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Iron Deficiency and Other Hypoproliferative Anemias: Introduction

Anemias associated with normocytic and normochromic red cells and an inappropriately low reticulocyte response (reticulocyte index <2.0–2.5) are *hypoproliferative anemias*. This category includes early iron deficiency (before hypochromic microcytic red cells develop), acute and chronic inflammation (including many malignancies), renal disease, hypometabolic states such as protein malnutrition and endocrine deficiencies, and anemias from marrow damage. Marrow damage states are discussed in Chap. 102.

Hypoproliferative anemias are the most common anemias, and anemia associated with acute and chronic inflammation is the most common of these. The anemia of inflammation, like iron deficiency, is related in part to abnormal iron metabolism. The anemias associated with renal disease, inflammation, cancer, and hypometabolic states are characterized by an abnormal erythropoietin response to the anemia.

Iron Metabolism

Iron is a critical element in the function of all cells, although the amount of iron required by individual tissues varies during development. At the same time, the body must protect itself from free iron, which is highly toxic in that it participates in chemical reactions that generate free radicals such as singlet O_2 or OH^-. Consequently, elaborate mechanisms have evolved that allow iron to be made available for physiologic functions while at the same time conserving this element and handling it in such a way that toxicity is avoided.

The major role of iron in mammals is to carry O_2 as part of hemoglobin. O_2 is also bound by myoglobin in muscle. Iron is a critical element in iron-containing enzymes, including the cytochrome system in mitochondria. Iron distribution in the body is shown in Table 98-1. Without iron, cells lose their capacity for electron transport and energy metabolism. In erythroid cells, hemoglobin synthesis is impaired, resulting in anemia and reduced O_2 delivery to tissue.

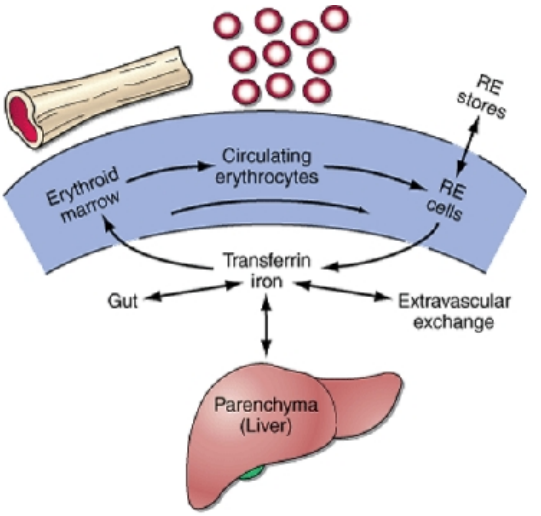
Table 98-1 Body Iron Distribution

	Iron Content, mg	
	Adult Male, 80 kg	**Adult Female, 60 kg**
Hemoglobin	2500	1700
Myoglobin/enzymes	500	300
Transferrin iron	3	3
Iron stores	600–1000	0–300

The Iron Cycle in Humans

Figure 98-1 outlines the major pathways of internal iron exchange in humans. Iron absorbed from the diet or released from stores circulates in the plasma bound to *transferrin*, the iron transport protein. Transferrin is a bilobed glycoprotein with two iron binding sites. Transferrin that carries iron exists in two forms– *monoferric* (one iron atom) or *diferric* (two iron atoms). The turnover (half-clearance time) of transferrin-bound iron is very rapid– typically 60–90 min. Because almost all of the iron transported by transferrin is delivered to the erythroid marrow, the clearance time of transferrin-bound iron from the circulation is affected most by the plasma iron level and the erythroid marrow activity. When erythropoiesis is markedly stimulated, the pool of erythroid cells requiring iron increases and the clearance time of iron from the circulation decreases. The half-clearance time of iron in the presence of iron deficiency is as short as 10–15 min. With suppression of erythropoiesis, the plasma iron level typically increases and the half-clearance time may be prolonged to several hours. Normally, the iron bound to transferrin turns over 10–20 times per day. Assuming a normal plasma iron level of 80–100 μg/dL, the amount of iron passing through the transferrin pool is 20–24 mg/d.

Figure 98-1



Internal iron exchange. Normally about 80% of iron passing through the plasma transferrin pool is recycled from broken-down red cells. Absorption of about 1 mg/d is required from the diet in men, 1.4 mg/d in women to maintain homeostasis. As long as transferrin saturation is maintained between 20–60% and erythropoiesis is not increased, iron stores are not required. However, in the event of blood loss, dietary iron deficiency, or inadequate iron absorption, up to 40 mg/d of iron can be mobilized from stores. RE, reticuloendothelial.

The iron-transferrin complex circulates in the plasma until it interacts with specific *transferrin receptors* on the surface of marrow erythroid cells. Diferric transferrin has the highest affinity for transferrin receptors; apotransferrin (transferrin not carrying iron) has very little affinity. While transferrin receptors are found on cells in many tissues within the body– and all cells at some time during development will display transferrin receptors– the cell having the greatest number of receptors (300,000 to 400,000/cell) is the developing erythroblast.

Once the iron-bearing transferrin interacts with its receptor, the complex is internalized via clathrin-coated pits and transported to an acidic endosome, where the iron is released at the low pH. The iron is then made available for heme synthesis while the transferrin-receptor complex is recycled to the surface of the cell, where the bulk of the transferrin is released back into circulation and the transferrin receptor reanchors into the cell membrane. At this point a certain amount of the transferrin receptor protein may be released into circulation and can be measured as soluble transferrin receptor protein. Within the erythroid cell, iron in excess of the amount needed for hemoglobin synthesis binds to a storage protein, *apoferritin*, forming *ferritin*. This mechanism of iron exchange also takes place in other cells of the body expressing transferrin receptors, especially liver parenchymal cells where the iron can be incorporated into heme-containing enzymes or stored. The iron incorporated into hemoglobin subsequently enters the circulation as new red cells are released from the bone marrow. The iron is then part of the red cell mass and will not become available for reutilization until the red cell dies.

In a normal individual, the average red cell life span is 120 days. Thus, 0.8–1.0% of red cells turn over each day. At the end of its life span, the red cell is recognized as senescent by the cells of the *reticuloendothelial (RE) system*, and the cell undergoes phagocytosis. Once within the RE cell, the hemoglobin from the ingested red cell is broken down, the globin and other proteins are returned to the amino acid pool, and the iron is shuttled back to the surface of the RE cell, where it is presented to circulating transferrin. It is the efficient and highly conserved recycling of iron from senescent red cells that supports steady state (and even mildly accelerated) erythropoiesis.

Since each milliliter of red cells contains 1 mg of elemental iron, the amount of iron needed to replace those red cells lost through senescence amounts to 16–20 mg/d (assuming an adult with a red cell mass of 2 L). Any additional iron required for daily red cell production comes from the diet. Normally, an adult male will need to absorb at least 1 mg of elemental iron daily to meet needs, while females in the childbearing years will need to absorb an average of 1.4 mg/d. However, to achieve a maximum proliferative erythroid marrow response to anemia, additional iron must be available. With markedly stimulated erythropoiesis, demands for iron are increased by as much as six- to eightfold. With extravascular hemolytic anemia, the rate of red cell destruction is increased, but the iron recovered from the red cells is efficiently reutilized for hemoglobin synthesis. In contrast, with intravascular hemolysis or blood loss anemia, the rate of red cell production is limited by the amount of iron that can be mobilized from stores. Typically, the rate of mobilization under these circumstances will not support red cell production more than 2.5 times normal. If the delivery of iron to the stimulated marrow is suboptimal, the marrow's proliferative response is blunted, and hemoglobin synthesis is impaired. The result is a hypoproliferative marrow accompanied by microcytic, hypochromic anemia.

While blood loss or hemolysis places a demand on the iron supply, conditions associated with inflammation interfere with iron release from stores and can result in a rapid decrease in the serum iron (see below).

Nutritional Iron Balance

The balance of iron in humans is tightly controlled and designed to conserve iron for reutilization. There is no regulated excretory pathway for iron, and the only mechanisms by which iron is lost from the body are blood loss (via gastrointestinal bleeding, menses, or other forms

of bleeding) and the loss of epithelial cells from the skin, gut, and genitourinary tract. Normally, the only route by which iron comes into the body is via absorption from food or from medicinal iron taken orally. Iron may also enter the body through red-cell transfusions or injection of iron complexes. The margin between the amount of iron available for absorption and the requirement for iron in growing infants and the adult female is narrow; this accounts for the great prevalence of iron deficiency worldwide– currently estimated at one-half billion people.

The amount of iron required from the diet to replace losses averages about 10% of body iron content a year in men and 15% in women of childbearing age. Dietary iron content is closely related to total caloric intake (approximately 6 mg of elemental iron per 1000 calories). Iron bioavailability is affected by the nature of the foodstuff, with heme iron (e.g., red meat) being most readily absorbed. In the United States, the average iron intake in an adult male is 15 mg/d with 6% absorption; for the average female, the daily intake is 11 mg/d with 12% absorption. An individual with iron deficiency can increase iron absorption to about 20% of the iron present in a meat-containing diet but only 5–10% of the iron in a vegetarian diet. As a result, one-third of the female population in the United States has virtually no iron stores. Vegetarians are at an additional disadvantage because certain foodstuffs that include phytates and phosphates reduce iron absorption by about 50%. When ionizable iron salts are given together with food, the amount of iron absorbed is reduced. When the percentage of iron absorbed from individual food items is compared with the percentage for an equivalent amount of ferrous salt, iron in vegetables is only about one-twentieth as available, egg iron one-eighth, liver iron one-half, and heme iron one-half to two-thirds.

Infants, children, and adolescents may be unable to maintain normal iron balance because of the demands of body growth and lower dietary intake of iron. During the last two trimesters of pregnancy, daily iron requirements increase to 5–6 mg. That is the reason why iron supplements are strongly recommended for pregnant women in developed countries. Enthusiasm for supplementing foods such as bread and cereals with iron has waned in the face of concerns that the very prevalent hemochromatosis gene would result in an unacceptable risk of iron overload.

Iron absorption takes place largely in the proximal small intestine and is a carefully regulated process. For absorption, iron must be taken up by the luminal cell. That process is facilitated by the acidic contents of the stomach, which maintains the iron in solution. At the brush border of the absorptive cell, the ferric iron is converted to the ferrous form by a ferrireductase. Transport across the membrane is accomplished by divalent metal transporter 1 (DMT-1, also known as Nramp 2 or DCT-1). DMT-1 is a general cation transporter. Once inside the gut cell, iron may be stored as ferritin or transported through the cell to be released at the basolateral surface to plasma transferrin through the membrane-embedded iron exporter, ferroportin. The function of ferroportin is negatively regulated by hepcidin, the principal iron regulatory hormone. In the process of release, iron interacts with another ferroxidase, hephaestin, which oxidizes the iron to the ferric form for transferrin binding. Hephaestin is similar to ceruloplasmin, the copper-carrying protein.

Iron absorption is influenced by a number of physiologic states. Erythroid hyperplasia, for example, stimulates iron absorption, even in the face of normal or increased iron stores, and hepcidin levels are inappropriately low. The molecular mechanism underlying this relationship is not known. Thus, patients with anemias associated with high levels of ineffective erythropoiesis absorb excess amounts of dietary iron. Over time, this may lead to iron overload and tissue damage. In iron deficiency, hepcidin levels are low and iron is much more efficiently absorbed from a given diet; the contrary is true in states of secondary iron overload. The normal individual can reduce iron absorption in situations of excessive intake or medicinal iron intake; however, while the percentage of iron absorbed goes down, the absolute amount goes up. This accounts for the acute iron toxicity occasionally seen when children ingest large numbers of iron tablets. Under these circumstances, the amount of iron absorbed exceeds the transferrin binding capacity of the plasma, resulting in free iron that affects critical organs such as cardiac muscle cells.

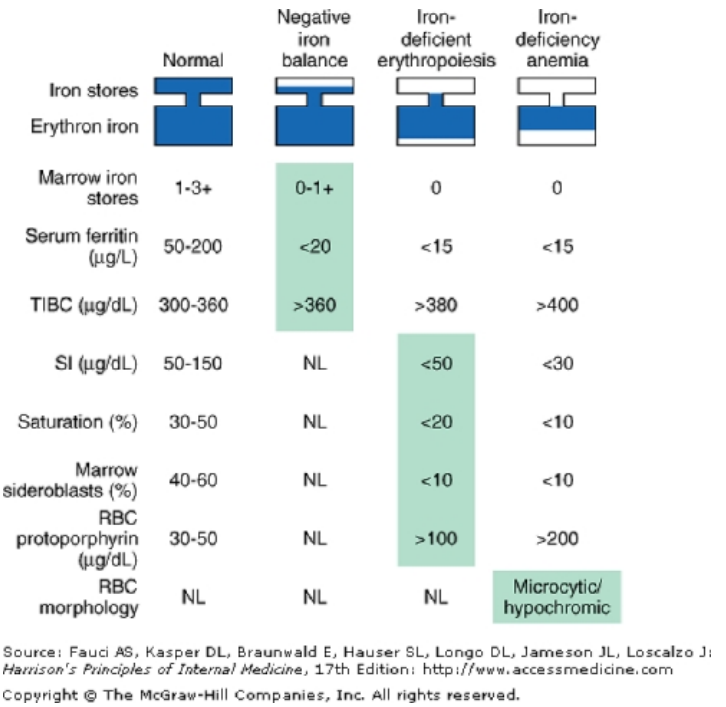
Iron-Deficiency Anemia

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributable to iron deficiency and accounts for around 841,000 deaths annually worldwide. Africa and parts of Asia bear 71% of the global mortality burden; North America represents only 1.4% of the total morbidity and mortality associated with iron deficiency.

Stages of Iron Deficiency

Iron-deficiency anemia is the condition in which there is anemia and clear evidence of iron lack. The progression to iron deficiency can be divided into three stages (Fig. 98-2). The first stage is *negative iron balance*, in which the demands for (or losses of) iron exceed the body's ability to absorb iron from the diet. This stage results from a number of physiologic mechanisms, including blood loss, pregnancy (in which the demands for red cell production by the fetus outstrip the mother's ability to provide iron), rapid growth spurts in the adolescent, or inadequate dietary iron intake. Blood loss in excess of 10–20 mL of red cells per day is greater than the amount of iron that the gut can absorb from a normal diet. Under these circumstances the iron deficit must be made up by mobilization of iron from RE storage sites. During this period, iron stores– reflected by the serum ferritin level or the appearance of stainable iron on bone marrow aspirations– decrease. As long as iron stores are present and can be mobilized, the serum iron, total iron-binding capacity (TIBC), and red cell protoporphyrin levels remain within normal limits. At this stage, red cell morphology and indices are normal.

Figure 98-2



Laboratory studies in the evolution of iron deficiency. Measurements of marrow iron stores, serum ferritin, and total iron-binding capacity (TIBC) are sensitive to early iron-store depletion. Iron-deficient erythropoiesis is recognized from additional abnormalities in the serum iron (SI), percent transferrin saturation, the pattern of marrow sideroblasts, and the red cell protoporphyrin level. Patients with iron-deficiency anemia demonstrate all the same abnormalities plus hypochromic microcytic anemia. *(From Hillman and Finch, with permission.)*

When iron stores become depleted, the serum iron begins to fall. Gradually, the TIBC increases, as do red cell protoporphyrin levels. By definition, marrow iron stores are absent when the serum ferritin level is <15 µg/L. As long as the serum iron remains within the normal range, hemoglobin synthesis is unaffected despite the dwindling iron stores. Once the transferrin saturation falls to 15–20%, hemoglobin synthesis becomes impaired. This is a period of *iron-deficient erythropoiesis* . Careful evaluation of the peripheral blood smear reveals the first appearance of microcytic cells, and if the laboratory technology is available, one finds hypochromic reticulocytes in circulation. Gradually, the hemoglobin and hematocrit begin to fall, reflecting *iron-deficiency anemia*. The transferrin saturation at this point is 10–15%.

When moderate anemia is present (hemoglobin 10–13 g/dL), the bone marrow remains hypoproliferative. With more severe anemia (hemoglobin 7–8 g/dL), hypochromia and microcytosis become more prominent, target cells and misshapen red cells (poikilocytes) appear on the blood smear as cigar- or pencil-shaped forms, and the erythroid marrow becomes increasingly ineffective. Consequently, with severe prolonged iron-deficiency anemia, erythroid hyperplasia of the marrow develops, rather than hypoproliferation.

Causes of Iron Deficiency

Conditions that increase demand for iron, increase iron loss, or decrease iron intake or absorption can produce iron deficiency (Table 98-2).

Table 98-2 Causes of Iron Deficiency
Increased demand for iron and/or hematopoiesis
rapid growth in infancy or adolescence
pregnancy
erythropoietin therapy
Increased iron loss
chronic blood loss
menses

acute blood loss

blood donation

phlebotomy as treatment for polycythemia vera

Decreased iron intake or absorption

inadequate diet

malabsorption from disease (sprue, Crohn's disease)

malabsorption from surgery (post-gastrectomy)

acute or chronic inflammation

Clinical Presentation of Iron Deficiency

Certain clinical conditions carry an increased likelihood of iron deficiency. Pregnancy, adolescence, periods of rapid growth, and an intermittent history of blood loss of any kind should alert the clinician to possible iron deficiency. A cardinal rule is that the appearance of iron deficiency in an adult male means gastrointestinal blood loss until proven otherwise. Signs related to iron deficiency depend on the severity and chronicity of the anemia in addition to the usual signs of anemia– fatigue, pallor, and reduced exercise capacity. *Cheilosis* (fissures at the corners of the mouth) and *koilonychia* (spooning of the fingernails) are signs of advanced tissue iron deficiency. The diagnosis of iron deficiency is typically based on laboratory results.

Laboratory Iron Studies

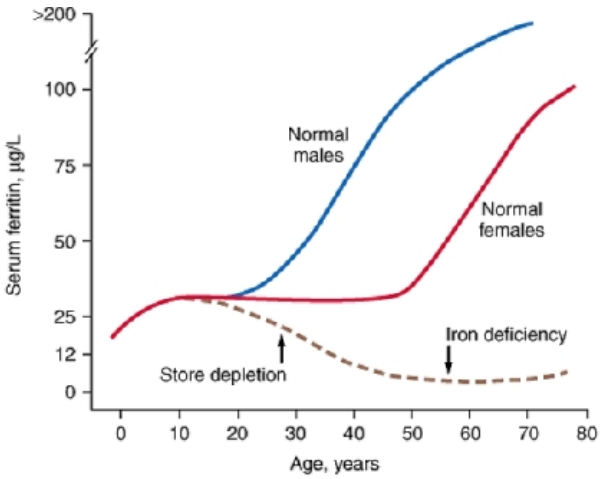
Serum Iron and Total Iron-Binding Capacity

The serum iron level represents the amount of circulating iron bound to transferrin. The TIBC is an indirect measure of the circulating transferrin. The normal range for the serum iron is 50–150 μg/dL; the normal range for TIBC is 300–360 μg/dL. Transferrin saturation, which is normally 25–50%, is obtained by the following formula: serum iron × 100 ÷ TIBC. Iron-deficiency states are associated with saturation levels below 18%. In evaluating the serum iron, the clinician should be aware that there is a diurnal variation in the value. A transferrin saturation >50% indicates that a disproportionate amount of the iron bound to transferrin is being delivered to nonerythroid tissues. If this persists for an extended time, tissue iron overload may occur.

Serum Ferritin

Free iron is toxic to cells, and the body has established an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the RE system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells, although hemosiderin is less readily available. Under steady-state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores. The normal value for ferritin varies according to the age and gender of the individual (Fig. 98-3). Adult males have serum ferritin values averaging about 100 μg/L, while adult females have levels averaging 30 μg/L. As iron stores are depleted, the serum ferritin falls to <15 μg/L. Such levels are diagnostic of absent body iron stores.

Figure 98-3



Serum ferritin levels as a function of sex and age. Iron store depletion and iron deficiency are accompanied by a fall in serum ferritin level below 20 µg/L. *(From Hillman et al, with permission.)*

Evaluation of Bone Marrow Iron Stores

Although RE cell iron stores can be estimated from the iron stain of a bone marrow aspirate or biopsy, the measurement of serum ferritin has largely supplanted bone marrow aspirates for determination of storage iron (Table 98-3). The serum ferritin level is a better indicator of iron overload than the marrow iron stain. However, in addition to storage iron, the marrow iron stain provides information about the effective delivery of iron to developing erythroblasts. Normally, when the marrow smear is stained for iron, 20–40% of developing erythroblasts- called *sideroblasts*- will have visible ferritin granules in their cytoplasm. This represents iron in excess of that needed for hemoglobin synthesis. In states in which release of iron from storage sites is blocked, RE iron will be detectable, and there will be few or no sideroblasts. In the myelodysplastic syndromes, mitochondrial dysfunction can occur, and accumulation of iron in mitochondria appears in a necklace fashion around the nucleus of the erythroblast. Such cells are referred to as *ringed sideroblasts*.

Table 98-3 Iron Store Measurements

Iron Stores	Marrow Iron Stain, 0–4+	Serum Ferritin, µg/L
0	0	<15
1–300 mg	Trace to 1+	15–30
300–800 mg	2+	30–60
800–1000 mg	3+	60–150
1–2 g	4+	>150
Iron overload	-	> 500–1000

Red Cell Protoporphyrin Levels

Protoporphyrin is an intermediate in the pathway to heme synthesis. Under conditions in which heme synthesis is impaired, protoporphyrin accumulates within the red cell. This reflects an inadequate iron supply to erythroid precursors to support hemoglobin synthesis. Normal values are <30 µg/dL of red cells. In iron deficiency, values in excess of 100 µg/dL are seen. The most common causes of increased red cell protoporphyrin levels are absolute or relative iron deficiency and lead poisoning.

Serum Levels of Transferrin Receptor Protein

Because erythroid cells have the highest numbers of transferrin receptors on their surface of any cell in the body, and because transferrin receptor protein (TRP) is released by cells into the circulation, serum levels of TRP reflect the total erythroid marrow mass. Another condition in which TRP levels are elevated is absolute iron deficiency. Normal values are 4–9 µg/L determined by immunoassay. This laboratory test is becoming increasingly available and, along with the serum ferritin, has been proposed to distinguish between iron deficiency and the anemia of chronic inflammation (see below).

Differential Diagnosis

Other than iron deficiency, only three conditions need to be considered in the differential diagnosis of a hypochromic microcytic anemia

(Table 98-4). The first is an inherited defect in globin chain synthesis: the thalassemias. These are differentiated from iron deficiency most readily by serum iron values; normal or increased serum iron levels and transferrin saturation are characteristic of the thalassemias.

Table 98-4 Diagnosis of Microcytic Anemia

Tests	Iron Deficiency	Inflammation	Thalassemia	Sideroblastic Anemia
Smear	Micro/hypo	Normal micro/hypo	Micro/hypo with targeting	Variable
SI	<30	<50	Normal to high	Normal to high
TIBC	>360	<300	Normal	Normal
Percent saturation	<10	10–20	30–80	30–80
Ferritin (μg/L)	<15	30–200	50–300	50–300
Hemoglobin pattern	Normal	Normal	Abnormal	Normal

Note: SI, serum iron; TIBC, total iron-binding capacity.

The second condition is the anemia of chronic inflammation with inadequate iron supply to the erythroid marrow. The distinction between true iron-deficiency anemia and the anemia associated with chronic inflammation is among the most common diagnostic problems encountered by clinicians (see below). Usually the anemia of chronic inflammation is normocytic and normochromic. The iron values usually make the differential diagnosis clear, as the ferritin level is normal or increased and the percent transferrin saturation and TIBC are typically below normal.

Finally, the myelodysplastic syndromes represent the third and least common condition. Occasionally, patients with myelodysplasia have impaired hemoglobin synthesis with mitochondrial dysfunction, resulting in impaired iron incorporation into heme. The iron values again reveal normal stores and more than an adequate supply to the marrow, despite the microcytosis and hypochromia.

Iron-Deficiency Anemia: Treatment

The severity and cause of iron-deficiency anemia will determine the appropriate approach to treatment. As an example, symptomatic elderly patients with severe iron-deficiency anemia and cardiovascular instability may require red cell transfusions. Younger individuals who have compensated for their anemia can be treated more conservatively with iron replacement. The foremost issue for the latter patient is the precise identification of the cause of the iron deficiency.

For the majority of cases of iron deficiency (pregnant women, growing children and adolescents, patients with infrequent episodes of bleeding, and those with inadequate dietary intake of iron), oral iron therapy will suffice. For patients with unusual blood loss or malabsorption, specific diagnostic tests and appropriate therapy take priority. Once the diagnosis of iron-deficiency anemia and its cause is made, there are three major therapeutic approaches.

Red Cell Transfusion

Transfusion therapy is reserved for individuals who have symptoms of anemia, cardiovascular instability, continued and excessive blood loss from whatever source, and require immediate intervention. The management of these patients is less related to the iron deficiency than it is to the consequences of the severe anemia. Not only do transfusions correct the anemia acutely, but the transfused red cells provide a source of iron for reutilization, assuming they are not lost through continued bleeding. Transfusion therapy will stabilize the patient while other options are reviewed.

Oral Iron Therapy

In the asymptomatic patient with established iron-deficiency anemia, treatment with oral iron is usually adequate. Multiple preparations are available, ranging from simple iron salts to complex iron compounds designed for sustained release throughout the small intestine (Table 98-5). While the various preparations contain different amounts of iron, they are generally all absorbed well and are effective in treatment. Some come with other compounds designed to enhance iron absorption, such as ascorbic acid. It is not clear whether the benefits of such compounds justify their costs. Typically, for iron replacement therapy, up to 300 mg of elemental iron per day is given, usually as three or four iron tablets (each containing 50–65 mg elemental iron) given over the course of the day. Ideally, oral iron preparations should be taken on an empty stomach, since foods may inhibit iron absorption. Some patients with gastric disease or prior gastric surgery require special treatment with iron solutions, since the retention capacity of the stomach may be reduced. The retention capacity is necessary for dissolving the shell of the iron tablet before the release of iron. A dose of 200–300 mg of elemental iron per day should result in the absorption of iron up to 50 mg/d. This supports a red cell production level of two to three times normal in an individual with a normally functioning marrow and appropriate erythropoietin stimulus. However, as the hemoglobin level rises, erythropoietin stimulation decreases, and the amount of iron absorbed is reduced. The goal of therapy in individuals with iron-deficiency anemia is not only to repair the anemia, but also to provide stores of at least 0.5–1.0 g of iron. Sustained treatment for a period of 6–12 months after correction of the anemia will be necessary to achieve this.

Table 98-5 Oral Iron Preparations

Generic Name	Tablet (Iron Content), mg	Elixir (Iron Content), mg in 5 mL
Ferrous sulfate	325 (65)	300 (60)
	195 (39)	90 (18)
Extended release	525 (105)	
Ferrous fumarate	325 (107)	
	195 (64)	100 (33)
Ferrous gluconate	325 (39)	300 (35)
Polysaccharide iron	150 (150)	100 (100)
	50 (50)	

Of the complications of oral iron therapy, gastrointestinal distress is the most prominent and is seen in 15–20% of patients. Abdominal pain, nausea, vomiting, or constipation may lead to noncompliance. Although small doses of iron or iron preparations with delayed release may help somewhat, the gastrointestinal side effects are a major impediment to the effective treatment of a number of patients.

The response to iron therapy varies, depending on the erythropoietin (EPO) stimulus and the rate of absorption. Typically, the reticulocyte count should begin to increase within 4–7 days after initiation of therapy and peak at 1½ weeks. The absence of a response may be due to poor absorption, noncompliance (which is common), or a confounding diagnosis. A useful test in the clinic to determine the patient's ability to absorb iron is the *iron tolerance test*. Two iron tablets are given to the patient on an empty stomach, and the serum iron is measured serially over the subsequent 2 hours. Normal absorption will result in an increase in the serum iron of at least 100 μg/dL. If iron deficiency persists despite adequate treatment, it may be necessary to switch to parenteral iron therapy.

Parenteral Iron Therapy

Intravenous iron can be given to patients who are unable to tolerate oral iron; whose needs are relatively acute; or who need iron on an ongoing basis, usually due to persistent gastrointestinal blood loss. Parenteral iron use has been rising rapidly in the last several years with the recognition that recombinant erythropoietin therapy induces a large demand for iron– a demand that frequently cannot be met through the physiologic release of iron from RE sources. The safety of parenteral iron– particularly iron dextran– has been a concern. The serious adverse reaction rate to intravenous iron dextran is 0.7%. Fortunately, newer iron complexes are available in the United States, such as sodium ferric gluconate (Ferrlecit) and iron sucrose (Venofer), that have a much lower rate of adverse effects.

Parenteral iron is used in two ways: one is to administer the total dose of iron required to correct the hemoglobin deficit and provide the patient with at least 500 mg of iron stores; the second is to give repeated small doses of parenteral iron over a protracted period. The latter approach is common in dialysis centers, where it is not unusual for 100 mg of elemental iron to be given weekly for 10 weeks to augment the response to recombinant EPO therapy. The amount of iron needed by an individual patient is calculated by the following formula:

$$\text{Body weight (kg)} \times 2.3 \times (15 - \text{patient's hemoglobin, g/dL}) + 500$$
$$\text{or } 1000 \text{ mg (for stores)}.$$

In administering intravenous iron dextran, anaphylaxis is a concern. Anaphylaxis is much rarer with the newer preparations. The factors that have correlated with an anaphylactic-like reaction include a history of multiple allergies or a prior allergic reaction to dextran (in the case of iron dextran). Generalized symptoms appearing several days after the infusion of a large dose of iron can include arthralgias, skin rash, and low-grade fever. This may be dose-related, but it does not preclude the further use of parenteral iron in the patient. To date, patients with sensitivity to iron dextran have been safely treated with iron gluconate. If a large dose of iron dextran is to be given (>100 mg), the iron preparation should be diluted in 5% dextrose in water or 0.9% NaCl solution. The iron solution can then be infused over a 60- to 90-min period (for larger doses) or at a rate convenient for the attending nurse or physician. While a test dose (25 mg) of parenteral iron dextran is recommended, in reality a slow infusion of a larger dose of parenteral iron solution will afford the same kind of early warning as a separately injected test dose. Early in the infusion of iron, if chest pain, wheezing, a fall in blood pressure, or other systemic symptoms occur, the infusion of iron should be stopped immediately.

Other Hypoproliferative Anemias

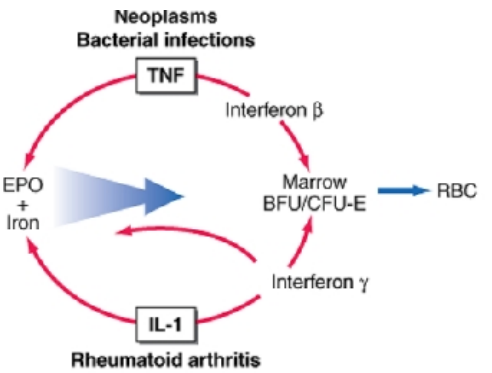
In addition to mild to moderate iron-deficiency anemia, the hypoproliferative anemias can be divided into four categories: (1) chronic inflammation, (2) renal disease, (3) endocrine and nutritional deficiencies (hypometabolic states), and (4) marrow damage (Chap. 102). With chronic inflammation, renal disease, or hypometabolism, endogenous EPO production is inadequate for the degree of anemia observed. For the anemia of chronic inflammation, the erythroid marrow also responds inadequately to stimulation, due in part to defects in *iron reutilization*. As a result of the lack of adequate EPO stimulation, an examination of the peripheral blood smear will disclose only an occasional polychromatophilic ("shift") reticulocyte. In cases of iron deficiency or marrow damage, appropriate elevations in endogenous EPO levels are typically found, and shift reticulocytes will be present on the blood smear.

Anemia of Acute and Chronic Inflammation/Infection (the Anemia of Chronic Disease)

The anemia of chronic disease– which encompasses inflammation, infection, tissue injury, and conditions (such as cancer) associated with the release of proinflammatory cytokines– is one of the most common forms of anemia seen clinically and probably the most important in the differential diagnosis of iron deficiency, since many of the features of the anemia are brought about by inadequate iron delivery to the marrow, despite the presence of normal or increased iron stores. This is reflected by a low serum iron, increased red cell protoporphyrin, a hypoproliferative marrow, transferrin saturation in the range of 15–20%, and a normal or increased serum ferritin. The serum ferritin values

are often the most distinguishing feature between true iron-deficiency anemia and the iron-deficient erythropoiesis associated with inflammation. Typically, serum ferritin values increase threefold over basal levels in the face of inflammation. All of these changes are due to the effects of inflammatory cytokines and hepcidin, the key iron regulatory hormone, acting at several levels of erythropoiesis (Fig. 98-4).

