46

Anemias

Linda K. Hendricks and Abdullah Kutlar

Introduction

Hematopoiesis is the process whereby mature blood cells (red cells, white cells, and platelets) are produced from the pluripotent hematopoietic stem cells in the bone marrow. The process involves the proliferation and differentiation of stem cells into different lineages (megakaryocytic, erythroid, lymphoid, granulocytic/marophage) with the ultimate production of mature blood cells. This process is influenced by many complex factors including the bone marrow microenvironment, an elaborate network of cytokines and hematopoietic growth factors, and an adequate supply of nutrients, vitamins, and some trace elements (Table 46.1). Erythropoiesis refers to the production of red blood cells whose major function is oxygen transport and delivery. A decrease in red blood cell mass and oxygen-carrying capacity results in anemia.

This section will provide an overview of the classification and pathogenesis of anemia, and will primarily focus on a detailed discussion of nutritional deficiencies leading to various types of anemia.

Anemia: Definition and Classification

Anemia is best defined as a reduction in the oxygen-carrying capacity of blood. Since this function is carried out by hemoglobin (Hb) in the red blood cells, measurement of Hb provides an accurate, reproducible means of detecting anemia. The normal values for Hb for different age groups as well as for adult men and women are shown in Table 46.2. Initial laboratory approach to anemia is summarized in Table 46.3.

Anemias can be classified morphologically and functionally. The morphologic classification is based upon the mean corpuscular volume (MCV), average volume of the red cell (Table 46.4). The diameter of a red cell is close to the size of the nucleus of a normal lymphocyte, approximately 7 μm (Color Figure 46.1*). Normal red cells are formed in the shape of two biconcave discs that are flexible and change shape according to a variety of

* Color figures follow page 992.

TABLE 46.1

Nutrients Important for Normal Red Blood Cell (RBC) Production

Protein/Calories
Vitamin B_{12} (cobalamin)
Folate
Iron
Vitamin B_6 (pyridoxine)
Riboflavin
Nicotinic acid
Ascorbic acid
Vitamin A (retinol)
Vitamin E (α-tocopherol)
Copper

TABLE 46.2

Criteria for Anemia and Normal MCV Values

	Hgb (g/dl)	**Hct (%)**	**MCV (fl)**
Infants			
1-3 days	<14.5	<45	95-108
One month	<10	<31	85-104
Two months	<9	<28	77-96
0.5-2 years	<11	<33	70-78
Children			
2-6 years	<11.5	<34	75-81
6-12 years	<11.5	<35	77-86
12-18 yrs			
Female	<12	<36	78-90
Male	<13	<37	78-88
Women	<12	<37	80-90
Men	<14	<40	80-90

MCV = Mean Corpuscular Volume

TABLE 46.3

Initial Laboratory Data in the Evaluation of Anemia

CBC (complete blood count)
White count and differential
Platelet count
Hemoglobin and hematocrit
MCV (mean cell volume)
Reticulocyte count
Red cell morphology on peripheral blood smear

factors. The amount of volume inside the cell membrane is the MCV. The normal MCV of a red cell is 80 to 100 fl. When evaluating either the MCV or the Hb of an individual, it is important to consider the patient's age and sex.

The functional classification takes the pathogenesis of anemia into consideration. Thus, anemias can be hypoproliferative (bone marrow or stem cell defects, decreased stimulation of erythropoiesis), or result from maturation defects (nuclear or cytoplasmic) or from blood loss or destruction (hemorrhage, hemolysis, and sequestration) (Table 46.5).

TABLE 46.4

Classification of Anemias by Morphology

Low MCV (Microcytic)	High MCV (Macrocytic)	Normal MCV (Normocytic)
Fe deficiency	Nonmegaloblastic	Bone marrow failure
Thalassemia	Liver disease	Aplastic anemia
Sideroblastic anemia	Hypothyroidism	Red cell aplasia (acquired and congenital)
Chronic disease	Reticulocytosis	Marrow infiltration
Lead poisoning	Aplastic anemia	Chronic renal failure
Protein deficiency		Endocrine abnormalities
	Megaloblastic	Hypothyroidism
	Vitamin B_{12} deficiency	Adrenal insufficiency
	Folate deficiency	HIV
	Myelodysplastic syndromes	Chronic disease
	Drug Induced	
	Chemotherapeutic agents	
	Nitrous oxide (laughing gas)	

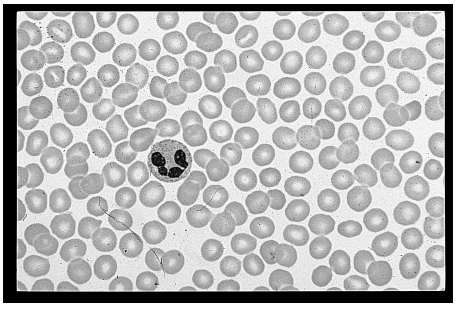

FIGURE 46.1
(See Color Figure 46.1) A normal peripheral blood smear.

Normal Erythropoiesis

A good understanding of normal erythropoiesis will enable one to better understand and appreciate the pathogenesis of different types of anemias (Color Figure 46.2). The earliest red cell precursor, the proerythroblast, is very large (12 to 20 μm) with a large nucleus. The nucleus contains DNA necessary for cell division, and the cytoplasm contains RNA necessary for hemoglobin synthesis. As the cells divide and mature, the nucleus becomes very small and condensed. Eventually the nucleus is extruded and the mature red cell remaining is only the cytoplasm full of hemoglobin. The normal red cell is smaller than the precursor cells and is pink-colored from the hemoglobin.

Perturbations of either the nuclear (DNA) or cytoplasmic (RNA and hemoglobin) maturation leads to anemia. The nucleus of the red cell precursor utilizes cobalamin (vitamin B_{12}) and folate in the synthesis of DNA. When either of these nutrients is sparse the nucleus cannot divide or mature normally, despite normal cytoplasmic development. The red cells formed are large (macrocytic). The bone marrow reveals red cell precursors that are *megaloblastic*, that is, large with fine, sparse nuclear chromatin. Other nucleated bone marrow cells are also affected such as the white cells, resulting in hypersegmented neutrophils in the peripheral blood (Color Figure 46.3).

