Julie L. Meredith, MD
Nancy S. Rosenthal, MD

Differential Diagnosis of Microcytic Anemias

ABSTRACT *Anemias are classified on the basis of size of the RBCs. In this scheme, the microcytic anemias are defined as those in which the mean cell volume is <80 fL. This review describes the common types—iron deficiency anemia, thalassemia, anemia of chronic inflammation, and sideroblastic anemia—as well as the clinical and laboratory findings in each. Diagnosis depends on the CBC, cell morphology (as observed on the peripheral blood smear), and, in rare cases, the bone marrow findings. Levels of iron, total iron binding capacity, free erythrocyte protoporphyrin, and ferritin in serum, as well as electrophoretic separation of hemoglobins may also be included.*

This is the second article in a three-part continuing education series on anemias. On completion of this article, the reader will be able to identify the common types of microcytic anemias—iron deficiency anemia, thalassemia, anemia of chronic inflammation, and sideroblastic anemia—by reviewing appropriate laboratory test results.

From the Department of Pathology, University of Iowa, Iowa City, Iowa.

Reprint requests to Dr Rosenthal, Department of Pathology, University of Iowa Hospitals and Clinics, 200 Hawkins Dr, 6233 RCP, Iowa City, IA 52242-1009; or e-mail: nancy-rosenthal@uiowa.edu

The microcytic anemias are associated with a mean corpuscular volume (MCV) below the lower limit of normal, which is 80 fL in adults and 70 fL in young children (ie, 1–12 years). Examination of the peripheral blood smear generally reveals the presence of microcytes and hypochromia. A decrease in the mean corpuscular hemoglobin concentration (MCHC) correlates with the degree of hypochromia.

The causes of microcytic anemias are diverse and include iron deficiency, thalassemia, chronic inflammation, and sideroblastic anemia (shown in decreasing order of prevalence). Microcytosis occurs as a result of an important underlying feature: quantitatively deficient hemoglobin synthesis that results from defects in the production of either heme or the globin chains that comprise the hemoglobin molecule. We will discuss the characteristic CBC and peripheral blood smear findings, diagnostic laboratory procedures, and the pathogenesis for each disorder.

Iron Deficiency Anemia

Iron deficiency anemia (IDA) is the most common cause of a microcytic anemia. Iron deficiency is also the most common nutritional deficiency in the world, although its prevalence is decreasing in developed countries. In the United States, at-risk populations include children, adolescents, women with pregnancy or menstrual blood loss,[1] and individuals with blood loss due to gastrointestinal malignancy and peptic ulcer disease. The hemoglobin level usually ranges from 8 g/dL to 12 g/dL (80–120 g/L), and the MCV is reduced.

In the early stages of iron depletion disease, iron stores are low, although hemoglobin synthesis is not disrupted. With the onset of deficiency, the hemoglobin level falls, and microcytosis and hypochromia are evident on the peripheral blood smear (Fig 1).

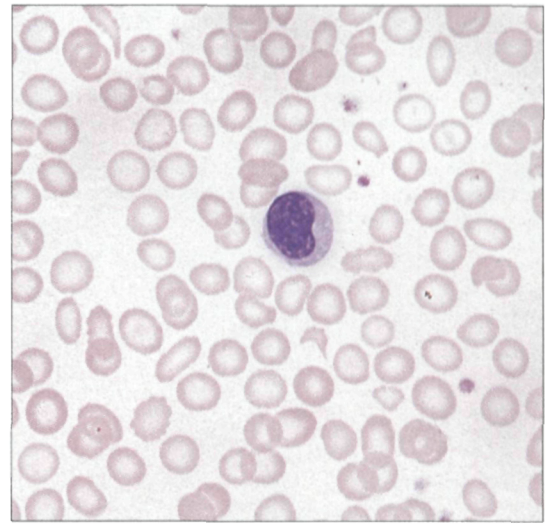


Fig 1. Peripheral blood smear from a patient with iron deficiency anemia showing microcytic, hypochromic RBCs (Wright stain, original magnification × 1,000).