Figure 98-4



Suppression of erythropoiesis by inflammatory cytokines. Through the release of tumor necrosis factor (TNF) and interferon γ (IFN-γ), neoplasms and bacterial infections suppress erythropoietin (EPO) production and the proliferation of erythroid progenitors [erythroid burst-forming units and erythroid colony-forming units (BFU/CFU-E)]. The mediators in patients with vasculitis and rheumatoid arthritis include interleukin 1 (IL-1) and IFN-γ. The red arrows indicate sites of inflammatory cytokine inhibitory effects.

Interleukin 1 (IL-1) directly decreases EPO production in response to anemia. IL-1, acting through accessory cell release of interferon γ (IFN-γ), suppresses the response of the erythroid marrow to EPO– an effect that can be overcome by EPO administration in vitro and in vivo. In addition, tumor necrosis factor (TNF), acting through the release of IFN-γ by marrow stromal cells, also suppresses the response to EPO. Hepcidin, made by the liver, is increased in inflammation and acts to suppress iron absorption and iron release from storage sites. The overall result is a chronic hypoproliferative anemia with classic changes in iron metabolism. The anemia is further compounded by a mild to moderate shortening in red cell survival.

With chronic inflammation, the primary disease will determine the severity and characteristics of the anemia. For instance, many patients with cancer also have anemia that is typically normocytic and normochromic. In contrast, patients with long-standing active rheumatoid arthritis or chronic infections such as tuberculosis will have a microcytic, hypochromic anemia. In both cases, the bone marrow is hypoproliferative, but the differences in red cell indices reflect differences in the availability of iron for hemoglobin synthesis. Occasionally, conditions associated with chronic inflammation are also associated with chronic blood loss. Under these circumstances, a bone marrow aspirate stained for iron may be necessary to rule out absolute iron deficiency. However, the administration of iron in this case will correct the iron deficiency component of the anemia and leave the inflammatory component unaffected.

The anemia associated with acute infection or inflammation is typically mild but becomes more pronounced over time. Acute infection can produce a fall in hemoglobin levels of 2–3 g/dL within 1 or 2 days; this is largely related to the hemolysis of red cells near the end of their natural life span. The fever and cytokines released exert a selective pressure against cells with more limited capacity to maintain the red cell membrane. In most individuals the mild anemia is reasonably well tolerated, and symptoms, if present, are associated with the underlying disease. Occasionally, in patients with preexisting cardiac disease, moderate anemia (hemoglobin 10–11 g/dL) may be associated with angina, exercise intolerance, and shortness of breath. The erythropoietic profile that distinguishes the anemia of inflammation from the other causes of hypoproliferative anemias is shown in Table 98-6.

Table 98-6 Diagnosis of Hypoproliferative Anemias

Tests	Iron Deficiency	Inflammation	Renal Disease	Hypometabolic States
Anemia	Mild to severe	Mild	Mild to severe	Mild
MCV (fL)	60–90	80–90	90	90
Morphology	Normo-microcytic	Normocytic	Normocytic	Normocytic
SI	<30	<50	Normal	Normal
TIBC	>360	<300	Normal	Normal
Saturation (%)	<10	10–20	Normal	Normal
Serum ferritin (μg/L)	<15	30–200	115–150	Normal

Iron stores	0	2–4+	1–4+	Normal

Note: MCV, mean corpuscular volume; SI, serum iron; TIBC, total iron-binding capacity.

Anemia of Renal Disease

Chronic renal failure is usually associated with a moderate to severe hypoproliferative anemia; the level of the anemia correlates with the severity of the renal failure. Red cells are typically normocytic and normochromic, and reticulocytes are decreased. The anemia is primarily due to a failure to produce adequate amounts of EPO and a reduction in red cell survival. In certain forms of acute renal failure, the correlation between the anemia and renal function is weaker. Patients with the hemolytic-uremic syndrome increase erythropoiesis in response to the hemolysis, despite renal failure requiring dialysis. Polycystic kidney disease also shows a smaller degree of EPO deficiency for a given level of renal failure. By contrast, patients with diabetes or myeloma have more severe EPO deficiency for a given level of renal failure.

Assessment of iron status provides information to distinguish the anemia of renal disease from the other forms of hypoproliferative anemia (Table 98-6) and to guide management. Patients with the anemia of renal disease usually present with normal serum iron, TIBC, and ferritin levels. However, those maintained on chronic hemodialysis may develop iron deficiency from blood loss through the dialysis procedure. Iron must be replenished in these patients to ensure an adequate response to EPO therapy (see below).

Anemia in Hypometabolic States

Patients who are starving, particularly for protein, and those with a variety of endocrine disorders that produce lower metabolic rates, may develop a mild to moderate hypoproliferative anemia. The release of EPO from the kidney is sensitive to the need for O_2, not just O_2 levels. Thus, EPO production is triggered at lower levels of blood O_2 content in disease states (such as hypothyroidism and starvation) where metabolic activity, and thus O_2 demand, is decreased.

Endocrine Deficiency States

The difference in the levels of hemoglobin between men and women is related to the effects of androgen and estrogen on erythropoiesis. Testosterone and anabolic steroids augment erythropoiesis; castration and estrogen administration to males decrease erythropoiesis. Patients who are hypothyroid or have deficits in pituitary hormones also may develop a mild anemia. Pathogenesis may be complicated by other nutritional deficiencies since iron and folic acid absorption can be affected by these disorders. Usually, correction of the hormone deficiency reverses the anemia.

Anemia may be more severe in Addison's disease, depending on the level of thyroid and androgen hormone dysfunction; however, anemia may be masked by decreases in plasma volume. Once such patients are given cortisol and volume replacement, the hemoglobin level may fall rapidly. Mild anemia complicating hyperparathyroidism may be due to decreased EPO production as a consequence of the renal effects of hypercalcemia or to impaired proliferation of erythroid progenitors.

Protein Starvation

Decreased dietary intake of protein may lead to mild to moderate hypoproliferative anemia; this form of anemia may be prevalent in the elderly. The anemia can be more severe in patients with a greater degree of starvation. In marasmus, where patients are both protein- and calorie-deficient, the release of EPO is impaired in proportion to the reduction in metabolic rate; however, the degree of anemia may be masked by volume depletion and becomes apparent after refeeding. Deficiencies in other nutrients (iron, folate) may also complicate the clinical picture but may not be apparent at diagnosis. Changes in the erythrocyte indices on refeeding should prompt evaluation of iron, folate, and B_{12} status.

Anemia in Liver Disease

A mild hypoproliferative anemia may develop in patients with chronic liver disease from nearly any cause. The peripheral blood smear may show spur cells and stomatocytes from the accumulation of excess cholesterol in the membrane from a deficiency of lecithin cholesterol acyltransferase. Red cell survival is shortened, and the production of EPO is inadequate to compensate. In alcoholic liver disease, nutritional deficiencies are common and complicate the management. Folate deficiency from inadequate intake, as well as iron deficiency from blood loss and inadequate intake, can alter the red cell indices.

Hypoproliferative Anemias: Treatment

Many patients with hypoproliferative anemias experience recovery of normal hemoglobin levels when the underlying disease is appropriately treated. For those in whom such reversals are not possible- such as patients with end-stage kidney disease, cancer, and chronic inflammatory diseases- symptomatic anemia requires treatment. The two major forms of treatment are transfusions and EPO.

Transfusions

Thresholds for transfusion should be altered based on the patient's symptoms. In general, patients without serious underlying cardiovascular or pulmonary disease can tolerate hemoglobin levels above 8 g/dL and do not require intervention until the hemoglobin falls below that level. Patients with more physiologic compromise may need to have their hemoglobin levels kept above 11 g/dL. A typical unit of packed

red cells increases the hemoglobin level by 1 g/dL. Transfusions are associated with certain infectious risks (Chap. 107), and chronic transfusions can produce iron overload. Importantly, the liberal use of blood has been associated with increased morbidity and mortality, particularly in the intensive care setting. Therefore, in the absence of documented tissue hypoxia, a conservative approach to the use of red cell transfusions is preferable.

Erythropoietin (Epo)

EPO is particularly useful in anemias in which endogenous EPO levels are inappropriately low, such as the hypoproliferative anemias. Iron status must be evaluated and iron repleted to obtain optimal effects from EPO. In patients with chronic renal failure, the usual dose of EPO is 50–150 U/kg three times a week intravenously. Hemoglobin levels of 10–12 g/dL are usually reached within 4–6 weeks if iron levels are adequate; 90% of these patients respond. Once a target hemoglobin level is achieved, the EPO dose can be decreased. A fall in hemoglobin level occurring in the face of EPO therapy usually signifies the development of an infection or iron depletion. Aluminum toxicity and hyperparathyroidism can also compromise the EPO response. When an infection intervenes, it is best to interrupt the EPO therapy and rely on transfusion to correct the anemia until the infection is adequately treated. The dose needed to correct the anemia in patients with cancer is higher, up to 300 U/kg three times a week, and only about 60% of patients respond.

Longer-acting preparations of EPO can reduce the frequency of injections. Darbepoetin alfa, a molecularly modified EPO with additional carbohydrate, has a half-life in the circulation that is 3–4 times longer than epoetin alfa, permitting weekly or every other week dosing.

Acknowledgment

Dr. Robert S. Hillman was the author of this chapter in the 14th edition, and material from his chapter has been retained.

Further Readings

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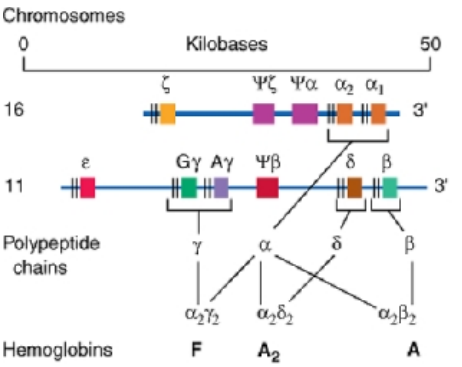
Disorders of Hemoglobin: Introduction

Hemoglobin is critical for normal oxygen delivery to tissues; it is also present in erythrocytes in such high concentrations that it can alter red cell shape, deformability, and viscosity. Hemoglobinopathies are disorders affecting the structure, function, or production of hemoglobin. These conditions are usually inherited and range in severity from asymptomatic laboratory abnormalities to death in utero. Different forms may present as hemolytic anemia, erythrocytosis, cyanosis, or vasoocclusive stigmata.

Hemoglobin Structure

Different hemoglobins are produced during embryonic, fetal, and adult life (Fig. 99-1). Each consists of a tetramer of globin polypeptide chains: a pair of α-like chains 141 amino acids long and a pair of β-like chains 146 amino acids long. The major adult hemoglobin, HbA, has the structure $\alpha_2\beta_2$. HbF ($\alpha_2\gamma_2$) predominates during most of gestation, and HbA_2 ($\alpha_2\delta_2$) is minor adult hemoglobin. Embryonic hemoglobins need not be considered here.

Figure 99-1



The globin genes. The α-like genes (α, ζ) are encoded on chromosome 16; the β-like genes (β, γ, δ, ε) are encoded on chromosome 11. The ζ and ε genes encode embryonic globins.

Each globin chain enfolds a single heme moiety, consisting of a protoporphyrin IX ring complexed with a single iron atom in the ferrous state (Fe^{2+}). Each heme moiety can bind a single oxygen molecule; a molecule of hemoglobin can transport up to four oxygen molecules.

The amino acid sequences of the various globins are highly homologous to one another. Each has a highly helical *secondary structure*. Their globular *tertiary structures* can cause the exterior surfaces to be rich in polar (hydrophilic) amino acids that enhance solubility and the interior to be lined with nonpolar groups, forming a hydrophobic pocket into which heme is inserted. The tetrameric *quaternary structure* of HbA contains two αβ dimers. Numerous tight interactions (i.e., $\alpha_1\beta_1$ contacts) hold the α and β chains together. The complete tetramer is held together by interfaces (i.e., $\alpha_1\beta_2$ contacts) between the α-like chain of one dimer and the non-α chain of the other dimer.

The hemoglobin tetramer is highly soluble but individual globin chains are insoluble. Unpaired globin precipitates, forming inclusions that damage the cell. Normal globin chain synthesis is balanced so that each newly synthesized α or non-α globin chain will have an available partner with which to pair.

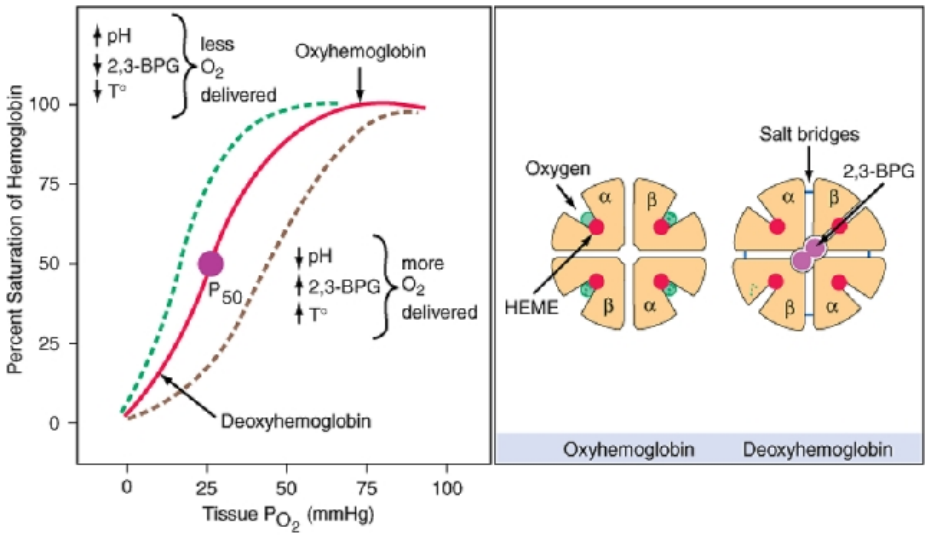
Solubility and reversible oxygen binding are the key properties deranged in hemoglobinopathies. Both depend most on the hydrophilic surface amino acids, the hydrophobic amino acids lining the heme pocket, a key histidine in the F helix, and the amino acids forming the $\alpha_1\beta_1$ and $\alpha_1\beta_2$ contact points. Mutations in these strategic regions tend to be the ones that alter clinical behavior.

Function of Hemoglobin

To support oxygen transport, hemoglobin must bind O_2 efficiently at the partial pressure of oxygen (P_{O_2}) of the alveolus, retain it, and release it to tissues at the P_{O_2} of tissue capillary beds. Oxygen acquisition and delivery over a relatively narrow range of oxygen tensions depend on a property inherent in the tetrameric arrangement of heme and globin subunits within the hemoglobin molecule called *cooperativity* or *heme-heme interaction*.

At low oxygen tensions, the hemoglobin tetramer is fully deoxygenated (Fig. 99-2). Oxygen binding begins slowly as O_2 tension rises. However, as soon as some oxygen has been bound by the tetramer, an abrupt increase occurs in the slope of the curve. Thus, hemoglobin molecules that have bound some oxygen develop a higher oxygen affinity, greatly accelerating their ability to combine with more oxygen. This S-shaped oxygen equilibrium curve (Fig. 99-2), along which substantial amounts of oxygen loading *and unloading* can occur over a narrow range of oxygen tensions, is physiologically more useful than the high-affinity hyperbolic curve of individual monomers.

Figure 99-2



Hemoglobin-oxygen dissociation curve. The hemoglobin tetramer can bind up to four molecules of oxygen in the iron-containing sites of the heme molecules. As oxygen is bound, 2,3-BPG and CO_2 are expelled. Salt bridges are broken, and each of the globin molecules changes its conformation to facilitate oxygen binding. Oxygen release to the tissues is the reverse process, salt bridges being formed and 2,3-BPG and CO_2 bound. Deoxyhemoglobin does not bind oxygen efficiently until the cell returns to conditions of higher pH, the most important modulator of O_2 affinity (Bohr effect). When acid is produced in the tissues, the dissociation curve shifts to the right, facilitating oxygen release and CO_2 binding. Alkalosis has the opposite effect, reducing oxygen delivery.

Oxygen affinity is modulated by several factors. The Bohr effect is the ability of hemoglobin to deliver more oxygen to tissues at low pH. It arises from the stabilizing action of protons on deoxyhemoglobin, which binds protons more readily than oxyhemoglobin because it is a weaker acid (Fig. 99-2). Thus, hemoglobin has a lower oxygen affinity at low pH. The major small molecule that alters oxygen affinity in humans is 2,3-bisphosphoglycerate (2,3-BPG, formerly 2,3-DPG), which lowers oxygen affinity when bound to hemoglobin. HbA has a reasonably high affinity for 2,3-BPG. HbF does not bind 2,3-BPG, so it tends to have a higher oxygen affinity in vivo. Hemoglobin also binds nitric oxide reversibly; this interaction may influence vascular tone, but its physiologic relevance remains unclear.

Proper oxygen transport depends on the tetrameric structure of the proteins, the proper arrangement of the charged amino acids, and interaction with protons or 2,3-BPG.

Developmental Biology of Human Hemoglobins

Red cells first appearing at about 6 weeks after conception contain the embryonic hemoglobins Hb Portland ($\zeta_2\gamma_2$), Hb Gower I ($\zeta_2\varepsilon_2$), and Hb Gower II ($\alpha_2\varepsilon_2$). At 10–11 weeks, fetal hemoglobin (HbF; $\alpha_2\gamma_2$) becomes predominant. The switch to nearly exclusive synthesis of adult hemoglobin (HbA; $\alpha_2\beta_2$) occurs at about 38 weeks (Fig. 99-1). Fetuses and newborns therefore require α-globin but not β-globin for normal gestation. Small amounts of HbF are produced during postnatal life. A few red cell clones called *F cells* are progeny of a small pool of immature committed erythroid precursors (BFU-e) that retain the ability to produce HbF. Profound erythroid stresses, such as severe hemolytic anemias, bone marrow transplant, or cancer chemotherapy, cause more of the F-potent BFU-e to be recruited. HbF levels thus tend to rise in some patients with sickle cell anemia or thalassemia. This phenomenon is also important because it probably explains the ability of hydroxyurea to increase levels of HbF in adults. Agents such as butyrate that inhibit histone deacetylase and modify chromatin structure can also activate fetal globin genes partially after birth.

Genetics and Biosynthesis of Human Hemoglobin

The human hemoglobins are encoded in two tightly linked gene clusters; the α-like globin genes are clustered on chromosome 16, and the β-like genes on chromosome 11 (Fig. 99-1). The α-like cluster consists of two α-globin genes and a single copy of the ζ gene. The non-α gene cluster consists of a single ε gene, the G γ and A γ fetal globin genes, and the adult δ and β genes.

Important regulatory sequences flank each gene. Immediately upstream are typical promoter elements needed for the assembly of the transcription initiation complex. Sequences in the 5' flanking region of the γ and the β genes appear to be crucial for the correct developmental regulation of these genes, while elements that function like classic enhancers and silencers are in the 3' flanking regions. The locus control region (LCR) elements located far upstream appear to control the overall level of expression of each cluster. These elements achieve their regulatory effects by interacting with *trans*-acting transcription factors. Some of these factors are ubiquitous (e.g., Sp1 and YY1), while others are more or less limited to erythroid cells or hematopoietic cells (e.g., GATA-1, NFE-2, and EKLF). The LCR controlling the α-globin gene cluster is modulated by a SWI/SNF-like protein called *ATRX*; this protein appears to influence chromatin remodeling and DNA methylation. The association of α thalassemia with mental retardation and myelodysplasia in some families appears to be related to mutations in the ATRX pathway. This pathway also modulates genes specifically expressed during erythropoiesis, such as those that encode the enzymes for heme biosynthesis. Normal red blood cell (RBC) differentiation requires the coordinated expression of

the globin genes with the genes responsible for heme and iron metabolism. RBC precursors contain a protein, α-hemoglobin stabilizing protein (AHSP), that enhances the folding and solubility of α globin, which is otherwise easily denatured, leading to insoluble precipitates. These precipitates play an important role in the thalassemia syndromes and certain unstable hemoglobin disorders. Polymorphic variation in the amounts and/or functional capacity of AHSP might explain some of the clinical variability seen in patients inheriting identical thalassemia mutations. AHSP may be a therapeutic target, particularly in syndromes of intermediate severity.

Classes

There are five major classes of hemoglobinopathies (Table 99-1). *Structural hemoglobinopathies* occur when mutations alter the amino acid sequence of a globin chain, altering the physiologic properties of the variant hemoglobins and producing the characteristic clinical abnormalities. The most clinically relevant variant hemoglobins polymerize abnormally, as in sickle cell anemia, or exhibit altered solubility or oxygen-binding affinity. *Thalassemia syndromes* arise from mutations that impair production or translation of globin mRNA, leading to deficient globin chain biosynthesis. Clinical abnormalities are attributable to the inadequate supply of hemoglobin and the imbalances in the production of individual globin chains, leading to premature destruction of erythroblasts and RBC. *Thalassemic hemoglobin variants* combine features of thalassemia (e.g., abnormal globin biosynthesis) and of structural hemoglobinopathies (e.g., an abnormal amino acid sequence). *Hereditary persistence of fetal hemoglobin* (HPFH) is characterized by synthesis of high levels of fetal hemoglobin in adult life. *Acquired hemoglobinopathies* include modifications of the hemoglobin molecule by toxins (e.g., acquired methemoglobinemia) and abnormal hemoglobin synthesis (e.g., high levels of HbF production in preleukemia and α thalassemia in myeloproliferative disorders).

Table 99-1 Classification of Hemoglobinopathies

I. Structural hemoglobinopathies– hemoglobins with altered amino acid sequences that result in deranged function or altered physical or chemical properties

A. Abnormal hemoglobin polymerization– HbS, hemoglobin sickling

B. Altered O_2 affinity

1. High affinity– polycythemia

2. Low affinity– cyanosis, pseudoanemia

C. Hemoglobins that oxidize readily

1. Unstable hemoglobins– hemolytic anemia, jaundice

2. M hemoglobins– methemoglobinemia, cyanosis

II. Thalassemias– defective biosynthesis of globin chains

A. α Thalassemias

B. β Thalassemias

C. αβ, ταβ, μβ Thalassemias

III. Thalassemic hemoglobin variants– structurally abnormal Hb associated with co-inherited thalassemic phenotype

A. HbE

B. Hb Constant Spring

C. Hb Lepore

IV. Hereditary persistence of fetal hemoglobin– persistence of high levels of HbF into adult life

V. Acquired hemoglobinopathies

A. Methemoglobin due to toxic exposures

B. Sulfhemoglobin due to toxic exposures

C. Carboxyhemoglobin

D. HbH in erythroleukemia

E. Elevated HbF in states of erythroid stress and bone marrow dysplasia

Epidemiology

Hemoglobinopathies are especially common in areas in which malaria is endemic. This clustering of hemoglobinopathies is assumed to reflect a selective survival advantage for the abnormal RBC, which presumably provide a less hospitable environment during the obligate

RBC stages of the parasitic life cycle. Very young children with α thalassemia are *more* susceptible to infection with the nonlethal *Plasmodium vivax*. Thalassemia might then favor a natural protection against infection with the more lethal *P. falciparum*.

Thalassemias are the most common genetic disorders in the world, affecting nearly 200 million people worldwide. About 15% of American blacks are silent carriers for α thalassemia; α-thalassemia trait (minor) occurs in 3% of American blacks and in 1–15% of persons of Mediterranean origin. β Thalassemia has a 10–15% incidence in individuals from the Mediterranean and Southeast Asia and 0.8% in American blacks. The number of severe cases of thalassemia in the United States is about 1000. Sickle cell disease is the most common structural hemoglobinopathy occurring in heterozygous form in ~8% of American blacks and in homozygous form in 1 in 400. Between 2 and 3% of American blacks carry a hemoglobin C allele.

Inheritance and Ontogeny

Hemoglobinopathies are autosomal co-dominant traits– compound heterozygotes who inherit a different abnormal mutant allele from each parent exhibit composite features of each. For example, patients inheriting sickle β thalassemia exhibit features of β thalassemia and sickle cell anemia. The α chain is present in HbA, HbA_2, and HbF; α-chain mutations thus cause abnormalities in all three. The α-globin hemoglobinopathies are symptomatic in utero and after birth because normal function of the α-globin gene is required throughout gestation and adult life. In contrast, infants with β-globin hemoglobinopathies tend to be asymptomatic until 3–9 months of age, when HbA has largely replaced HbF.

Detection and Characterization of Hemoglobinopathies– General Methods

Of the many methods available for hemoglobin analysis, electrophoretic techniques are used for routine clinical purposes. Electrophoresis at pH 8.6 on cellulose acetate membranes is especially simple, inexpensive, and reliable for initial screening. Agar gel electrophoresis at pH 6.1 in citrate buffer is often used as a complementary method because each method detects different variants. Comparison of results obtained in each system usually allows unambiguous diagnosis, but some important variants are electrophoretically silent. These mutant hemoglobins can usually be characterized by more specialized techniques such as isoelectric focusing and/or high-pressure liquid chromatography (HPLC).

Quantitation of the hemoglobin profile is often desirable. HbA_2 is frequently elevated in β-thalassemia trait and depressed in iron deficiency. HbF is elevated in HPFH, some β-thalassemia syndromes, and occasional periods of erythroid stress or marrow dysplasia. For characterization of sickle cell trait, sickle thalassemia syndromes, or HbSC disease, and for monitoring the progress of exchange transfusion therapy to lower the percentage of circulating HbS, quantitation of individual hemoglobins is also required. In most laboratories, quantitation is performed only if the test is specifically ordered.

Because some variants can comigrate with HbA or HbS (sickle hemoglobin), electrophoretic assessment should always be regarded as incomplete unless functional assays for hemoglobin sickling, solubility, or oxygen affinity are also performed, as dictated by the clinical presentation. The best sickling assays involve measurement of the degree to which the hemoglobin sample becomes insoluble, or gelated, as it is deoxygenated (i.e., sickle solubility test). Unstable hemoglobins are detected by their precipitation in isopropanol or after heating to 50°C. High-O_2 affinity and low-O_2 affinity variants are detected by quantitating the P_{50}, the partial pressure of oxygen at which the hemoglobin sample becomes 50% saturated with oxygen. Direct tests for the percent carboxyhemoglobin and methemoglobin, employing spectrophotometric techniques, can readily be obtained from most clinical laboratories on an urgent basis.

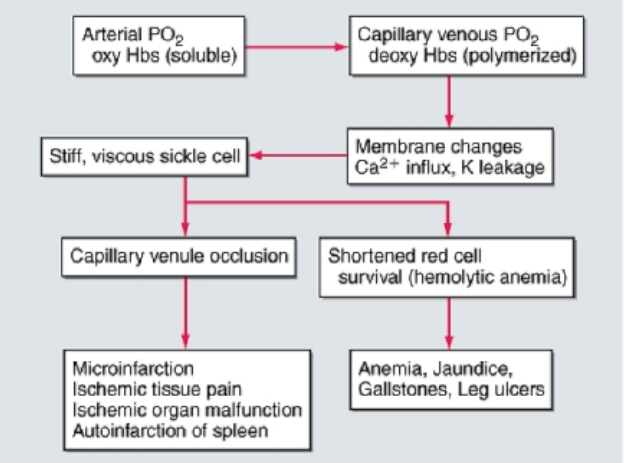
Complete characterization, including amino acid sequencing or gene cloning and sequencing, is available from several investigational laboratories around the world. Polymerase chain reaction (PCR), allele-specific oligonucleotide hybridization, and automated DNA sequencing allow identification of globin gene mutations in a few days.

Laboratory evaluation remains an adjunct, rather than the primary diagnostic aid. Diagnosis is best established by recognition of a characteristic history, physical findings, peripheral blood smear morphology, and abnormalities of the complete blood cell count (e.g., profound microcytosis with minimal anemia in thalassemia trait).

Sickle Cell Syndromes

The sickle cell syndromes are caused by a mutation in the β-globin gene that changes the sixth amino acid from glutamic acid to valine. HbS ($\alpha_2\beta_2^{6\ Glu\rightarrow Val}$) polymerizes reversibly when deoxygenated to form a gelatinous network of fibrous polymers that stiffen the RBC membrane, increase viscosity, and cause dehydration due to potassium leakage and calcium influx (Fig. 99-3). These changes also produce the sickle shape. Sickled cells lose the pliability needed to traverse small capillaries. They possess altered sticky membranes (especially reticulocytes) that are abnormally adherent to the endothelium of small venules. These abnormalities provoke unpredictable episodes of microvascular vasoocclusion and premature RBC destruction (hemolytic anemia). Hemolysis occurs because the spleen destroys the abnormal RBC. The rigid adherent cells also clog small capillaries and venules, causing tissue ischemia, acute pain, and gradual end-organ damage. This venoocclusive component usually dominates the clinical course. Prominent manifestations include episodes of ischemic pain (i.e., painful crises) and ischemic malfunction or frank infarction in the spleen, central nervous system, bones, liver, kidneys, and lungs (Fig. 99-3).

Figure 99-3



Pathophysiology of sickle cell crisis.

Several sickle syndromes occur as the result of inheritance of HbS from one parent and another hemoglobinopathy, such as β thalassemia or HbC ($\alpha_2\beta_2^{6\ Glu\rightarrow Lys}$), from the other parent. The prototype disease, sickle cell anemia, is the homozygous state for HbS (Table 99-2).

Table 99-2 Clinical Features of Sickle Hemoglobinopathies

Condition	Clinical Abnormalities	Hemoglobin Level g/L (g/dL)	MCV, fL	Hemoglobin Electrophoresis
Sickle cell trait	None; rare painless hematuria	Normal	Normal	Hb S/A:40/60
Sickle cell anemia	Vasoocclusive crises with infarction of spleen, brain, marrow, kidney, lung; aseptic necrosis of bone; gallstones; priapism; ankle ulcers	70–100 (7–10)	80–100	Hb S/A:100/0 Hb F:2–25%
S/β^0 thalassemia	Vasoocclusive crises; aseptic necrosis of bone	70–100 (7–10)	60–80	Hb S/A:100/0 Hb F:1–10%
S/β^+ thalassemia	Rare crises and aseptic necrosis	100–140 (10–14)	70–80	Hb S/A:60/40
Hemoglobin SC	Rare crises and aseptic necrosis; painless hematuria	100–140 (10–14)	80–100	Hb S/A:50/0 Hb C:50%

Clinical Manifestations of Sickle Cell Anemia

Most patients with sickling syndromes suffer from hemolytic anemia, with hematocrits from 15–30%, and significant reticulocytosis. Anemia was once thought to exert protective effects against vasoocclusion by reducing blood viscosity. However, natural history and drug therapy trials suggest that an *increase* in the hematocrit and feedback inhibition of reticulocytosis might be beneficial, even at the expense of increased blood viscosity. The role of adhesive reticulocytes in vasoocclusion might account for these paradoxical effects.

Granulocytosis is common. The white count can fluctuate substantially and unpredictably during and between painful crises, infectious episodes, and other intercurrent illnesses.

Vasoocclusion causes protean manifestations. Intermittent episodes of vasoocclusion in connective and musculoskeletal structures produce painful ischemia manifested by acute pain and tenderness, fever, tachycardia, and anxiety. These recurrent episodes, called *painful crises*, are the most common clinical manifestation. Their frequency and severity vary greatly. Pain can develop almost anywhere in the body and may last from a few hours to 2 weeks. Repeated crises requiring hospitalization (>3 per year) correlate with reduced survival in adult life, suggesting that these episodes are associated with accumulation of chronic end-organ damage. Provocative factors include infection, fever, excessive exercise, anxiety, abrupt changes in temperature, hypoxia, or hypertonic dyes.