In the cytoplasm, hemoglobin synthesis proceeds normally as long as both heme and globin are manufactured normally. Defective heme synthesis can occur by two mecha-

TABLE 46.5

Functional Causes of Anemia

Blood loss
Gastrointestinal bleeding
Menses/Menorrhagia
Internal bleeding
Decreased production of red blood cells
Nutritional deficiencies
Primary bone marrow failure
(myelodysplastic syndromes, leukemias, infiltrative processes)
Secondary bone marrow failure
(drugs, toxins, metabolic processes, infections)
Increased destruction of red blood cells
Immune hemolytic anemia
Mechanical hemolysis
(heart valve, microangiopathic hemolytic anemia, TTP, DIC)
Hereditary hemolysis
hemoglobinopathies
enzyme defects (G6PD, pyruvate kinase)
membrane defects
Acquired membrane defects
paroxysmal nocturnal hemoglobinuria
spur cell anemia
Infection related hemolysis
Clostridia, malaria, babesiosis
Splenic sequestration
Increased plasma volume
Pregnancy

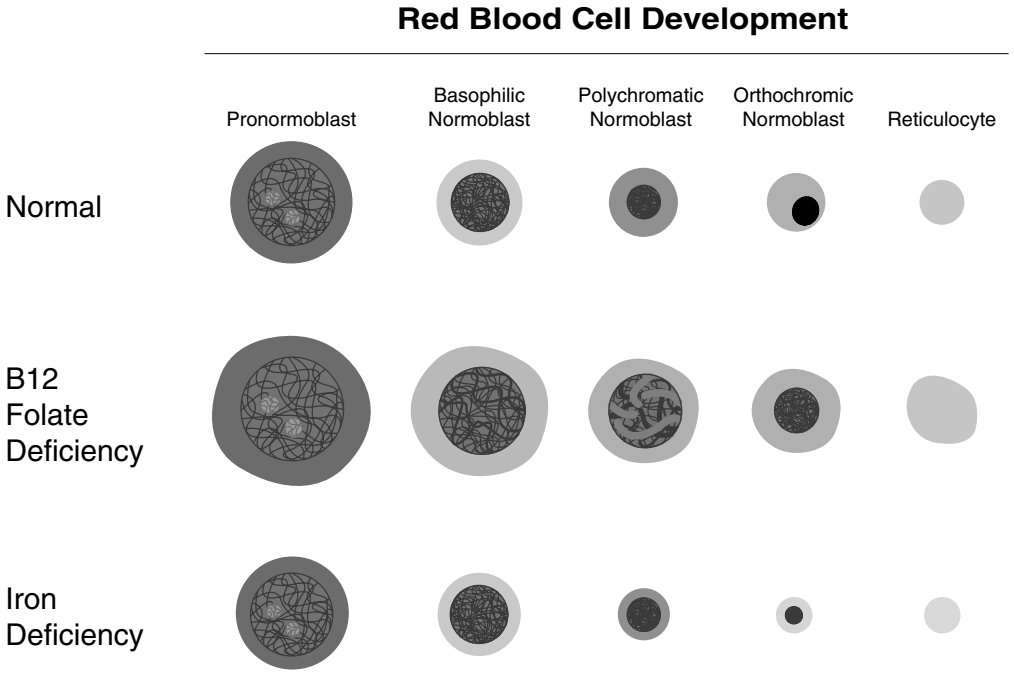

FIGURE 46.2
(See Color Figure 46.2) Erythropoiesis as it is affected by states of iron deficiency and vitamin B_{12} or folate deficiency.

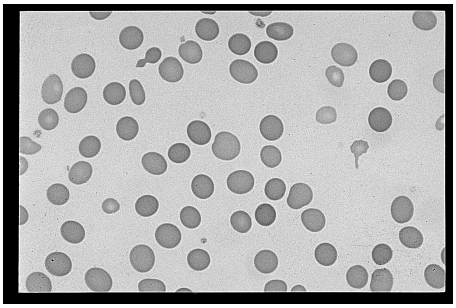

FIGURE 46.3
(See Color Figure 46.3) Macrocytic RBCs. Red blood cells that are larger than normal (macrocytic) and white blood cells that have nuclei with multiple segments (hypersegmented neutrophils) are characteristic of a vitamin B_{12} deficiency anemia.

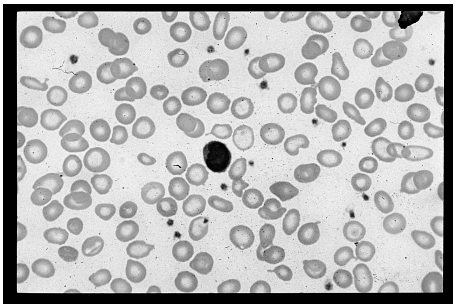

FIGURE 46.4
(See Color Figure 46.4) Microcytic RBCs. Red blood cells that are smaller than normal and have pale features (microcytic and hypochromic) are characteristic of Fe deficiency anemia.

nisms: faulty iron metabolism (iron deficiency anemia) or defective porphyrin metabolism (sideroblastic anemia). A deletion in or defect of the genes encoding globin (thalassemia) causes defective globin synthesis. All of these anemias are characterized by small red cells, *microcytosis*, because the nucleus divides and matures normally (Color Figure 46.4).

The Reticulocyte

The normal red blood cell (RBC) lives about 120 days in the peripheral blood: when the senescent RBC are destroyed they have to be replaced by new RBCs to maintain RBC homeostasis. A newly produced, young RBC still has residual RNA and mitochondria in the cytoplasm and is slightly larger than a normal red cell, appearing a little bluish on the peripheral blood smear. This is called a reticulocyte. A special stain is performed using new methylene blue, which stains the residual RNA, making a reticulocyte easy to identify and count in the blood (Color Figure 46.5). The *reticulocyte count* is the number of reticulocytes counted in 1000 red cells, reported as a percentage. A reticulocyte lives normally two days before it matures into a fully developed RBC. In a normal person with normal red cells and normal bone marrow the reticulocyte count will range from 0.5 to 1.5% (1/120 of RBCs replaced daily). Thus, the reticulocyte count is a sensitive method to detect how rigorous the bone marrow is producing new RBCs. Two corrections need to be made after the reticulocyte percentage is obtained before a conclusion can be drawn about the bone marrow.

