Anemia in Adults: A Contemporary Approach to Diagnosis

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There are numerous ways of classifying the causes of anemia, and no one way is necessarily superior to another. It is equally important to appreciate the differences in the approaches to diagnosis between children and adults, men and women, and persons of different ethnic backgrounds. Regardless of the specific algorithm followed in evaluating anemia, it is essential that easily remediable causes such as nutritional deficiencies, hemolysis, and anemia of renal insufficiency are identified early and treated appropriately. In general, the differential diagnosis of anemia can be substantially narrowed by subcategorization into “microcytic,” “normocytic,” and “macrocytic” subtypes on the basis of mean corpuscular volume. However, such classification is a starting point and not infallible. Each category then can be deciphered using a stepwise approach that utilizes readily accessible laboratory tests.


A neemia is defined as a decrease in hemoglobin (or hematocrit) level from an individual’s baseline value. Because individual baseline hemoglobin levels often are not readily accessible, physicians use sex-specific and race-specific reference ranges to make a working diagnosis of anemia. In general, “normal” hemoglobin levels are 1 to 2 g/dL lower in women and African American men than in white men.

In routine clinical practice, laboratory, rather than clinical, parameters are most useful in formulating a practical diagnostic approach.1 Accordingly, the mean red blood cell volume (mean corpuscular volume [MCV]) is used first to classify the anemic process as microcytic, normocytic, or macrocytic (Table 1).

MICROCYTIC ANEMIA
Step 1. Rule Out Iron Deficiency Anemia

Iron deficiency is the most common cause of microcytic anemia. The definitive test for iron deficiency anemia (IDA) is measurement of serum ferritin. A low serum ferritin level is diagnostic of an iron-depleted state.2 Contrary to current dogma that says the serum ferritin level may be spuriously elevated in the presence of acute phase reaction, a diagnosis of IDA is extremely unlikely in the presence of either normal or elevated serum ferritin levels. However, such classification is a starting point and not infallible. Each category then can be deciphered using a stepwise approach that utilizes readily accessible laboratory tests.

ACD = anemia of chronic disease; CD = Castleman disease; HL = Hodgkin lymphoma; IDA = iron deficiency anemia; MCV = mean corpuscular volume; MMM = myelofibrosis with myeloid metaplasia; RCC = renal cell carcinoma; RDW = red blood cell distribution width

The other serum iron studies (serum iron, total iron-binding capacity, transferrin saturation) do not accurately distinguish IDA from anemia of chronic disease (ACD) and therefore have limited value in the evaluation of microcytic anemia. Similarly, assessment of iron stores through bone marrow biopsy is neither necessary nor accurate for evaluation of IDA.3 Instead, a finite treatment trial with iron supplementation is both a cost-effective and definitive way of addressing the issue in equivocal cases.

Although not definitive, there are other clues for diagnosing IDA. For example, microcytic anemia associated with increased red blood cell distribution width (RDW) favors a diagnosis of IDA over that of ACD. In contrast, microcytic anemia associated with increased red blood cell count is characteristic of the thalassemia trait. However, it should be remembered that microcytosis without anemia could occur in the thalassemia trait and in polycythemia associated with iron deficiency.4 The peripheral blood smear in IDA usually shows anisocytosis and poikilocytosis. In severe cases, cigar-shaped red blood cells and elliptocytes are characteristically present. In contrast, polychromasia (the Wright-Giemsa stain equivalent of reticulocytosis), basophilic stippling, and target cells are conspicuously absent in IDA but are characteristic features in thalassemia. Finally, IDA may be associated with reactive thrombocytosis.

Step 2. Evaluation of Microcytic Anemia With Normal Serum Ferritin

If the serum ferritin level is normal, the next step in evaluating microcytic anemia depends on whether the microcytosis is new or previously recognized. If the microcyt-
tosis is preexisting, it implies a congenital disorder, and a diagnosis of thalassemia should be considered. If the microcytosis is new, a nonthalassemic condition associated with acquired microcytosis is a possibility.