Repeated micro-infarction can destroy tissues having microvascular beds that promote sickling. Thus, the spleen is frequently lost within the first 18–36 months of life, causing susceptibility to infection, particularly by pneumococci. Acute venous obstruction of the spleen (*splenic sequestration crisis*), a rare occurrence in early childhood, may require emergency transfusion and/or splenectomy to prevent trapping of the entire arterial output in the obstructed spleen. Occlusion of retinal vessels can produce hemorrhage, neovascularization, and eventual detachments. Renal papillary necrosis invariably produces isosthenuria. More widespread renal necrosis leads to renal failure in adults, a common late cause of death. Bone and joint ischemia can lead to aseptic necrosis, especially of the femoral or humeral heads; chronic arthropathy; and unusual susceptibility to osteomyelitis, which may be caused by organisms, such as *Salmonella*, rarely encountered in other settings. The *hand-foot syndrome* is caused by painful infarcts of the digits and dactylitis. Stroke is especially common in children; a small subset tend to suffer repeated episodes. Stroke is less common in adults and is often hemorrhagic. A particularly painful complication in males is priapism, due to infarction of the penile venous outflow tracts; permanent impotence is a frequent consequence. Chronic lower leg ulcers probably arise from ischemia and superinfection in the distal circulation.

Acute chest syndrome is a distinctive manifestation characterized by chest pain, tachypnea, fever, cough, and arterial oxygen desaturation. It can mimic pneumonia, pulmonary emboli, bone marrow infarction and embolism, myocardial ischemia, or in situ lung infarction. Acute chest syndrome is thought to reflect in situ sickling within the lung producing pain and temporary pulmonary dysfunction. Often it is difficult or impossible to distinguish among other possibilities. Pulmonary infarction and pneumonia are the most frequent underlying or concomitant conditions in patients with this syndrome. Repeated episodes of acute chest pain correlate with reduced survival. Acutely, reduction in arterial oxygen saturation is especially ominous because it promotes sickling on a massive scale. Chronic acute or subacute pulmonary crises lead to pulmonary hypertension and cor pulmonale, an increasingly common cause of death as patients survive longer.

Sickle cell syndromes are remarkable for their clinical heterogeneity. Some patients remain virtually asymptomatic into or even through adult life, while others suffer repeated crises requiring hospitalization from early childhood. Patients with sickle thalassemia and sickle-HbE tend to have similar, slightly milder, symptoms, perhaps because of the ameliorating effects of production of other hemoglobins within the RBC. Hemoglobin SC disease, one of the more common variants of sickle cell anemia, is frequently marked by lesser degrees of hemolytic anemia and a greater propensity for the development of retinopathy and aseptic necrosis of bones. In most respects, however, the clinical manifestations resemble sickle cell anemia. Some rare hemoglobin variants actually aggravate the sickling phenomenon. The clinical variability in different patients inheriting the same disease-causing mutation (sickle hemoglobin) has made sickle cell disease the focus of efforts to identify modifying genetic polymorphisms in other genes that might account for the heterogeneity. To date, these genome screening efforts have not yielded modifying genes, other than those known to affect the hemoglobin profile directly: e.g., persistence of fetal hemoglobin in adult life, α thalassemia, or co-inheritance of other hemoglobin structural variants. The complexity of the data obtained thus far undermines the expectation that genome-wide analysis will yield individualized profiles that predict a patient's clinical course.

Nevertheless, a number of interesting patterns have emerged from these modifying gene analyses. For example, genes affecting the inflammatory response or cytokine expression appear to be modifying candidates. Genes that affect transcriptional regulation of lymphocytes may be involved. Thus, it appears likely that key polymorphic changes in the patient's inflammatory response to the damages provoked by sickle red cells or in the response to chronic or recurrent infections may prove to be important for prognosticating the clinical severity of disease.

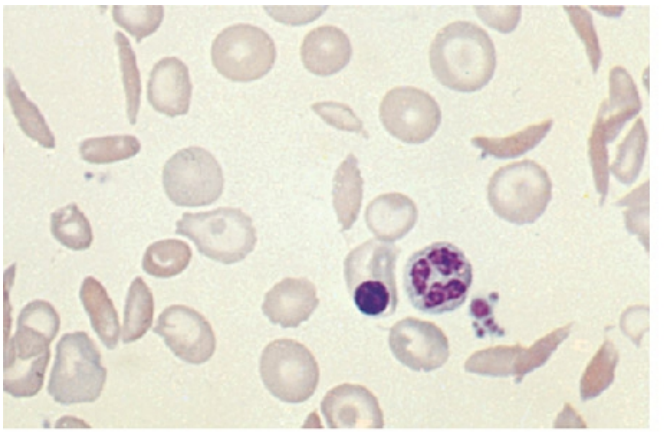
Clinical Manifestations of Sickle Cell Trait

Sickle cell trait is usually asymptomatic. Anemia and painful crises are exceedingly rare. An uncommon but highly distinctive symptom is painless hematuria often occurring in adolescent males, probably due to papillary necrosis. Isosthenuria is a more common manifestation of the same process. Sloughing of papillae with urethral obstruction has been reported, as have isolated cases of massive sickling or sudden death due to exposure to high altitudes or extremes of exercise and dehydration.

Diagnosis

Sickle cell syndromes are suspected on the basis of hemolytic anemia, RBC morphology (Fig. 99-4), and intermittent episodes of ischemic pain. Diagnosis is confirmed by hemoglobin electrophoresis and the sickling tests already discussed. Thorough characterization of the exact hemoglobin profile of the patient is important, because sickle thalassemia and hemoglobin SC disease have distinct prognoses or clinical features. Diagnosis is usually established in childhood, but occasional patients, often with compound heterozygous states, do not develop symptoms until the onset of puberty, pregnancy, or early adult life. Genotyping of family members and potential parental partners is critical for genetic counseling. Details of the childhood history establish prognosis and need for aggressive or experimental therapies. Factors associated with increased morbidity and reduced survival are more than three crises requiring hospitalization per year, chronic neutrophilia, a history of splenic sequestration or hand-foot syndrome, and second episodes of acute chest syndrome. Patients with a history of cerebrovascular accidents are at higher risk for repeated episodes and require especially close monitoring using Doppler carotid flow measurements. Patients with severe or repeated episodes of acute chest syndrome may need lifelong transfusion support, utilizing partial exchange transfusion, if possible.

Figure 99-4



Sickle cell anemia. The elongated and crescent-shaped red blood cells seen on this smear represent circulating irreversibly sickled cells. Target cells and a nucleated red blood cell are also seen.

Sickle Cell Syndromes: Treatment

Patients with sickle cell syndromes require ongoing continuity of care. Familiarity with the pattern of symptoms provides the best safeguard against excessive use of the emergency room, hospitalization, and habituation to addictive narcotics. Additional preventive measures include regular slit-lamp examinations to monitor development of retinopathy; antibiotic prophylaxis appropriate for splenectomized patients during dental or other invasive procedures; and vigorous oral hydration during or in anticipation of periods of extreme exercise, exposure to heat or cold, emotional stress, or infection. Pneumococcal and *Haemophilus influenzae* vaccines are less effective in splenectomized individuals. Thus, patients with sickle cell anemia should be vaccinated early in life.

The management of acute painful crisis includes vigorous hydration, thorough evaluation for underlying causes (such as infection), and aggressive analgesia administered by a standing order and/or patient-controlled analgesia (PCA) pump. Morphine (0.1–0.15 mg/kg every 3–4 h) or meperidine (0.75–1.5 mg/kg every 2–4 h) should control severe pain. Meperidine should be used only for acute short-term pain control; as a chronic analgesic, it is unsuitable. Bone pain may respond as well to ketorolac (30–60 mg initial dose, then 15–30 mg every 6–8 h). Inhalation of nitrous oxide can provide short-term pain relief, but great care must be exercised to avoid hypoxia and respiratory depression. Nitrous oxide also elevates O_2 affinity, reducing O_2 delivery to tissues. Its use should be restricted to experts. Many crises can be managed at home with oral hydration and oral analgesia. Use of the emergency room should be reserved for especially severe symptoms or circumstances in which other processes, e.g., infection, are strongly suspected. Nasal oxygen should be employed as appropriate to protect arterial saturation. Most crises resolve in 1–7 days. Use of blood transfusion should be reserved for extreme cases: transfusions do not shorten the duration of the crisis.

No tests are definitive to diagnose acute painful crisis. Critical to good management is an approach that recognizes that most patients reporting crisis symptoms do indeed have crisis or another significant medical problem. Diligent diagnostic evaluation for underlying causes is imperative, even though these are found infrequently. In adults, the possibility of aseptic necrosis or sickle arthropathy must be considered, especially if pain and immobility become repeated or chronic at a single site. Nonsteroidal anti-inflammatory agents are often effective for sickle cell arthropathy.

Acute chest syndrome is a medical emergency that may require management in an intensive care unit. Hydration should be monitored carefully to avoid the development of pulmonary edema, and oxygen therapy should be especially vigorous for protection of arterial saturation. Diagnostic evaluation for pneumonia and pulmonary embolism should be especially thorough, since these may occur with atypical symptoms. Critical interventions are transfusion to maintain a hematocrit > 30, and emergency exchange transfusion if arterial saturation drops to <90%. As patients with sickle cell syndrome increasingly survive into their fifth and sixth decades, end-stage renal failure and pulmonary hypertension are becoming increasingly prominent causes of end-stage morbidity. A sickle cell cardiomyopathy and/or premature coronary artery disease may compromise cardiac function in later years. Sickle cell patients have received kidney transplants, but they often experience an increase in the frequency and severity of crises, possibly due to increased infection as a consequence of immunosuppression.

The most significant advance in the therapy of sickle cell anemia has been the introduction of hydroxyurea as a mainstay of therapy for patients with severe symptoms. Hydroxyurea (10–30 mg/kg per day) increases fetal hemoglobin and may also exert beneficial affects on RBC hydration, vascular wall adherence, and suppression of the granulocyte and reticulocyte counts; dosage is titrated to maintain a white cell count between 5000 and 8000 per μL. White cells and reticulocytes may play a major role in the pathogenesis of sickle cell crisis, and their suppression may be an important benefit of hydroxyurea therapy.

Hydroxyurea should be considered in patients experiencing repeated episodes of acute chest syndrome or with more than three crises per year requiring hospitalization. The utility of this agent for reducing the incidence of other complications (priapism, retinopathy) is under evaluation, as are the long-term side effects. Hydroxyurea offers broad benefits to most patients whose disease is severe enough to impair their functional status, and it may improve survival. HbF levels increase in most patients within a few months.

The antitumor drug, 5-azacytidine, was the first agent found to elevate HbF. It never achieved widespread use because of concerns about acute toxicity and carcinogenesis. However, low doses of the related agent, 5-deoxyazacytidine (decitabine) can elevate HbF with acceptable toxicity.

Bone marrow transplantation can provide definitive cures but is known to be effective and safe only in children. Prognostic features justifying bone marrow transplant are the presence of repeated crises early in life, a high neutrophil count, or the development of hand-foot syndrome. Children at risk for stroke can now be identified through the use of Doppler ultrasound techniques. Prophylactic exchange transfusion appears to substantially reduce the risk of stroke in this population. Children who do suffer a cerebrovascular accident should be maintained for at least 3–5 years on a program of vigorous exchange transfusion, as the risk of second strokes is extremely high.

Gene therapy for sickle cell anemia is being intensively pursued, but no safe measures are currently available. Agents blocking RBC dehydration or vascular adhesion, such as clotrimazole or magnesium, may have value as an adjunct to hydroxyurea therapy, pending the completion of ongoing trials. Combinations of clotrimazole and magnesium are being evaluated.

Unstable Hemoglobins

Amino acid substitutions that reduce solubility or increase susceptibility to oxidation produce unstable hemoglobins that precipitate, forming inclusion bodies injurious to the RBC membrane. Representative mutations are those that interfere with contact points between the α and β subunits [e.g., Hb Philly ($\beta^{35Tyr \rightarrow Phe}$)], alter the helical segments [e.g., Hb Genova ($\beta^{28Leu \rightarrow Pro}$)], or disrupt interactions of the hydrophobic pockets of the globin subunits with heme [e.g., Hb Koln ($\beta^{98Val \rightarrow Met}$)] (Table 99-3). The inclusions, called *Heinz bodies*, are clinically detectable by staining with supravital dyes such as crystal violet. Removal of these inclusions by the spleen generates pitted, rigid cells that have shortened life spans, producing hemolytic anemia of variable severity, sometimes requiring chronic transfusion support. Splenectomy may be needed to correct the anemia. Leg ulcers and premature gallbladder disease due to bilirubin load are frequent stigmata.

Table 99-3 Representative Abnormal Hemoglobins with Altered Synthesis or Function

Designation	Mutation	Population	Main Clinical Effects[a]
Sickle or S	$\beta^{6Glu \rightarrow Val}$	African	Anemia, ischemic infarcts
C	$\beta^{6Glu \rightarrow Lys}$	African	Mild anemia; interacts with HbS
E	$\beta^{26Glu \rightarrow Lys}$	Southeast Asian	Microcytic anemia, splenomegaly, thalassemic phenotype
Köln	$\beta^{98Val \rightarrow Met}$	Sporadic	Hemolytic anemia, Heinz bodies when splenectomized
Yakima	$\beta^{99Asp \rightarrow His}$	Sporadic	Polycythemia
Kansas	$\beta^{102Asn \rightarrow Lys}$	Sporadic	Mild anemia
M. Iwata	$\alpha^{87His \rightarrow Tyr}$	Sporadic	Methemoglobinemia

[a]See text for details.

Unstable hemoglobins occur sporadically, often by spontaneous new mutations. Heterozygotes are often symptomatic because a significant Heinz body burden can develop even when the unstable variant accounts for a portion of the total hemoglobin. Symptomatic unstable hemoglobins tend to be β-globin variants, because sporadic mutations affecting only one of the four α globins would generate only 20–30% abnormal hemoglobin.

Hemoglobins with Altered Oxygen Affinity

High-affinity hemoglobins [e.g., Hb Yakima ($\beta^{99Asp \rightarrow His}$)] bind oxygen more readily but deliver less O_2 to tissues at normal capillary P_{O_2} levels (Fig. 99-2). Mild tissue hypoxia ensues, stimulating RBC production and erythrocytosis (Table 99-3). In extreme cases, the hematocrits can rise to 60–65%, increasing blood viscosity and producing typical symptoms (headache, somnolence, or dizziness). Phlebotomy may be required. Typical mutations alter interactions within the heme pocket or disrupt the Bohr effect or salt-bond site. Mutations that impair the interaction of HbA with 2,3-BPG can increase O_2 affinity because 2,3-BPG binding lowers O_2 affinity.

Low-affinity hemoglobins [e.g., Hb Kansas ($\beta^{102Asn \rightarrow Lys}$)] bind sufficient oxygen in the lungs, despite their lower oxygen affinity, to achieve nearly full saturation. At capillary oxygen tensions, they lose sufficient amounts of oxygen to maintain homeostasis at a low hematocrit (Fig. 99-2) (*pseudoanemia*). Capillary hemoglobin desaturation can also be sufficient to produce clinically apparent cyanosis. Despite these findings, patients usually require no specific treatment.

Methemoglobinemias

Methemoglobin is generated by oxidation of the heme iron moieties to the ferric state, causing a characteristic bluish-brown muddy color resembling cyanosis. Methemoglobin has such high oxygen affinity that virtually no oxygen is delivered. Levels >50–60% are often fatal.

Congenital methemoglobinemia arises from globin mutations that stabilize iron in the ferric state [e.g., HbM Iwata ($\alpha^{87His \rightarrow Tyr}$), Table 99-3] or from mutations that impair the enzymes that reduce methemoglobin to hemoglobin (e.g., methemoglobin reductase, NADP diaphorase). Acquired methemoglobinemia is caused by toxins that oxidize heme iron, notably nitrate and nitrite-containing compounds.
Diagnosis and Management of Patients with Unstable Hemoglobins, High-Affinity Hemoglobins, and Methemoglobinemia

Unstable hemoglobin variants should be suspected in patients with nonimmune hemolytic anemia, jaundice, splenomegaly, or premature biliary tract disease. Severe hemolysis usually presents during infancy as neonatal jaundice or anemia. Milder cases may present in adult life with anemia or only as unexplained reticulocytosis, hepatosplenomegaly, premature biliary tract disease, or leg ulcers. Because spontaneous mutation is common, family history of anemia may be absent. The peripheral blood smear often shows anisocytosis, abundant cells with punctate inclusions, and irregular shapes (i.e., poikilocytosis).

The two best tests for diagnosing unstable hemoglobins are the Heinz body preparation and the isopropanol or heat stability test. Many unstable Hb variants are electrophoretically silent. A normal electrophoresis does not rule out the diagnosis.

Severely affected patients may require transfusion support for the first 3 years of life, because splenectomy before age 3 is associated with a significantly higher immune deficit. Splenectomy is usually effective thereafter, but occasional patients may require lifelong transfusion support. Even after splenectomy, patients can develop cholelithiasis and leg ulcers. Splenectomy can also be considered in patients exhibiting severe secondary complications of chronic hemolysis, even if anemia is absent. Precipitation of unstable hemoglobins is aggravated by oxidative stress, e.g., infection, antimalarial drugs.

High-O_2 affinity hemoglobin variants should be suspected in patients with erythrocytosis. The best test for confirmation is measurement of the P_{50}. A high-O_2 affinity Hb causes a significant left shift (i.e., lower numeric value of the P_{50}); confounding conditions, e.g., tobacco smoking or carbon monoxide exposure, can also lower the P_{50}.

High-affinity hemoglobins are often asymptomatic; rubor or plethora may be telltale signs. When the hematocrit reaches to 55–60%, symptoms of high blood viscosity and sluggish flow (headache, lethargy, dizziness, etc.) may be present. These persons may benefit from judicious phlebotomy. Erythrocytosis represents an appropriate attempt to compensate for the impaired oxygen delivery by the abnormal variant. Overzealous phlebotomy may stimulate increased erythropoiesis or aggravate symptoms by thwarting this compensatory mechanism. The guiding principle of phlebotomy should be to improve oxygen delivery by reducing blood viscosity and increasing blood flow rather than restoration of a normal hematocrit. Modest iron deficiency may aid in control.

Low-affinity hemoglobins should be considered in patients with cyanosis or a low hematocrit with no other reason apparent after thorough evaluation. The P_{50} test confirms the diagnosis. Counseling and reassurance are the interventions of choice.

Methemoglobin should be suspected in patients with hypoxic symptoms who appear cyanotic but have a Pa_{O_2} sufficiently high that hemoglobin should be fully saturated with oxygen. A history of nitrite or other oxidant ingestions may not always be available; some exposures may be unapparent to the patient, and others may result from suicide attempts. The characteristic muddy appearance of freshly drawn blood can be a critical clue. The best diagnostic test is methemoglobin assay, which is usually available on an emergency basis.

Methemoglobinemia often causes symptoms of cerebral ischemia at levels >15%; levels >60% are usually lethal. Intravenous injection of 1 mg/kg of methylene blue is effective emergency therapy. Milder cases and follow-up of severe cases can be treated orally with methylene blue (60 mg three to four times each day) or ascorbic acid (300–600 mg/d).
Thalassemia Syndromes: Introduction

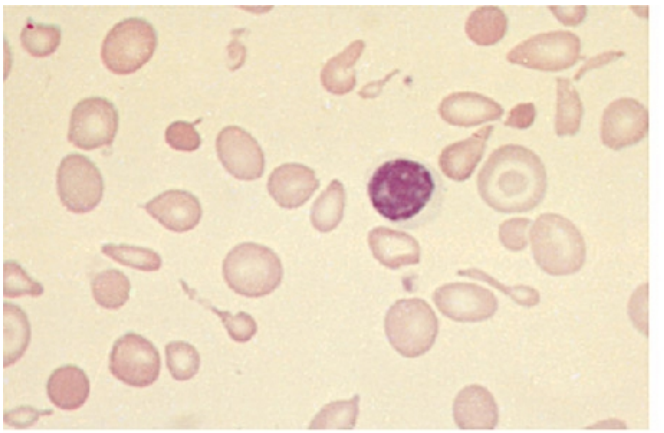
The thalassemia syndromes are inherited disorders of α- or β-globin biosynthesis. The reduced supply of globin diminishes production of hemoglobin tetramers, causing hypochromia and microcytosis. Unbalanced accumulation of α and β subunits occurs because the synthesis of the unaffected globins proceeds at a normal rate. Unbalanced chain accumulation dominates the clinical phenotype. Clinical severity varies widely, depending on the degree to which the synthesis of the affected globin is impaired, altered synthesis of other globin chains, and co-inheritance of other abnormal globin alleles.
Clinical Manifestations of β-Thalassemia Syndromes

Mutations causing thalassemia can affect any step in the pathway of globin gene expression: transcription, processing of the mRNA precursor, translation, and posttranslational metabolism of the β-globin polypeptide chain. The most common forms arise from mutations that derange splicing of the mRNA precursor or prematurely terminate translation of the mRNA.

Hypochromia and microcytosis characterize all forms of β thalassemia because of the reduced amounts of hemoglobin tetramers (Fig. 99-5). In heterozygotes (β-thalassemia trait), this is the only abnormality seen. Anemia is minimal. In more severe homozygous states, unbalanced α- and β-globin accumulation causes accumulation of highly insoluble unpaired α chains. They form toxic inclusion bodies that kill developing erythroblasts in the marrow. Few of the proerythroblasts beginning erythroid maturation survive. The few resulting RBCs bear a burden of inclusion bodies that are detected in the spleen, shortening the RBC life span and producing severe hemolytic anemia. The resulting profound anemia stimulates erythropoietin release and compensatory erythroid hyperplasia, but the marrow response is sabotaged by ineffective erythropoiesis. Anemia persists. Erythroid hyperplasia can become exuberant and produce masses of extramedullary

erythropoietic tissue in the liver and spleen.

Figure 99-5



β-Thalassemia intermedia. Microcytic and hypochromic red blood cells are seen that resemble the red blood cells of severe iron deficiency anemia. Many elliptical and teardrop-shaped red blood cells are noted.

Massive bone marrow expansion deranges growth and development. Children develop characteristic "chipmunk" facies due to maxillary marrow hyperplasia and frontal bossing. Thinning and pathologic fracture of long bones and vertebrae may occur due to cortical invasion by erythroid elements and profound growth retardation. Hemolytic anemia causes hepatosplenomegaly, leg ulcers, gallstones, and high-output congestive heart failure. The conscription of caloric resources to support erythropoiesis leads to inanition, susceptibility to infection, endocrine dysfunction, and in the most severe cases, death during the first decade of life. Chronic transfusions with RBCs improves oxygen delivery, suppresses the excessive ineffective erythropoiesis, and prolongs life, but the inevitable side effects, notably iron overload, usually prove fatal by age 30.

Severity is highly variable. Known modulating factors are those that ameliorate the burden of unpaired α-globin inclusions. Alleles associated with milder synthetic defects and co-inheritance of α-thalassemia trait reduce clinical severity by reducing accumulation of excess α globin. HbF persists to various degrees in β thalassemias. γ-Globin gene chains can substitute for β chains, generating more hemoglobin and reducing the burden of α-globin inclusions. The terms **β-*thalassemia major*** and **β-*thalassemia intermedia*** are used to reflect the clinical heterogeneity. Patients with β-thalassemia major require intensive transfusion support to survive. Patients with β-thalassemia intermedia have a somewhat milder phenotype and can survive without transfusion. The terms **β-*thalassemia minor*** and **β-*thalassemia trait*** describe asymptomatic heterozygotes for β thalassemia.

α-Thalassemia Syndromes

The four classic α thalassemias, most common in Asians, are α-thalassemia-2 trait, in which one of the four α-globin loci is deleted; α-thalassemia-1 trait, with two deleted loci; HbH disease, with three loci deleted; and hydrops fetalis with Hb Bart's, with all four loci deleted (Table 99-4). Nondeletion forms of α thalassemia also exist.

Table 99-4 The α Thalassemias

Condition	Hemoglobin A, %	Hemoglobin H (β^4), %	Hemoglobin Level, g/L (g/dL)	MCV, fL
Normal	97	0	150 (15)	90
Silent thalassemia: –α/αα	98–100	0	150 (15)	90
Thalassemia trait: –α/–α homozygous α-thal-2[a] or ––/αα heterozygous α-thal-1[a]	85–95	Rare red blood cell inclusions	120–130 (12–13)	70–80
Hemoglobin H disease: ––/–α heterozygous α-thal-1/α-thal-2	70–95	5–30	60–100 (6–10)	60–70
Hydrops fetalis: ––/–– homozygous α-thal-1	0	5–10[b]	Fatal in utero or at birth	

[a]When both α alleles on one chromosome are deleted, the locus is called α-thal-1; when only a single α allele on one chromosome is deleted, the locus is called α-thal-2.

[b]90–95% of the hemoglobin is hemoglobin Barts (tetramers of γ chains).

α-*Thalassemia-2 trait* is an asymptomatic, silent carrier state. α-*Thalassemia-1 trait* resembles β-thalassemia minor. Offspring doubly heterozygous for α-thalassemia-2 and α-thalassemia-1 exhibit a more severe phenotype called *HbH disease*. Heterozygosity for a deletion that removes both genes from the same chromosome (*cis* deletion) is common in Asians and in those from the Mediterranean region, as is homozygosity for α-thalassemia-2 (*trans* deletion). Both produce asymptomatic hypochromia and microcytosis.

In *HbH disease,* HbA production is only 25–30% normal. Fetuses accumulate some unpaired β chains. In adults, unpaired β chains accumulate and are soluble enough to form β_4 tetramers called HbH. HbH forms few inclusions in erythroblasts and precipitates in circulating RBC. Patients with HbH disease have thalassemia intermedia characterized by moderately severe hemolytic anemia but milder ineffective erythropoiesis. Survival into mid-adult life without transfusions is common.

The homozygous state for the α-thalassemia-1 *cis* deletion (hydrops fetalis) causes total absence of α-globin synthesis. No physiologically useful hemoglobin is produced beyond the embryonic stage. Excess γ globin forms tetramers called *Hb Barts* (γ_4), which has a very high oxygen affinity. It delivers almost no O_2 to fetal tissues, causing tissue asphyxia, edema (hydrops fetalis), congestive heart failure, and death in utero. α-Thalassemia-2 trait is common (15–20%) among people of African descent. The *cis* α-thalassemia-1 deletion is almost never seen, however. Thus, α-thalassemia-2 and the *trans* form of α-thalassemia-1 are very common, but HbH disease and hydrops fetalis are almost never encountered.

It has been known for some time that some patients with myelodysplasia or erythroleukemia produce RBC clones containing HbH. This phenomenon is due to mutations in the ATRX pathway that affect the LCR of the α-globin gene cluster.

Diagnosis and Management of Thalassemias

The diagnosis of β-thalassemia major is readily made during childhood on the basis of severe anemia accompanied by the characteristic signs of massive ineffective erythropoiesis: hepatosplenomegaly, profound microcytosis, a characteristic blood smear (Fig. 99-5), and elevated levels of HbF, HbA_2, or both. Many patients require chronic hypertransfusion therapy designed to maintain a hematocrit of at least 27–30% so that erythropoiesis is suppressed. Splenectomy is required if the annual transfusion requirement (volume of RBCs per kilogram of body weight per year) increases by >50%. Folic acid supplements may be useful. Vaccination with Pneumovax in anticipation of eventual splenectomy is advised, as is close monitoring for infection, leg ulcers, and biliary tract disease. Many patients develop endocrine deficiencies as a result of iron overload. Early endocrine evaluation is required for glucose intolerance, thyroid dysfunction, and delayed onset of puberty or secondary sexual characteristics.

Patients with β-thalassemia intermedia exhibit similar stigmata but can survive without chronic hypertransfusion. Management is particularly challenging because a number of factors can aggravate the anemia, including infection, onset of puberty, and development of splenomegaly and hypersplenism. Some patients may eventually benefit from splenectomy. The expanded erythron can cause absorption of excessive dietary iron and hemosiderosis, even without transfusion.

β-Thalassemia minor (i.e., thalassemia trait) usually presents as profound microcytosis and hypochromia with target cells, but only minimal or mild anemia. The mean corpuscular volume is rarely >75 fL; the hematocrit is rarely <30–33%. Hemoglobin electrophoresis classically reveals an elevated HbA_2 (3.5–7.5%), but some forms are associated with normal HbA_2 and/or elevated HbF. Genetic counseling and patient education are essential. Patients with β-thalassemia trait should be warned that their blood picture resembles iron deficiency and can be misdiagnosed. They should eschew empirical use of iron; yet iron deficiency can develop during pregnancy or from chronic bleeding.

Persons with α-thalassemia trait may exhibit mild hypochromia and microcytosis usually without anemia. HbA_2 and HbF levels are normal. Affected individuals usually require only genetic counseling. HbH disease resembles β-thalassemia intermedia, with the added complication that the HbH molecule behaves like moderately unstable hemoglobin. Patients with HbH disease should undergo splenectomy if excessive anemia or a transfusion requirement develops. Oxidative drugs should be avoided. Iron overload leading to death can occur in more severely affected patients.

Prevention

Antenatal diagnosis of thalassemia syndromes is now widely available. DNA diagnosis is based on PCR amplification of fetal DNA, obtained by amniocentesis or chorionic villus biopsy followed by hybridization to allele-specific oligonucleotides probes. The probes can be designed to detect simultaneously the subset of mutations that account for 95–99% of the α- or β-thalassemias that occur in a particular group.

Structure

Thalassemic structural variants are characterized by both defective synthesis and abnormal structure.

Hemoglobin Lepore

Hb Lepore [$\alpha_2(\delta\beta)_2$] arises by an unequal crossover and recombination event that fuses the proximal end of the δ-gene with the distal end of the closely linked β-gene. The resulting chromosome contains only the fused δβ gene. The Lepore (δβ) globin is synthesized poorly because the fused gene is under the control of the weak δ-globin promoter. Hb Lepore alleles have a phenotype like β thalassemia, except for the added presence of 2–20% Hb Lepore. Compound heterozygotes for Hb Lepore and a classic β-thalassemia allele may also have severe thalassemia.

Hemoglobin E

HbE (i.e., $\alpha_2\beta_2^{26Glu\rightarrow Lys}$) is extremely common in Cambodia, Thailand, and Vietnam. The gene has become far more prevalent in the United States as a result of immigration of Asian persons, especially in California, where HbE is the most common variant detected. HbE is mildly unstable but not enough to affect RBC life span significantly. The high frequency of the HbE gene may be a result of the thalassemia phenotype associated with its inheritance. Heterozygotes resemble individuals with mild β-thalassemia trait. Homozygotes have somewhat more marked abnormalities but are asymptomatic. Compound heterozygotes for HbE and a β-thalassemia gene can have β-thalassemia intermedia or β-thalassemia major, depending on the severity of the coinherited thalassemic gene.

The β^E allele contains a single base change in codon 26 that causes the amino acid substitution. However, this mutation activates a cryptic RNA splice site generating a structurally abnormal globin mRNA that cannot be translated from about 50% of the initial pre-mRNA molecules. The remaining 40–50% are normally spliced and generate functional mRNA that is translated into β^E-globin because the mature mRNA carries the base change that alters codon 26.

Genetic counseling of the persons at risk for HbE should focus on the interaction of HbE with β thalassemia rather than HbE homozygosity, a condition associated with asymptomatic microcytosis, hypochromia, and hemoglobin levels rarely <1 g/L (<10 g/dL).

Hereditary Persistence of Fetal Hemoglobin

HPFH is characterized by continued synthesis of high levels of HbF in adult life. No deleterious effects are apparent, even when all of the hemoglobin produced is HbF. These rare patients demonstrate convincingly that prevention or reversal of the fetal to adult hemoglobin switch would provide effective therapy for sickle cell anemia and β thalassemia.

Acquired Hemoglobinopathies

The two most important acquired hemoglobinopathies are carbon monoxide poisoning and methemoglobinemia (see above). Carbon monoxide has a higher affinity for hemoglobin than does oxygen; it can replace oxygen and diminish O_2 delivery. Chronic elevation of carboxyhemoglobin levels to 10 or 15%, as occurs in smokers, can lead to secondary polycythemia. Carboxyhemoglobin is cherry red in color and masks the development of cyanosis usually associated with poor O_2 delivery to tissues.

Abnormalities of hemoglobin biosynthesis have also been described in blood dyscrasias. In some patients with myelodysplasia, erythroleukemia, or myeloproliferative disorders, a mild form of HbH disease may also be seen. The abnormalities are not severe enough to alter the course of the underlying disease.

Transfusional Hemosiderosis: Treatment

Chronic blood transfusion can lead to blood-borne infection, alloimmunization, febrile reactions, and lethal iron overload (Chap. 107). A unit of packed RBCs contains 250–300 mg iron (1 mg/mL). The iron assimilated by a single transfusion of two units of packed RBCs is thus equal to a 1- to 2-year intake of iron. Iron accumulates in chronically transfused patients because no mechanisms exist for increasing iron excretion: an expanded erythron causes especially rapid development of iron overload because accelerated erythropoiesis promotes excessive absorption of dietary iron. Vitamin C should not be supplemented because it generates free radicals in iron excess states.