One, if a patient has decreased numbers of red cells to begin with, then a reticulocyte count of 0.5 to 1.5% would not be considered an appropriate bone marrow response.

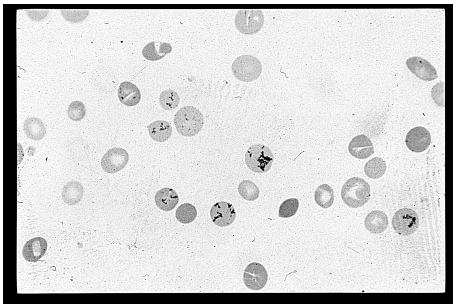

FIGURE 46.5
(See Color Figure 46.5) Reticulocytes. The appearance of young, newly produced red blood cells, reticulocytes, as seen in the peripheral blood smear that has been stained with new methylene blue.

Because of the fewer number of RBCs present, a reticulocyte percentage within that range would actually reflect a much smaller absolute reticulocyte number; i.e., two percent of a hundred cells is less than two percent of a thousand cells. The bone marrow should be producing more reticulocytes in the setting of anemia. Thus, when reticulocytes are counted it is important to take into account the degree of anemia present to avoid a misleading "normal" percentage of reticulocytes. Dividing the patient's hematocrit or hemoglobin by the normal hematocrit or hemoglobin does this. This gives us the fraction of normal red cells the patient has. That fraction is then multiplied by the reticulocyte count giving us the *corrected reticulocyte count*.

Second, the faster the bone marrow is producing reticulocytes, the younger they are when they are released into the peripheral blood. When the reticulocytes are counted on the special stain, both older reticulocytes and younger reticulocytes are counted. Therefore, adjustments for this can be made when calculating a patient's *reticulocyte index* (Color Figure 46.6). The corrected reticulocyte count is divided by the number of days the reticulocyte lives in the peripheral blood. This number, of course, depends on the degree of anemia. The more severely anemic someone is, the earlier the reticulocyte is pushed into the peripheral blood and the longer it stays as a reticulocyte in the blood. (The usual number used in the setting of anemia, however, is two.) After these two corrections have been made to the reticulocyte count, then one has a fair estimate of how well the bone marrow is able to make new RBCs. In the case of nutritionally caused anemias, the reticulocyte index will invariably be low (Table 46.6).

Erythropoietin

Erythropoietin, a glycoprotein hormone produced in the kidney in response to tissue hypoxia, is the major regulator of erythropoiesis. In cases of renal failure the erythropoietin level may be low, causing anemia, usually normocytic. Measuring the erythropoietin level may be a useful step in the evaluation of anemia; however, in cases of nutritionally deficient anemia the level is normal or elevated and is not, therefore, routinely measured.

Symptoms of Anemia

Patients with anemia will often present with similar symptoms regardless of the cause. Low RBCs decrease the oxygen-carrying capacity of the blood, producing generalized symptoms (Table 46.7). The severity of the symptoms, however, is directly related to how rapidly the anemia develops. Anemias that develop rapidly such as in acute bleeding,

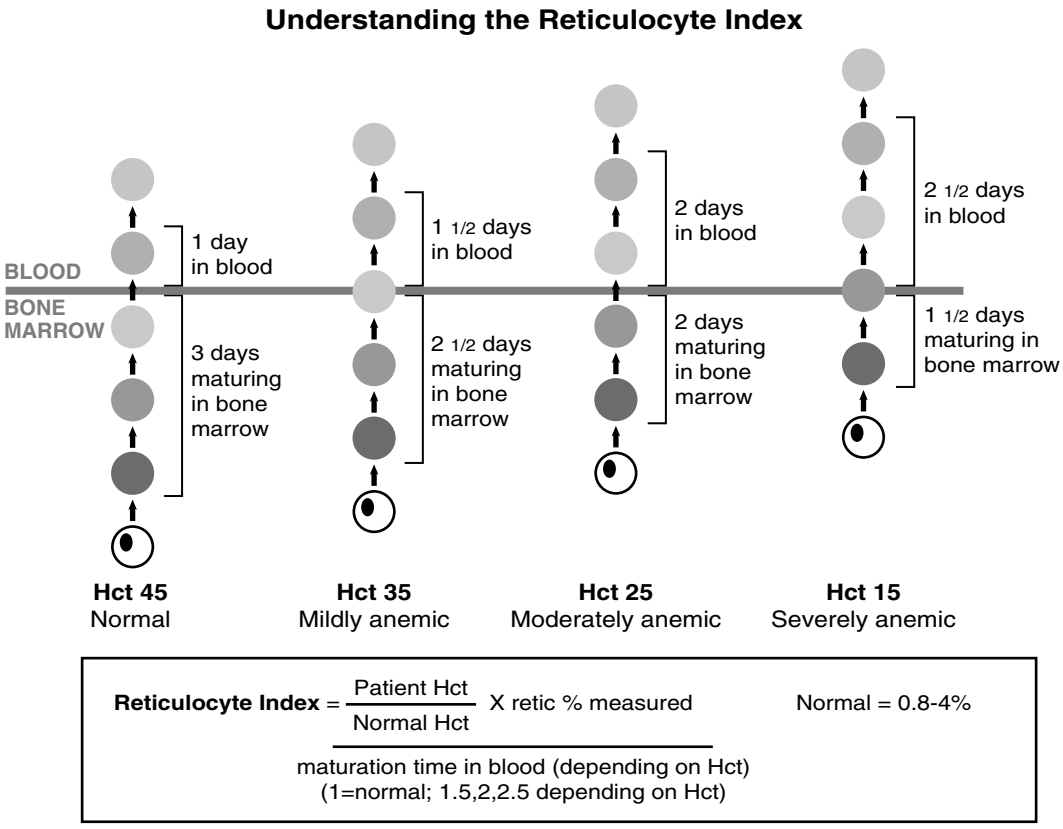

FIGURE 46.6
Understanding the reticulocyte index.