**Thalassemic Syndromes.**—Approximately 97% of normal hemoglobin in adults (hemoglobin A) consists of equal quantities of α-globin and β-globin chains (α₂β₂). Thalassemia is defined as a hemoglobinopathy associated with decreased production of either of the 2 normal globin chains (eg, α-thalassemia or β-thalassemia) or a structurally abnormal globin chain (eg, hemoglobin E). The resulting unbalanced globin chain production results in microcytosis and an alteration in the normal hemoglobin electrophoresis pattern. Therefore, hemoglobin electrophoresis is the initial test of choice in the investigation of thalassemia. However, it is important to note that hemoglobin electrophoresis does not always detect the presence of thalassemia. To appreciate the utility of both hemoglobin electrophoresis and genetic testing in thalassemia, each of the thalassemic syndromes (α-thalassemia, β-thalassemia, and structurally abnormal globin chain thalassemia) are considered separately.

**α-Thalassemia.**—The production of α-globin chains is controlled by 4 genes (2 per haploid chromosome). Mutation of all 4 genes is incompatible with life (hydrops fetalis). Mutation of only 1 of the 4 genes causes neither anemia nor microcytosis (silent carrier). Mutation of 2 of the 4 genes results in both microcytosis and mild anemia (α-thalassemia trait). Mutation of 3 of the 4 genes allows excess β-chains to form tetramers (hemoglobin H) and results in severe anemia in addition to microcytosis. Hemoglobin electrophoresis is normal in the α-thalassemia trait and abnormal in hemoglobin H disease. Genetic testing (polymerase chain reaction–based DNA tests and Southern blot analysis) can reveal the molecular defect in the α-thalassemia trait. However, a working diagnosis can be made and genetic counseling can be initiated on the basis of family history and ethnic origin and without resorting to DNA testing.

**β-Thalassemia.**—β-Globin chain production is controlled by 2 genes (1 per haploid chromosome). β-Thalassemia occurs as a trait (1 of 2 gene mutations) or symptomatic disease (mutations of both genes). In the β-thalassemia trait, the level of hemoglobin A₂ (α₂δ₂) may increase from the normal value of 2% to a value of 3% to 6%. However, if iron deficiency coexists, the expected increase in hemoglobin A₂ may not occur. Therefore, a normal hemoglobin A₂ level may not exclude the possibility of the β-thalassemia trait unless a simultaneously measured normal serum ferritin level is documented. In β-thalassemia disease, the hemoglobin electrophoresis reveals mostly hemoglobin F (α₂γ₂). A slight or moderate increase in hemoglobin F may also be seen in the β-thalassemia trait and in compound heterozygotes. Therefore, in general, hemoglobin electrophoresis is often adequate for evaluating β-thalassemia, and genetic testing may be unnecessary.