Patients who receive >100 units of packed RBCs usually develop hemosiderosis. The ferritin level rises, followed by early endocrine dysfunction (glucose intolerance and delayed puberty), cirrhosis, and cardiomyopathy. Liver biopsy shows both parenchymal and reticuloendothelial iron. The superconducting quantum-interference device (SQUID) is accurate at measuring hepatic iron but not widely available. Cardiac toxicity is often insidious. Early development of pericarditis is followed by dysrhythmia and pump failure. The onset of heart failure is ominous, often presaging death within a year (Chap. 351).

The decision to start long-term transfusion support should also prompt one to institute therapy with iron-chelating agents. Desferoxamine (Desferal) is for parenteral use. Its iron-binding kinetics require chronic slow infusion via a metering pump. The constant presence of the drug improves the efficiency of chelation and protects tissues from occasional releases of the most toxic fraction of iron– low-molecular-weight iron– which may not be sequestered by protective proteins.

Desferoxamine is relatively nontoxic. Occasional cataracts, deafness, and local skin reactions, including urticaria, occur. Skin reactions can usually be managed with antihistamines. Negative iron balance can be achieved, even in the face of a high transfusion requirement, but this alone does not prevent long-term morbidity and mortality in chronically transfused patients. Irreversible end-organ deterioration develops at relatively modest levels of iron overload, even if symptoms do not appear for many years thereafter. To enjoy a significant survival advantage, chelation must begin before 5–8 years of age in β-thalassemia major.

Deferasirox is a promising oral iron-chelating agent. Single daily doses of 20 or 30 mg deferasirox produced reductions in liver iron concentration comparable to desferoxamine in chronically transfused adult and pediatric patients. Deferasirox produces some elevations in liver enzymes and slight but persistent increases in serum creatinine, without apparent clinical consequence. Other toxicities are similar to those of desferoxamine. Its toxicity profile is acceptable, although long-term effects are still being evaluated.

Bone Marrow Transplantation, Gene Therapy, and Manipulation of HbF

Bone marrow transplantation provides stem cells able to express normal hemoglobin; it has been used in a large number of patients with β thalassemia and a smaller number of patients with sickle cell anemia. Early in the course of disease, before end-organ damage occurs, transplantation is curative in 80–90% of patients. In highly experienced centers, the treatment-related mortality is <10%. Since survival into adult life is possible with conventional therapy, the decision to transplant is best made in consultation with specialized centers.

Gene therapy of thalassemia and sickle cell disease has proved to be an elusive goal. Uptake of gene vectors into the nondividing hematopoietic stem cells has been inefficient. Lentiviral-type vectors that can transduce nondividing cells may solve this problem.

Reestablishing high levels of fetal hemoglobin synthesis should ameliorate the symptoms of β thalassemia. Cytotoxic agents such as hydroxyurea and cytarabine promote high levels of HbF synthesis, probably by stimulating proliferation of the primitive HbF-producing progenitor cell population (i.e., F cell progenitors). Unfortunately, no regimen has yet been identified that ameliorates the clinical manifestations of β thalassemia. Butyrates stimulate HbF production, but only transiently. Pulsed or intermittent administration has been found to sustain HbF induction in the majority of patients with sickle cell disease. It is unclear whether butyrates will have similar activity in patients with β thalassemia.

Aplastic and Hypoplastic Crisis in Patients with Hemoglobinopathies

Patients with hemolytic anemias sometimes exhibit an alarming decline in hematocrit during and immediately after acute illnesses. Bone marrow suppression occurs in almost everyone during acute inflammatory illnesses. In patients with short RBC life spans, suppression can affect RBC counts more dramatically. These hypoplastic crises are usually transient and self-correcting before intervention is required.

Aplastic crisis refers to a profound cessation of erythroid activity in patients with chronic hemolytic anemias. It is associated with a rapidly falling hematocrit. Episodes are usually self-limited. Aplastic crises are caused by infection with a particular strain of parvovirus, B19A. Children infected with this virus usually develop permanent immunity. Aplastic crises do not often recur and are rarely seen in adults. Management requires close monitoring of the hematocrit and reticulocyte count. If anemia becomes symptomatic, transfusion support is indicated. Most crises resolve spontaneously within 1–2 weeks.

Further Readings

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Megaloblastic Anemias: Introduction

The megaloblastic anemias are a group of disorders characterized by the presence of distinctive morphologic appearances of the developing red cells in the bone marrow. The cause is usually deficiency of either cobalamin (vitamin B_{12}) or folate, but megaloblastic anemia may arise because of genetic or acquired abnormalities affecting the metabolism of these vitamins or because of defects in DNA synthesis not related to cobalamin or folate (Table 100-1). Cobalamin and folate absorption and metabolism are described next and then the biochemical basis, clinical and laboratory features, causes, and treatment of megaloblastic anemia. The marrow is usually cellular, and the anemia is based on ineffective erythropoiesis.

Table 100-1 Causes of Megaloblastic Anemia

Cobalamin deficiency or abnormalities of cobalamin metabolism (see Tables 100-3, 100-4)
Folate deficiency or abnormalities of folate metabolism (see Table 100-5)
Therapy with antifolate drugs (e.g., methotrexate)
Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy:
Some cases of acute myeloid leukemia, myelodysplasia
Therapy with drugs interfering with synthesis of DNA [e.g., cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine (AZT)]
Orotic aciduria (responds to uridine)
Thiamine-responsive

Cobalamin

Cobalamin (vitamin B_{12}) exists in a number of different chemical forms. All have a cobalt atom at the center of a corrin ring. In nature, the vitamin is mainly in the 2-deoxyadenosyl (ado) form, which is located in mitochondria. It is the cofactor for the enzyme methylmalonyl CoA mutase. The other major natural cobalamin is methylcobalamin, the form in human plasma and in cell cytoplasm. It is the cofactor for methionine synthase. There are also minor amounts of hydroxocobalamin to which methyl- and adocobalamin are rapidly converted by exposure to light.

Dietary Sources and Requirements

Cobalamin is synthesized solely by microorganisms. Ruminants obtain cobalamin from the foregut, but the only source for humans is food of animal origin, e.g., meat, fish, and dairy products. Vegetables, fruits, and other foods of non-animal origin are free from cobalamin unless they are contaminated by bacteria. A normal Western diet contains between 5 and 30 μg of cobalamin daily. Adult daily losses (mainly in the urine and feces) are between 1 and 3 μg (~0.1% of body stores) and, as the body does not have the ability to degrade cobalamin, daily requirements are also about 1–3 μg. Body stores are of the order of 2–3 mg, sufficient for 3–4 years if supplies are completely cut off.

Absorption

Two mechanisms exist for cobalamin absorption. One is passive, occurring equally through buccal, duodenal, and ileal mucosa; it is rapid but extremely inefficient, <1% of an oral dose being absorbed by this process. The normal physiologic mechanism is active; it occurs through the ileum and is efficient for small (a few micrograms) oral doses of cobalamin and is mediated by gastric intrinsic factor (IF). Dietary cobalamin is released from protein complexes by enzymes in the stomach, duodenum, and jejunum; it combines rapidly with a salivary glycoprotein that belongs to the family of cobalamin-binding proteins known as haptocorrins (HCs). In the intestine, the haptocorrin is digested by pancreatic trypsin and the cobalamin transferred to IF.

IF is produced in the gastric parietal cells of the fundus and body of the stomach, and its secretion parallels that of hydrochloric acid. The IF-cobalamin complex passes to the ileum, where IF attaches to a specific receptor (cubilin) on the microvillus membrane of the enterocytes. Cubilin is also present in yolk sac and renal proximal tubular epithelium. Cubulin appears to traffic by means of amnionless (AMN), an endocytic receptor protein that directs sublocalization and endocytosis of cubulin with its ligand IF-cobalamin complex. The cobalamin-IF complex enters the ileal cell where IF is destroyed. After a delay of about 6 h, the cobalamin appears in portal blood attached to transcobalamin (TC) II.

Between 0.5 and 5.0 μg of cobalamin enters the bile each day. This binds to IF, and a major portion of biliary cobalamin is normally reabsorbed together with cobalamin derived from sloughed intestinal cells. Because of the appreciable amount of cobalamin undergoing enterohepatic circulation, cobalamin deficiency develops more rapidly in individuals who malabsorb cobalamin than it does in vegans, in whom reabsorption of biliary cobalamin is intact.

Transport

Two main cobalamin transport proteins exist in human plasma; they both bind cobalamin– one molecule for one molecule. One HC, known as TC I, is closely related to other cobalamin-binding HCs in milk, gastric juice, bile, saliva, and other fluids. These HCs differ from each other only in the carbohydrate moiety of the molecule. TC I is derived primarily from the specific granules in neutrophils. Normally, it is about two-thirds saturated with cobalamin, which it binds tightly. TC I does not enhance cobalamin entry into tissues. Glycoprotein receptors on liver cells are involved in the removal of TC I from plasma, and TC I may have a role in the transport of cobalamin analogues to the liver for excretion in bile.

The other major cobalamin transport protein in plasma is TC II. This is synthesized by liver and by other tissues, including macrophages, ileum, and endothelium. It normally carries only 20–60 ng of cobalamin per liter of plasma and readily gives up cobalamin to marrow, placenta, and other tissues, which it enters by receptor-mediated endocytosis.

Folate

Dietary Folate

Folic (pteroylglutamic) acid is a yellow, crystalline, water-soluble substance. It is the parent compound of a large family of natural folate compounds, which differ from it in three respects: (1) they are partly or completely reduced to di- or tetrahydrofolate (THF) derivatives; (2) they usually contain a single carbon unit (Table 100-2), and (3) 70–90% of natural folates are folate-polyglutamates.

Table 100-2 Biochemical Reactions of Folate Coenzymes

Reaction	Coenzyme Form of Folate Involved	Single Carbon Unit Transferred	Importance
Formate activation	THF	–CHO	Generation of 10-formyl-THF
Purine synthesis			
Formation of glycinamide ribonucleotide	5,10-MethyleneTHF	–CHO	Formation of purines needed for DNA, RNA synthesis, but reactions probably not rate limiting
Formylation of aminoimidazolecarboxamide-ribonucleotide (AICAR)	10-Formyl (CHO) THF		
Pyrimidine synthesis			
Methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP)	5,10-MethyleneTHF	$-CH_3$	Rate limiting in DNA synthesis Oxidizes THF to DHF Some breakdown of folate at the C-9–N-10 bond
Amino acid interconversion			
Serine–glycine interconversion	THF	$=CH_2$	Entry of single carbon units into active pool
Homocysteine to methionine	5-Methyl(M)THF	$-CH_3$	Demethylation of 5-MTHF to THF; also requires cobalamin, flavine adenine dinucleotide, ATP, and adenosylmethionine
Forminoglutamic acid to glutamic acid in histidine catabolism	THF	–HN–CH=	

DHF, dihydrofolate; THF, tetrahydrofolate.

Most foods contain some folate. The highest concentrations are found in liver, yeast, spinach, other greens, and nuts (>100 μg/100 g). The total folate content of an average Western diet is ~250 μg daily, but the amount varies widely according to the type of food eaten and the method of cooking. Folate is easily destroyed by heating, particularly in large volumes of water. Total-body folate in the adult is ~10 mg, the liver containing the largest store. Daily adult requirements are ~100 μg, so stores are only sufficient for 3–4 months in normal adults and severe folate deficiency may develop rapidly.

Absorption

Folates are absorbed rapidly from the upper small intestine. The absorption of folate polyglutamates is less efficient than for monoglutamates; on average, ~50% of food folate is absorbed. Polyglutamate forms are hydrolysed to the monoglutamate derivatives, either in the lumen of the intestine or within the mucosa. All dietary folates are converted to 5-methylTHF (5-MTHF) within the small-intestinal mucosa before entering portal plasma. The monoglutamates are actively transported across the enterocyte by a carrier-mediated mechanism. Pteroylglutamic acid at doses >400 μg is absorbed largely unchanged and converted to natural folates in the liver. Lower doses are converted to 5-MTHF during absorption through the intestine.

About 60–90 μg of folate enters the bile each day and is excreted into the small intestine. Loss of this folate, together with the folate of sloughed intestinal cells, accelerates the speed with which folate deficiency develops in malabsorption conditions.

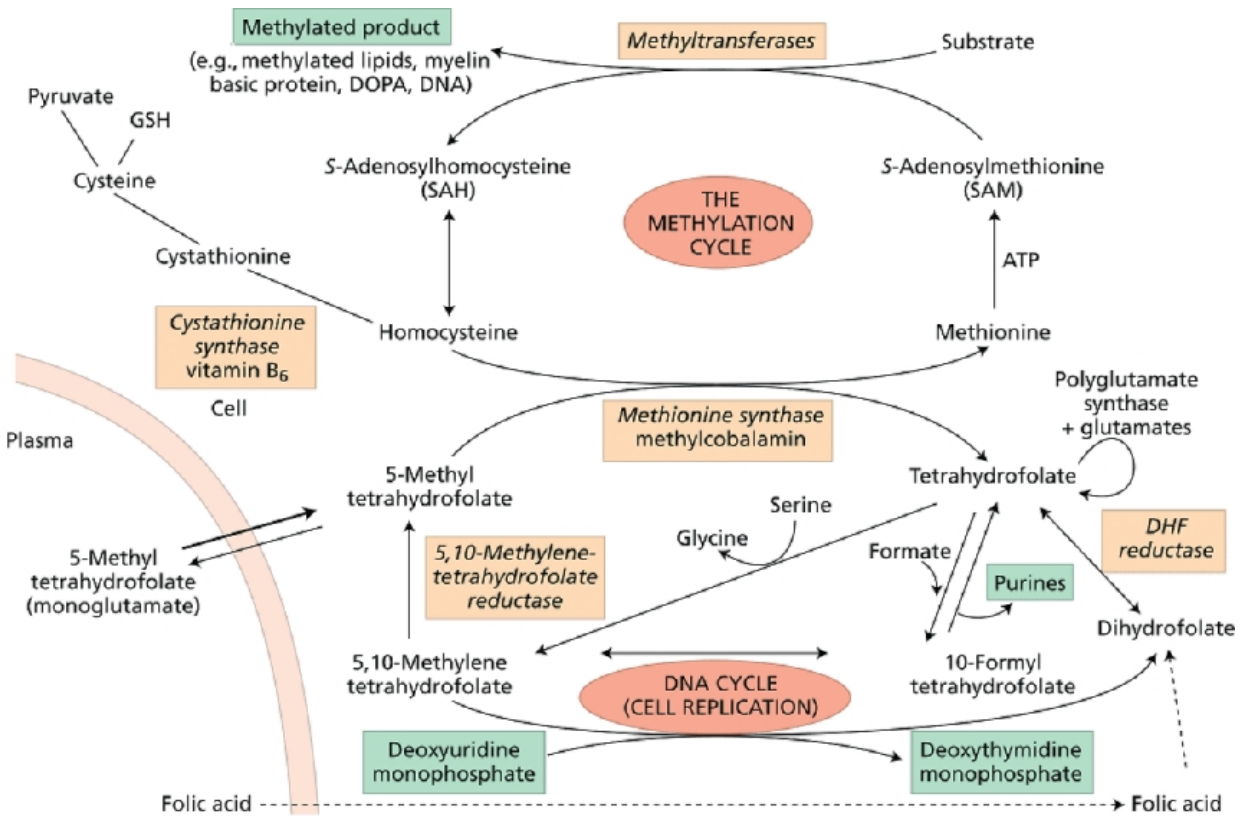
Transport

Folate is transported in plasma; about one-third is loosely bound to albumin and two-thirds unbound. In all body fluids (plasma, cerebrospinal fluid, milk, bile) folate is largely, if not entirely, 5-MTHF in the monoglutamate form. Two types of folate-binding protein are involved in entry of MTHF into cells. A high-affinity folate receptor takes folate into cells by endocytosis, is internalized by clathrin-coated pits or in a vesicle (caveola), which is then acidified, releasing folate. Folate is then carried by the membrane folate transporter into the cytoplasm. The high-affinity receptor is attached to the outer surface of the cell membrane by glycosyl phosphatidylinositol linkages. It may be involved in transport of oxidized folates and folate breakdown products to the liver for excretion in bile. An independent low-affinity reduced-folate carrier also mediates uptake of physiologic folates into cells but also of methotrexate.

Biochemical Functions

Folates (as the intracellular polyglutamate derivatives) act as coenzymes in the transfer of single-carbon units (Fig. 100-1 and Table 100-2). Two of these reactions are involved in purine and one in pyrimidine synthesis necessary for DNA and RNA replication. Folate is also a coenzyme for methionine synthesis, in which methylcobalamin is also involved and in which THF is regenerated. THF is the acceptor of single carbon units newly entering the active pool via conversion of serine to glycine. Methionine, the other product of the methionine synthase reaction, is the precursor for *S*-adenosylmethionine (SAM), the universal methyl donor involved in >100 methyltransferase reactions (Fig. 100-1).

Figure 100-1



The role of folates in DNA synthesis and in formation on *S*-adenosylmethionine (SAM), which is involved in numerous methylation reactions. *[Reprinted from Hoffbrand AV et al (eds), Postgraduate Haematology, 5th ed, Blackwell Publishing, Oxford, UK 2005; with permission.]*

During thymidylate synthesis, 5,10-methylene-THF is oxidized to DHF (dihydrofolate). The enzyme DHF reductase converts this to THF. The drugs methotrexate, pyrimethamine, and (mainly in bacteria) trimethoprim inhibit DHF reductase and so prevent formation of active THF coenzymes from DHF. A small fraction of the folate coenzyme is not recycled during thymidylate synthesis but is degraded.

Biochemical Basis of Megaloblastic Anemia

The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow. All conditions that give rise to megaloblastic changes share in common a disparity in the rate of synthesis or availability of the four immediate precursors of DNA: the deoxyribonucleoside triphosphates (dNTPs): dA(adenine)TP and dG(guanine)TP (purines), dT(thymine)TP and dC

(cytosine)TP (pyrimidines). In deficiencies of either folate or cobalamin, there is failure to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), the precursor of dTTP (Fig. 100-1). This is because folate is needed as the coenzyme 5,10-methylene-THF polyglutamate for conversion of dUMP to dTMP; the availability of 5,10-methylene-THF is reduced in either cobalamin or folate deficiency. An alternative theory for megaloblastic anemia in cobalamin or folate deficiency is misincorporation of uracil into DNA because of a build-up of deoxyuridine triphosphate (dUTP) at the DNA replication fork as a consequence of the block in conversion of dUMP to dTMP.

Cobalamin-Folate Relations

Folate is required for many reactions in mammalian tissues. Only two reactions in the body are known to require cobalamin. Methylmalonyl CoA isomerization, which requires adocobalamin, and the methylation of homocysteine to methionine requires both methylcobalamin and both 5-MTHF (Fig. 100-1). This reaction is the first step in the pathway by which 5-MTHF, which enters bone marrow and other cells from plasma, is converted into all the intracellular folate coenzymes. The coenzymes are all polyglutamated (the larger size aiding retention in the cell), but the enzyme folate polyglutamate synthase can use only THF, not MTHF, as substrate. In cobalamin deficiency, MTHF accumulates in plasma, while intracellular folate concentrations fall due to failure of formation of THF, the substrate on which folate polyglutamates are built. This has been termed *THF starvation*, or the *methylfolate trap*.

This theory explains the abnormalities of folate metabolism that occur in cobalamin deficiency [high serum folate, low cell folate, positive purine precursor aminomidazole carboxamide ribonucleotide (AICAR) excretion; Table 100-2] and also why the anemia of cobalamin deficiency will respond to folic acid in large doses.

Clinical Features

Many symptomless patients are detected through the finding of a raised mean corpuscular volume (MCV) on a routine blood count. The main clinical features in more severe cases are those of anemia. Anorexia is usually marked and there may be weight loss, diarrhea, or constipation. Glossitis, angular cheilosis, a mild fever in the more severely anemic patients, jaundice (unconjugated), and reversible melanin skin hyperpigmentation may also occur with deficiency of either folate or cobalamin. Thrombocytopenia sometimes leads to bruising, and this may be aggravated by vitamin C deficiency or alcohol in malnourished patients. The anemia and low leukocyte count may predispose to infections, particularly of the respiratory or urinary tracts. Cobalamin deficiency has also been associated with impaired bactericidal function of phagocytes.

General Tissue Effects of Cobalamin and Folate Deficiencies

Epithelial Surfaces

After the marrow, the next most affected tissues are the epithelial cell surfaces of the mouth, stomach, and small intestine and the respiratory, urinary, and female genital tracts. The cells show macrocytosis, with increased numbers of multinucleate and dying cells. The deficiencies may cause cervical smear abnormalities.

Complications of Pregnancy

The gonads are also affected, and infertility is common in both men and women with either deficiency. Maternal folate deficiency has been implicated as a cause of prematurity, and both folate and cobalamin deficiency have been implicated in recurrent fetal loss and neural tube defects, discussed below.

Neural Tube Defects

Folic acid supplements at the time of conception and in the first 12 weeks of pregnancy reduce by ~70% the incidence of neural tube defects (NTDs) (anencephaly, meningomyelocele, encephalocele, and spina bifida) in the fetus. Most of this protective effect can be achieved by taking folic acid, 0.4 mg daily at the time of conception.

The incidence of cleft palate and harelip can also be reduced by prophylactic folic acid. There is no clear simple relationship between maternal folate status and these fetal abnormalities, although overall the lower the maternal folate, the greater the risk to the fetus. NTDs can also be caused by antifolate and antiepileptic drugs.

An underlying maternal folate metabolic abnormality has also been postulated. One abnormality has been identified: reduced activity of the enzyme 5,10-methylene-THF reductase (MTHFR) (Fig. 100-1) caused by a common 677CÆT polymorphism in the *MTHFR* gene. In one study, the prevalence of this polymorphism was found to be higher in the parents of NTD fetuses and in the fetuses themselves: homozygosity for the TT mutation was found in 13% compared with 5% in control subjects. The polymorphism codes for a thermolabile form of MTHFR. The homozygous state results in a lower mean serum and red cell folate level compared with control subjects, as well as significantly higher serum homocysteine levels. Tests for mutations in other enzymes possibly associated with NTDs, e.g., methionine synthase or serine–glycine hydroxymethylase, have been negative.

Autoantibodies to folate receptors have, however, been detected in 9 of 12 women who were or had been pregnant with a fetus with a NTD, but in only 2 of 20 control women. Antiserum to folate receptors results in resorption or multiple developmental abnormalities in mouse embryos. It is possible, therefore, that the association of antibodies to maternal folate receptors and NTDs reflects a causal relation.

Cardiovascular Disease

Children with severe homocystinuria (blood levels ≥100 μmol/L) due to deficiency of one of three enzymes, methionine synthase, MHTFR,

or cystathionine synthase (Fig. 100-1), suffer from vascular disease, e.g., ischemic heart disease, cerebrovascular disease, or pulmonary embolus as teenagers or in young adulthood. Lesser degrees of raised serum homocysteine and low levels of serum folate have been found to be associated with cerebrovascular, peripheral vascular, and coronary heart disease and with deep vein thrombosis. Prospective randomized trials of lowering homocysteine levels with supplements of folic acid, vitamin B_{12}, and vitamin B_6 against placebo over a 5-year period in patients with vascular disease or diabetes have not, however, shown a reduction of major cardiovascular events, nor have these supplements reduced the risk of recurrent cardiovascular disease after an acute myocardial infarct. It is possible that these trials were not sufficiently powered to detect a small (e.g., 10%) benefit or that some other underlying factor is responsible for both the vascular damage and the raised homocysteine. Alternatively, the beneficial effects of lowering homocysteine were offset in these trials by the vitamins stimulating endothelial cell proliferation. The results of longer and larger trials are needed to resolve these uncertainties.

Malignancy

Prophylactic folic acid in pregnancy has been found to reduce the subsequent incidence of acute lymphoblastic leukemia (ALL) in childhood. A significant negative association has also been found with the *MTHFR* 677(C→T) polymorphism and leukemias with mixed lineage leukemia (MLL) translocations, but a positive association with hyperdiploidy in infants with ALL or acute myeloid leukemia or with childhood ALL. A second polymorphism in the *MHTFR* gene, A1298C, is also strongly associated with hyperdiploid leukemia. There are various positive and negative associations between polymorphisms in folate-dependent enzymes and the incidence of adult ALL. The C677T polymorphism is thought to lead to increased thymidine pools and "better quality" of DNA synthesis by shunting 1-carbon groups towards thymidine and purine synthesis. This may explain its reported association with a lower risk for colorectal cancer. Other tumors that have been associated with folate polymorphisms or status include follicular lymphoma, breast cancer, and gastric cancer.

Neurologic Manifestations

Cobalamin deficiency may cause a bilateral peripheral neuropathy or degeneration (demyelination) of the posterior and pyramidal tracts of the spinal cord and, less frequently, optic atrophy or cerebral symptoms.

The patient, more frequently male, presents with paresthesias, muscle weakness, or difficulty in walking and sometimes dementia, psychotic disturbances, or visual impairment. Long-term nutritional cobalamin deficiency in infancy leads to poor brain development and impaired intellectual development. Folate deficiency has been suggested to cause organic nervous disease but this is uncertain, although methotrexate injected into the cerebrospinal fluid may cause brain or spinal cord damage.

An important clinical problem is the nonanemic patient with neurologic or psychiatric abnormalities and a low or borderline serum cobalamin level. In such patients, it is necessary to try to establish whether or not there is significant cobalamin deficiency, e.g., by careful examination of the blood film, cobalamin absorption studies, tests for antibodies to IF or parietal cells, and serum methylmalonic acid (MMA) measurement if available. A trial of cobalamin therapy for at least 3 months will also usually be needed to determine whether the symptoms improve.

The biochemical basis for cobalamin neuropathy remains obscure. Its occurrence in the absence of methylmalonic aciduria in TC II deficiency suggests that the neuropathy is related to the defect in homocysteine-methionine conversion. Accumulation of *S*-adenosylhomocysteine in the brain, resulting in inhibition of transmethylation reactions, has been suggested.

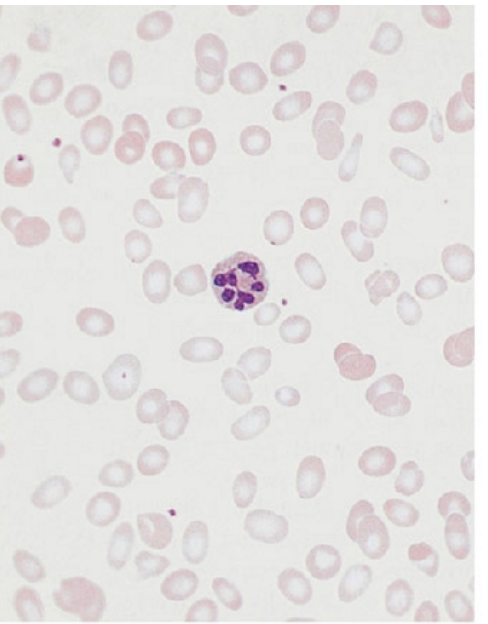
Psychiatric disturbance is common in both folate and cobalamin deficiencies. This, like the neuropathy, has been attributed to a failure of the synthesis of SAM, which is needed in methylation of biogenic amines (e.g., dopamine) as well as of proteins, phospholipids, and neurotransmitters in the brain (Fig. 100-1). Associations between lower serum folate or cobalamin levels and higher homocysteine levels and the development of Alzheimer's disease have been reported. A 2-year double-blind placebo-controlled randomized clinical trial involving healthy subjects >65 years old given folate, cobalamin, and vitamin B_6 supplements showed no benefit on cognitive performance, whereas a 3-year (FACIT) study did show benefit.

Hematologic Findings

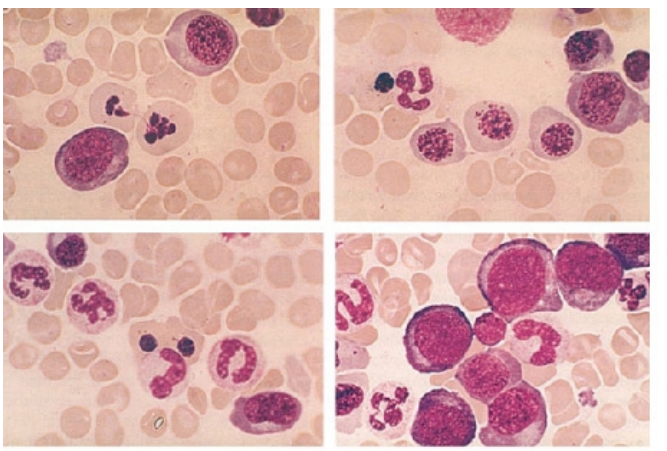
Peripheral Blood

Oval macrocytes, usually with considerable anisocytosis and poikilocytosis, are the main feature (Fig. 100-2A). The MCV is usually >100 fL unless a cause of microcytosis (e.g., iron deficiency or thalassemia trait) is present. Some of the neutrophils are hypersegmented (more than five nuclear lobes). There may be leukopenia due to a reduction in granulocytes and lymphocytes, but this is usually >1.5 x 10^9/L; the platelet count may be moderately reduced, rarely to <40 x 10^9/L. The severity of all these changes parallels the degree of anemia. In the nonanemic patient, the presence of a few macrocytes and hypersegmented neutrophils in the peripheral blood may be the only indication of the underlying disorder.

Figure 100-2



A



B

A. The peripheral blood in severe megaloblastic anemia. ***B***. The bone marrow in severe megaloblastic anemia. *[Reprinted from Hoffbrand AV et al (eds) Postgraduate Haematology, 5th ed, Blackwell Publishing, Oxford, UK 2005; with permission.]*

Bone Marrow

In the severely anemic patient, the marrow is hypercellular with an accumulation of primitive cells due to selective death by apoptosis of more mature forms. The erythroblast nucleus maintains a primitive appearance despite maturation and hemoglobinization of the cytoplasm. The cells are larger than normoblasts, and an increased number of cells with eccentric lobulated nuclei or nuclear fragments may be present (Fig. 100-2B). Giant and abnormally shaped metamyelocytes and enlarged hyperpolyploid megakaryocytes are characteristic. In less anemic patients, the changes in the marrow may be difficult to recognize. The terms *intermediate*, *mild*, and *early* have been used. The term *megaloblastoid* does not mean mildly megaloblastic. It is used to describe cells with both immature appearing nuclei and defective hemoglobinization and is usually seen in myelodysplasia.

Chromosomes

Bone marrow cells, transformed lymphocytes, and other proliferating cells in the body show a variety of changes including random breaks, reduced contraction, spreading of the centromere, and exaggeration of secondary chromosomal constrictions and overprominent satellites. Similar abnormalities may be produced by antimetabolite drugs (e.g., cytosine arabinoside, hydroxyurea, and methotrexate) that either

interfere with DNA replication or folate metabolism and that also cause megaloblastic appearances.

Ineffective Hemopoiesis

There is an accumulation of unconjugated bilirubin in plasma due to the death of nucleated red cells in the marrow (ineffective erythropoiesis). Other evidence for this includes raised urine urobilinogen, reduced haptoglobins and positive urine hemosiderin, and a raised serum lactate dehydrogenase. A weakly positive direct antiglobulin test due to complement can lead to a false diagnosis of autoimmune hemolytic anemia.

Causes of Cobalamin Deficiency

Cobalamin deficiency is usually due to malabsorption. The only other cause is inadequate dietary intake.

Inadequate Dietary Intake

Adults

Dietary cobalamin deficiency arises in vegans who omit meat, fish, eggs, cheese, and other animal products from their diet. The largest group in the world consists of Hindus, and it is likely that many millions of Indians are at risk of deficiency of cobalamin on a nutritional basis. Subnormal serum cobalamin levels are found in up to 50% of randomly selected, young, adult Indian vegans, but the deficiency usually does not progress to megaloblastic anemia since the diet of most vegans is not totally lacking cobalamin and the enterohepatic circulation of cobalamin is intact. Dietary cobalamin deficiency may also arise rarely in nonvegetarian individuals who exist on grossly inadequate diets because of poverty or psychiatric disturbance.

Infants

Cobalamin deficiency has been described in infants born to severely cobalamin-deficient mothers. These infants develop megaloblastic anemia at about 3–6 months of age, presumably because they are born with low stores of cobalamin and because they are fed breast milk of low cobalamin content. The babies have also shown growth retardation, impaired psychomotor development, and other neurologic sequelae.