TABLE 46.6

Categorizing Anemias by Reticulocyte Count

Low Reticulocytes	High Reticulocytes
Decreased production:	Acute blood loss
Fe deficiency	Splenic seqestration
B_{12} deficiency	Increased destruction
Folate deficiency	(hemolysis)

TABLE 46.7

Symptoms of Anemia

Dyspnea with exertion
Dizziness
Lightheadedness
Throbbing headaches
Tinnitus
Palpitations
Syncope
Fatigue
Disrupted sleep patterns
Decreased libido
Mood disturbances
Difficulty concentrating

TABLE 46.8

Physical Findings in Anemia

Pallor of skin
Pallor of mucous membranes
Mild to moderate tachycardia
Widened pulse pressure
Systolic ejection murmur
Venous hums
Mild peripheral edema

will be more symptomatic than anemias that progress slowly over months to years. The heart must increase the rate of blood flow to the body to compensate for anemia. This may precipitate palpitations, shortness of breath, and throbbing headaches. Syncope occurs with severe anemia due to decreased oxygenation of the brain in the upright position. Often patients with chronic anemia such as those with sickle cell disease will maintain a hemoglobin level much lower than normal and have few symptoms, due to compensation by the body.

Physical Findings

Anemia causes general physical findings independent of the cause of the anemia (Table 46.8). These findings are due to the decrease in hemoglobin; skin and mucous membranes may be pale and the heart rate may be increased. As in symptoms, the physical findings may be influenced by the chronicity of the anemia.

Vitamin B_{12} Deficiency

Mechanism of Anemia

Erythropoiesis depends on proper nuclear (DNA) and cytoplasmic (RNA and hemoglobin) maturation. Vitamin B_{12} (or cobalamin) is important in DNA synthesis. When vitamin B_{12} is not available, the conversion of homocysteine to methionine is impaired. DNA is not synthesized properly and megaloblastic anemia ensues.

Etiology of Vitamin B_{12} Deficiency

The most common cause of vitamin B_{12} deficiency is not the dietary lack of vitamin B_{12} but the inability to absorb the vitamin from food due to a condition known as pernicious anemia. Normally, vitamin B_{12} is released from food in the acidic environment of the stomach and is bound to intrinsic factor formed by the parietal cells of the stomach that allow absorption in the ileum. In pernicious anemia, the stomach does not make intrinsic factor, and the vitamin cannot be absorbed. Pernicious anemia is more commonly seen in people of Northern European descent, but can be seen in all racial groups. Women are affected more than men are. A family history of pernicious anemia may indicate increased risk.

Other causes of vitamin B_{12} deficiency are related to the absorption mechanism (Table 46.9). For example, antibodies to intrinsic factor or to the parietal cells of the stomach may inhibit proper function. Gastrectomy will result in lack of intrinsic factor. Conditions that

TABLE 46.9

Causes of Vitamin B_{12} Deficiency

Pernicious anemia (most common cause)
Gastrectomy
Zollinger Ellison syndrome
Intestinal causes
Ileal resection or disease
Blind loop syndrome
Fish tapeworm
Pancreatic insufficiency
Strict vegetarianism (those who exclude meats, eggs, and milk)

TABLE 46.10

Clinical Features of B_{12} Deficiency

Megaloblastic anemia
Pallor/icterus
Sore tongue or mouth
Gastrointestinal symptoms
Neurologic symptoms
Finger and toe paresthesias
Disturbance of vibration and position sense
Spastic ataxia — subacute combined degeneration
Somnolence, impairment of taste, smell, vision
Dementia, psychosis, "megaloblastic madness"
Well nourished, generally

interfere with the binding of intrinsic factor to the ileum include Crohn's disease, ileal resection, and tropical sprue. The fish tapeworm, Diphyllobothrium latum, competes for vitamin B_{12} and can be a cause of deficiency in infected patients. If pancreatic enzymes are not sufficient to aid in the binding of B_{12} to intrinsic factor, the absorption will be impaired. Only very rarely is dietary deficiency of vitamin B_{12} seen in the United States. Those cases are usually seen in very strict vegans, those who exclude meats, eggs, and milk from their diets. The deficiency develops slowly over many years.

The Clinical Features of B_{12} Deficiency

Full-blown cases of deficiency present with megaloblastic anemia. Sore tongue or mouth and gastrointestinal complaints can occur. Neurologic effects can be devastating (Table 46.10). Demyelination of nerves leads to axonal degeneration and highly variable clinical symptoms. Subacute combined degeneration of the spinal cord and cerebral abnormalities include symptoms of finger and toe paresthesias, disturbance of vibration and position sense, spastic ataxia, impairment of taste, smell, vision, and even psychosis often referred to as "megaloblastic madness." Importantly, the anemia and neurologic effects are not always seen together. A patient could have severe megaloblastic anemia with no neurologic effects, and vice versa. The mechanism of neurologic effects is poorly understood. Unfortunately, treatment with vitamin B_{12} does not always correct the neurologic effects despite correction of the anemia.

The Hematologic Effects of B_{12} Deficiency

The nuclear maturation defect results in a macrocytic anemia (Table 46.11). The mean cell volume is usually greater than 110 fl and can vary depending on the degree of deficiency.