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**Table 1. An Operational Classification of Anemia**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Microcytic anemia (MCV, &lt;80 fL)</td>
<td>Iron deficiency anemia</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>Nonthalassemic conditions associated with microcytosis other than iron deficiency anemia</td>
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<tr>
<td>Anemia of chronic disease (eg, rheumatoid arthritis, Hodgkin lymphoma)</td>
<td>Sideroxoblast anemia (eg, hereditary, lead poisoning)</td>
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<tr>
<td>Normocytic anemias (MCV, 80-100 fL)</td>
<td>Nutritional anemias (eg, iron deficiency anemia)</td>
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<tr>
<td>Anemia of renal insufficiency</td>
<td>Hemolytic anemias</td>
</tr>
<tr>
<td>Red cell intrinsic causes</td>
<td>Membranopathies (eg, hereditary spherocytosis)</td>
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<td>Enzymopathies (eg, glucose-6-phosphate dehydrogenase deficiency)</td>
<td>Hemoglobinopathies (eg, sickle cell disease)</td>
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<tr>
<td>Hemolysis</td>
<td>Red cell extrinsic causes</td>
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<tr>
<td>Immune mediated</td>
<td>Autoimmune</td>
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<tr>
<td>Drug associated</td>
<td>Enzyme-mediated thrombocytopenia</td>
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<tr>
<td>Virus associated</td>
<td>Idiopathic</td>
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<td>Lymphoid disorder associated</td>
<td>Alloimmune</td>
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<tr>
<td>Idiopathic</td>
<td>Immediate transfusion reaction</td>
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<tr>
<td>Immune mediated</td>
<td>Delayed transfusion reaction</td>
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<td>Neutropenic anemia</td>
<td>Neonatal hemolytic anemia</td>
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<td>Microangiopathic (eg, thrombotic thrombocytopenic purpura/ hemolytic uremic syndrome)</td>
<td>Infection associated (eg, falciparum malaria)</td>
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<td>Chemical agent associated (eg, spider venoms)</td>
<td>Anemia of chronic disease (believed to be cytokine mediated)</td>
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<tr>
<td>Primary bone marrow disorder</td>
<td>Causes that are intrinsic to the hematopoietic stem cell</td>
</tr>
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<td>Aplastic anemia (idiopathic, paroxysmal nocturnal hemoglobinuria, Fanconi anemia)</td>
<td>Pure red cell aplasia (acquired, congenital [Diamond-Blackfan syndrome])</td>
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<td>Pure red cell aplasia (acquired, congenital [Diamond-Blackfan syndrome])</td>
<td>Ineffective erythropoiesis (myelodysplastic syndrome and other myeloid disorders)</td>
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<td>Ineffective erythropoiesis (myelodysplastic syndrome and other myeloid disorders)</td>
<td>Extrinsic causes</td>
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<tr>
<td>Drugs, toxins, radiation, virus (parovirus, etc)</td>
<td>Immune mediated (contributes to aplastic anemia and pure red cell aplasia)</td>
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<tr>
<td>Immune mediated (contributes to aplastic anemia and pure red cell aplasia)</td>
<td>Bone marrow infiltrating processes such as metastatic cancer and lymphoma</td>
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<td>Macrophage anemias (MCV, &gt;100 fL)</td>
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<td>Drug induced (hydroxyurea, methotrexate, etc)</td>
<td>Nutritional (vitamin B₉, folic acid deficiency)</td>
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<td>Nutritional (vitamin B₉, folic acid deficiency)</td>
<td>Non–drug induced, nonnutritional macrocytic anemia</td>
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<td>Marked macrocytosis (MCV, &gt;110 fL)</td>
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<td>Clonal hematologic disorder (myelodysplastic syndrome, aplastic anemia, large granular lymphocyte disorder)</td>
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<td>Clonal hematologic disorder (myelodysplastic syndrome, aplastic anemia, large granular lymphocyte disorder)</td>
<td>Mild macrocytosis (MCV, 100-110 fL)</td>
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<td>Mild macrocytosis (MCV, 100-110 fL)</td>
<td>Oval macrocytosis (clonal hematologic disorder)</td>
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<td>Oval macrocytosis (clonal hematologic disorder)</td>
<td>Round macrocytes (excess alcohol usage, liver disease)</td>
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<td>Round macrocytes (excess alcohol usage, liver disease)</td>
<td>Reticulocytes (hemolytic anemia)</td>
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<tr>
<td>Reticulocytes (hemolytic anemia)</td>
<td>Spurious (presence of cold agglutinins, hyperglycemia)</td>
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*MCV = mean corpuscular volume.*
Structurally Abnormal Globin Chain Thalassemia.— Some structural hemoglobinopathies can produce a thalassemic (microcytic) phenotype as a result of decreased globin synthesis. Examples include hemoglobin E (a structural hemoglobinopathy, prevalent in Southeast Asia, that results from an RNA splice site mutation associated with the production of an alternative messenger RNA that is not effectively translated), hemoglobin Lepore (resulting from a stop codon mutation and decreased transcription efficiency), and hemoglobin Constant Spring (resulting from a stop codon mutation and synthesis of a longer, unstable globin chain messenger RNA). These thalassemic syndromes usually are identified by routine hemoglobin electrophoresis, and genetic testing may not be required.