Gastric Causes of Cobalamin Malabsorption

See Tables 100-3 and 100-4.

Table 100-3 Causes of Cobalamin Deficiency Sufficiently Severe to Cause Megaloblastic Anemia

Nutritional	Vegans
Malabsorption	Pernicious anemia
Gastric causes	Congenital absence of intrinsic factor or functional abnormality
	Total or partial gastrectomy
Intestinal causes	Intestinal stagnant loop syndrome: jejunal diverticulosis, ileocolic fistula, anatomic blind loop, intestinal stricture, etc.
	Ileal resection and Crohn's disease
	Selective malabsorption with proteinuria
	Tropical sprue
	Transcobalamin II deficiency
	Fish tapeworm

Table 100-4 Malabsorption of Cobalamin May Occur in the Following Conditions But Is Not Usually Sufficiently Severe and Prolonged to Cause Megaloblastic Anemia

Gastric causes
Simple atrophic gastritis (food cobalamin malabsorption)
Zollinger–Ellison syndrome
Gastric bypass surgery
Use of proton pump inhibitors
Intestinal causes
Gluten-induced enteropathy
Severe pancreatitis
HIV infection

Radiotherapy

Graft-versus-host disease

Deficiencies of cobalamin, folate, protein, ?riboflavin, ?nicotinic acid

Therapy with colchicine, para-aminosalicylate, neomycin, slow-release potassium chloride, anticonvulsant drugs, metformin, phenformin, cytotoxic drugs

Alcohol

Pernicious Anemia

Pernicious anemia (PA) may be defined as a severe lack of IF due to gastric atrophy. It is a common disease in north Europeans but occurs in all countries and ethnic groups. The overall incidence is about 120 per 100 000 population in the United Kingdom (UK). The ratio of incidence in men and women in Caucasians is ~1:1.6 and the peak age of onset is 60 years, with only 10% of patients being <40 years of age. However, in some ethnic groups, notably black individuals and Latin Americans, the age of onset of PA is generally lower. The disease occurs more commonly than by chance in close relatives and in persons with other organ-specific autoimmune diseases, e.g., thyroid diseases, vitiligo, hypoparathyroidism, and Addison's disease. It is also associated with hypogammaglobulinemia, with premature graying or blue eyes, and in persons of blood group A. An association with human leukocyte antigen (HLA) 3 has been reported in some but not all series and, in those with endocrine disease, with HLA-B8, -B12, and -BW15. The life expectancy is normal in women once regular treatment has begun. Men have a slightly subnormal life expectancy as a result of a higher incidence of carcinoma of the stomach than in control subjects. Gastric output of hydrochloric acid, pepsin, and IF are severely reduced. The serum gastrin level is raised, and serum pepsinogen I levels are low.

Gastric Biopsy

This usually shows atrophy of all layers of the body and fundus, with loss of glandular elements, an absence of parietal and chief cells and replacement by mucous cells, a mixed inflammatory cell infiltrate, and perhaps intestinal metaplasia. The infiltrate of plasma cells and lymphocytes contains an excess of CD4 cells. The antral mucosa is usually well preserved. *Helicobacter pylori* infection is infrequent in PA, but it has been suggested that *H. pylori* gastritis occurs at an early phase of atrophic gastritis and presents in younger patients as iron deficiency anemia but in older patients as PA. *H. pylori* is suggested to stimulate an autoimmune process directed against parietal cells, the *H. pylori* infection then being gradually replaced, in some individuals, by an autoimmune process.

Serum Antibodies

Two types of IF immunoglobulin G antibody may be found in the sera of patients with PA. One, the "blocking," or type I, antibody, prevents the combination of IF and cobalamin, whereas the "binding," or type II, antibody prevents attachment of IF to ileal mucosa. Type I occurs in the sera of ~55% of patients and type II in 35%. IF antibodies cross the placenta and may cause temporary IF deficiency in the newborn infant. Patients with PA also show cell-mediated immunity to IF. Type I antibody has been detected rarely in the sera of patients without PA but with thyrotoxicosis, myxedema, Hashimoto's disease, or diabetes mellitus and in relatives of PA patients. IF antibodies have also been detected in gastric juice in ~80% of PA patients. These gastric antibodies may reduce absorption of dietary cobalamin by combining with small amounts of remaining IF.

Parietal cell antibody is present in the sera of almost 90% of adult patients with PA but is frequently present in other subjects. Thus, it occurs in as many as 16% of randomly selected female subjects aged >60 years. The parietal cell antibody is directed against the α and β subunits of the gastric proton pump (H^+,K^+-ATPase).

Juvenile Pernicious Anemia

This usually occurs in older children and resembles PA of adults. Gastric atrophy, achlorhydria, and serum IF antibodies are all present, although parietal cell antibodies are usually absent. About one-half of these patients show an associated endocrinopathy such as autoimmune thyroiditis, Addison's disease, or hypoparathyroidism; in some, mucocutaneous candidiasis occurs.

Congenital Intrinsic Factor Deficiency or Functional Abnormality

The affected child usually presents with megaloblastic anemia in the first to third year of life; a few have presented as late as the second decade. The child has no demonstrable IF but has a normal gastric mucosa and normal secretion of acid. The inheritance is autosomally recessive. Parietal cell and IF antibodies are absent. Variants have been described in which the child is born with IF that can be detected immunologically but is unstable or functionally inactive.

Gastrectomy

Following total gastrectomy, cobalamin deficiency is inevitable, and prophylactic cobalamin therapy should be commenced immediately following the operation. After partial gastrectomy, 10–15% of patients also develop this deficiency. The exact incidence and time of onset are most influenced by the size of the resection and the preexisting size of cobalamin body stores.

Food Cobalamin Malabsorption

Failure of release of cobalamin from binding proteins in food is believed to be responsible for this condition, more common in the elderly.

It is associated with low serum cobalamin levels, with or without raised serum levels of MMA and homocysteine. Typically, these patients have normal cobalamin absorption, as measured with crystalline cobalamin, but show malabsorption when a modified test using food-bound cobalamin is used. The frequency of progression to severe cobalamin deficiency and reasons for this progression are not clear.

Intestinal Causes of Cobalamin Malabsorption

Intestinal Stagnant Loop Syndrome

Malabsorption of cobalamin occurs in a variety of intestinal lesions in which there is colonization of the upper small intestine by fecal organisms. This may occur in patients with jejunal diverticulosis, enteroanastomosis, or intestinal stricture or fistula or with an anatomic blood loop due to Crohn's disease, tuberculosis, or an operative procedure.

Ileal Resection

Removal of ≥1.2 m of terminal ileum causes malabsorption of cobalamin. In some patients following ileal resection, particularly if the ileocecal valve is incompetent, colonic bacteria may contribute further to the onset of cobalamin deficiency.

Selective Malabsorption of Cobalamin with Proteinuria (Imerslund Syndrome: Imerslund-Gräsbeck Syndrome; Congenital Cobalamin Malabsorption; Autosomal Recessive Megaloblastic Anemia, MGA1)

This autosomally recessive disease is the most common cause of megaloblastic anemia due to cobalamin deficiency in infancy in Western countries. More than 200 cases have been reported, with familial clusters in Finland, Norway, the Middle East, and North Africa. The patients secrete normal amounts of IF and gastric acid but are unable to absorb cobalamin. In Finland, impaired synthesis, processing, or ligand binding of cubilin due to inherited mutations is found. In Norway, mutation of the gene for *AMN* has been reported. Other tests of intestinal absorption are normal. Over 90% of the patients show nonspecific proteinuria, but renal function is otherwise normal and renal biopsy has not shown any consistent renal defect. A few have shown aminoaciduria and congenital renal abnormalities, such as duplication of the renal pelvis.

Tropical Sprue

Nearly all patients with acute and subacute tropical sprue show malabsorption of cobalamin; this may persist as the principal abnormality in the chronic form of the disease, when the patient may present with megaloblastic anemia or neuropathy due to cobalamin deficiency. Absorption of cobalamin usually improves after antibiotic therapy and, in the early stages, folic acid therapy.

Fish Tapeworm Infestation

The fish tapeworm (*Diphyllobothrium latum*) lives in the small intestine of humans and accumulates cobalamin from food, rendering this unavailable for absorption. Individuals acquire the worm by eating raw or partly cooked fish. Infestation is common around the lakes of Scandinavia, Germany, Japan, North America, and Russia. Megaloblastic anemia or cobalamin neuropathy occurs only in those with a heavy infestation.

Gluten-Induced Enteropathy

Malabsorption of cobalamin occurs in ~30% of untreated patients (presumably those in whom the disease extends to the ileum). Cobalamin deficiency is not severe in these patients and is corrected with a gluten-free diet.

Severe Chronic Pancreatitis

In this condition, lack of trypsin is thought to cause dietary cobalamin attached to gastric non-IF (R) binder to be unavailable for absorption. It has also been proposed that in pancreatitis, the concentration of calcium ions in the ileum falls below the level needed to maintain normal cobalamin absorption.

HIV Infection

Serum cobalamin levels tend to fall in patients with HIV infection and are subnormal in 10–35% of those with AIDS. Malabsorption of cobalamin not corrected by IF has been shown in some, but not all, patients with subnormal serum cobalamin levels. Cobalamin deficiency sufficiently severe to cause megaloblastic anemia or neuropathy is rare.

Zollinger–Ellison Syndrome

Malabsorption of cobalamin has been reported in the Zollinger–Ellison syndrome. It is thought that there is a failure to release cobalamin from R-binding protein due to inactivation of pancreatic trypsin by high acidity, as well as interference with IF binding of cobalamin.

Radiotherapy

Both total-body irradiation and local radiotherapy to the ileum (e.g., as a complication of radiotherapy for carcinoma of the cervix) may cause malabsorption of cobalamin.

Graft-versus-Host Disease

This commonly affects the small intestine. Malabsorption of cobalamin due to abnormal gut flora, as well as damage to ileal mucosa, is frequent.

Drugs

The drugs that have been reported to cause malabsorption of cobalamin are listed in Table 100-4. Megaloblastic anemia due to these drugs is, however, rare.

Abnormalities of Cobalamin Metabolism

Congenital Transcobalamin II Deficiency or Abnormality

Infants with TC II deficiency usually present with megaloblastic anemia within a few weeks of birth. Serum cobalamin and folate levels are normal, but the anemia responds to massive (e.g., 1 mg three times weekly) injections of cobalamin. Some cases show neurologic complications. The protein may be present but functionally inert. Genetic abnormalities found include mutations of an intra-exonic cryptic splice site, extensive deletion, single nucleotide deletion, nonsense mutation, and an RNA editing defect. Malabsorption of cobalamin occurs in all cases and serum immunoglobulins are usually reduced. Failure to institute adequate cobalamin therapy or treatment with folic acid may lead to neurologic damage.

Congenital Methylmalonic Acidemia and Aciduria

The infants with this abnormality are ill from birth with vomiting, failure to thrive, severe metabolic acidosis, ketosis, and mental retardation. Anemia, if present, is normocytic and normoblastic. The condition may be due to a functional defect in either mitochondrial methylmalonyl CoA mutase or its cofactor adocobalamin. Mutations in the methylmalonyl CoA mutase are not responsive, or only poorly responsive, to treatment with cobalamin. A proportion of the infants with failure of adocobalamin synthesis respond to cobalamin in large doses. Some children have combined methylmalonic aciduria and homocystinuria due to defective formation of both cobalamin coenzymes. This usually presents in the first year of life with feeding difficulties, developmental delay, microcephaly, seizures, hypotonia, and megaloblastic anemia.

Acquired Abnormality of Cobalamin Metabolism: Nitrous Oxide Inhalation

Nitrous oxide irreversibly oxidizes methylcobalamin to an inactive precursor; this inactivates methionine synthase. Megaloblastic anemia has occurred in patients undergoing prolonged N_2O anesthesia (e.g., in intensive care units). A neuropathy resembling cobalamin neuropathy has also been described in dentists and anesthetists who are repeatedly exposed to N_2O. Methylmalonic aciduria does not occur as adocobalamin is not inactivated by N_2O.

Causes of Folate Deficiency

(Table 100-5)

Table 100-5 Causes of Folate Deficiency

Dietary[a]
Particularly in: old age, infancy, poverty, alcoholism, chronic invalids, and the psychiatrically disturbed; may be associated with scurvy or kwashiorkor
Malabsorption
Major causes of deficiency
Tropical sprue, gluten-induced enteropathy in children and adults, and in association with dermatitis herpetiformis, specific malabsorption of folate, intestinal megaloblastosis caused by severe cobalamin or folate deficiency
Minor causes of deficiency
Extensive jejunal resection, Crohn's disease, partial gastrectomy, congestive heart failure, Whipple's disease, scleroderma, amyloid, diabetic enteropathy, systemic bacterial infection, lymphoma, salazopyrine
Excess utilization or loss
Physiologic
Pregnancy and lactation, prematurity
Pathologic
Hematologic diseases: chronic hemolytic anemias, sickle cell anemia, thalassemia major, myelofibrosis
Malignant diseases: carcinoma, lymphoma, leukemia, myeloma
Inflammatory diseases: tuberculosis, Crohn's disease, psoriasis, exfoliative dermatitis, malaria
Metabolic disease: homocystinuria

Excess urinary loss: congestive heart failure, active liver disease

Hemodialysis, peritoneal dialysis

Antifolate drugs[b]

Anticonvulsant drugs (phenytoin, primidone, barbiturates), sulphasalazine

Nitrofurantoin, tetracycline, anti-tuberculosis (less well documented)

Mixed causes

Liver diseases, alcoholism, intensive care units

[a]In severely folate-deficient patients with causes other than those listed under Dietary, poor dietary intake is often present.

[b]Drugs inhibiting dihydrofolate reductase are discussed in the text.

Nutritional

Dietary folate deficiency is common. Indeed, in most patients with folate deficiency a nutritional element is present. Certain individuals are particularly prone to have diets containing inadequate amounts of folate (Table 100-5). In the United States and other countries where fortification of the diet with folic acid has been adopted, the prevalence of folate deficiency has dropped dramatically and is now almost restricted to high-risk groups with increased folate needs. Nutritional folate deficiency occurs in kwashiorkor and scurvy and in infants with repeated infections or who are fed solely on goats' milk, which has a low folate content.

Malabsorption

Malabsorption of dietary folate occurs in tropical sprue and in gluten-induced enteropathy. In the rare congenital syndrome of selective malabsorption of folate, there is an associated defect of folate transport into the cerebrospinal fluid, and these patients show megaloblastic anemia, which responds to physiologic doses of folic acid given parenterally but not orally. They also show mental retardation, convulsions, and other central nervous system abnormalities. Minor degrees of malabsorption may also occur following jejunal resection or partial gastrectomy, in Crohn's disease, and in systemic infections but, in these conditions, if severe deficiency occurs, it is usually largely due to poor nutrition. Malabsorption of folate has been described in patients receiving salazopyrine, cholestyramine, and triamterene.

Excess Utilization or Loss

Pregnancy

Folate requirements are increased by 200–300 μg to ~400 μg daily in a normal pregnancy, partly because of transfer of the vitamin to the fetus, but mainly because of increased folate catabolism due to cleavage of folate coenzymes in rapidly proliferating tissues. Megaloblastic anemia due to this deficiency is prevented by prophylactic folic acid therapy. It occurred in 0.5% of pregnancies in the UK and other Western countries before prophylaxis with folic acid, but the incidence is much higher in countries where the general nutritional status is poor.

Prematurity

The newborn infant, whether full term or premature, has higher serum and red cell folate concentrations than the adult. However, the newborn infant's demand for folate has been estimated to be up to 10 times that of adults on a weight basis, and the neonatal folate level falls rapidly to the lowest values at about 6 weeks of age. The falls are steepest and are liable to reach subnormal levels in premature babies, a number of whom develop megaloblastic anemia responsive to folic acid at about 4–6 weeks of age. This occurs particularly in the smallest babies (<1500 g birth weight) and in those who have feeding difficulties or infections or who have undergone multiple exchange transfusions. In these babies, prophylactic folic acid should be given.

Hematologic Disorders

Folate deficiency frequently occurs in chronic hemolytic anemia, particularly in sickle cell disease, autoimmune hemolytic anemia, and congenital spherocytosis. In these and other conditions of increased cell turnover (e.g., myelofibrosis, malignancies) folate deficiency arises because it is not completely reutilized after performing coenzyme functions.

Inflammatory Conditions

Chronic inflammatory diseases, such as tuberculosis, rheumatoid arthritis, Crohn's disease, psoriasis, exfoliative dermatitis, bacterial endocarditis, and chronic bacterial infections, cause deficiency by reducing the appetite and by increasing the demand for folate. Systemic infections may also cause malabsorption of folate. Severe deficiency is virtually confined to the patients with the most active disease and the poorest diet.

Homocystinuria

This is a rare metabolic defect in the conversion of homocysteine to cystathionine. Folate deficiency occurring in most of these patients may be due to excessive utilization because of compensatory increased conversion of homocysteine to methionine.

Long-Term Dialysis

As folate is only loosely bound to plasma proteins, it is easily removed from plasma by dialysis. In patients with anorexia, vomiting, infections, and hemolysis, folate stores are particularly likely to become depleted. Routine folate prophylaxis is now given.

Congestive Heart Failure, Liver Disease

Excess urinary folate losses of >100 μg per day may occur in some of these patients. The explanation appears to be release of folate from damaged liver cells.

Antifolate Drugs

A large number of epileptics, who are receiving long-term therapy with phenytoin or primidone, with or without barbiturates, develop low serum and red cell folate levels. The exact mechanism is unclear. Alcohol may also be a folate antagonist, as patients who are drinking spirits may develop megaloblastic anemia that will respond to normal quantities of dietary folate or to physiologic doses of folic acid only if alcohol is withdrawn. Macrocytosis of red cells is associated with chronic alcohol intake even when folate levels are normal. Inadequate folate intake is the major factor in the development of deficiency in spirit-drinking alcoholics. Beer is relatively folate-rich in some countries, depending on the technique used for brewing.

The drugs that inhibit DHF reductase include methotrexate, pyrimethamine, and trimethoprim. Methotrexate has the most powerful action against the human enzyme, whereas trimethoprim is most active against the bacterial enzyme and is only likely to cause megaloblastic anemia when used in conjunction with sulphamethoxazole in patients with preexisting folate or cobalamin deficiency. The activity of pyrimethamine is intermediate. The antidote to these drugs is folinic acid (5-formyl-THF).

Congenital Abnormalities of Folate Metabolism

Some infants with congenital defects of folate enzymes (e.g., cyclohydrolase or methionine synthase) have had megaloblastic anemia.
Diagnosis of Cobalamin and Folate Deficiencies

The diagnosis of cobalamin or folate deficiency has traditionally depended on the recognition of the relevant abnormalities in the peripheral blood and analysis of the blood levels of the vitamins.

Serum Cobalamin

This is measured by an automated enzyme-linked immunoadsorbent (ELISA) assay. Normal serum levels range 118–148 pmol/L (160–200 ng/L) to ~738 pmol/L (1000 ng/L). In patients with megaloblastic anemia due to cobalamin deficiency, the level is usually <74 pmol/L (100 ng/L). In general, the more severe the deficiency, the lower the serum cobalamin level. In patients with spinal cord damage due to the deficiency, levels are very low even in the absence of anemia. Values of between 74 and 148 pmol/L (100 and 200 ng/L) are regarded as borderline. They may occur, for instance, in pregnancy, in patients with megaloblastic anemia due to folate deficiency. The serum cobalamin level is generally considered to be sufficiently robust, cost-effective, and most convenient to rule out cobalamin deficiency in the vast majority of patients suspected of having this problem.

Serum Methylmalonate and Homocysteine

In patients with cobalamin deficiency sufficient to cause anemia or neuropathy, the serum MMA level is raised. Sensitive methods for measuring MMA and homocysteine in serum have been introduced and recommended for the early diagnosis of cobalamin deficiency, even in the absence of hematologic abnormalities or subnormal levels of serum cobalamin. Serum MMA levels fluctuate, however, in patients with renal failure. Mildly elevated serum MMA and/or homocysteine levels occur in up to 30% of apparently healthy volunteers, with serum cobalamin levels up to 258 pmol/L (350 ng/L) and normal serum folate levels; 15% of elderly subjects, even with cobalamin levels >258 pmol/L (>350 ng/L), have this pattern of raised metabolite levels. These findings bring into question the exact cut-off points for normal MMA and homocysteine levels. It is also unclear at present whether these mildly raised metabolite levels have clinical consequences.

Serum homocysteine is raised in both early cobalamin and folate deficiency but may be raised in other conditions, e.g., chronic renal disease, alcoholism, smoking, pyridoxine deficiency, hypothyroidism, therapy with steroids, cyclosporine, and other drugs. Levels are also higher in serum than in plasma, in men than in premenopausal women, in women taking hormone replacement therapy, or in oral contraceptive users and in elderly persons and patients with several inborn errors of metabolism affecting enzymes in trans-sulfuration pathways of homocysteine metabolism. Thus, homocysteine levels are not used for diagnosis of cobalamin or folate deficiency.

Cobalamin Absorption

Studies of cobalamin absorption have been widely used, but difficulty in obtaining radioactive cobalamin and of ensuring IF preparations are free of viruses have led to reduced availability. For the urinary excretion (Schilling) test the patient is fasted overnight. Radioactive cyanocobalamin is given orally. Then, 2 h later an IM injection of cyanocobalamin or hydroxocobalamin (1 mg) is given ("flushing dose"). A 24-h urine specimen is collected for determination of radioactivity; low excretion shows malabsorption; the oral dose is then given again after 48 h with IF. The results distinguish between gastric and intestinal causes of cobalamin malabsorption.

Serum Folate

This is also measured by an ELISA technique. In most laboratories, the normal range is from 11 nmol/L (2.0 μg/L) to ~82 nmol/L (15 μg/L). The serum folate level is low in all folate-deficient patients. It also reflects recent diet. Because of this, serum folate may be low before there is hematologic or biochemical evidence of deficiency. Serum folate rises in severe cobalamin deficiency because of the block in conversion of MTHF to THF inside cells; raised levels have also been reported in the intestinal stagnant loop syndrome, due to absorption of bacterially synthesized folate.

Red Cell Folate

The red cell folate assay is a valuable test of body folate stores. It is less affected than the serum assay by recent diet and traces of hemolysis. In normal adults, concentrations range 880–3520 μmol/L (160–640 μg/L) of packed red cells. Subnormal levels occur in patients with megaloblastic anemia due to folate deficiency but also in nearly two-thirds of patients with severe cobalamin deficiency. False-normal results may occur if the folate-deficient patient has received a recent blood transfusion or if the patient has a raised reticulocyte count.

Megaloblastic Anemia: Treatment

It is usually possible to establish which of the two deficiencies, folate or cobalamin, is the cause of the anemia and to treat only with the appropriate vitamin. In patients who enter hospital severely ill, however, it may be necessary to treat with both vitamins in large doses once blood samples have been taken for cobalamin and folate assays and a bone marrow biopsy has been performed (if deemed necessary). Transfusion is usually unnecessary and inadvisable. If it is essential, packed red cells should be given slowly, one or two units only, with the usual treatment for heart failure if present. Potassium supplements have been recommended to obviate the danger of the hypokalemia that has been recorded in some patients during the initial hematologic response. Occasionally, an excessive rise in platelets occurs after 1–2 weeks of therapy. Antiplatelet therapy, e.g., aspirin should be considered if the platelet count rises to >800 × 10^9/L.

Treatment of Cobalamin Deficiency

It is usually necessary to treat patients who have developed cobalamin deficiency with lifelong regular cobalamin injections. In the UK, the form used is hydroxocobalamin; in the United States, cyanocobalamin. In a few instances, the underlying cause of cobalamin deficiency can be permanently corrected, e.g., the fish tapeworm, tropical sprue, or an intestinal stagnant loop that is amenable to surgery. The indications for starting cobalamin therapy are a well-documented megaloblastic anemia or other hematologic abnormalities or neuropathy due to the deficiency. Patients with borderline serum cobalamin levels but no hematologic or other abnormality should be followed, e.g., at yearly intervals to make sure that the cobalamin deficiency does not progress. If malabsorption of cobalamin or rises in serum MMA levels have also been demonstrated, however, they should also be given regular maintenance cobalamin therapy. Cobalamin should be given routinely to all patients who have had a total gastrectomy or ileal resection. Patients who have undergone gastric reduction for control of obesity or who are receiving long-term treatment with proton pump inhibitors should be screened and, if necessary, given cobalamin replacement.

Replenishment of body stores should be complete with six 1000-μg IM injections of hydroxocobalamin given at 3- to 7-day intervals. More frequent doses are usually used in patients with cobalamin neuropathy, but there is no evidence that these produce a better response. For maintenance therapy, 1000 μg hydroxocobalamin IM once every 3 months is satisfactory. Because of the poorer retention of cyanocobalamin, protocols generally use higher and more frequent doses, e.g., 1000 μg IM, monthly, for maintenance treatment.

Toxic reactions are extremely rare and are usually due to contamination in its preparation rather than to cobalamin itself. Because a small fraction of cobalamin can be absorbed passively through mucous membranes even when there is complete failure of physiological IF-dependent absorption, large daily oral doses (1000–2000 μg) of cyanocobalamin can be used in PA for replacement and maintenance of normal cobalamin status. Sublingual therapy has also been proposed for those in whom injections are difficult because of a bleeding tendency and may not tolerate oral therapy. If oral therapy is used, it is important to monitor compliance, particularly with elderly, forgetful patients.

Treatment of Folate Deficiency

Oral doses of 5–15 mg folic acid daily are satisfactory, as sufficient folate is absorbed from these extremely large doses even in patients with severe malabsorption. The length of time therapy must be continued depends on the underlying disease. It is customary to continue therapy for about 4 months, when all folate-deficient red cells will have been eliminated and replaced by new folate-replete populations.

Before large doses of folic acid are given, cobalamin deficiency must be excluded and, if present, corrected, otherwise cobalamin neuropathy may develop, despite a response of the anemia of cobalamin deficiency to folate therapy. Studies in the United States, however, suggest that there is no increase in the proportion of individuals with low serum cobalamin levels and no anemia since food fortification with folic acid, but it is unknown if there has been a change in incidence of cobalamin neuropathy.

Long-term folic acid therapy is required when the underlying cause of the deficiency cannot be corrected and the deficiency is likely to recur, for instance, in chronic dialysis or hemolytic anemias. It may also be necessary in gluten-induced enteropathy if this does not respond to a gluten-free diet. Where mild but chronic folate deficiency occurs, it is preferable to encourage improvement in the diet after correcting the deficiency with a short course of folic acid. In any patient receiving long-term folic acid therapy, it is important to measure the serum cobalamin level at regular (e.g., once yearly) intervals to exclude the coincidental development of cobalamin deficiency.

Folinic Acid (5-Formyl-Thf)

This is a stable form of fully reduced folate. It is given orally or parenterally to overcome the toxic effects of methotrexate or other DHF reductase inhibitors.

Prophylactic Folic Acid

In many countries, food is fortified with folic acid (in grain or flour) to prevent neural tube defects. It is also used in chronic dialysis patients and in parenteral feeds. Prophylactic folic acid has been used to reduce homocysteine levels to prevent cardiovascular disease, but further data are needed to assess the benefit for this and for cognitive function in the elderly.

Pregnancy

Folic acid, 400 μg daily, should be given as a supplement before and throughout pregnancy. In women who have had a previous fetus with a neural tube defect, 5 mg daily is recommended when pregnancy is contemplated and throughout the subsequent pregnancy.

Infancy and Childhood

The incidence of folate deficiency is so high in the smallest premature babies during the first 6 weeks of life that folic acid (e.g., 1 mg daily) should be given routinely to those weighing <1500 g at birth and to larger premature babies who require exchange transfusions or develop feeding difficulties, infections, or vomiting and diarrhea.

The World Health Organization currently recommends routine supplementation with iron and folic acid in children in countries where iron deficiency is common and child mortality, largely due to infectious diseases, is high. However, some studies suggest that where malaria rates are high, this approach may increase the incidence of severe illness and death. Even where malaria is rare, there appears to be no survival benefit.

Megaloblastic Anemia Not Due to Cobalamin or Folate Deficiency or Altered Metabolism

This may occur with many antimetabolic drugs (e.g.,. hydroxyurea, cytosine arabinoside, 6-mercaptopurine) that inhibit DNA replication. Antiviral nucleoside analogues used in treatment of HIV infection may also cause macrocytosis and megaloblastic marrow changes. In the rare disease orotic aciduria, two consecutive enzymes in purine synthesis are defective. The condition responds to therapy with uridine, which by-passes the block. In thiamine-responsive megaloblastic anemia, there is a genetic defect in the high-affinity thiamine transport (*SLC19A2*) gene. This causes defective RNA ribose synthesis through impaired activity of transketolase, a thiamine-dependent enzyme in the pentose cycle. This leads to reduced nucleic acid production. It may be associated with diabetes mellitus and deafness and the presence of many ringed sideroblasts in the marrow. The explanation is unclear for megaloblastic changes in the marrow in some patients with acute myeloid leukemia and myelodysplasia.

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Note: Large images and tables on this page may necessitate printing in landscape mode.

Definitions

A finite life span is a distinct characteristic of red cells. Hence, a logical, time-honored classification of anemias comprises three groups: decreased production of red cells, increased destruction of red cells, and acute blood loss. Red cell destruction and acute loss, both associated with increased reticulocyte production, are covered in this chapter. Red cell production defects are discussed in Chaps. 98, 99, and 100.

Physical loss of red cells from the bloodstream– which in most cases also means physical loss *from* the body– is fundamentally different from destruction of red cells *within* the body. Therefore the clinical aspects and the pathophysiology of anemia in these two groups of patients are quite different, and they will be considered separately.

Hemolytic Anemias

Anemias due to increased destruction of red cells, or hemolytic anemias (HAs), may be *inherited* or *acquired*. From the clinical point of view, they may be more *acute* or more *chronic*, and they may vary from mild to very severe. The site of hemolysis may be predominantly *intravascular* or *extravascular*. With respect to mechanisms, HAs may be due to *intracorpuscular* or *extracorpuscular* causes (Table 101-1); however, before reviewing the individual types of HAs, it is appropriate to consider what they have in common.

Table 101-1 Classification of Hemolytic Anemias[a]

	Intracorpuscular Defects	**Extracorpuscular Factors**
Hereditary	Hemoglobinopathies	Familial hemolytic uremic syndrome (HUS)
	Enzymopathies	
	Membrane-cytoskeletal defects	
Acquired	Paroxysmal nocturnal hemoglobinuria (PNH)	Mechanical destruction (microangiopathic)
		Toxic agents
		Drugs
		Infectious
		Autoimmune

[a]There is a strong correlation between hereditary causes and intracorpuscular defects, because such defects are due to inherited mutations; the one exception is PNH, because the defect is due to an acquired somatic mutation. There is also a strong correlation between acquired causes and extracorpuscular factors; the one exception is familial HUS, because here an inherited abnormality allows excessive complement activation, with bouts of production of membrane attack complex capable of severely damaging normal cells.

General Clinical and Laboratory Features

The clinical presentation of a patient with anemia is greatly influenced by whether the onset is abrupt or gradual, and HA is no exception. A patient with autoimmune hemolytic anemia or with favism may be a medical emergency, whereas a patient with mild hereditary spherocytosis or with cold agglutinin disease may be diagnosed after years. This is due in large measure to the remarkable ability of the body to adapt to anemia when it is slowly progressing (Chap. 58).

What differentiates HA from other anemias is that the patient has signs and symptoms arising directly from hemolysis (Table 101-2). At the clinical level, the main sign is *jaundice;* in addition, the patient may report discoloration of the urine. In many cases of HA, the spleen is enlarged because it is a preferential site of hemolysis; in some cases the liver may be enlarged as well. In all severe congenital forms of HA, skeletal changes may be noted due to over-activity of the bone marrow (although they are never as severe as in thalassemia).

Table 101-2 General Features of Hemolytic Disorders

General examination	Jaundice, pallor
Other physical findings	Spleen may be enlarged; bossing of skull in severe congenital cases
Hemoglobin	From normal to severely reduced
MCV, MCH	Usually increased
Reticulocytes	Increased
Bilirubin	Increased (mostly unconjugated)
LDH	Increased (up to 10× normal with intravascular hemolysis)
Haptoglobin	Reduced to absent

Note: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; LDH, lactate dehydrogenase.