Table 1. Laboratory Findings in Various Microcytic Anemias*

Test	Microcytic Anemia Due to			
	Iron Deficiency	Thalassemia	Chronic Inflammation	Sideroblastic Anemia
FEP level	High	Normal	Normal	Normal
Ferritin level	Low	Normal to increased	High	Normal to increased
MCV	Low	Low	Low or normal	Low, normal, or high
Percentage saturation	Low	Increased	Normal	Normal
RDW	High	Normal to high	Normal	High
Serum iron level	Low	Normal	Low	Normal
Serum TfR level	High	Normal	Normal	Normal
TIBC level	High	Normal	Low	Normal

FEP represents free erythrocyte protoporphyrin; MCV, mean corpuscular volume; RDW, red cell distribution width; TfR, serum transferrin receptor; TIBC, total iron binding capacity.
*The microcytic anemias are presented in decreasing order of prevalence.

Poikilocytosis often occurs, and elliptocytes, target cells, and bizarre RBCs are present. The red cell distribution width (RDW) is elevated in the later stages of iron deficiency. Thrombocytosis is also commonly seen, but the WBCs are unaffected. The CBC and cell morphology (as shown on peripheral blood smear), particularly in severe cases of iron deficiency, suggest the presence of IDA; a low iron level, an elevated total iron binding capacity (TIBC), and a reduced percentage saturation of iron must be present to confirm the diagnosis (Table 1). The ferritin level, an indicator of total body iron stores, is usually low. As an acute-phase reactant, ferritin may increase in concentration with infections and chronic inflammation, and in these settings may be falsely elevated as an indicator of iron stores. The free erythrocyte protoporphyrin (FEP) level is increased before the serum iron and TIBC results become abnormal and is therefore an early indicator of the disease. If the iron study results are inconclusive, a bone marrow examination (the gold standard for diagnosis of IDA) may be performed, although this is rare.

Recently, serum transferrin receptor (TfR) concentration was evaluated for diagnostic efficacy in iron deficiency.[2] The TfR is a transmembrane protein present on the cell surface of virtually all cells, and is required for iron binding and entry into the cell. The number of TfR molecules on the cell surface reflects the iron requirement, and iron deprivation induces the cell to synthesize more receptors. A form of the receptor is detectable in serum, and when its level is elevated, has been shown to be a reliable and early indicator of depleted iron stores in IDA. Unlike ferritin, TfR is not an acute phase reactant and is therefore not elevated in the anemia of chronic inflammation.

The goal of IDA treatment is to supply sufficient iron to remove the hemoglobin deficit and replenish iron stores. Although oral administration of iron is the treatment of choice, parenteral therapy is sometimes required. Only rarely are RBC transfusions necessary to prevent cardiac or cerebral ischemia, such as in severe anemia or to support patients whose rate of chronic iron loss exceeds the rate of replacement possible with oral or parenteral therapy.

Thalassemia

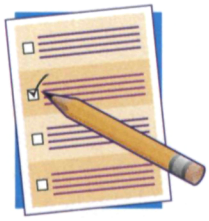
Common in Asian, Mediterranean, and African populations, the thalassemia syndromes are hereditary disorders of globin synthesis. Classification of the two main subtypes, alpha (α) and beta (β), is based on which of the globin chains is affected.

Thalassemia trait is often confused with IDA on the basis of the CBC and peripheral blood smear findings. To distinguish thalassemia from other causes of microcytosis, several indices based on parameters derived solely from the CBC have

Glossary

Cell hemoglobin distribution width—Red cell index that quantifies cell hemoglobin concentration as related to cell volume; decreased in patients with thalassemia trait.
Cytokine—Protein released by specific cells, which on contact with specific antigens, acts as an intercellular mediator, as in the generation of an immune response.
Serum transferrin receptor (TfR)—Transmembrane protein present on all cells that facilitates the entry of iron into the cell; increased in patients with iron deficiency anemia.