Nonthalassemic Conditions Associated With Microcytosis Other ThanIDA.—The differential diagnosis of nonthalassemic, non-IDA microcytic anemia includes ACD and hereditary or acquired sideroblastic anemia. The latter is a rare disorder that is characterized by increased RDW, dimorphic red blood cells, and bone marrow ring sideroblasts.

Anemia in ACD is usually normocytic. However, some systemic diseases (eg, rheumatoid arthritis, polymyalgia rheumatica, diabetes mellitus, connective tissue disease, chronic infection, Hodgkin lymphoma [HL], Castleman disease [CD], renal cell carcinoma [RCC], and myelofibrosis with myeloid metaplasia [MMM]) can be accompanied by microcytic anemia. The first 5 aforementioned diseases often are associated with mild microcytosis characterized by a normal RDW and an unremarkable peripheral blood smear. Diagnosis is made on clinical grounds, and bone marrow examination is unnecessary.

Microcytic anemia associated with HL, CD, or RCC often is accompanied by systemic manifestations including fever and other constitutional symptoms. Other symptoms include pruritus (HL), lymphadenopathy (HL, CD), monoclonal gammopathy (CD), hematuria (RCC), or splenomegaly (MMM, HL, CD). Peripheral blood smear shows leukoerythroblastosis (the presence of nucleated red blood cells and immature myeloid cells) in MMM. Obviously, bone marrow examination is indicated if these disorders are suspected.

### NORMOCYTIC ANEMIA

**Step 1. Rule Out Readily Treatable Causes**

The critical issue in evaluating any form of anemia is to recognize treatable causes early. In normocytic anemia, treatable causes include nutritional anemias, anemia of renal insufficiency, and hemolytic anemia.

**Nutritional Anemias.**—In patients with normocytic anemia, both iron and vitamin $B_{12}$/folate deficiencies are possible causes despite their usual association with microcytic and macrocytic anemias, respectively. Therefore, the initial investigation of normocytic anemia should include determination of both serum ferritin and serum vitamin $B_{12}$/folate levels (see “Microcytic Anemia” and “Macrocytic Anemia” for further information regarding the evaluation of nutritional anemias).

**Anemia of Renal Insufficiency.**—Anemia of renal insufficiency is associated with an unremarkable peripheral blood smear and an inappropriately normal serum erythropoietin level. Although anemia is severe and symptomatic only with advanced kidney disease (serum creatinine, >3 mg/dL), mild to moderate anemia may be seen in moderate renal insufficiency (serum creatinine, 1.5-3 mg/dL), especially in diabetic patients with nephrotic syndrome. If initial tests are unrevealing, the possibility of hemolysis should be considered.

**Hemolytic Anemia.**—In all types of hemolytic anemia, laboratory evidence of increased cell destruction (suggested by increased lactate dehydrogenase), increased hemoglobin catabolism (suggested by increased levels of indirect bilirubin), decreased levels of haptoglobin (a serum protein that clears free hemoglobin), and bone marrow regenerative effort (suggested by reticulocytosis) may be appreciated. Therefore, when a hemolytic process is suspected, initial tests should include measurement of lactate dehydrogenase, indirect bilirubin, haptoglobin, and reticulocyte count. None of these tests are specific or able to distinguish among the various causes of hemolytic anemia.

As with the anemia classification as a whole, the hemolytic anemias can be classified in many ways. One practical classification separates causes that are inherent to the red blood cell from causes that are extrinsic to the red blood cell (Table 1). However, in routine clinical practice, it may be preferable to first distinguish extravascular (occurring in the monocyte-macrophage system of the spleen and liver) from intravascular (occurring by lysis inside the blood vessels) hemolytic anemia using the urinary hemosiderin test (Table 2). In general, red cell–intrinsic and immune-mediated hemolytic anemia are
extravascular, whereas microangiopathic, infection-associated, and chemical-induced hemolytic anemias are extravascular (Table 1). Figures 1 and 2 provide practical information to investigate the specific cause of either intravascular (Figure 1) or extravascular (Figure 2) hemolysis. Further elaboration on hemolytic anemia, including immune hemolytic anemia, enzymopathies, and hemoglobinopathies, is beyond the scope of this review. However, it is important to note that the possibility of a drug-induced mechanism always should be considered in any hemolytic process.