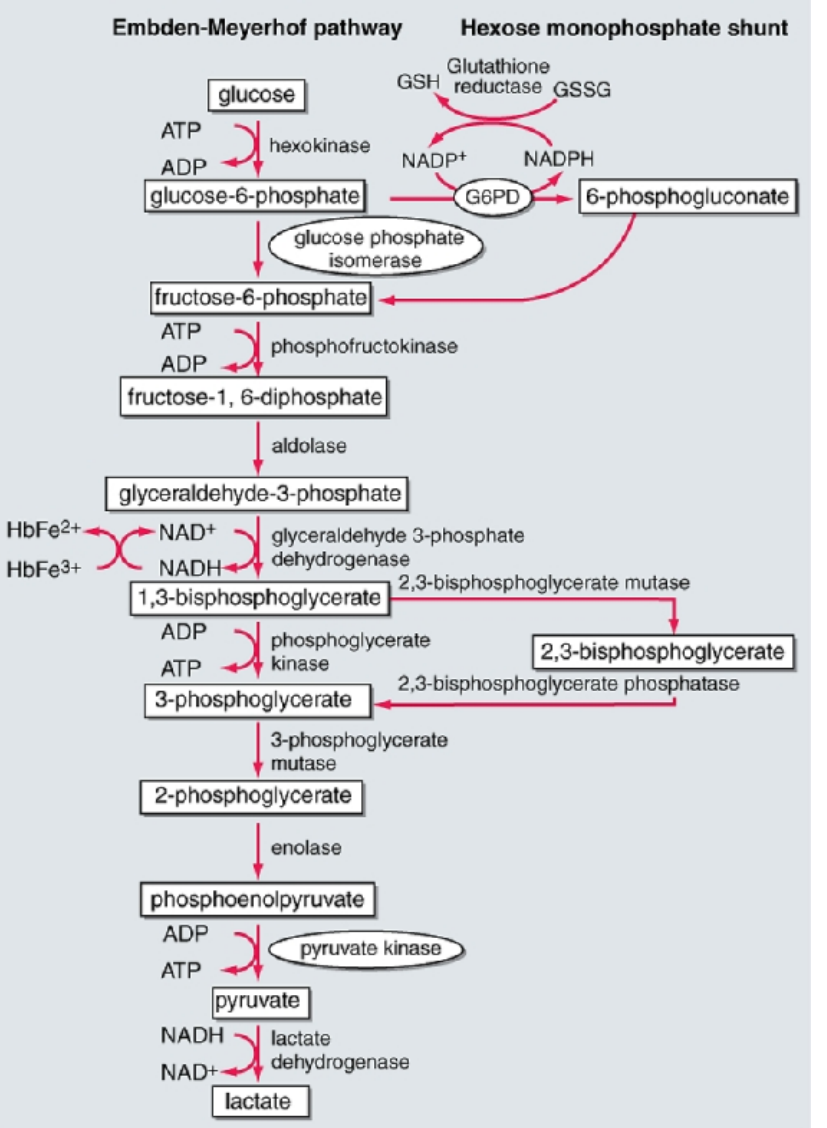
The laboratory features of HA are related to (1) hemolysis per se and (2) the erythropoietic response of the bone marrow. In the serum, hemolysis regularly produces an increased unconjugated bilirubin, increased lactate dehydrogenase (LDH), increased aspartate transaminase, and reduced haptoglobin. Urobilinogen will be increased in both urine and stool. If hemolysis is mainly intravascular, the telltale sign is hemoglobinuria, often associated with hemosiderinuria and an increase in serum hemoglobin; in contrast, the bilirubin level may be normal or only mildly elevated. The main sign of the erythropoietic response by the bone marrow is an increase in reticulocytes (a test all too often neglected in the initial workup of a patient with anemia). Usually the increase will be reflected in both the percentage of reticulocytes (the more commonly quoted figure) and the absolute reticulocyte count (the more definitive parameter). The increased number of reticulocytes is associated with an increased mean corpuscular volume (MCV) in the blood count. On the blood smear this is reflected in the presence of macrocytes; there is also polychromasia and sometimes nucleated red cells. In most cases a bone marrow aspirate is not necessary in the diagnostic workup; if it is done, it will show erythroid hyperplasia. In practice, once an HA is suspected, specific tests will usually be required for a definitive diagnosis of the specific type of HA.

General Pathophysiology

The mature red cell is the product of a developmental pathway that brings the phenomenon of differentiation to an extreme. An orderly sequence of events produces synchronous changes whereby the gradual accumulation of a huge amount of hemoglobin in the cytoplasm (to a final level of 340 g/L, i.e., about 5 mM) goes hand in hand with the gradual loss of cellular organelles and of biosynthetic abilities. In the end the erythroid cell undergoes a process that has features of apoptosis, including nuclear pyknosis and actual loss of the nucleus. However, the final result is more altruistic than suicidal; the cytoplasmic body, instead of disintegrating, is now able to provide oxygen to all cells in the human organism for some remaining 120 days of the red cell "life" span.

As a result of this unique process of differentiation and maturation, intermediary metabolism is drastically curtailed in mature red cells (Fig. 101-1); for instance, cytochrome-mediated oxidative phosphorylation has been lost with the loss of mitochondria; therefore there is no backup to anaerobic glycolysis for the production of adenosine triphosphate (ATP). Also, the capacity of making protein has been lost with the loss of ribosomes. This places the cell's limited metabolic apparatus at risk because if any protein component deteriorates, it cannot be replaced as in most other cells; and in fact the activity of most enzymes gradually decreases as red cells age. Another consequence of the relative simplicity of red cells is that they have a very limited range of ways to manifest distress under hardship: in essence, any sort of metabolic failure will eventually lead either to structural damage to the membrane or to failure of the cation pump. In either case the life span of the red cell is reduced, which is the definition of a *hemolytic disorder*. If the rate of red cell destruction exceeds the capacity of the bone marrow to produce more red cells, the hemolytic disorder will manifest as *hemolytic anemia*.

Figure 101-1



RBC metabolism. The Embden-Meyerhof pathway (glycolysis) generates ATP for energy and membrane maintenance. The generation of NADPH maintains hemoglobin in a reduced state. The hexose monophosphate shunt generates NADPH that is used to reduce glutathione, which protects the red cell against oxidant stress. Regulation of 2,3-bisphosphoglycerate levels is a critical determinant of oxygen affinity of hemoglobin. Enzyme deficiency states in order of prevalence: glucose-6-phosphate dehydrogenase (G6PD) >>> pyruvate kinase > glucose-6-phosphate isomerase > rare deficiencies of other enzymes in the pathway. The more common enzyme deficiencies are encircled.

Thus, the essential pathophysiologic process common to all HAs is an increased red cell turnover. The gold standard for proving that the life span of red cells is reduced (compared to the normal value of about 120 days) is a *red cell survival* study, which can be carried out by labeling the red cells with ^{51}Cr and measuring residual radioactivity over several days or weeks; however, this classic test is now available in very few centers and is rarely necessary. If the hemolytic event is transient, it does not usually cause any long-term consequences. However, if hemolysis is recurrent or persistent, the increased bilirubin production favors the formation of gallstones. If a considerable proportion of hemolysis takes place in the spleen, as is often the case, splenomegaly may become a prominent feature and hypersplenism may develop, with consequent neutropenia and/or thrombocytopenia.

The increased red cell turnover also has metabolic consequences. In normal subjects, the iron from effete red cells is very efficiently recycled by the body; however, with chronic intravascular hemolysis, the persistent hemoglobinuria will cause considerable iron loss, needing replacement. With chronic extravascular hemolysis, the opposite problem, iron overload, is more common, especially if the patient needs frequent blood transfusions. Chronic iron overload will cause secondary hemochromatosis; this will cause damage, particularly to the liver, eventually leading to cirrhosis, and to the heart muscle, eventually causing heart failure. The increased activity of the bone marrow

also entails an increased requirement for erythropoietic factors, particularly folic acid.

Compensated Hemolysis versus HA

Red cell destruction is a potent stimulus for erythropoiesis, which is mediated by erythropoietin (EPO) produced by the kidney. This mechanism is so effective that in many cases the increased output of red cells from the bone marrow can fully balance an increased destruction of red cells. In such cases we say that hemolysis is *compensated*. The pathophysiology of compensated hemolysis is similar to that just described, except there is no anemia. This notion is important from the diagnostic point of view, because a patient with a hemolytic condition, even an inherited one, may present without anemia. It is also important from the point of view of management because compensated hemolysis may become "decompensated"– i.e., anemia may suddenly appear– in certain circumstances– for instance, pregnancy, folate deficiency, renal failure interfering with adequate EPO production, or an acute infection depressing erythropoiesis. Another general feature of chronic HA is seen when any intercurrent condition depresses erythropoiesis. When this happens, in view of the increased rate of red cell turnover, the effect will be predictably much more marked than in a person who does not have hemolysis. The most dramatic example is infection by parvovirus B19, which may cause a rather precipitous fall in hemoglobin, an occurrence sometimes referred to as *aplastic crisis*.

Inherited Hemolytic Anemias

There are three essential components in the red cell: (1) hemoglobin, (2) the membrane-cytoskeleton complex, and (3) the metabolic machinery necessary to keep (1) and (2) in working order. Here we will discuss diseases of the latter two components. Diseases caused by abnormalities of hemoglobin are discussed in Chap. 99.

Hemolytic Anemias Due to Abnormalities of the Membrane-Cytoskeleton Complex

The detailed architecture of the red cell membrane is complex, but its basic design is relatively simple (Fig. 101-2). The lipid bilayer, which incorporates phospholipids and cholesterol, is spanned by a number of proteins that have their hydrophobic transmembrane domains embedded in the membrane. Most of these proteins have hydrophilic domains extending toward both the outside and the inside of the cell. Other proteins are tethered to the membrane through a glycosylphosphatidylinositol (GPI) anchor, and they have only an extracellular domain. These proteins are arranged roughly perpendicular to or lying across the membrane; they include ion channels, receptors for complement components, receptors for other ligands, and some of unknown function. The most abundant of these proteins are glycophorins and the so-called band 3, an anion transporter. The extracellular domains of many of these proteins are heavily glycosylated, and they carry antigenic determinants that correspond to blood groups. Underneath the membrane, and tangential to it, is a network of other proteins that make up the cytoskeleton. The main cytoskeletal protein is spectrin, the basic unit of which is a dimer of α-spectrin and β-spectrin. The membrane is physically linked to the cytoskeleton by a third set of proteins (including ankyrin and the so-called band 4.1 and band 4.2), which thus connect these two structures intimately.

Figure 101-2

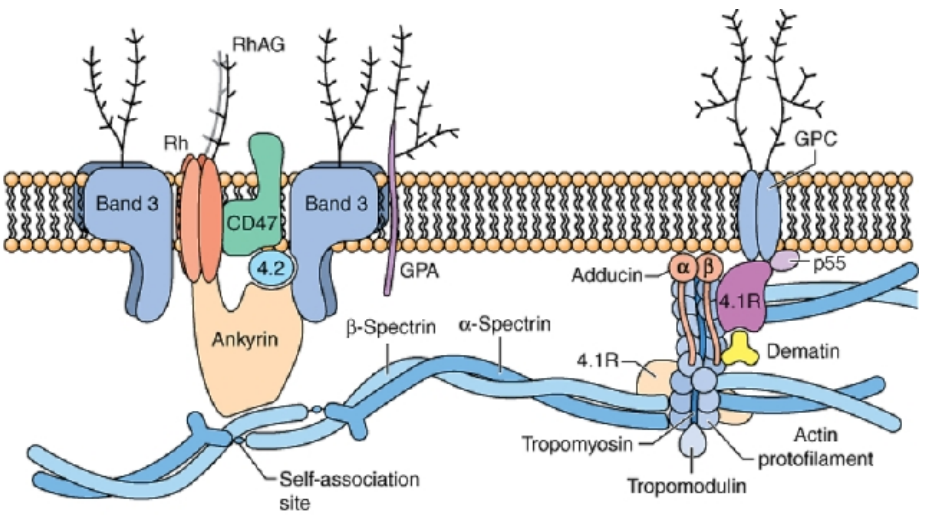


Diagram of red cell membrane/cytoskeleton. (For explanation see text.) *(From N Young et al: Clinical Hematology. Copyright Elsevier, 2006; with permission.)*

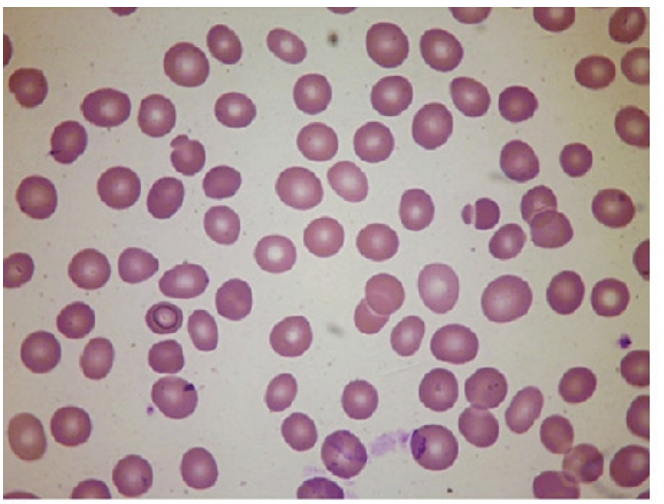
The membrane-cytoskeleton complex is indeed so integrated that, not surprisingly, an abnormality of almost any of its components will be disturbing or disruptive, causing structural failure, which results ultimately in hemolysis. These abnormalities are almost invariably

inherited mutations, and thus diseases of the membrane-cytoskeleton complex belong to the category of inherited hemolytic anemias. Before the red cells lyse, they often exhibit more or less specific morphologic changes that alter the normal biconcave disc shape. Thus, the majority of the diseases in this group have been known for over a century as *hereditary spherocytosis* (HS) and *hereditary elliptocytosis* (HE). Their molecular basis has been elucidated.

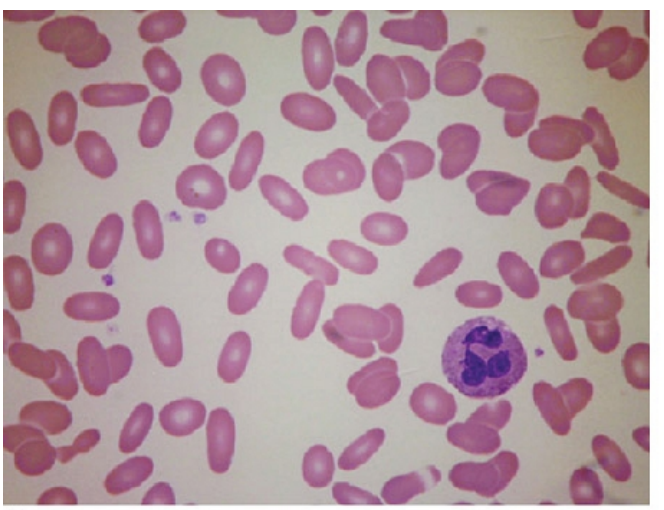
Hereditary Spherocytosis

This is a relatively common type of hemolytic anemia, with an estimated frequency of at least 1 in 5000. Its identification is credited to Minkowksy and Chauffard, who at the end of the 19th century reported families in whom HS was inherited as an autosomal dominant condition. From this seminal work, HS came to be defined as an inherited form of HA associated with the presence of spherocytes in the peripheral blood (Fig. 101-3A). In addition, in vitro studies revealed that the red cells were abnormally susceptible to lysis in hypotonic media; indeed, the presence of *osmotic fragility* became the main diagnostic test for HS. Today we know that HS, thus defined, is genetically heterogeneous, i.e., it can arise from a variety of mutations in one of several genes (Table 101-3). Whereas classically the inheritance of HS is autosomal dominant (with the patients being heterozygous), some severe forms are instead autosomal recessive (with the patient being homozygous).

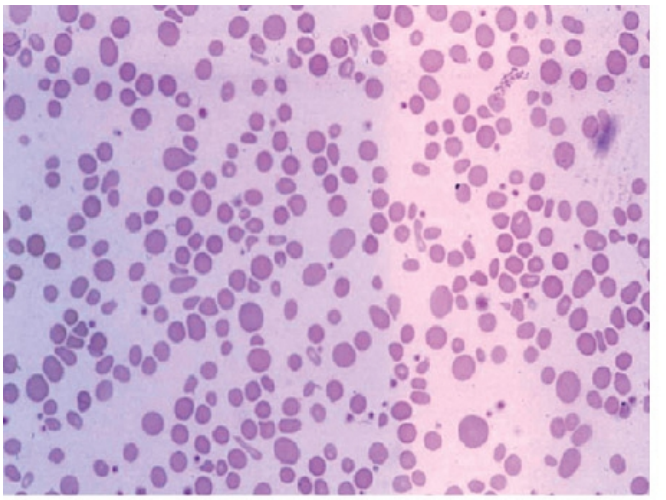
Figure 101-3



A



B



C

Peripheral blood smear from patients with membrane-cytoskeleton abnormalities. ***A***. Hereditary spherocytosis. ***B***. Hereditary elliptocytosis, heterozygote. ***C***. Elliptocytosis, with both alleles of the α-spectrin gene mutated. *[From L Luzzatto, in J Gribben and D Pravan (eds): Molecular Hematology, 2d edition. Oxford, Blackwell, 2005; with permission.]*

Table 101-3 Inherited Diseases of the Red Cell Membrane-Cytoskeleton

Gene	Chromosomal Location	Protein Produced	Disease(s) with Certain Mutations (Inheritance)	Comments
SPTA1	1q22-q23	α-Spectrin	HS (recessive)	Rare.
			HE (dominant)	Mutations of this gene account for about 65% of HE. More severe forms may be due to coexistence of an otherwise silent mutant allele.
SPTB	14q23-q24.1	β-Spectrin	HS (dominant)	Rare.
			HE (dominant)	Mutations of this gene account for ~30% of HE, including some severe forms.
ANK1	8p11.2	Ankyrin	HS (dominant)	May account for majority of HS.
SLC4A1	17q21	Band 3 (anion channel)	HS (dominant)	Mutations of this gene may account for ~25% of HS.
			Southeast Asian ovalocytosis (dominant)	Polymorphic mutation (deletion of 9 amino acids); clinically asymptomatic; protective against *Plasmodium falciparum*.
EPB41	1p33-p34.2	Band 4.1	HE (dominant)	Mutations of this gene account for about 5% of HE, mostly with prominent morphology but no hemolysis in heterozygotes; severe hemolysis in homozygotes.
EPB42	15q15-q21	Band 4.2	HS (recessive)	Mutations of this gene account for about 3% of HS.
RHAG	6p21.1-p11	Rhesus antigen	Chronic nonspherocytic hemolytic anemia	Very rare; associated with total loss of all Rh antigens.

Note: HS, hereditary spherocytosis; HE, hereditary elliptocytosis.

Clinical Presentation and Diagnosis

The spectrum of clinical severity of HS is broad. Severe cases may present in infancy with severe anemia, whereas mild cases may present in young adults or even later in life. In women, HS is sometimes first diagnosed when anemia is investigated during pregnancy. The main clinical findings are jaundice, an enlarged spleen, and often gallstones; frequently it is the finding of gallstones in a young person that triggers diagnostic investigations.

The variability in clinical manifestations that is observed among patients with HS is largely due to the different underlying molecular lesions (Table 101-3). Not only are mutations of several genes involved, but individual mutations of the same gene can also give very different clinical manifestations. In milder cases, hemolysis is often compensated (see above), and this may cause variation even in the same patient, due to the fact that intercurrent conditions (e.g., infection) cause decompensation. The anemia is usually normocytic, with the

characteristic morphology that gives the disease its name. A characteristic feature is an increase in mean corpuscular hemoglobin concentration (MCHC): this is almost the only condition in which high MCHC is seen.

When there is a family history, it is usually easy to suspect the diagnosis, but there may be no family history for at least two reasons: (1) The patient may have a de novo mutation, i.e., a mutation that has taken place in a germ cell of one of his parents or early after zygote formation; and (2) the patient may have a recessive form of HS (Table 101-3). In most cases the diagnosis is confirmed on the basis of red cell morphology and a test for osmotic fragility, a modified version of which is called the "pink test." In some cases a definitive diagnosis can be obtained only by molecular studies demonstrating a mutation in one of the genes underlying HS. This is carried out only in laboratories with special expertise in this area.

Hereditary Spherocytosis: Treatment

There is currently no treatment aimed at the cause of HS; no way has yet been found to correct the basic defect in the membrane-cytoskeleton structure. However, it has been apparent for a long time that the spleen plays a special role in HS, through a dual mechanism. On one hand, as in many other HAs, the spleen itself is a major site of destruction; on the other hand, transit through the splenic circulation makes the defective red cells more spherocytic and therefore accelerates their demise, even though lysis may take place elsewhere. For these reasons, splenectomy has long been regarded as a prime, almost obligatory therapeutic measure in HS. However, it also increases the risk of certain infections, and therefore current guidelines (not evidence-based) are as follows.

1. Avoid splenectomy in mild cases.
2. Delay splenectomy until at least 4 years of age, after the risk of severe sepsis has peaked.
3. Antipneumococcal vaccination before splenectomy is imperative, whereas penicillin prophylaxis postsplenectomy is controversial.
4. HS patients often may require cholecystectomy. It used to be considered mandatory to combine this procedure with splenectomy, but this may not be always necessary.

Hereditary Elliptocytosis

HE is at least as heterogeneous as HS, both from the genetic (Table 101-3) and from the clinical point of view. Again it is the shape of the red cells that gives the name to these conditions, but there is no direct correlation between elliptocytic morphology and clinical severity. In fact, some mild or even asymptomatic cases may have nearly 100% elliptocytes, whereas in severe cases, all sorts of bizarre poikilocytes may predominate (Fig. 101-3B, C). Clinical features and recommended management are similar to those for HS. Although the spleen may not have the specific role it has in HS, in severe cases splenectomy may be beneficial. The prevalence of HE causing clinical disease is similar to that of HS. However, an asymptomatic form, referred to as *Southeast Asian ovalocytosis*, has a frequency of up to 7% in certain populations, presumably as a result of malaria selection.

Stomatocytosis

This rare condition with autosomal dominant inheritance draws its name (mouth-like cells) from the fact that the normally round-shaped central pallor of red cells is replaced by a linear-shaped central pallor. Hemolysis is usually relatively mild. Splenectomy is contraindicated as it has been followed in a majority of cases by severe thromboembolic complications.

Enzyme Abnormalities

When there is an important defect in the membrane or in the cytoskeleton, hemolysis is a direct consequence of the fact that the very structure of the red cell is abnormal. Instead, when one of the enzymes is defective, the consequences will depend on the precise role of that enzyme in the metabolic machinery of the red cell, which, in its first approximation, has two important functions: (1) to provide energy in the form of ATP, and (2) to prevent oxidative damage to hemoglobin and to other proteins.

Abnormalities of the Glycolytic Pathway

(Fig. 101-1) Since red cells, in the course of their differentiation, have sacrificed not only their nucleus and their ribosomes but also their mitochondria, they rely exclusively on the anaerobic portion of the glycolytic pathway for producing energy in the form of ATP. Most of the ATP is required by the red cell for cation transport against a concentration gradient across the membrane. If this fails, due to a defect of any of the enzymes of the glycolytic pathway, the result will be hemolytic disease.

Pyruvate Kinase Deficiency

Abnormalities of the glycolytic pathway are all inherited and all rare (Table 101-4). Among them, deficiency of pyruvate kinase (PK) is the least rare, with an estimated prevalence of 1:10,000. The clinical picture is that of an HA that often presents in the newborn with neonatal jaundice; the jaundice persists and is usually associated with a very high reticulocytosis. The anemia is of variable severity; sometimes it is so severe as to require regular blood transfusions; sometimes it is mild, bordering on a nearly compensated hemolytic disorder. As a result, the diagnosis may be delayed, and in some cases it is made in young adults– for instance, in a woman during her first pregnancy, when the anemia may get worse. In part the delay in diagnosis is due to the fact that the anemia is remarkably well-tolerated because the metabolic block at the last step in glycolysis causes an increase in bisphosphoglycerate (or DPG), a major effector of the hemoglobin-oxygen dissociation curve. Thus, the oxygen delivery to the tissues is increased.

Table 101-4 Red Cell Enzyme Abnormalities Causing Hemolysis

	Enzyme (Acronym)	Chromosomal Location	Prevalence of Enzyme Deficiency (Rank)	Clinical Manifestations Extra-Red Cell	Comments
Glycolytic pathway	*Hexokinase* (HK)	10q22	Very rare		Other isoenzymes known.
	Glucose 6-phosphate isomerase (G6PI)	19q31.1	Rare (4)	NM, CNS	
	Phosphofructokinase (PFK)	12q13	Very rare	Myopathy	
	Aldolase	16q22-24	Very rare		
	Triose phosphate isomerase (TPI)	12p13	Very rare	CNS (severe), NM	
	Glyceraldehyde 3-phosphate dehydrogenase (GAPD)	12p13.31–p13.1	Very rare	Myopathy	
	Diphosphoglycerate mutase (DPGM)	7q31-q34	Very rare		Erythrocytosis rather than hemolysis.
	Phosphoglycerate kinase (PGK)	Xq13	Very rare	CNS, NM	May benefit from splenectomy.
	Pyruvate kinase (PK)	1q21	Rare (2)		May benefit from splenectomy.
Redox	*Glucose 6-phosphate dehydrogenase* (G6PD)	Xq28	Common (1)	Very rarely granulocytes	In almost all cases only AHA from exogenous trigger.
	Glutathione synthase	20q11.2	Very rare	CNS	
	γ-Glutamylcysteine synthase	6p12	Very rare	CNS	
	Cytochrome b5 reductase	22q13.31–qter	Rare	CNS	Methemoglobinemia rather than hemolysis.
Nucleotide metabolism	*Adenylate kinase* (AK)	9q34.1	Very rare	CNS	
	Pyrimidine 5′-nucleotidase (P5N)	3q11–q12	Rare (3)		May benefit from splenectomy.

Note: CNS, central nervous system; AHA, acquired hemolytic anemia.

Pyruvate Kinase Deficiency: Treatment

Management of PK deficiency is mainly supportive. In view of the marked increase in red cell turnover, oral folic acid supplements should be given constantly. Blood transfusion should be used as necessary, and iron chelation may have to be added if the blood transfusion requirement is high enough to cause iron overload. In these patients, who have more severe disease, splenectomy may be beneficial. There is a single case report of curative treatment of PK deficiency by bone marrow transplantation from an HLA-identical PK normal sib: this seems a viable option for severe cases when a sib donor is available.

Other Glycolytic Enzyme Abnormalities

All of these defects are rare to very rare (Table 101-4), and all cause HA of varying degrees of severity. It is not unusual for the presentation to be in the guise of severe neonatal jaundice, which may require exchange transfusion; if the anemia is less severe, it may present later in life or may even remain asymptomatic and be detected incidentally when a blood count is done for unrelated reasons. The spleen is often enlarged. When other systemic manifestations occur, they involve the central nervous system, sometimes entailing severe mental retardation (particularly in the case of triose phosphate isomerase deficiency) or the neuromuscular system, or both. The *diagnosis* of HA is usually not difficult, thanks to the triad of normo-macrocytic anemia, reticulocytosis, and hyperbilirubinemia. Enzymopathies should be considered in the differential diagnosis of any chronic Coombs-negative HA. In most cases of glycolytic enzymopathies, the morphologic abnormalities of red cells characteristically seen in membrane disorders are absent. A definitive diagnosis can be made only by demonstrating the deficiency of an individual enzyme by quantitative assays carried out in only a few specialized laboratories. If a particular molecular abnormality is already known in the family, then of course one could test directly for that defect at the DNA level, bypassing the need for enzyme assays.

Abnormalities of Redox Metabolism

G6PD Deficiency

Glucose 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme critical in the redox metabolism of all aerobic cells (Fig. 101-4). In

red cells, its role is even more critical because it is the only source of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which, directly and via reduced glutathione (GSH), defends these cells against oxidative stress. G6PD deficiency is a prime example of an HA due to interaction between an intracorpuscular and an extracorpuscular cause, because in the majority of cases hemolysis is triggered by an exogenous agent. Although in G6PD-deficient subjects there is a decrease in G6PD activity in most tissues, this is less marked than in red cells, and it does not seem to produce symptoms.

Figure 101-4

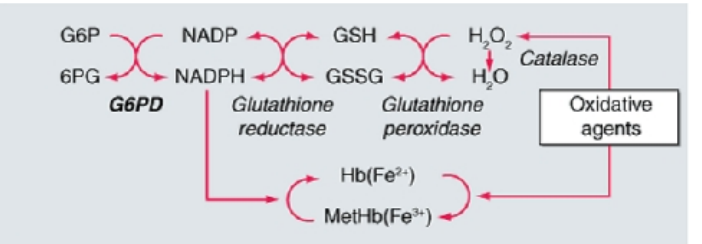


Diagram of redox metabolism in the red cell. G6P, glucose 6-phosphate; 6PG, 6-phosphogluconate; G6PD, glucose 6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; Hb, hemoglobin; MetHb, methemoglobin; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

Genetic Considerations

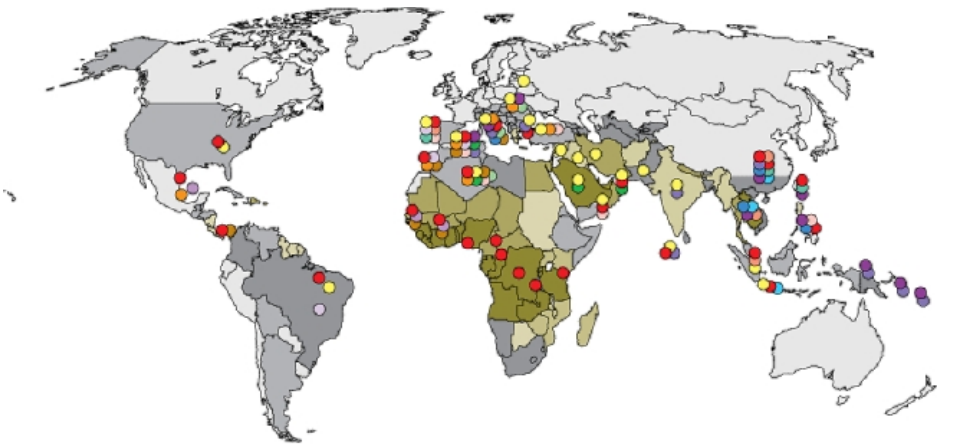
The *G6PD* gene is X-linked, and this has important implications. First, as males have only one *G6PD* gene (i.e., they are hemizygous for this gene), they must be either normal or G6PD-deficient. By contrast, females, having two *G6PD* genes, can be normal, deficient (homozygous), or intermediate (heterozygous). As a result of the phenomenon of X-chromosome inactivation, heterozygous females are genetic mosaics, with a highly variable ratio of G6PD-normal to G6PD-deficient cells and an equally variable degree of clinical expression; some heterozygotes can be just as affected as hemizygous males. The enzymatically active form of G6PD is either a dimer or a tetramer of a single protein subunit of 514 amino acids. G6PD-deficient subjects have been found invariably to have mutations in the coding region of the *G6PD* gene. Almost all of the 140 different mutations known are single missense point mutations, entailing single amino acid replacements in the G6PD protein. In most cases these mutations cause G6PD deficiency by decreasing the in vivo stability of the protein, and thus the physiologic decrease in G6PD activity that takes place with red cell ageing is greatly accelerated. In some cases an amino acid replacement can also affect the catalytic function of the enzyme.

Among the mutations, those underlying *chronic nonspherocytic hemolytic anemia* (CNSHA; see below) are a discrete subset. This much more severe clinical phenotype can be ascribed in some cases to adverse qualitative changes (for instance, a decreased affinity for the substrate, glucose 6-phosphate); or simply to the fact that the enzyme deficit is more extreme because it is more unstable. For instance, a cluster of mutations map at or near the dimer interface, and they prevent dimer formation.

