well as an alcohol block to the recycling of folate from liver stores to the tissues. A diagnosis of the deficiencies can be made by assays of plasma activity of the two factors. When a vitamin B_{12} deficiency is demonstrated, it is important to

identify its cause. A positive gastric acidity test indirectly excludes gastric atrophy. In the patient with vitamin B_{12} deficiency, correction of an abnormal Schilling test with added intrinsic factor is diagnostic of gastric atrophy, while noncorrection indicates a defect of the small intestine. In the malabsorption of sprue, there may be simultaneous deficiencies of both vitamin B_{12} and folic acid as well as iron. When this occurs, the MCV may not be increased.

Therapy consists of specific replacement. A response to vitamin B_{12} injection therapy (100 μg every three to four weeks) or folic acid orally (1 mg daily) is shown by a sharp reticulocytosis in the first week, followed by an increase in hemoglobin of 2 to 3 g/dL in two weeks. A single dose of vitamin B_{12} of 100 μg will completely convert megaloblastic bone marrow changes to normoblastic morphology with correction of the anemia.[33] A lack of response is an indication to discontinue therapy and to reevaluate the patient for other causes. If there is a partial response, one must consider coexisting iron deficiency as well as other causes of a macrocytic or megaloblastic anemia such as preleukemia or exposure to drugs affecting nucleic acid metabolism.

HEMOLYTIC ANEMIAS

The MCV as such does not provide as definite a clue to the hemolytic states as it does in the hypoproliferative anemias. It can be increased (macrocytosis up to 140 μm^3) in patients with autoimmune hemolytic anemia or abnormalities of glycolytic enzymes in whom reticulocytosis is marked, or it can be normal (85 to 100 μm^3) as seen in patients with hereditary membrane abnormalities such as spherocytosis and ovalocytosis or hemoglobinopathies S and C with reduced hemoglobin synthesis, or the MCV can be low (less than 80 μm^3) in patients with hemoglobin H disease in which hemoglobin synthesis is significantly impaired.

To distinguish hemolytic anemia from the other anemias discussed above, it is necessary to have some means of detecting increased red cell destruction. Such detection is provided by the reticulocyte count (Figure 5). Because red cell production and destruction are usually in equilibrium, the reticulocyte count, as a measure of red cell production, can conveniently be used to indicate red cell destruction as well.

The reticulocyte percentage reported in most blood counts relates the number of reticulocytes to the number of red cells in circulation. To translate the reticulocyte count to red cell production for comparison to the basal state, a correction for the degree of anemia is first made. In the anemic patient, a second correction is needed for the early release of marrow reticulocytes into the circulation.[17,34] A reticulocyte index greater than 3 indicates hemolysis.

As with other anemias, after the initial identification of a hemolytic state, the patient's history usually provides significant clues. Genetic disorders, infections, drugs and toxic exposures, and autoimmune disease are but some of the many causes.

To maintain a perspective, however, it must be realized that hemolytic anemia is much less common than iron deficiency anemia; therefore, the initial approach to the differential diagnosis of the unexplained anemia rests heavily on a few select laboratory tests. One is the MCV for the identification of microcytic anemia. With either microcytic or normocytic anemias, the further identification of iron-deficient erythropoiesis is made by a determination of the transferrin saturation. Only after iron deficiency has been excluded is it necessary to probe further, in which case marrow function and hemolysis can be evaluated by the reticulocyte index.

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