Step 2. Normocytic Anemia Not Associated With Nutritional Deficiency, Renal Insufficiency, or Hemolysis

The primary consideration in normocytic anemia not associated with nutritional deficiency, renal insufficiency, or hemolysis is either ACD or a primary bone marrow disorder; differentiating between the two is not always easy. Obviously, the patient’s history is critical for differentiation and for excluding other causes of normocytic anemia, including drug effect, alcoholism, radiation therapy, chemical exposure, and recent trauma or surgery. The presence of comorbid conditions, an increased erythrocyte sedimentation rate, and an unremarkable peripheral blood smear study support the diagnosis of ACD.

**Anemia of Chronic Disease.**—Anemia of chronic disease is usually normocytic but can be microcytic. Current understanding suggests a cytokine-mediated process that inhibits red blood cell production or interferes with erythropoietin production and/or function. Anemia of chronic disease, frequently associated with diabetes mellitus, connective tissue disease, chronic infections, and malignancy, may be mistaken for IDA because low serum iron and decreased transferrin saturation are seen in both conditions. Therefore, serum ferritin is the single best noninvasive test to differentiate IDA from ACD.
Anemia Due to Primary Bone Marrow Disorder.—
The peripheral blood smear is most helpful in providing clues for the presence of a primary bone marrow disease. In myelodysplastic syndrome, for example, the RDW often is increased, and the smear may show the presence of oval macrocytes, hyposegmented neutrophils (pseudo-Pelger-Huët anomaly), or monocytosis. In bone marrow infiltrating processes such as MMM and bone marrow involvement with metastatic cancer, nucleated red blood cells and immature myeloid cells are noted. Red blood cell rouleaux formation may be seen in multiple myeloma. Severe anemia associated with an extremely low reticulocyte count suggests pure red cell aplasia or aplastic anemia. Finally, a primary bone marrow disease often is associated with both quantitative and qualitative abnormalities of white blood cells and platelets.

The physician deciding whether to obtain a bone marrow biopsy should consider the likelihood of discovering a primary bone marrow disease and the therapeutic and prognostic value of the information derived from the procedure. For example, performing a bone marrow biopsy in an elderly patient with mild anemia is unnecessary, even if the peripheral blood smear suggests a primary hematologic disease because the results may not affect overall management decisions. In contrast, a younger patient with a history of chemotherapy or an abnormal peripheral blood smear should undergo bone marrow biopsy before a diagnosis of ACD is established.

MACROCYTIC ANEMIA
Step 1. Rule Out the Presence of Drugs That Cause Macrocytosis
To evaluate macrocytic anemia, the first step is to exclude substance (alcohol) or drug (hydroxyurea, methotrexate, trimethoprim, zidovudine, 5-fluorouracil) use associated with macrocytosis. Among the offenders, hydroxyurea is the most notorious and induces the largest increases in MCV (oval macrocytosis >110 fL). A lesser degree of macrocytosis (100-110 fL) may result from use of zidovudine (oval macrocytosis), chemotherapy (oval macrocytosis), or alcohol (round macrocytosis).

Step 2. Rule Out Nutritional Causes of Macrocytic Anemia
In patients with macrocytosis, vitamin $B_{12}$, and/or folate deficiencies must be ruled out. In folate deficiency, serum folate levels are usually low. However, because recent dietary changes may affect the serum folate level, red blood
cell folate levels are sometimes used to document chronic folate deficiency (red blood cells acquire folate at birth, and the cellular concentration does not change during their life span). Because red blood cell folate assays are not precise, the serum homocysteine level may be used instead to evaluate folate deficiency. (The serum homocysteine level is increased during folate deficiency due to impaired folate-dependent conversion of homocysteine to methionine.) A normal homocysteine level makes the diagnosis of folate deficiency extremely unlikely.