Epidemiology

G6PD deficiency is widely distributed in tropical and subtropical parts of the world (Africa, Southern Europe, the Middle East, Southeast Asia, and Oceania) (Fig. 101-5) and wherever people from those areas have migrated; a conservative estimate is that at least 400 million people have a G6PD-deficiency gene. In several of these areas, the frequency of a G6PD-deficiency gene may be as high as 20% or more. It would be quite extraordinary for a trait that causes significant pathology to spread widely and reach high frequencies in many populations without conferring some biologic advantage. Indeed, G6PD is one of the best characterized examples of genetic polymorphisms in the human species. Clinical field studies and in vitro experiments strongly support the view that G6PD deficiency has been selected by *Plasmodium falciparum* malaria, by virtue of the fact that it confers a relative resistance against this highly lethal infection. Whether this protective effect is exerted mainly in hemizygous males or in females heterozygous for G6PD deficiency is still not clear. Different G6PD variants underlie G6PD deficiency in different parts of the world. Some of the more widespread variants are G6PD Mediterranean on the shores of the Mediterranean Sea, in the Middle East, and in India; G6PD A– in Africa and in Southern Europe; G6PD Vianchan and G6PD Mahidol in Southeast Asia; G6PD Canton in China; and G6PD Union worldwide. The heterogeneity of polymorphic G6PD variants is proof of their independent origin, and it supports the notion that they have been selected by a common environmental agent, in keeping with the concept of convergent evolution.

Figure 101-5



Epidemiology of G6PD deficiency throughout the world. The different shadings indicate increasingly high levels of prevalence, up to about 20%; the different colored symbols indicate individual genetic variants of G6PD, each one having a different mutation. *[From L Luzzatto et al in C Scriver et al (eds): The Metabolic & Molecular Bases of Inherited Disease, 8th edition. New York, McGraw-Hill, 2001.]*

Clinical Manifestations

The vast majority of people with G6PD deficiency remain clinically asymptomatic throughout their lifetime. However, all of them have an increased risk of developing neonatal jaundice (NNJ) and a risk of developing acute HA when challenged by a number of oxidative agents. NNJ related to G6PD deficiency is very rarely present at birth: the peak incidence of clinical onset is between day 2 and day 3, and in most cases the anemia is not severe. However, NNJ can be very severe in some G6PD-deficient babies, especially in association with prematurity, infection, and/or environmental factors (such as naphthalene-camphor balls used in babies' bedding and clothing). In these cases, if inadequately managed, NNJ associated with G6PD deficiency can produce kernicterus and permanent neurologic damage.

Acute HA can develop as a result of three types of triggers: (1) fava beans, (2) infections, and (3) drugs (Table 101-5). Typically, a hemolytic attack starts with malaise, weakness, and abdominal or lumbar pain. After an interval of several hours to 2–3 days, the patient develops jaundice and often dark urine, due to hemoglobinuria (Table 101-6). The onset can be extremely abrupt, especially with favism in children. The anemia is moderate to extremely severe, usually normocytic and normochromic, and due partly to intravascular hemolysis; hence, it is associated with hemoglobinemia, hemoglobinuria, and low or absent plasma haptoglobin. The blood film shows anisocytosis, polychromasia, and spherocytes (Fig. 101-6). The most typical feature is the presence of bizarre poikilocytes with red cells that appear to have unevenly distributed hemoglobin (hemighosts) and red cells that appear to have had parts of them bitten away (bite cells or blister cells). A classic test, now rarely carried out, is supravital staining with methyl violet, which, if done promptly, reveals the presence of Heinz bodies, consisting of precipitates of denatured hemoglobin and regarded as a signature of oxidative damage to red cells (except for the rare occurrence of an unstable hemoglobin). LDH is high and so is the unconjugated bilirubin, indicating that there is also extravascular hemolysis. The most serious threat from acute HA in adults is the development of acute renal failure (exceedingly rare in children). Once the threat of acute anemia is over, and in the absence of comorbidity, full recovery from acute HA associated with G6PD deficiency is the rule.

Table 101-5 Drugs that Carry Risk of Clinical Hemolysis in Persons with G6PD Deficiency

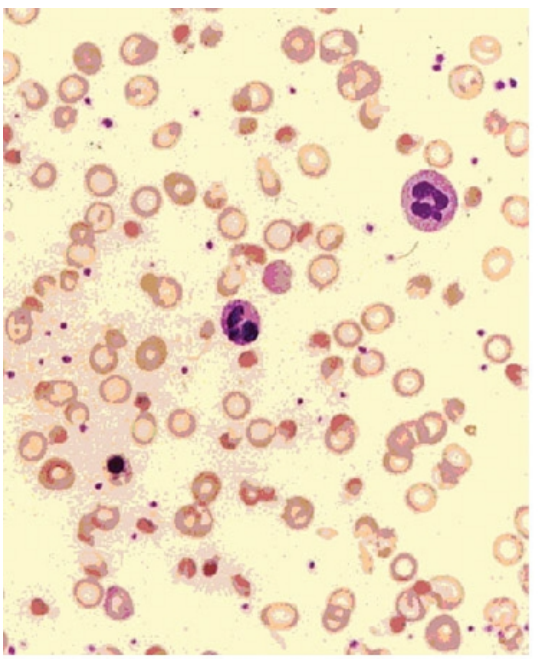
	Definite Risk	**Possible Risk**	**Doubtful Risk**
Antimalarials	Primaquine	Chloroquine	Quinine
	Dapsone/chlorproguanil		
Sulphonamides/sulphones	Sulphametoxazole	Sulfasalazine	Sulfisoxazole
	Others	Sulfadimidine	Sulfadiazine
	Dapsone		
Antibacterial/antibiotics	Cotrimoxazole	Ciprofloxacin	Chloramphenicol
	Nalidixic acid	Norfloxacin	*p*-Aminosalicylic acid
	Nitrofurantoin		
	Niridazole		
Antipyretic/analgesics	Acetanilide	Acetylsalicylic acid high dose (>3 g/d)	Acetylsalicylic acid <3 g/d
	Phenazopyridine (Pyridium)		Acetaminophen

			Phenacetin
Other	Naphthalene	Vitamin K analogues	Doxorubicin
	Methylene blue	Ascorbic acid >1 g	Probenecid
		Rasburicase	

Table 101-6 Diseases/Clinical Situations with Predominantly Intravascular Hemolysis

	Onset/Time Course	**Main Mechanism**	**Appropriate Diagnostic Procedure**	**Comments**
Mismatched blood transfusion	Abrupt	Nearly always ABO incompatibility	Repeat cross match	
Paroxysmal nocturnal hemoglobinuria (PNH)	Chronic with acute exacerbations	Complement (C)-mediated destruction of CD59(–) red cells	Flow cytometry to display a CD59(–) red cell population	Exacerbations due to C activation through any pathway
Paroxysmal cold hemoglobinuria (PCH)	Acute	Immune lysis of normal red cells	Test for Donath-Landsteiner antibody	Often triggered by viral infection
Septicemia	Very acute	Exotoxins produced by *Clostridium perfringens*	Blood cultures	Other organisms may be responsible
Microangiopathic	Acute or chronic	Red cell fragmentation	Red cell morphology on blood smear	Different causes ranging from endothelial damage to hemangioma to leaky prosthetic heart valve
March hemoglobinuria	Abrupt	Mechanical destruction	Targeted history taking	
Favism	Acute	Destruction of older fraction of G6PD-deficient red cells	G6PD assay	Triggered by ingestion of large dish of fava beans; but trigger can be infection or drug instead

Figure 101-6



Peripheral blood smear from a 5-year-old G6PD-deficient boy with acute favism.

A very small minority of subjects with G6PD deficiency have CNSHA of variable severity. The patient is always a male, usually with a history of NNJ, who may present with anemia or unexplained jaundice, or because of gallstones later in life. The spleen may be enlarged.

The severity of anemia ranges from borderline to transfusion-dependent. The anemia is usually normo-macrocytic, with reticulocytosis. Bilirubin and LDH are increased. Although hemolysis is, by definition, chronic in these patients, they are also vulnerable to acute oxidative damage, and therefore the same agents (see Table 101-5) that can cause acute HA in people with the ordinary type of G6PD deficiency will cause severe exacerbations in people with the severe form of G6PD deficiency. In some cases of CNSHA, the deficiency of G6PD is so severe in granulocytes that it becomes rate-limiting for their oxidative burst, with consequent increased susceptibility to bacterial infections.

Laboratory Diagnosis

The suspicion of G6PD deficiency can be confirmed by semiquantitative methods often referred to as screening tests, which are suitable for population studies and can correctly classify male subjects, in the steady state, as G6PD-normal or G6PD-deficient. However, in clinical practice a diagnostic test is usually needed when the patient has had a hemolytic attack: this implies that the oldest, most G6PD-deficient red cells have been selectively destroyed, and young red cells, having higher G6PD activity, are being released into the circulation. Under these conditions, only a quantitative test can give a definitive result. In males this test will identify normal hemizygotes and G6PD-deficient hemizygotes; among females some heterozygotes will be missed, but those who are at most risk of hemolysis will be identified.

G6PD Deficiency: Treatment

The acute HA of G6PD deficiency is largely preventable by avoiding exposure to triggering factors of previously screened subjects. Of course, the practicability and cost-effectiveness of screening depends on the prevalence of G6PD deficiency in each individual community. Favism is entirely preventable by not eating fava beans. Prevention of drug-induced hemolysis is possible in most cases by choosing alternative drugs. When acute HA develops and once its cause is recognized, no specific treatment is needed in most cases. However, if the anemia is severe, it may be a medical emergency, especially in children, requiring immediate action, including blood transfusion. If acute renal failure develops, hemodialysis may be necessary, but if there is no previous kidney disease, full recovery is the rule. The management of NNJ associated with G6PD deficiency is no different from that of NNJ due to other causes.

In cases with CNSHA, if the anemia is not severe, regular folic acid supplements and regular hematologic surveillance will suffice. It will be important to avoid exposure to potentially hemolytic drugs, and blood transfusion may be indicated when exacerbations occur, mostly in concomitance with intercurrent infection. In rare patients, regular blood transfusions may be required; appropriate iron chelation should be instituted in such cases. Unlike in hereditary spherocytosis, there is no evidence of selective red cell destruction in the spleen: however, in practice splenectomy has proven beneficial in severe cases.

Other Abnormalities of the Redox System

As mentioned above, GSH is a key player in the defense against oxidative stress (Fig. 101-4). Inherited defects of GSH metabolism are exceedingly rare, but each one of them can give rise to chronic HA (Table 101-4). A rare, peculiar, usually self-limited severe HA of the first month of life, called *infantile poikilocytosis*, may be associated with deficiency of glutathione peroxidase (GSHPx) due not to an inherited abnormality but to transient nutritional deficiency of selenium, an element essential for the activity of GSHPx.

Pyrimidine 5'-Nucleotidase (P5N) Deficiency

P5N is a key enzyme in the catabolism of nucleotides arising from the degradation of nucleic acids that takes place in the final stages of red cell maturation. How exactly its deficiency causes HA is not well understood, but a highly distinctive feature of this condition is a morphologic abnormality of the red cells known as *basophilic stippling*. The condition is rare, but it probably ranks third in frequency among red cell enzyme defects (after G6PD deficiency and PK deficiency). The anemia is lifelong, of variable severity, and may benefit from splenectomy.

Familial Hemolytic Uremic Syndrome (HUS)

This disorder is unique because, now that its basis has been elucidated, we can clearly see that hemolysis is due to an inherited defect, but this is external to red cells. HUS is defined as a microangiopathic hemolytic anemia with fragmented erythrocytes in the peripheral blood smear, thrombocytopenia (usually mild), and acute renal failure. An infection is usually the trigger of the syndrome, which tends to recur. When it does, the prognosis is serious. Although familial HUS is rare, studies of affected members from more than 100 families have revealed numerous mutations in any of three complement regulatory proteins: membrane cofactor protein, factor H, and factor I. It is thought that when complement is activated through the alternative pathway following damage to endothelial cells in the kidney, one of the results will be brisk hemolysis. Thus, the much more common Shiga toxin–related HUS can be regarded as a phenocopy of familial HUS.

Acquired Hemolytic Anemia

Mechanical Destruction of Red Cells

Although red cells are characterized by the remarkable deformability that enables them to squeeze through capillaries narrower than themselves thousands of times in their lifetime, there are at least two situations in which they succumb to shear, if not to wear and tear; the result is intravascular hemolysis resulting in hemoglobinuria. One situation, *march hemoglobinuria*, is acute and self-inflicted. Why a marathon runner may sometimes develop this complication and at another time does not is unclear (perhaps the footwear needs attention). A similar syndrome may develop after prolonged barefoot ritual dancing or vigorous bongo drumming. The other situation, which has been called *microangiopathic hemolytic anemia*, (Table 101-6) is chronic and iatrogenic; it takes place in patients with prosthetic heart valves, especially when paraprosthetic regurgitation is present. If the hemolysis consequent to mechanical trauma to the red cells is mild, and provided the supply of iron is adequate, it may be largely compensated. If more than mild anemia develops, reintervention to correct

regurgitation may be required.

Toxic Agents and Drugs

A number of chemicals with oxidative potential, whether medicinal or not, can cause hemolysis even in people who are not G6PD-deficient (see above). Examples are hyperbaric oxygen (or 100% oxygen), nitrates, chlorates, methylene blue, dapsone, cisplatin, and numerous aromatic (cyclic) compounds. Other chemicals may be hemolytic through nonoxidative, largely unknown mechanisms; examples are arsine, stibine, copper, and lead. The HA caused by lead poisoning is characterized by basophilic stippling: it is in fact a phenocopy of that seen in P5N deficiency (see above), suggesting it is mediated at least in part by lead inhibiting this enzyme.

In these cases hemolysis appears to be mediated by a direct chemical action on red cells. But drugs can cause hemolysis through at least two other mechanisms. (1) A drug can behave as a hapten and induce antibody production. In rare subjects this happens, for instance, with penicillin. Upon a subsequent exposure, red cells are caught as innocent bystanders in the reaction between penicillin and antipenicillin antibodies. Hemolysis will subside as soon as penicillin administration is stopped. (2) A drug can trigger, perhaps through mimicry, the production of an antibody against a red cell antigen. The best-known example is methyldopa, an antihypertensive agent no longer in use, which in a small fraction of patients stimulated the production of the Rhesus antibody anti-e. In patients who have this antigen, the anti-e is a true autoantibody, which would then cause an autoimmune HA (see below). Usually HA would gradually subside once methyldopa was discontinued.

Nucleosides may also cause hemolysis by depletion of ATP. Ribavirin, a drug used in the treatment of hepatitis C, causes the destruction of red cells through this mechanism. Severe intravascular hemolysis can be caused by the venom of certain snakes (cobras and vipers), and HA can also follow spider bites.

Infection

By far the most frequent infectious cause of hemolytic anemia in endemic areas is malaria (Chap. 203). In other parts of the world, the most frequent cause is probably Shiga toxin–producing *Escherichia coli* O157:H7, now recognized as the main etiologic agent of HUS, more common in children than in adults (Chap. 143). Life-threatening intravascular hemolysis due to a toxin with lecithinase activity occurs with *Clostridium perfringens* sepsis (Table 101-6), particularly with open wounds, following septic abortion, or as a disastrous accident due to a contaminated blood unit. Occasionally HA is seen, especially in children, with sepsis or endocarditis from a variety of organisms.

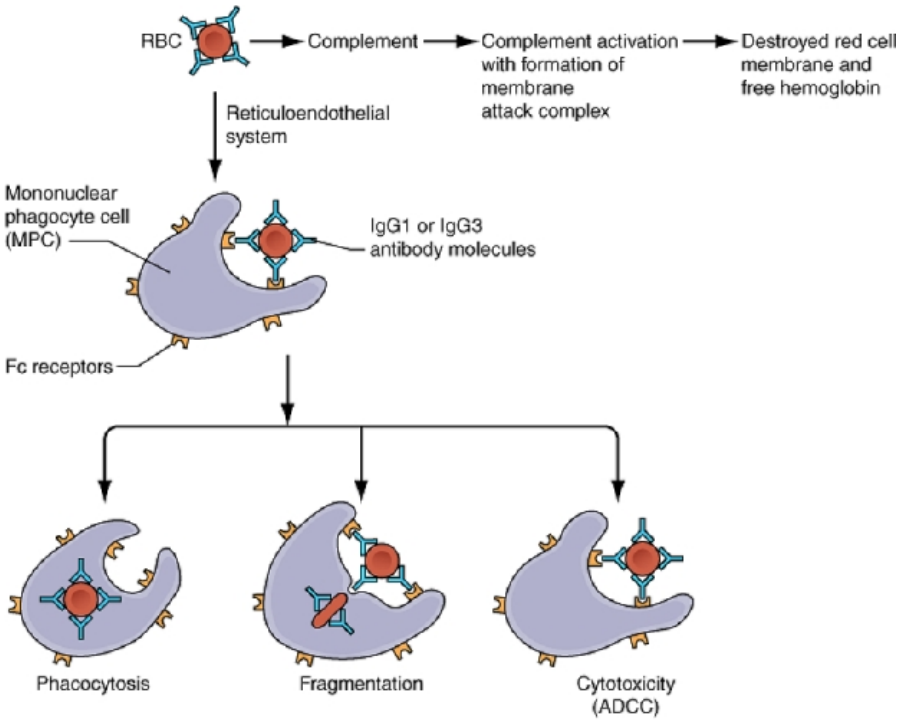
Autoimmune Hemolytic Anemia (AIHA)

Except for countries where malaria is endemic, AIHA is the most common form of acquired hemolytic anemia. In fact, not quite appropriately, the two phrases are sometimes used synonymously.

Pathophysiology

AIHA is caused by an autoantibody directed against a red cell antigen, i.e., a molecule present on the surface of red cells. The autoantibody binds to the red cells. Once a red cell is coated by antibody, it will be destroyed by one or more mechanisms. In most cases the Fc portion of the antibody will be recognized by the Fc receptor of macrophages, and this will trigger erythrophagocytosis (Fig. 101-7). Thus, destruction of red cells will take place wherever macrophages are abundant– i.e., in the spleen, liver, and bone marrow. Because of the special anatomy of the spleen, this organ is particularly efficient in trapping antibody-coated red cells, and often this is the predominant site of red cell destruction. Although in severe cases even circulating monocytes can take part in this process, most of the phagocytosis-mediated red cell destruction takes place in the spleen and liver, and it is therefore called *extravascular hemolysis*. In some cases the nature of the antibody is such (usually an IgM antibody) that the antigen-antibody complex on the surface of red cells is able to activate complement (C). As a result, a large amount of membrane attack complex will form, and the red cells may be destroyed directly, known as *intravascular hemolysis*.

Figure 101-7



Mechanism of antibody-mediated immune destruction of red cells. *(From N Young et al: Clinical Hematology. Copyright Elsevier, 2006; with permission.)*

Clinical Features

The onset of AIHA is very often abrupt and can be dramatic. The hemoglobin level can drop, within days, to as low as 4 g/dL; the massive red cell removal will produce jaundice, and often the spleen will be enlarged. When this triad is present, the suspicion of AIHA must be high. When hemolysis is (in part) intravascular, the telltale sign will be hemoglobinuria, which the patient may report or for which the physician must test. The diagnostic test for AIHA is the antiglobulin test worked out in 1945 by R.R.A. Coombs and known since by his name. The beauty of this test is that it directly detects the pathogenetic mediator of the disease, i.e., the presence of antibody on the red cells themselves. When the test is positive, it clinches the diagnosis; when it is negative, the diagnosis is unlikely. However, the sensitivity of the Coombs test varies depending on the technology that is used, and in doubtful cases a repeat in a specialized lab is advisable; the term *Coombs-negative AIHA* is a last resort. In some cases the autoantibody has a defined identity: it may be specific for a Rhesus system antigen (often anti-e). In many cases it is regarded as "unspecific" because it reacts with virtually all types of red cells.

As in autoimmune diseases in general, the real cause of AIHA remains obscure. However, from the clinical point of view, an important feature is that AIHA can appear to be isolated, or it can develop as part of a more general autoimmune disease, particularly systemic lupus erythematosus (SLE), of which sometimes it may be the first manifestation. Therefore, when AIHA is diagnosed, a full screen for autoimmune disease is imperative. In some cases AIHA can be associated, on first presentation or subsequently, with autoimmune thrombocytopenia (Evans's syndrome).

Autoimmune Hemolytic Anemia: Treatment

The first-line treatment of AIHA is glucocorticoids. A dose of prednisone of 1 mg/kg per day will cause a prompt remission in at least one-half of cases. Whereas some patients are apparently cured, relapses are not uncommon. For patients who do not respond, and for those who have relapsed, second-line treatment measures include long-term immunosuppression with low-dose prednisone, azathioprine, or cyclosporine. In patients whose AIHA has become chronic, and sometimes even earlier, splenectomy is a viable option: although it does not cure the disease, it can produce significant benefit by removing a major site of hemolysis, thus improving the anemia and/or reducing the need for immunosuppressive agents. Most of the management of AIHA is not evidence-based. However, the anti-CD20 antibody rituximab has produced responses. Anecdotal reports suggest response to intravenous immunoglobulin. In severe refractory cases, either auto- or allohematopoietic stem cell transplantation has been used, sometimes successfully.

Severe acute AIHA can be a medical emergency. The immediate treatment almost invariably includes transfusion of red cells. This may pose a special problem because if the antibody involved is "unspecific," all the blood units cross-matched will be incompatible. In these cases it is often correct, paradoxically, to transfuse incompatible blood, the rationale being that the transfused red cells will be destroyed no less but no more than the patient's own red cells, and in the meantime the patient stays alive. Clearly this rather unique situation requires good liaison and understanding between the clinical unit treating the patient and the blood transfusion/serology lab.

Paroxysmal Cold Hemoglobinuria (PCH)

PCH is a rather rare form of AIHA occurring mostly in children, usually triggered by a viral infection, usually self-limited, and characterized by involvement of the so-called Donath-Landsteiner antibody. In vitro this antibody has unique serologic features: it has anti-P specificity and binds to red cells only at a low temperature (optimally at 4°C), but when the temperature is shifted to 37°C, lysis of red cells takes place in the presence of complement. Consequently, in vivo there is intravascular hemolysis, resulting in hemoglobinuria. Clinically, the differential diagnosis must include other causes of hemoglobinuria (Table 101-2), but the presence of the Donath-Landsteiner antibody will prove PCH. Active supportive treatment, including blood transfusion, is needed to control the anemia; subsequently, recovery is the rule.

Cold Agglutinin Disease (CAD)

This designation is used for a form of chronic AIHA that usually affects the elderly and has special clinical and pathologic features. First, the term *cold* refers to the fact that the autoantibody involved reacts with red cells poorly or not at all at 37°C, whereas it reacts strongly at lower temperatures.[1] As a result, hemolysis is more prominent the more the body is exposed to cold. The antibody is usually an IgM, usually has an anti-I specificity (the I antigen is present on the red cells of almost everyone), and may have a very high titer (1:100,000 or more has been observed). Second, the antibody is produced by an expanded clone of B lymphocytes, and sometimes its concentration in the plasma is high enough to show up as a spike in plasma protein electrophoresis– i.e., as a monoclonal gammopathy. Third, since the antibody is IgM, CAD is related to Waldenström macroglobulinemia (WM; Chap. 106), although in most cases the other clinical features of this disease are not present. Thus, CAD must be regarded as a form of WM, i.e., as a low-grade mature B-cell lymphoma that manifests at an earlier stage because the unique biologic properties of the IgM that it produces give the clinical picture of chronic HA.

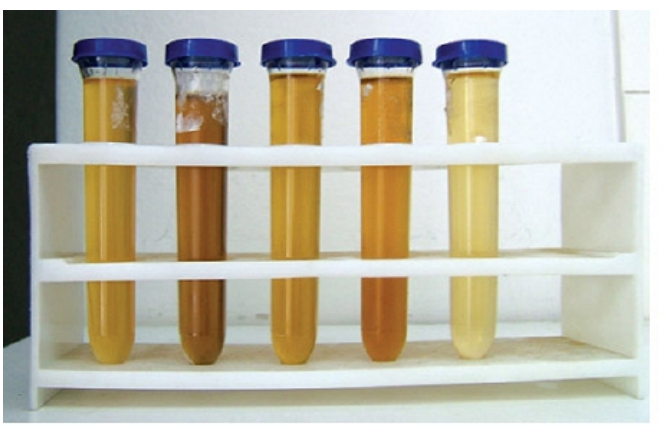
[1]In the past, this type of antibody was called a cold antibody, whereas the antibodies causing the more common form of AIHA were called warm antibodies.

In mild forms of CAD, avoidance of exposure to cold may be all that is needed to enable the patient to live with a reasonably comfortable quality of life, but in more severe forms the management of CAD is not easy. Blood transfusion is not very effective because donor red cells are I-positive and will be removed rapidly. Immunosuppressive/cytotoxic treatment with prednisone, azathioprine, or cyclophosphamide can reduce the antibody titer, but clinical efficacy is limited, and in view of the chronic nature of the disease, the side effects may prove unacceptable. Plasma exchange is a rational approach, but it is laborious and must be carried out, in some patients, at very frequent intervals. The picture may be changing, as in a recent study rituximab gave a response rate of 60%. Given the long clinical course of CAD, it remains to be seen with what periodicity this agent will need to be administered.

Paroxysmal Nocturnal Hemoglobinuria (PNH)

PNH is an acquired chronic HA characterized by persistent intravascular hemolysis subject to recurrent exacerbations (Table 101-6; Fig. 101-8). In addition to hemolysis, there is often pancytopenia and a risk of venous thrombosis. This triad makes PNH a truly unique clinical condition; however, when not all of these three features are manifest on presentation, the diagnosis is often delayed, although it can be always made by appropriate laboratory investigations (see below).

Figure 101-8



Consecutive urine samples from a patient with paroxysmal nocturnal hemoglobinuria (PNH). The variation in the severity of hemoglobinuria within hours is probably unique to this condition.

PNH has about the same frequency in men and women, and it is encountered in all populations throughout the world, but it is a rare disease: its prevalence is 1–5 per million (it may be somewhat less rare in Southeast Asia and in the Far East). There is no evidence of

inherited susceptibility. PNH has never been reported as a congenital disease, but it can present in small children or in people in their seventies, although most patients are young adults.

Clinical Features

The patient may seek medical attention because one morning she or he has passed "blood instead of urine." This distressing event may be regarded as the classical presentation; however, more frequently this symptom is not noticed or is suppressed. Indeed, the patient often presents simply as a problem in the differential diagnosis of *anemia*, whether symptomatic or discovered incidentally. Sometimes the anemia is associated from the outset with neutropenia or thrombocytopenia, or both. Some patients may present with recurrent attacks of severe abdominal pain, defying a specific diagnosis and eventually found to be caused by thrombosis. When thrombosis affects the hepatic veins, it may produce acute hepatomegaly and ascites, i.e., a full-fledged Budd-Chiari syndrome, which, in the absence of liver disease, ought to raise the suspicion of PNH.

The *natural history* of PNH can extend over decades. Without treatment, the median survival is ~8–10 years; in the past the commonest cause of death has been venous thrombosis followed by infection secondary to severe neutropenia and hemorrhage secondary to severe thrombocytopenia. PNH may evolve into aplastic anemia (AA), and PNH may manifest itself in patients who previously had AA. Rarely (estimated 1–2% of all cases), PNH may terminate in acute myeloid leukemia. On the other hand, full spontaneous recovery from PNH has been well documented, albeit rarely.

Laboratory Investigations and Diagnosis

The most consistent blood finding is anemia, which may range from mild to moderate to very severe. The anemia is usually normo-macrocytic, with unremarkable red cell morphology; if the MCV is high, it is usually largely accounted for by reticulocytosis, which may be quite marked (up to 20%, or up to 400,000/μL). The anemia may become microcytic if the patient is allowed to become iron-deficient as a result of chronic urinary blood loss through hemoglobinuria. Neutropenia and/or thrombocytopenia may or may not be present from the outset or may develop subsequently. Unconjugated bilirubin is mildly or moderately elevated, LDH is typically markedly elevated (values in the thousands are common), and haptoglobin is usually undetectable. All these findings make the diagnosis of HA compelling. Hemoglobinuria may be overt in a random urine sample; if it is not, it may be helpful to obtain serial urine samples, since hemoglobinuria can vary dramatically from day to day, and even from hour to hour (Fig. 101-8). The bone marrow is usually cellular with marked to massive erythroid hyperplasia, often with mild to moderate dyserythropoietic features (these do not justify confusing PNH with MDS). At some stage of the disease the marrow may become hypocellular or even frankly aplastic (see below).

The definitive diagnosis of PNH must be based on the demonstration that a substantial proportion of the patient's red cells have an increased susceptibility to complement (C), due to the deficiency on their surface of proteins (particularly CD59 and CD55) that normally protect the red cells from activated C. The sucrose hemolysis test is unreliable, and the acidified serum (Ham) test is carried out in few labs. The gold standard today is flow cytometry, which can be carried out on granulocytes as well as on red cells. A bimodal distribution of cells, with a discrete population that is CD59–, CD55–, is diagnostic of PNH. Usually this population is at least 5% of the total in the case of red cells and at least 20% of the total in the case of granulocytes.

Pathophysiology

Hemolysis in PNH is due to an intrinsic abnormality of the red cell, which makes it exquisitely sensitive to activated C, whether it is activated through the alternative pathway or through an antigen-antibody reaction. The former mechanism is mainly responsible for intravascular hemolysis in PNH. The latter mechanism explains why the hemolysis can be dramatically exacerbated in the course of a viral or bacterial infection. Hypersusceptibility to C is due to deficiency of several protective membrane proteins, of which CD59 is the most important because it hinders the insertion of C9 polymers into the membrane. The molecular basis for the deficiency of these proteins has been pinpointed not to a defect in any of the respective genes but rather to the shortage of a unique glycolipid molecule, GPI, which, through a peptide bond, anchors these proteins to the surface membrane of cells. The shortage of GPI is due in turn to a mutation in an X-linked gene, called *PIG-A*, required for an early step in GPI biosynthesis. In virtually each patient, the PIG-A mutation is different. This is not surprising, since these mutations are not inherited. Rather, each one takes place de novo in a hemopoietic stem cell (i.e., they are somatic mutations). As a result, the patient's marrow is a mosaic of mutant and nonmutant cells, and the peripheral blood always contains both PNH cells and normal (non-PNH) cells. Thrombosis is one of the most immediately life-threatening complications of PNH and yet one of the least understood in its pathogenesis. It could be that deficiency of CD59 on the PNH platelet causes inappropriate platelet activation; however, other mechanisms are possible.

Bone Marrow Failure- Relationship between PNH and AA

It is not unusual that patients with firmly established PNH have a previous history of well-documented AA. On the other hand, sometimes a patient with PNH becomes less hemolytic and more pancytopenic and ultimately has the clinical picture of AA. Since AA is probably an organ-specific autoimmune disease in which T cells cause damage to hematopoietic stem cells, the same may be true of PNH, with the specific proviso that the damage spares PNH stem cells. Skewing of the T cell repertoire in patients with PNH supports this notion. In addition, in mouse models, PNH stem cells do not expand when the rest of the bone marrow is normal, and by high-sensitivity flow cytometry technology, very rare PNH cells harboring PIG-A mutations can be demonstrated in normal people. In view of these facts, it seems that an element of bone marrow failure (BMF) in PNH is the rule rather than the exception. An extreme view is that PNH is a form of AA in which BMF is masked by the massive expansion of the PNH clone that populates the patient's bone marrow. The mechanism whereby PNH stem cells escape the damage suffered by non-PNH stem cells is not yet known.

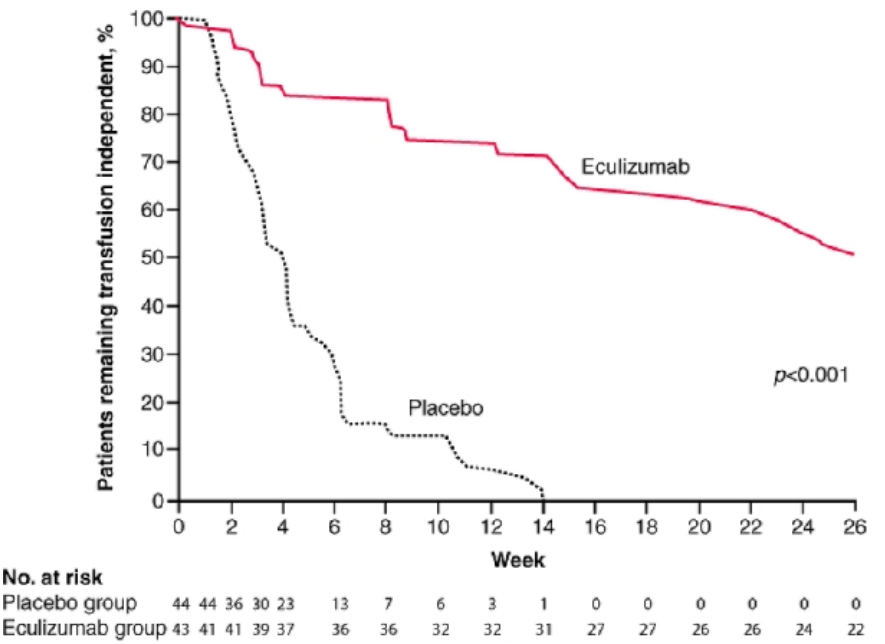
Paroxysmal Nocturnal Hemoglobinuria: Treatment

Unlike other acquired HAs, PNH may be lifelong and most patients receive supportive treatment only, including transfusion of filtered red cells[2] whenever necessary. Folic acid supplements (at least 3 mg/d) are mandatory; the serum iron should be checked periodically and iron supplements administered as appropriate. Long-term glucocorticoids are not indicated because there is no evidence that they have any effect on chronic hemolysis, and their side effects are considerable and potentially dangerous. The only form of treatment that can provide a cure for PNH is allogeneic bone marrow transplantation (BMT); when an HLA-identical sibling is available, BMT should be offered to any young patient with severe PNH.