TABLE 46.11

Laboratory Features of B_{12} Deficiency

Hematologic
Megaloblastic anemia (MCV>110)
Leukopenia and thrombocytopenia
Bone marrow
Megaloblastic appearance
Ineffective erythropoiesis
Biochemical
Serum B_{12} level is decreased, i.e., <223 pg/ml
Methylmalonic acid is elevated, i.e., >0.4 μmol/L
Homocysteine is elevated, i.e., >14 μm
Marked increase in LDH, i.e., >3000 units/ml (normal 260 U/ml)
Serum folate levels may be elevated
Schilling Test

In vitamin B_{12} deficiency the red cell precursors in the bone marrow are large with immature nuclei, megaloblastic. Ineffective erythropoiesis (destruction of RBC precursors in the marrow) can be quite significant and is responsible for the elevated indirect bilirubin level and high lactate dehydrogenase (LDH). In fact, up to 90% of the red cells may actually be destroyed in the bone marrow before being released to the peripheral blood as compared to the 10 to 15% seen in normal subjects. Furthermore, the white cell and platelet lineages are affected, and the hematologic picture is often one of pancytopenia.

The Laboratory Diagnosis of B_{12} Deficiency

The serum vitamin B_{12} level reflects the vitamin stores in the body and is the standard method in determining vitamin B_{12} deficiency usually ranging from 200 to 1000 pg/ml. Some conditions such as folic acid deficiency, pregnancy, oral contraceptive use, multiple myeloma, and possibly antibiotic therapy can cause falsely low vitamin B_{12} levels. Because it is important to differentiate true B_{12} deficiency from folic acid deficiency, the serum methylmalonic acid level and serum homocysteine level can be measured (Table 46.12). While the homocysteine level may be elevated in both vitamin B_{12} deficiency and folate deficiency due to interruption of the formation of succinyl CoA, methylmalonic acid will be elevated only in vitamin B_{12} deficiency because it utilizes vitamin B_{12} to form methionine, a process not dependent on folate.

TABLE 46.12

The Laboratory Difference between Negative Nutritional Balance and True Folate and B_{12} Deficiency

Lab Value	Serum Folate	RBC Folate	Serum B_{12}	Serum HCYS	Serum MMA
Condition					
Negative folate balance	⇓	NL	NL	NL	NL
Folate deficiency	⇓	⇓	NL	⇑	NL
Negative B_{12} balance	NL	NL	NL	NL	NL
B_{12} deficiency	NL/⇑	NL/⇓	⇓	⇑	⇑
Combined deficiency	⇓/NL	⇓	⇓	⇑	⇑

HCYS = Homocysteine
MMA = Methylmalonic acid

TABLE 46.13

Treatment of B_{12} Deficiency

Standard dose is 1000 μg intramuscularly
Anemia only: 1000 μg daily for a week, then weekly until Hgb normalizes; maintenance dose is 1000 μg monthly
Neurologic deficits: 1000 μg daily for two weeks, then weekly until Hgb normalizes followed by twice a month for 6 months, then monthly for life
If injections are not an option, oral dose is 1000 μg daily
For the rare case of decreased oral intake from malnutrition, replacement of 50 μg daily is adequate

The Schilling Test

Once vitamin B_{12} deficiency is diagnosed, the cause can be determined by the Schilling Test. The test will differentiate between dietary deficiency, absence of intrinsic factor, and ileal malabsorption. The test consists of two parts: the first part involves administering radiolabeled cobalamin orally to the patient. The urine is collected for 24 hours and radioactivity is measured. If at least 7.5% of the oral dose is excreted in the urine, this means that the patient was able to absorb the vitamin and subsequently excrete the unused amount in the urine. However, if less than 7.5% of radiolabeled cobalamin is excreted in the urine, this means that the vitamin was not absorbed adequately. These patients go on to part two of the test: patients are given the radiolabeled cobalamin orally again. This time they are also given intrinsic factor. The urine collection is repeated. If the excretion is above 7.5% this time, this means that the addition of intrinsic factor corrected the absorption and the patient has pernicious anemia. If the urine excretion is still less than 7.5%, this points to a problem with absorption such as sprue or intrinsic factor receptor abnormalities. Before the test, all patients are given a dose of unlabeled vitamin B_{12} intramuscularly to saturate the cobalamin receptors in the tissues and plasma. This way, the body will absorb and then excrete the unused radiolabeled cobalamin and the test will be valid.

Nutritional Requirements of Vitamin B_{12} and Treatment

The normal total body pool of vitamin B_{12} is 3000 μgs. The daily dietary requirement is approximately 1 μg. The recommended daily allowance is 2 μg, and the average daily diet contains 5 to 15 μg a day (ranging from 1 to 100 μg a day). Thus it is very difficult to become vitamin B_{12} deficient based on poor diet alone. When deficiency is present the treatment requires only 1 μg of B_{12} a day; however, in the setting of neurologic deficiencies, higher doses are often given. The standard maintenance dose is 100 μg intramuscularly each month after an initial loading dose is given (Table 46.13).

Folate Deficiency

Mechanism of Anemia

Like vitamin B_{12}, folate is utilized in the synthesis of DNA in the nucleus. The deficiency of folate causes megaloblastic anemia due to the arrested nuclear maturation. The main function of folate in the biochemistry of hematopoiesis is the transfer of carbon groups to various compounds. The most important compounds formed by this carbon donation are

TABLE 46.14

Causes of Folate Deficiency

Decreased nutritional intake
Poverty
Old age
Alcoholism/ cirrhosis
Children on synthetic diets
Goat's milk anemia
Premature infants
Hemodialysis
Hyperalimentation
Nontropical sprue (gluten-sensitive enteropathy)
Tropical sprue
Congenital folate malabsorption
Other small intestine disease
Pregnancy
Increased cell turnover (chronic hemolysis, exfoliative dermatitis)
Drug induced
Alcohol
Trimethoprim and Pyrimethamine
Methotrexate
Sulfasalazine
Oral contraceptives
Anticonvulsants

the purines, dTMP and methionine. As explained above, methionine formation also requires vitamin B_{12}. Without vitamin B_{12}, methionine is not formed and the prospective folate cannot be recycled, causing a "folate trap." Thus, vitamin B_{12} deficiency can also give a laboratory picture of folate deficiency.