been applied. A normal RDW is thought to be more consistent with the presence of thalassemia.[3] Other indices are based on the discrepancies among the MCV, RBC count, and the hemoglobin levels.[4,5] The finding of an MCV ≤72 fL was recently shown to be more effective than these indices in selecting patients with a high probability of thalassemia trait.[6] Another index of RBC heterogeneity, the cell hemoglobin distribution width, may differentiate thalassemia trait from other causes of microcytosis, and is available on some hematology instruments. A cell hemoglobin distribution width (defined as the standard deviation of the product of the RBC volume and hemoglobin concentration) of less than 3.05 suggests the presence of thalassemia trait.[7]



Test Your Knowledge
Look for the CE Update exam on Anemias (906) in the September issue of *Laboratory Medicine*. Participants will earn 3 CMLE credit hours.

α-Thalassemia

α-Thalassemia results from deletion of one or more of the four α-chain genes on chromosome 16. This leads to abnormal α-chain synthesis, a relative excess of β chains, and a reduction in the amount of hemoglobin in the RBC.

The severity of the disease is correlated with the number of genes deleted (Table 2). A single deletion, the silent carrier state, causes no clinical disease. Deletion of two genes causes a mild microcytic anemia, α-thalassemia minor (or trait). Bart's hemoglobin, consisting of tetramers of γ chains derived from fetal hemoglobin, is present at birth but disappears soon after. In older children and adults, the hemoglobin electrophoresis pattern is normal, and diagnosis is often made on the basis of exclusion. Although rarely needed, molecular diagnostic techniques can assist in diagnosis.

A condition associated with deletion of three α genes, hemoglobin H (HbH) disease, causes a mild to moderate hemolytic anemia. Although HbH disease may be severe, it generally does not require blood transfusions. The peripheral blood smear shows microcytic, hypochromic RBCs with polychromasia, as well as rare teardrop cells and fragmented cells. The reticulocyte count is elevated. Hemoglobin electrophoresis reveals a fast-moving hemoglobin, HbH, composed of β tetramers that form as a result of the lack of α chains. HbH levels may be decreased during the concomitant iron deficiency. The presence of HbH may also be established by incubating blood with brilliant cresyl blue, which results in diffuse stippling of the RBCs.

The deletion of all four α-chain genes results in the production of only Bart's hemoglobin, which binds oxygen so tightly that it is unable to release it to the tissues, resulting in fetal death due to hydrops fetalis.

Table 2. Subtypes and Laboratory Features of α-Thalassemia

Subtype	No. of Gene Deletions	Clinical Findings	Hemoglobin Electrophoresis Result
Silent carrier	1	Normal	1%–2% Bart's*
α-Thalassemia trait	2	Microcytosis; + or − mild anemia	5% Bart's*
HgH disease	3	Microcytosis; mild to moderate anemia	25% Bart's; 3%–30% HgH
Hydrops fetalis	4	Lethal in utero	100% Bart's

HgH represents hemoglobin H.
*Amount of Bart's hemoglobin present at birth.

β-Thalassemia

Like the α thalassemias, β-thalassemia also may arise from gene deletions. More often, however, the condition develops as a result of point mutations of the β-globin gene located on chromosome 11. As with α-thalassemia, the severity of the disease depends on the number of abnormal genes inherited. With only one abnormal gene, β-thalassemia minor (or trait), a benign microcytic anemia similar to IDA, is seen. When two β genes are markedly abnormal, the more severe β-thalassemia major occurs. A third clinical syndrome, thalassemia intermedia, results from (1) a homozygous disorder with less impaired β-chain production or (2) severe variants of a heterozygous disorder. Transfusions may be required for this condition. Although no treatment is necessary for β-thalassemia trait, β-thalassemia major requires transfusion support.

The peripheral blood smear in patients with β-thalassemia major shows severely hypochromic, microcytic RBCs, as well as marked anisopoikilocytosis with teardrop and target cells, nucleated RBCs, and prominent basophilic stippling (Fig 2). β-Thalassemia trait is associated with mild anemia, and often the MCV is lower than expected for the degree of anemia. Although the RBC count is often elevated, the RDW may be normal.