In vitamin B₁₂ deficiency, serum vitamin B₁₂ levels are usually low. However, vitamin B₁₂ levels may be spuriously low during pregnancy, in elderly patients, and in patients with low white blood cell counts. In these instances and in patients with borderline-low vitamin B₁₂ levels, a more sensitive and highly specific test is the measurement of serum methylmalonic acid level (vitamin B₁₂ cofactor activity is required to convert methylmalonyl coenzyme A to succinyl coenzyme A). A normal level makes the diagnosis of vitamin B₁₂ deficiency extremely unlikely. However, an increased serum methylmalonic acid level is not specific to vitamin B₁₂ deficiency and can be seen in renal insufficiency or as a result of an inborn metabolic disorder.

Once vitamin B₁₂ deficiency is confirmed, the next step is to determine the cause. The initial test is to screen for the presence of intrinsic factor antibodies; if they are present, a working diagnosis of pernicious anemia is made, and additional testing may be unnecessary. Otherwise, the Schilling test is performed to help differentiate pernicious anemia from primary intestinal malabsorptive disorders (tropical sprue and celiac sprue, inflammatory bowel disease, amyloidosis, and intestinal lymphoma) (Table 3).

Step 3. Evaluating Non–Drug Induced, Nonnutritional Macrocytic Anemia

If neither vitamin deficiency nor drug (including ethyl alcohol) exposure can be implicated in a macrocytic process, it might be helpful to subclassify the process into mild (MCV, 100-110 fL) or marked (MCV, >110 fL) macrocytosis. Marked macrocytosis that is not secondary to either nutritional deficiency or drug effect almost always is associated with a primary bone marrow disease (eg, myelodysplastic syndrome, aplastic anemia, pure red cell aplasia, or large granular lymphocyte disorder), and a bone marrow biopsy is indicated if the specific hematologic diagnosis affects management decisions. However, with mild macrocytosis it is important to obtain detailed information from the peripheral blood smear before proceeding to bone marrow biopsy. For example, substantial polychromasia (indicative of reticulocytosis) suggests hemolysis as the cause of macrocytosis, whereas round morphology of red blood cells, as opposed to oval macrocytosis, suggests liver disease (target cells are also evident) or hypothyroidism.

CONCLUSIONS

The guidelines outlined in this review may not be used effectively when the patient has either a recent history of red blood cell transfusion or multiple medical problems. The latter is especially true regarding hospitalized patients. These confounding factors must be considered during the process of anemia investigation. Reference to previous laboratory records may provide the most cost-effective clarification.

REFERENCEs


**Questions About Anemia in Adults**

1. Which one of the following is inconsistent with a diagnosis of ACD?
   a. Decreased serum iron level
   b. Decreased serum transferrin saturation level
   c. Decreased serum ferritin level
   d. Increased serum erythrocyte sedimentation rate
   e. Peripheral blood smear showing microcytes

2. Which one of the following usually is not seen on the peripheral blood smear of patients with IDA?
   a. Basophilic stippling
   b. Anisocytosis and poikilocytosis
   c. Cigar-shaped red blood cells
   d. Paucity of reticulocytes
   e. Microcytosis

3. Which one of the following does not present with microcytic anemia?
   a. Autoimmune hemolytic anemia
   b. ACD
   c. HL
   d. MMM
   e. Sideroblastic anemia

4. Which one of the following is not associated with macrocytic anemia?
   a. Hemolytic anemia
   b. Myelodysplastic syndrome
   c. Excess alcohol use without obvious liver disease
   d. ACD
   e. Treatment with hydroxyurea

5. Which one of the following regarding the Schilling test is incorrect?
   a. The test can distinguish pernicious anemia from other causes of vitamin B₁₂ deficiency
   b. In pernicious anemia, results from stage 2 of the test are always normal
   c. A normal test result does not rule out malabsorption as a cause of vitamin B₁₂ deficiency
   d. Results from stage 1 of the test are always abnormal in pernicious anemia
   e. In a patient with pernicious anemia who has been treated adequately, stage 2 of the test should provide normal results

Correct answers:
1. c, 2. a, 3. a, 4. d, 5. b