Etiology of Folate Deficiency

Unlike vitamin B_{12} deficiency, nutritional factors play the major role in folate deficiency (Table 46.14). This is seen most commonly in the elderly, the poor, or in alcoholics. Financial reasons, poor nutritional education, and excessive alcohol consumption prohibiting good dietary habits all contribute to the development of folate deficiency. States such as pregnancy or hemolysis, which increase the requirements of folate, will often precipitate a folate deficiency. Drugs can interfere with the recycling of folate by interfering with enzymes necessary to transfer carbon groups to folate. These include methotrexate, some anticonvulsants, and oral contraceptives. Malabsorption of folate, while unusual, is seen in cases of sprue. Bacterial overgrowth in the intestine may utilize the folate before it can be absorbed, such as in blind loop syndrome.

Clinical Features of Folate Deficiency

Unlike patients with vitamin B_{12} deficiency, patients with folate deficiency are more apt to appear malnourished. While the neurologic deficits described above due to vitamin B_{12} deficiency are not seen in folate deficiency, patients with folate deficiency commonly complain of poor sleep, irritability, and depression, and may appear to have a blunted affect (Table 46.15). Folate-deficient patients may also have other nutrient deficiencies such as vitamins A, D, and K, and protein/calorie malnutrition. These concomitant deficiencies may contribute to skin pigmentation changes, sores at the corners of the mouth (angular cheilosis), and pallor such as lemon-tinted skin.

TABLE 46.15

Clinical Features of Folate Deficiency

Blunted affect/ mask-like facies
Depression
Irritability
Forgetfulness
Sleep deprivation
Weight loss from underlying GI disease
Diffuse blotchy brownish skin pigmentation in nail beds/ skin creases
Poor nutritional state
Pallor/icterus from anemia

TABLE 46.16

Laboratory Diagnosis of Folate Deficiency

Red blood cell folate level is reduced
Serum folate level may be reduced but this is not specific; it may also be elevated, depending on the recent folate intake
Serum B_{12} level is normal

The Hematologic Effects of Folate Deficiency

Macrocytosis and megaloblastosis due to folate deficiency are essentially identical to that seen in vitamin B_{12} deficiency.

The Laboratory Diagnosis of Folate Deficiency

The serum folate level reflects only the recent dietary intake of folate and does not provide an accurate assessment of folate stores (Table 46.16). The RBC folate level is low in true folate deficiency. The serum folate level will fall early in dietary restriction, and in true deficiency the red cell folate levels will fall. In vitamin B_{12} deficiency, the folate is trapped in the unconjugated form as described above, leading to a loss of folate in the RBC. Thus, vitamin B_{12} deficiency may give a falsely low RBC folate value, but the serum folate will be normal to increased. Serum homocysteine, the precursor to methionine, will be elevated in folate deficiency as it is in B_{12} deficiency. The methylmalonic acid, however, will be normal in folate deficiency.

Nutritional Requirements of Folate and Treatment

The total body pool of folate stores is 5 to 10 mg. The daily dietary requirement is 50 µg/day and the recommended daily allowance is 200 µg/day for men and 180 µg/day for women. The average daily diet contains 225 µg/day of folate. Foods rich in folate include green leafy vegetables, liver, kidney, fruits, dairy products, and cereals. Because the most common cause of folate deficiency is decreased nutritional intake, treatment is folate supplementation given in one-mg tablets daily (Tables 46.17 and 46.18).

TABLE 46.17

Treatment of Folate Deficiency

Dose is 1-5 mg orally, daily
Pregnancy maintenance dose is 1 mg daily

TABLE 46.18

Causes for Treatment Failure

Wrong diagnosis
Additional vitamin and/or mineral deficiency (concomitant B_{12} and folate, for example)
Additional iron deficiency
Additional hemoglobinopathy (sickle cell disease or thalassemia)
Associated anemia of chronic disease
Hypothyroidism

TABLE 46.19

Who Should Receive Prophylaxis for B_{12} and Folate Deficiency?

Vitamin B_{12}
Infants of mothers with pernicious anemia
Strict vegetarians
Patients who have had total gastrectomy
Folate
ALL women considering pregnancy (0.4 mg/day orally)
Pregnant women
Breastfeeding women
Premature infants
Chronic hemolytic states/ hyperproliferative states
Patients receiving methotrexate for a rheumatologic condition
Certain individuals with hyperhomocysteinemia

Prophylactic Administration of Folate and Vitamin B_{12}

Some patients can be at risk for either folate deficiency or vitamin B_{12} deficiency. These patients should receive supplementation prophylactically (Table 46.19). For vitamin B_{12}, these patients include those with gastrectomy, very strict vegetarians, and infants born to mothers with pernicious anemia, because of the possibility of placental transfer of parietal cell or intrinsic factor antibodies. Folate prophylaxis should be given to pregnant women and women considering pregnancy to prevent neural tube defects. Breastfeeding women and premature infants should also receive folate. Patients with chronic hemolytic states such as sickle cell disease should receive folate as well as patients receiving chronic methotrexate for rheumatologic conditions, as the methotrexate inhibits dihydrofolate reductase, creating a form of "folate trap." Certain individuals with hyperhomocysteinemia may benefit from prophylactic folate administration, because a concomitant folate deficiency would create an exacerbation of the homocysteinemia due to the inhibition of methionine synthesis. Elevated homocysteine levels increase the risk of coronary artery disease.

Iron Deficiency Anemia

Mechanism of Anemia

Every cell in the body requires iron. The cells utilize iron in oxidative metabolism, growth, and cellular proliferation as well as in oxygen transport. The major portion of body iron

is found in hemoglobin. Heme is the component of hemoglobin that binds the iron, thus other heme-containing compounds including myoglobin and enzymes also contain iron. Hemoglobin accounts for 85% of all the heme-containing compounds in the body. Iron exists in the body bound to proteins, both in storage and in heme compounds and enzymes (Hb, myoglobin, and cytochrome). Unbound iron is toxic, primarily by generating reactive oxygen species.