The diagnosis of β-thalassemia trait is confirmed by hemoglobin electrophoresis, which shows mildly elevated HbA_2 (3.5%–8.0%) and HbF (1%–2%) levels. Patients with β-thalassemia major produce no HbA and markedly increased amounts of HbF

(>94%) and HbA_2 (1%–6%). Quantitation of HbA_2 by anion exchange chromatography is necessary for accurate diagnosis. Concurrent IDA can cause a falsely reduced HbA_2 level. High-performance liquid chromatography has been applied to distinguish and quantitate hemoglobins, and several molecular methods can be used to detect the genetic defects in β-thalassemia.

Anemia of Chronic Inflammation

Anemia of chronic inflammation (also known as anemia of chronic disease) is often seen in the setting of malignancy (eg, carcinoma, lymphoma), chronic infection (eg, tuberculosis, fungal infection), inflammatory states (eg, rheumatoid arthritis, systemic lupus erythematosis), and other chronic diseases (eg, chronic renal failure). Although the peripheral blood smear often shows normocytic, normochromic RBCs without significant anisopoikilocytosis, significant numbers of microcytic, hypochromic RBCs are present in 25% of cases. The hemoglobin level usually ranges from 8 g/dL to 12 g/dL. The MCV, when abnormal, is usually mildly decreased, and RDW is normal. The lowest reported MCV in a patient with anemia of chronic inflammation is 61 fL.[8] Reticulocytes are not increased appropriately for the degree of anemia. Target cells, dimorphic RBCs, and RBCs with basophilic stippling are rarely seen. Examination of the bone marrow generally shows normal numbers and morphology of the erythroid elements. Storage iron is increased with decreased iron in sideroblasts. Serum iron level is generally decreased, TIBC level is low to normal, and ferritin concentration is normal to increased.

The cause of the anemia of chronic inflammation is thought to be multifactorial and due to failure of erythropoiesis, lack of iron for hemoglobin synthesis, and decreased RBC survival time. These defects can be attributed to sustained macrophage secretion of cytokines, whose broad effects result in iron being unavailable for hemoglobin synthesis.[9,10]

Because the anemia of chronic inflammation is usually mild, transfusions are rarely required. Treatment is usually limited to treating the underlying disorder, although some patients respond to recombinant erythropoietin therapy.[11]

Sideroblastic Anemia

Sideroblastic anemias are characterized by abnormal iron metabolism within the RBC and may be present as either acquired or hereditary disorders.[12] Iron accumulates within the mitochondria of the RBC and is unavailable for heme synthesis. Enzyme deficiencies and DNA abnormalities within the RBC lead to the iron accumulation. Acquired forms may be due to systemic, metabolic, or toxic disorders (Table 3). Excess alcohol intake is a relatively common underlying cause, and ringed sideroblasts may be seen as part of a myelodysplastic syndrome.

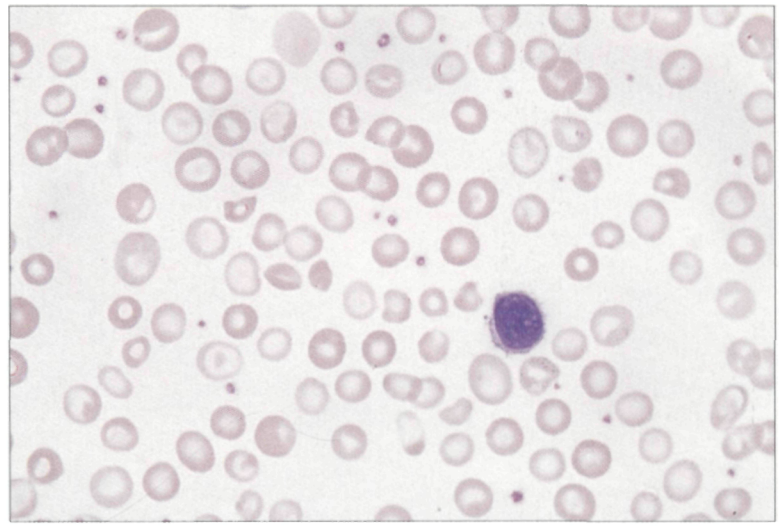


Fig 2. Peripheral blood smear from a patient with β-thalassemia major showing marked microcytosis and hypochromia; note presence of teardrop and target cells, and basophilic stippling (Wright stain, original magnification × 1,000).