Iron is absorbed from the duodenum and transported via transferrin to tissues such as RBC precursors. When not immediately utilized, the iron is stored mostly in the liver in the form of ferritin. During erythropoiesis iron is incorporated in the hemoglobin, where it functions as the transporter of oxygen. When senescent red cells are destroyed in the reticuloendothelial system, macrophages of the spleen and liver take up the iron and recycle it to transferrin to be transported back to the bone marrow.

While vitamin B_{12} and folate are used in DNA synthesis in red cell precursors, iron is utilized in cytoplasmic hemoglobin synthesis. Heme requires adequate iron and porphyrin metabolism for formation. Defective porphyrin metabolism (sideroblastic anemia) sometimes responds to pyridoxine treatment; however, the cause of anemia is not due to a nutritional deficiency of pyridoxine but to inherent enzyme mutations disrupting normal porphyrin metabolism. Thus, sideroblastic anemia is beyond the scope of this section. Deficiency of iron creates a cytoplasmic maturation defect while the nuclear maturation proceeds normally. The result is small (microcytic) cells with poorly hemoglobinized cytoplasm (hypochromia).

Etiology of Iron Deficiency

Iron deficiency is the most common nutritional deficiency in the U.S. In the late 1800s young menstruating females were considered fashionable if they were pale and chlorotic, a state of iron deficiency, though at the time the diagnosis of iron deficiency was not known. The loss of iron through chronic blood loss such as menses in a young female is the classic cause of iron deficiency. Any time iron is removed from the recycling process and lost from the body, iron deficiency can ensue (Table 46.20). Other conditions that remove iron include pregnancy and lactation. Increased iron requirements occur during periods of rapid growth such as in childhood. Adult men or menopausal women who are proven to have iron deficiency must be evaluated for blood loss, such as occurs in occult colon cancer, for example. Dietary causes of iron deficiency are unusual in developed countries except among infants, adolescents, and pregnant women, where the iron requirements are increased and the diet often compromised. The average adult man consumes more than adequate iron daily to make up for the normal iron loss. Therefore, in the U.S., iron deficiency seen in a patient not falling under one of these categories should be thoroughly evaluated for blood loss and not just merely treated.

TABLE 46.20

Causes of Iron Deficiency Anemia

Gastrointestinal bleed
Genitourinary bleed
Menses
Repeated blood donation
Growth
Pregnancy and lactation
Poor diets
Intestinal malabsorption
Hookworm/intestinal parasites
Gastric surgery

TABLE 46.21

Clinical Features of Iron Deficiency

Symptoms of anemia
Pagophagia (heavy ice consumption)
Koilonychia (brittle spoon nails)
Blue sclera
glossitis
Angular stomatitis
Postcricoid esophageal web/stricture (Plummer-Vinson syndrome)
Gastric atrophy
Impaired immunity
Decreased exercise tolerance
Neuropsychological abnormalities

The Clinical Features of Iron Deficiency

Children who develop iron deficiency may demonstrate irritability, memory loss, and learning difficulties. In adults, iron deficiency can develop slowly, and very low levels of hemoglobin may be attained before symptoms of anemia develop (Table 46.21). It is not unusual for a woman to present with hemoglobin of 2 or 3 g/dl with only moderate symptoms of fatigue or shortness of breath. Blood transfusion in these patients can be dangerous and should be performed very carefully, and only with one or two units to avoid congestive heart failure or stroke from rapid increase in intravascular volume and red cell numbers. Iron deficiency anemia may cause brittle "spoon" nails (koilonychia), blue-tinted sclera, and a painful tongue (glossitis). Immunity may be impaired due to the lack of iron needed by white blood cells and the enzymes used in host defense.

Pica is a fascinating manifestation of iron deficiency whereby the appetite is altered and patients crave unusual things to eat. Classic examples include starch, ice, or clay consumption. Pica in most cases is the symptom of iron deficiency and not the cause. However, clay inhibits the absorption of iron and may perpetuate the condition. Furthermore, excessive consumption of these items provides for a poor diet in general, thus exacerbating iron deficiency. In some cultures, pica is practiced as a norm, and in those cases iron deficiency may be the result of and not the cause of pica.

The Hematologic Effects of Iron Deficiency

As mentioned, the perturbation of cytoplasmic maturation during erythropoiesis (decreased Hb synthesis due to Fe deficiency) leads to small, underhemoglobinized red cells (microcytosis and hypochromia). In fact, the MCV can be as low as 50 fl in severe cases. The reticulocyte index is low. Often cells of various shapes and sizes are released from the bone marrow. Platelets may increase in iron deficiency and can even exceed one million (normal being 150 to 400 thousand.) If concurrent folate or vitamin B_{12} deficiency exists, then the red cells may not demonstrate the microcytosis as expected due to the concomitant macrocytosis. However, if folate or vitamin B_{12} is replaced the cells will become small.

The Laboratory Diagnosis of Fe Deficiency

Ferritin is the storage compartment for iron in the body; therefore, serum ferritin levels reflect the state of body Fe stores. A ferritin level of less than 12 μg/dl is diagnostic of

TABLE 46.22

Laboratory Features of Iron Deficiency

Hematologic
Microcytic anemia
Thrombocytosis
Bone marrow
Normal nuclear maturation
Cytoplasmic abnormalities
Absent iron stores on iron stain
Biochemical
Ferritin level is decreased
Total iron binding capacity is elevated
Iron saturation and serum iron are decreased

iron deficiency. Other laboratory features such as the transferrin level (or the iron-binding capacity), the serum iron level, and the transferrin saturation are summarized in Table 46.22. If iron stores are low the iron binding capacity will, of course, be elevated reflecting the vacant binding sites. The transferrin saturation will be decreased. The degree of iron deficiency present and the resultant hematologic effects is an important concept. By the time microcytosis is evident, the red cell hemoglobin content is decreased. Before that stage, however, the body stores of iron will be decreased but the red cell amount of iron will be maintained. This is why a patient may demonstrate a low ferritin, an elevated TIBC, and decreased iron saturation, yet have no evidence of anemia. These patients will develop symptoms of anemia in time if the iron loss is not corrected.

Ferritin is also an acute phase reactant. Thus, conditions such as renal failure, infection, liver disease, acute or chronic inflammatory states will lead to elevated ferritin levels. In these cases it may be necessary to ascertain iron stores directly with a bone marrow aspirate. The bone marrow should demonstrate iron in the interstitium when stained with an iron stain. If no iron is demonstrated in the marrow, the patient definitely has iron depletion. Iron deficiency anemia, however, is only diagnosed by the RBC iron studies.

Nutritional Requirements of Iron and Treatment

Adult men have 50 mg of iron per kilogram of body weight; women have 35 mg/kg. The minimal daily requirement is 1 mg for men and 2 mg for menstruating women. The recommended daily allowance is 12 mg/d for men and 15 mg/d for women. The average daily diet contains 6 mg of iron per 1000 kcal of food consumed (10 to 30 mg/day). Foods rich in iron include red meat. Remember the conditions that increase iron requirements such as pregnancy, childhood, and chronic blood loss. In pregnancy the daily requirement may increase to 5-6 mg. For infants the daily requirement is 0.5 mg, and for children 1 mg.

Oral iron replacement is sufficient in the majority of cases of iron deficiency anemia (Table 46.23). The usual dose is 60 mg of elemental iron administered as 325 mg of iron sulfate three times a day. The best available is in the ferrous form and heme iron as in red meat. The reticulocyte response will peak at day 8 to 10 after initiation of treatment, and the hemoglobin should normalize over 6 to 10 weeks. An improved sense of wellbeing may occur as soon as day 2 or 3 of treatment, however. Often patients complain of gastrointestinal side effects of oral iron (Table 46.24). These include constipation, diarrhea, epigastric discomfort, and nausea. Taking the iron with food can ameliorate the symptoms, though this decreases the absorption as much as 50%. Alternatively, smaller amounts may be given or different preparations tried. Some other iron formulations (iron-sorbitol) may be better tolerated in terms of gastrointestinal side effects. On very rare occasions it may

TABLE 46.23

Treatment of Iron Deficiency

Oral
150-200 mg elemental iron a day given in 3 divided doses on empty stomach (Children's dose is 3 mg iron/ kilogram body weight per day in 3 divided doses) Ferrous sulfate is best absorbed and least expensive. One tablet of 325 mg ferrous sulfate contains 60-70 mg of elemental iron. This given 3 times a day is a good standard treatment for adults. Hemoglobin should rise approximately 2 grams/dl every 3 weeks. Treatment should continue for 4-6 months after obtaining normal hemoglobin.
Intravenous
Iron dextran with dose depending on body weight and degree of anemia is the most widely used preparation. This is a one-time dose calculated from a chart included in the product insert. This solution of ferric oxyhydroxide and low-molecular-weight dextran contains 50 mg of elemental iron per ml. An average dose for a 70 kg patient with hemoglobin of 7 g/dl would be 40 ml of iron dextran (2000 mg of elemental iron.)

TABLE 46.24

Possible Side Effects of Iron Therapy

Oral
Constipation Diarrhea Nausea Epigastric discomfort Vomiting
Intravenous
Anaphylactic reaction (rare) Fever Urticaria Adenopathy Myalgias Arthralgias Phlebitis Pain at injection site

be necessary to administer intravenous or intramuscular iron. This can be associated with some untoward side effects, but will eliminate the need for oral iron. Oral iron therapy should be continued for six months once the hemoglobin is normalized. Parenteral iron need only be given once. It should be kept in mind that if the cause of the iron deficiency is blood loss and this continues iron deficiency may recur in the future, and chronic iron replacement may be indicated (Table 46.25).

TABLE 46.25

Nutritional Information on B_{12}, Folate, and Iron

	B_{12}	Folate	Iron
Total body pool	3000 µg	5-10 mg	Men: 50 mg/kg Women: 35 mg/kg
Minimal daily adult Requirement (dietary)	0.3-1.2 µg/day	50 µg/day	10-20 mg/day
Recommended dietary allowance (RDA)	2 µg/day	Men: 200 µg/day Women: 180 µg/day	Men: 12 mg/d Women: 15mg/d
Average daily diet	5-15 µg/day (range 1-100 µg/d)	225 µg/day	6 mg/1000 kcal (10-30 mg/d)
Prevalence of deficiency	0.2% of population	8% of men in NA 10-13% of women	2% adult men 8% women
Source foods	Animal origin liver, kidney, mollusks, muscle, eggs, cheese, milk Multivitamins	Green leafy vegs. liver, kidney fruits, breakfast cereals, dairy, tea Multivitamins	Red meat
Time to develop blood signs after abstinence of nutrient	5-6 years	3 weeks	Years

Additional Sources of Information

1. Wintrobe MM. *Clinical Hematology,* Lippincott Williams & Wilkins, Philadelphia, 1999.
2. Israels LG, Israels ED. *Mechanisms in Hematology,* Core Health Services Inc., Ontario, 1998.
3. Hoffman R. *Hematology,* Churchill Livingstone, New York, 1995.
4. Hercberg S, Galan P. Nutritional anemias, *Clinical Haematology,* Vol. 5, Fleming AF, Ed, Bailliere Tindall, London, 1992, pp 143-168.
5. Hughes-Jones NC, Wickramasinghe SN. Lecture Notes on Haematology, Blackwell Science, London, 1996.
6. Foucar K. *Bone Marrow Pathology,* ASCP Press, Chicago, 1995.
7. Jandl JH. *Blood*, Little, Brown, Boston, 1987.
8. Duffy TP. Normochromic, normocytic anemias, *Cecil Textbook of Medicine*, 20th ed, Bennett JC, Plum F, Eds, WB Saunders, Philadelphia, 1996, p 837.
9. Duffy TP. Microcytic and hypochromic anemias, *Cecil Textbook of Medicine*, 20th ed, Bennett JC, Plum F, Eds, WB Saunders, Philadelphia, 1996, p 839.
10. Allen RH, Megaloblastic anemias, *Cecil Textbook of Medicine*, 20th ed, Bennett JC, Plum F, Eds, WB Saunders, Philadelphia, 1996, p 843.