The CBC shows a markedly decreased MCV in the hereditary forms; however, the MCV may be normal or increased in the acquired forms. The RDW is elevated, and RBC count is low in both forms. In addition, serum iron, ferritin, and FEP concentrations may be normal, although ferritin and FEP levels may be increased. The characteristic finding of sideroblastic anemia on the peripheral blood smear is a dimorphic RBC population consisting of a microcytic, hypochromic population of cells mixed with a relatively normal population

Table 3. Causes of Sideroblastic Anemia

Acquired
Alcohol
Drugs (eg, antituberculous agents, chloramphenicol, chemotherapeutic agents)
Idiopathic (eg, refractory anemia with ringed sideroblasts [RARS], a myelodysplastic syndrome)
Lead poisoning
Hereditary
Autosomal dominant
Autosomal recessive
X-linked

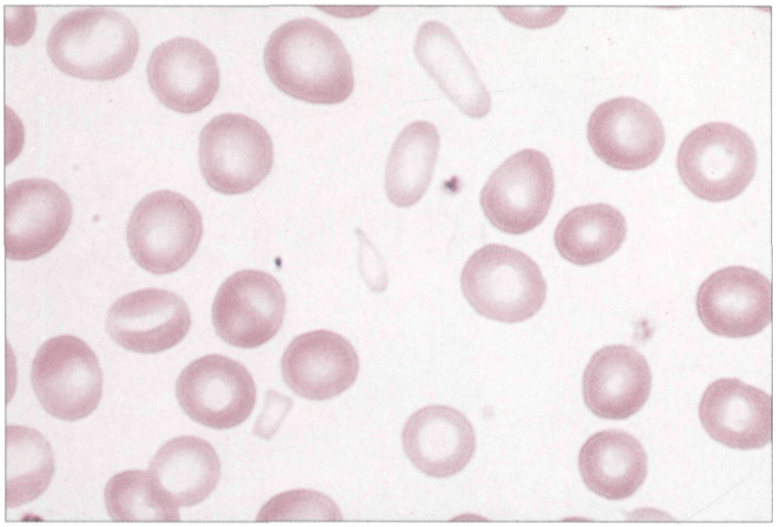


Fig 3. A peripheral blood smear from a patient with a sideroblastic anemia showing a dimorphic RBC population (Wright stain, original magnification × 1,000).

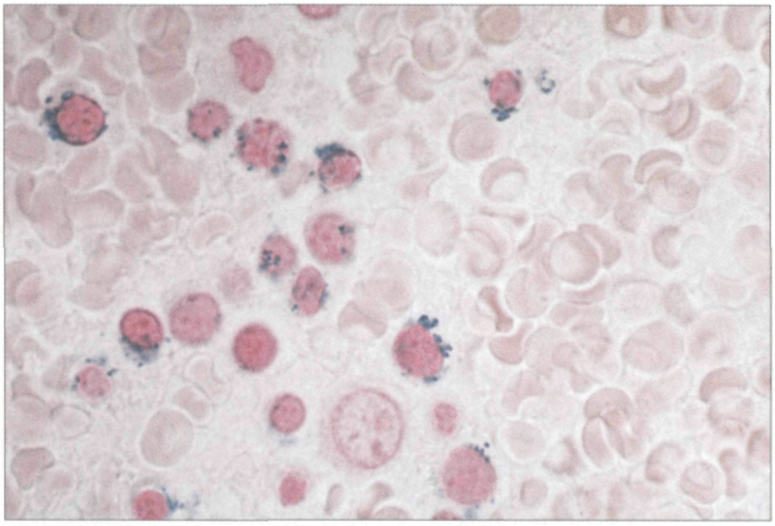


Fig 4. Bone marrow iron stain from a patient with sideroblastic anemia showing numerous ringed sideroblasts (Prussian blue, original magnification × 1,000).

(Fig 3). Target cells and RBCs with basophilic stippling are present in the hereditary forms. In the acquired forms, anisopoikilocytosis is more mild, and target cells may or may not be present. A bone marrow examination is required for the diagnosis. Erythroid hyperplasia is often seen, and the iron stain shows the presence of ringed sideroblasts (Fig 4), which have numerous iron granules surrounding the nucleus.

One form of acquired sideroblastic anemia is caused by lead poisoning. Lead interferes with heme synthesis by inhibiting enzymes involved in production of the heme molecule.[13] Iron deficiency may develop as lead impedes both iron absorption from the gastrointestinal tract and iron metabolism within the RBC.[14] The peripheral blood smear reveals microcytic, hypochromic RBCs, and prominent or coarse basophilic stippling of RBCs may be seen. Bone marrow examination may reveal erythroid hyperplasia and the presence of ringed sideroblasts. The diagnosis is confirmed by the presence of an elevated concentration of lead in serum.

Conclusion

Microcytic anemias are common abnormalities encountered routinely in the hematology laboratory. With careful evaluation of the CBC, peripheral blood smear, hemoglobin electrophoretic pattern, and several useful serum tests, determination of the underlying cause is usually straightforward. Bone marrow examination has limited usefulness except in the diagnosis of sideroblastic anemias.

References

1. Looker AC, Dallman PR, Carroll MD, et al. Prevalence of iron deficiency in the United States. *JAMA*.1997;277:973–976.
2. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its relation to serum ferritin in the diagnosis of iron deficiency. *Blood*. 1997;89:1052–1057.
3. Bessman JD, Gilmer PR, Gardner FH. Improved classification of anemias by MCV and RDW. *Am J Clin Pathol*. 1983;80:322–326.
4. England JM, Fraser PM. Differentiation of iron deficiency from thalassemia trait by routine blood count. *Lancet*. 1973;1:449–452.
5. Mentzler WC. Differentiation of iron deficiency from thalassemia trait. *Lancet*. 1973;1:882.
6. Lafferty JD, Crowther MA, Ali MA, et al. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and nonthalassemic microcytosis. *Am J Clin Pathol*. 1996;106:201–205.
7. Te-chih L, Seong PS, Lin TK. The erythrocyte cell hemoglobin distribution width segregates thalassemia traits from other nonthalassemic conditions with microcytosis. *Am J Clin Pathol*. 1997;107:601–607.
8. LoPresti PJ, Olson L, Auerbach M. Case report: severe microcytosis associated with the anemia of chronic disease. *Md Med J*. 1996;45:762–764.
9. Means RT, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood*. 1992;80:1639–1647.
10. Means RT Jr. Pathogenesis of the anemia of chronic disease: a cytokine-mediated anemia. *Stem Cells*. 1995;13:32–37.
11. Nordstrom D, Lindroth Y, Marsal L, et al. Availability of iron and degree of inflammation modifies the response to recombinant human erythropoietin when treating anemia of chronic disease patients with rheumatoid arthritis. *Rheumatol Int*. 1997;17:67–73.
12. Koc S, Haris JW. Sideroblastic anemias: variations on imprecision in diagnostic criteria, proposal for an extended classification of sideroblastic anemias. *Am J Hematol*. 1998;57:1–6.
13. Gibson SM, Goldberg A. Defects in haeme synthesis in mammalian tissues in experimental lead poisoning. *Clin Sci*. 1970;38:63.
14. Clark M, Trotsky MS, Pincus D. Interaction of iron deficiency and lead and the hematologic findings in children with severe lead poisoning. *Pediatrics*. 1988;81:247.

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