[2]Now that filters with excellent removal of white cells are routinely used, the traditional washing of red cells, which aimed to avoid white cell reactions triggering hemolysis, is no longer necessary and considered wasteful.

A major advance in the management of PNH has been the development of a humanized monoclonal antibody, eculizumab, directed against the complement protein C5 (Fig. 101-9). By blocking the complement cascade downstream of C5, this antibody provides a medical intervention capable of controlling complement-dependent hemolysis in PNH. In an international multicenter placebo-controlled randomized trial on 87 patients who had been selected on grounds of having severe transfusion-dependent hemolysis, eculizumab completely abolished the need for blood transfusion in about one-half of the patients. Eculizumab administered intravenously at q2wk intervals also ameliorated the anemia in most patients and dramatically improved their quality of life.

Figure 101-9



Therapeutic efficacy of an anti-C5 antibody on the anemia of paroxysmal nocturnal hemoglobinuria. *(From P Hillmen et al: N Engl J Med 355:1233, 2006; with permission.)*

For patients with PNH-AA syndrome, immunosuppressive treatment with antilymphocyte globulin (ALG or ATG) and cyclosporine A may be indicated. Although no formal trial has ever been conducted, this approach has helped particularly to relieve severe thrombocytopenia and/or neutropenia in patients in whom these were the main problem(s). By contrast, there is often little immediate effect on hemolysis. Thrombolytic therapy with tissue plasminogen activator may be indicated after severe thrombosis. Any patient who has had deep vein thrombosis at any site in the abdomen or in a limb should be on regular anticoagulant prophylaxis.

Anemia Due to Acute Blood Loss

Blood loss causes anemia by two main mechanisms: first, by the direct loss of red cells; second, because if the loss of blood is protracted, it will gradually deplete the iron stores, eventually resulting in iron deficiency. Iron-deficiency anemia is discussed in Chap. 98.

Here we are concerned with *post-hemorrhagic anemia*, which follows *acute blood loss*. This can be *external* (as after trauma or due to postpartum hemorrhage) or *internal* (e.g., from bleeding in the gastrointestinal tract, rupture of the spleen, rupture of an ectopic pregnancy). In any of these cases– i.e., after the sudden loss of a large amount of blood– three clinical/pathophysiologic stages are noted.

1. At first, the dominant feature is hypovolemia, which poses a threat particularly to organs that normally have a high blood supply, such as the brain and the kidneys; therefore, loss of consciousness and acute renal failure are major threats. It is important to note that

at this stage an ordinary blood count will not show anemia, as the hemoglobin concentration is not affected.

2. Next, as an emergency response, baroreceptors and stretch receptors will cause release of vasopressin and other peptides, and the body will shift fluid from the extravascular to the intravascular compartment, producing hemodilution. Thus, the hypovolemia gradually converts to anemia. The degree of anemia will reflect the amount of blood lost. If after 3 days the hemoglobin is, say, 7 g/dL, it means that about half of the entire blood volume had been lost.

3. Provided bleeding does not continue, the bone marrow response will gradually ameliorate the anemia if erythropoietin production, the erythroid progenitors, and iron supply are normal. Within about 2–3 days after acute hemorrhage, reticulocytes will increase in the blood and reach a maximum 7–10 days after the hemorrhage has been controlled. Reticulocyte counts of 20% may be achieved.

The diagnosis of acute post-hemorrhagic anemia (APHA) is usually straightforward, although sometimes internal bleeding episodes- after a traumatic injury or otherwise- may not be immediately obvious, even when large. Whenever an abrupt fall in hemoglobin has taken place, whatever history is given by the patient, APHA should be suspected. Supplementary history may have to be obtained by asking the appropriate questions, and appropriate investigations (e.g., a sonogram or an endoscopy) may have to be carried out. Internal bleeding may result in a rise in unconjugated bilirubin and a fall in serum haptoglobin.

Anemia Due to Blood Loss: Treatment

With respect to treatment, a two-pronged approach is imperative. First, in many cases the blood lost needs to be replaced promptly. With many chronic anemias, finding and correcting the cause of the anemia is the first priority, and blood transfusion may not be even necessary, because the body is adapted to the anemia; with acute blood loss the reverse is true. Since the body is not adapted to the anemia, blood transfusion takes priority. Although fluorocarbon synthetic chemicals have shown promise, no "blood substitute" has yet become standard treatment. Second, while the emergency is being confronted, it is imperative to stop the hemorrhage and to eliminate its source.

Acknowledgment

H. Frank Bunn and Wendell Rosse contributed this chapter in the last edition and material from that chapter has been used here.

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Aplastic Anemia, Myelodysplasia, and Related Bone Marrow Failure Syndromes: Introduction

The hypoproliferative anemias are normochromic, normocytic or macrocytic and are characterized by a low reticulocyte count. Deficient production of RBCs occurs with marrow damage and dysfunction, which may be secondary to infection, inflammation, and cancer. Hypoproliferative anemia is also a prominent feature of hematologic diseases that are described as bone marrow failure states; these include aplastic anemia, myelodysplasia (MDS), pure red cell aplasia (PRCA), and myelophthisis. Anemia in these disorders is often not a solitary or even the major hematologic finding. More frequent in bone marrow failure is pancytopenia: anemia, leukopenia, and thrombocytopenia. Low blood counts in the marrow failure diseases result from deficient hematopoiesis, as distinguished from blood count depression due to peripheral destruction of red cells (hemolytic anemias), platelets (idiopathic thrombocytopenic purpura or due to splenomegaly), and granulocytes (as in the immune leukopenias).

Hematopoietic failure syndromes are classified by dominant morphologic features of the bone marrow (Table 102-1). While practical distinction among these syndromes usually is clear, they can occur secondary to other diseases, and some processes are so closely related that the diagnosis may be complex. Patients may seem to suffer from two or three related diseases simultaneously, or one diagnosis may appear to evolve into another. Many of these syndromes share an immune-mediated mechanism of marrow destruction and some element of genomic instability resulting in a higher rate of malignant transformation.

Table 102-1 Differential Diagnosis of Pancytopenia

Pancytopenia with Hypocellular Bone Marrow	
Acquired aplastic anemia	
Constitutional aplastic anemia (Fanconi's anemia, dyskeratosis congenita)	
Some myelodysplasia	
Rare aleukemic leukemia (AML)	
Some acute lymphoid leukemia	
Some lymphomas of bone marrow	
Pancytopenia with Cellular Bone Marrow	
Primary bone marrow diseases	Secondary to systemic diseases
Myelodysplasia	Systemic lupus erythematosus
Paroxysmal nocturnal hemoglobinuria	Hypersplenism
Myelofibrosis	B_{12}, folate deficiency
Some aleukemic leukemia	Overwhelming infection
Myelophthisis	Alcohol
Bone marrow lymphoma	Brucellosis
Hairy cell leukemia	Sarcoidosis
	Tuberculosis
	Leishmaniasis
Hypocellular Bone Marrow ± Cytopenia	
Q fever	
Legionnaires' disease	
Anorexia nervosa, starvation	
Mycobacteria	

Aplastic Anemia

Definition

Aplastic anemia is pancytopenia with bone marrow hypocellularity. Acquired aplastic anemia is distinguished from iatrogenic marrow aplasia, marrow hypocellularity after intensive cytotoxic chemotherapy for cancer. Aplastic anemia can also be constitutional: the genetic

diseases Fanconi's anemia and dyskeratosis congenita, while frequently associated with typical physical anomalies and the development of pancytopenia early in life, can also present as marrow failure in normal-appearing adults. Acquired aplastic anemia is often stereotypical in its manifestations, with the abrupt onset of low blood counts in a previously well young adult; seronegative hepatitis or a course of an incriminated medical drug may precede the onset. The diagnosis in these instances is uncomplicated. Sometimes blood count depression is moderate or incomplete, resulting in anemia, leukopenia, and thrombocytopenia in some combination. Aplastic anemia is related to both paroxysmal nocturnal hemoglobinuria (PNH; Chap. 101) and to MDS, and in some cases a clear distinction among these disorders may not be possible.

Epidemiology

The incidence of acquired aplastic anemia in Europe and Israel is two cases per million persons annually. In Thailand and China, rates of five to seven per million have been established. In general, men and women are affected with equal frequency, but the age distribution is biphasic, with the major peak in the teens and twenties and a second rise in the elderly.

Etiology

The origins of aplastic anemia have been inferred from several recurring clinical associations (Table 102-2); unfortunately, these relationships are not reliable in an individual patient and may not be etiologic. In addition, while most cases of aplastic anemia are idiopathic, little other than history separates these cases from those with a presumed etiology such as a drug exposure.

Table 102-2 Classification of Aplastic Anemia and Single Cytopenias

Acquired	**Inherited**
Aplastic Anemia	
Secondary	Fanconi's anemia
Radiation	Dyskeratosis congenita
Drugs and chemicals	Shwachman-Diamond syndrome
Regular effects	Reticular dysgenesis
Idiosyncratic reactions	Amegakaryocytic thrombocytopenia
Viruses	Familial aplastic anemias
Epstein-Barr virus (infectious mononucleosis)	Preleukemia (monosomy 7, etc.)
Hepatitis (non-A, non-B, non-C hepatitis)	Nonhematologic syndrome (Down's, Dubowitz, Seckel)
Parvovirus B19 (transient aplastic crisis, PRCA)	
HIV-1 (AIDS)	
Immune diseases	
Eosinophilic fasciitis	
Hypoimmunoglobulinemia	
Thymoma/thymic carcinoma	
Graft-versus-host disease in immunodeficiency	
Paroxysmal nocturnal hemoglobinuria	
Pregnancy	
Idiopathic	
Cytopenias	
PRCA (see Table 102-4)	Congenital PRCA (Diamond-Blackfan anemia)
Neutropenia/Agranulocytosis	
Idiopathic	Kostmann's Syndrome
Drugs, toxins	Shwachman-Diamond syndrome
Pure white cell aplasia	Reticular dysgenesis
Thrombocytopenia	
Drugs, toxins	Amegakaryocytic thrombocytopenia
Idiopathic amegakaryocytic	Thrombocytopenia with absent radii

Note: PRCA, pure red cell aplasia.

Radiation

Marrow aplasia is a major acute sequela of radiation. Radiation damages DNA; tissues dependent on active mitosis are particularly susceptible. Nuclear accidents can involve not only power plant workers but also employees of hospitals, laboratories, and industry (food sterilization, metal radiography, etc.), as well as innocents exposed to stolen, misplaced, or misused sources. While the radiation dose can be approximated from the rate and degree of decline in blood counts, dosimetry by reconstruction of the exposure can help to estimate the patient's prognosis and also to protect medical personnel from contact with radioactive tissue and excreta. MDS and leukemia, but probably not aplastic anemia, are late effects of radiation.

Chemicals

Benzene is a notorious cause of bone marrow failure. Vast quantities of epidemiologic, clinical, and laboratory data link benzene to aplastic anemia, acute leukemia, and blood and marrow abnormalities. The occurrence of leukemia is roughly correlated with cumulative exposure, but susceptibility must also be important, as only a minority of even heavily exposed workers develop benzene myelotoxicity. The employment history is important, especially in industries where benzene is used for a secondary purpose, usually as a solvent. Benzene-related blood diseases have declined with regulation of industrial exposure. Although benzene is no longer generally available as a household solvent, exposure to its metabolites occurs in the normal diet and in the environment. The association between marrow failure and other chemicals is much less well substantiated.

Drugs

(Table 102-3) Many chemotherapeutic drugs have marrow suppression as a major toxicity; effects are dose-dependent and will occur in all recipients. In contrast, idiosyncratic reactions to a large and diverse group of drugs may lead to aplastic anemia without a clear dose-response relationship. These associations rested largely on accumulated case reports until a large international study in Europe in the 1980s quantitated drug relationships, especially for nonsteroidal analgesics, sulfonamides, thyrostatic drugs, some psychotropics, penicillamine, allopurinol, and gold. Not all associations necessarily reflect causation: a drug may have been used to treat the first symptoms of bone marrow failure (antibiotics for fever or the preceding viral illness) or provoked the first symptom of a preexisting disease (petechiae by nonsteroidal anti-inflammatory agents administered to the thrombocytopenic patient). In the context of total drug use, idiosyncratic reactions, while individually devastating, are rare events. Chloramphenicol, the most infamous culprit, reportedly produced aplasia in only about 1/60,000 therapy courses, and even this number is almost certainly an overestimate (risks are almost invariably exaggerated when based on collections of cases; although the introduction of chloramphenicol was perceived to have created an epidemic of aplastic anemia, its diminished use was not followed by a changed frequency of marrow failure). Risk estimates are usually lower when determined in population-based studies; furthermore, the low absolute risk is also made more obvious: even a ten- or twentyfold increase in risk translates, in a rare disease, to but a handful of drug-induced aplastic anemia cases among hundreds of thousands of exposed persons.

Table 102-3 Some Drugs and Chemicals Associated with Aplastic Anemia

Agents that regularly produce marrow depression as major toxicity in commonly employed doses or normal exposures:
Cytotoxic drugs used in cancer chemotherapy: *alkylating agents*, *antimetabolites*, *antimitotics*, some antibiotics
Agents that frequently but not inevitably produce marrow aplasia:
Benzene
Agents associated with aplastic anemia but with a relatively low probability:
Chloramphenicol
Insecticides
Antiprotozoals: *quinacrine* and chloroquine, mepacrine
Nonsteroidal anti-inflammatory drugs (including *phenylbutazone*, indomethacin, ibuprofen, sulindac, aspirin)
Anticonvulsants (*hydantoins*, *carbamazapine*, phenacemide, felbamate)
Heavy metals (*gold*, arsenic, bismuth, mercury)
Sulfonamides: some antibiotics, antithyroid drugs (methimazole, methylthiouracil, propylthiouracil), antidiabetes drugs (tolbutamide, chlorpropamide), carbonic anhydrase inhibitors (acetazolamide and methazolamide)
Antihistamines (*cimetidine*, chlorpheniramine)
-Penicillamine
Estrogens (in pregnancy and in high doses in animals)
Agents whose association with aplastic anemia is more tenuous:
Other antibiotics (streptomycin, tetracycline, methicillin, mebendazole, trimethoprim/sulfamethoxazole, flucytosine)
Sedatives and tranquilizers (chlorpromazine, prochlorperazine, piperacetazine, chlordiazepoxide, meprobamate, methyprylon)
Allopurinol
Methyldopa
Quinidine
Lithium
Guanidine

Potassium perchlorate

Thiocyanate

Carbimazole

Note: Terms set in italic show the most consistent association with aplastic anemia.

Infections

Hepatitis is the most common preceding infection, and posthepatitis marrow failure accounts for about 5% of etiologies in most series. Patients are usually young men who have recovered from a bout of liver inflammation 1 to 2 months earlier; the subsequent pancytopenia is very severe. The hepatitis is seronegative (non-A, non-B, non-C, non-G) and possibly due to a novel, as yet undiscovered, virus. Fulminant liver failure in childhood also follows seronegative hepatitis, and marrow failure occurs at a high rate in these patients. Aplastic anemia can rarely follow infectious mononucleosis, and Epstein-Barr virus has been found in the marrow of a few patients, some without a suggestive preceding history. Parvovirus B19, the cause of transient aplastic crisis in hemolytic anemias and of some PRCAs (see below), does not usually cause generalized bone marrow failure. Mild blood count depression is frequent in the course of many viral and bacterial infections but resolves with the infection.

Immunologic Diseases

Aplasia is a major consequence and the inevitable cause of death in *transfusion-associated graft-versus-host disease* (GVDH), which can occur after infusion of unirradiated blood products to an immunodeficient recipient. Aplastic anemia is strongly associated with the rare collagen vascular syndrome called *eosinophilic fasciitis*, which is characterized by painful induration of subcutaneous tissues (Chap. 316). Pancytopenia with marrow hypoplasia can also occur in systemic lupus erythematosus.

Pregnancy

Aplastic anemia very rarely may occur and recur during pregnancy and resolve with delivery or with spontaneous or induced abortion.

Paroxysmal Nocturnal Hemoglobinuria

An acquired mutation in the *PIG-A* gene in a hematopoietic stem cell is required for the development of PNH, but *PIG-A* mutations probably occur commonly in normal individuals. If the PIG-A mutant stem cell proliferates, the result is a clone of progeny deficient in glycosylphosphatidylinositol-linked cell surface membrane proteins (Chap. 101). Such PNH cells are now accurately enumerated using fluorescence-activated flow cytometry of CD55 or CD59 expression on granulocytes rather than Ham or sucrose lysis tests on red cells. Small clones of deficient cells can be detected in about half of patients with aplastic anemia at the time of presentation [and PNH cells are also seen in MDS (see below)]; frank hemolysis and thrombotic episodes occur in patients with large PNH clones (>50%). Functional studies of bone marrow from PNH patients, even those with mainly hemolytic manifestations, show evidence of defective hematopoiesis. Patients with an initial clinical diagnosis of PNH, especially younger individuals, may later develop frank marrow aplasia and pancytopenia; patients with an initial diagnosis of aplastic anemia may suffer from hemolytic PNH years after recovery of blood counts. One popular but unproven explanation for the aplastic anemia/PNH syndrome is selection of the deficient clones because they are favored for proliferation in the peculiar environment of immune-mediated marrow destruction.

Constitutional Disorders

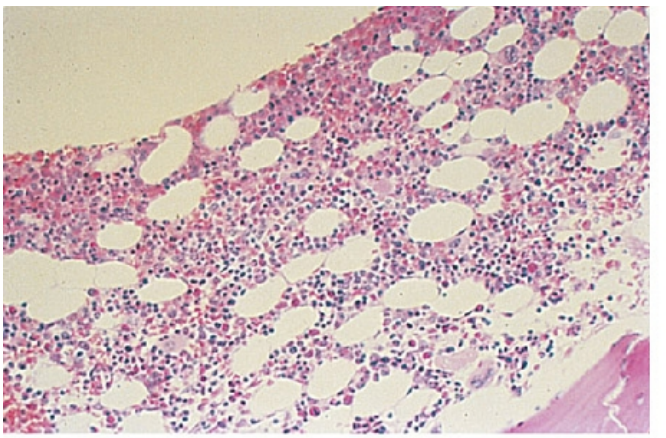
Fanconi's anemia, an autosomal recessive disorder, manifests as congenital developmental anomalies, progressive pancytopenia, and an increased risk of malignancy. Chromosomes in Fanconi's anemia are peculiarly susceptible to DNA cross-linking agents, the basis for a diagnostic assay. Patients with Fanconi's anemia typically have short stature, café au lait spots, and anomalies involving the thumb, radius, and genitourinary tract. At least 12 different genetic defects (all but one with an identified gene) have been defined; the most common, type A Fanconi's anemia, is due to a mutation in *FANCA*. Most of the Fanconi's anemia gene products form a protein complex that activates FANCD2 by monoubiquitination to play a role in the cellular response to DNA damage and especially interstrand cross-linking, a response that includes BRCA1, ATM, and NBS1.

Dyskeratosis congenita is characterized by mucous membrane leukoplasia, dystrophic nails, reticular hyperpigmentation, and the development of aplastic anemia during childhood. The X-linked variety is due to mutations in the *DKC1* (*dyskerin*) gene; the more unusual autosomal dominant type is due to mutation in *hTERC*, which encodes an RNA template, and *hTERT*, which encodes the catalytic reverse transcriptase, telomerase; these gene products cooperate in a repair complex to maintain telomere length. In Shwachman-Diamond syndrome, marrow failure is seen with pancreatic insufficiency and malabsorption; most patients have compound heterozygous mutations in *SBDS*, which has been implicated in RNA processing.

Pathophysiology

Bone marrow failure results from severe damage to the hematopoietic cell compartment. In aplastic anemia, replacement of the bone marrow by fat is apparent in the morphology of the biopsy specimen (Fig. 102-1) and MRI of the spine. Cells bearing the CD34 antigen, a marker of early hematopoietic cells, are greatly diminished, and in functional studies, committed and primitive progenitor cells are virtually absent; in vitro assays have suggested that the stem cell pool is reduced to ≤1% of normal in severe disease at the time of presentation.

Figure 102-1



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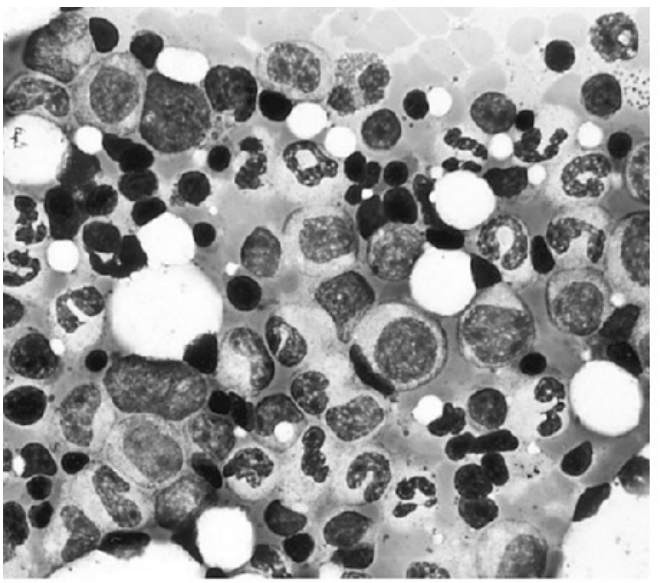

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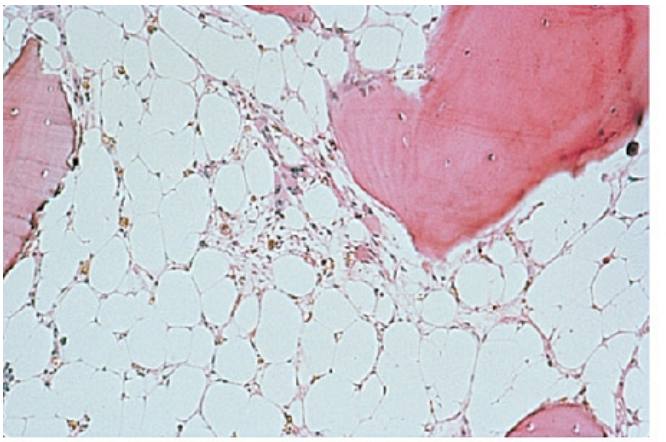

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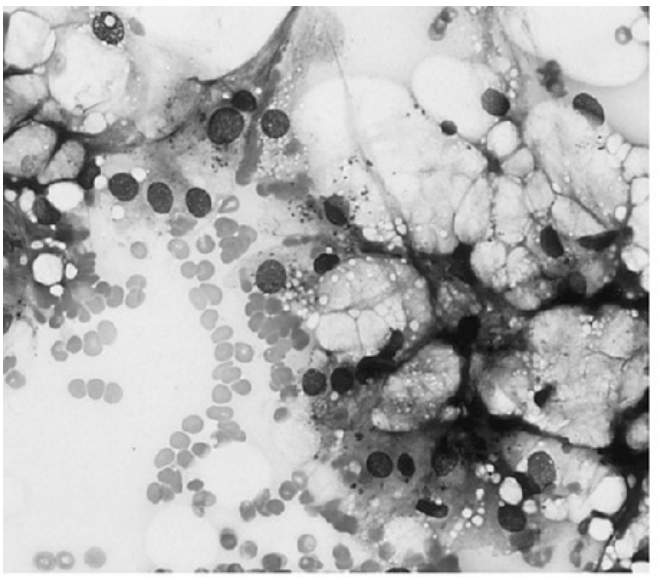


D

A. Normal bone marrow biopsy. ***B***. Normal bone marrow aspirate smear. The marrow is normally 30–70% cellular, and there is a heterogeneous mix of myeloid, erythroid, and lymphoid cells. ***C***. Aplastic anemia biopsy. ***D***. Marrow smear in aplastic anemia. The marrow shows replacement of hematopoietic tissue by fat and only residual stromal and lymphoid cells.

An intrinsic stem cell defect exists for the constitutional aplastic anemias: cells from patients with Fanconi's anemia exhibit chromosome damage and death on exposure to certain chemical agents. Telomeres are short in a large proportion of patients with aplastic anemia, and mutations in genes of the telomere repair complex (*TERC* and *TERT*) can be identified in some adults with apparently acquired marrow failure and without physical anomalies or typical family history.

Aplastic anemia does not appear to result from defective stroma or growth factor production.

Drug Injury

Extrinsic damage to the marrow follows massive physical or chemical insults such as high doses of radiation and toxic chemicals. For the more common idiosyncratic reaction to modest doses of medical drugs, altered drug metabolism has been invoked as a likely mechanism. The metabolic pathways of many drugs and chemicals, especially if they are polar and have limited water solubility, involve enzymatic degradation to highly reactive electrophilic compounds; these intermediates are toxic because of their propensity to bind to cellular macromolecules. For example, derivative hydroquinones and quinolones are responsible for benzene-induced tissue injury. Excessive generation of toxic intermediates or failure to detoxify the intermediates may be genetically determined and apparent only on specific drug challenge; the complexity and specificity of the pathways imply multiple susceptibility loci and would provide an explanation for the rarity

of idiosyncratic drug reactions.

Immune-Mediated Injury

The recovery of marrow function in some patients prepared for bone marrow transplantation with antilymphocyte globulin (ALG) first suggested that aplastic anemia might be immune-mediated. Consistent with this hypothesis was the frequent failure of simple bone marrow transplantation from a syngeneic twin, without conditioning cytotoxic chemotherapy, which also argued both *against* simple stem cell absence as the cause and *for* the presence of a host factor producing marrow failure. Laboratory data support an important role for the immune system in aplastic anemia. Blood and bone marrow cells of patients can suppress normal hematopoietic progenitor cell growth, and removal of T cells from aplastic anemia bone marrow improves colony formation in vitro. Increased numbers of activated cytotoxic T cells are observed in aplastic anemia patients and usually decline with successful immunosuppressive therapy; cytokine measurements show a T_H1 immune response (interferon γ and tumor necrosis factor). Interferon and tumor necrosis factor induce Fas expression on CD34 cells, leading to apoptotic cell death; localization of activated T cells to bone marrow and local production of their soluble factors are probably important in stem cell destruction.

Early immune system events in aplastic anemia are not well understood. Analysis of T cell receptor expression suggests an oligoclonal, antigen-driven cytotoxic T cell response. Many different exogenous antigens appear capable of initiating a pathologic immune response, but at least some of the T cells may recognize true self-antigens. The rarity of aplastic anemia despite common exposures (medicines, hepatitis virus) suggests that genetically determined features of the immune response can convert a normal physiologic response into a sustained abnormal autoimmune process, including polymorphisms in histocompatibility antigens, cytokine genes, and genes that regulate T cell polarization and effector function.

Clinical Features

History

Aplastic anemia can appear with seeming abruptness or have a more insidious onset. Bleeding is the most common early symptom; a complaint of days to weeks of easy bruising, oozing from the gums, nose bleeds, heavy menstrual flow, and sometimes petechiae will have been noticed. With thrombocytopenia, massive hemorrhage is unusual, but small amounts of bleeding in the central nervous system can result in catastrophic intracranial or retinal hemorrhage. Symptoms of anemia are also frequent, including lassitude, weakness, shortness of breath, and a pounding sensation in the ears. Infection is an unusual first symptom in aplastic anemia (unlike in agranulocytosis, where pharyngitis, anorectal infection, or frank sepsis occur early). A striking feature of aplastic anemia is the restriction of symptoms to the hematologic system, and patients often feel and look remarkably well despite drastically reduced blood counts. Systemic complaints and weight loss should point to other etiologies of pancytopenia. Prior drug use, chemical exposure, and preceding viral illnesses must often be elicited with repeated questioning. A family history of hematologic diseases or blood abnormalities may indicate a constitutional etiology of marrow failure.

Physical Examination

Petechiae and ecchymoses are typical, and retinal hemorrhages may be present. Pelvic and rectal examinations can often be deferred but, when performed, should be undertaken with great gentleness to avoid trauma; these will often show bleeding from the cervical os and blood in the stool. Pallor of the skin and mucous membranes is common except in the most acute cases or those already transfused. Infection on presentation is unusual but may occur if the patient has been symptomatic for a few weeks. Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. Café au lait spots and short stature suggest Fanconi's anemia; peculiar nails and leukoplakia suggest dyskeratosis congenita.

Laboratory Studies

Blood

The smear shows large erythrocytes and a paucity of platelets and granulocytes. Mean corpuscular volume (MCV) is commonly increased. Reticulocytes are absent or few, and lymphocyte numbers may be normal or reduced. The presence of immature myeloid forms suggests leukemia or MDS; nucleated red blood cells suggest marrow fibrosis or tumor invasion; abnormal platelets suggest either peripheral destruction or MDS.

Bone Marrow

The bone marrow is usually readily aspirated but dilute on smear, and the fatty biopsy specimen may be grossly pale on withdrawal; a "dry tap" instead suggests fibrosis or myelophthisis. In severe aplasia the smear of the aspirated specimen shows only red cells, residual lymphocytes, and stromal cells; the biopsy (which should be >1 cm in length) is superior for determination of cellularity and shows mainly fat under the microscope, with hematopoietic cells occupying <25% of the marrow space; in the most serious cases the biopsy is virtually 100% fat. The correlation between marrow cellularity and disease severity is imperfect, in part because marrow cellularity declines physiologically with aging. Additionally, some patients with moderate disease by blood counts will have empty iliac crest biopsies, while "hot spots" of hematopoiesis may be seen in severe cases. If an iliac crest specimen is inadequate, cells may also be obtained by aspiration from the sternum. Residual hematopoietic cells should have normal morphology, except for mildly megaloblastic erythropoiesis; megakaryocytes are invariably greatly reduced and usually absent. Areas adjacent to the spicule should be searched for myeloblasts. Granulomas (in cellular specimens) may indicate an infectious etiology of the marrow failure.

Ancillary Studies

Chromosome breakage studies of peripheral blood using diepoxybutane or mitomycin C should be performed on children and younger adults to exclude Fanconi's anemia. Genetic analysis applicable to the constitutional marrow failure states is available in some laboratories. Chromosome studies of bone marrow cells are often revealing in MDS and should be negative in typical aplastic anemia. Flow cytometric assays have replaced the Ham test for the diagnosis of PNH. Serologic studies may show evidence of viral infection, such as Epstein-Barr virus and HIV. Posthepatitis aplastic anemia is typically seronegative. The spleen size should be determined by CT scanning or ultrasound if the physical examination of the abdomen is unsatisfactory. MRI may be helpful to assess the fat content of a few vertebrae in order to distinguish aplasia from MDS.

Diagnosis

The diagnosis of aplastic anemia is usually straightforward, based on the combination of pancytopenia with a fatty, empty bone marrow. Aplastic anemia is a disease of the young and should be a leading diagnosis in the pancytopenic adolescent or young adult. When pancytopenia is secondary, the primary diagnosis is usually obvious from either history or physical examination: the massive spleen of alcoholic cirrhosis, the history of metastatic cancer or systemic lupus erythematosus, or miliary tuberculosis on chest radiograph (Table 102-1).

Diagnostic problems can occur with atypical presentations and among related hematologic diseases. While pancytopenia is most common, some patients with bone marrow hypocellularity have depression of only one or two of three blood lines, sometimes showing later progression to more recognizable aplastic anemia. The bone marrow in constitutional aplastic anemia is indistinguishable morphologically from the aspirate in acquired disease. The diagnosis can be suggested by family history, abnormal blood counts since childhood, or the presence of associated physical anomalies. Aplastic anemia may be difficult to distinguish from the hypocellular variety of MDS: MDS is favored by finding morphologic abnormalities, particularly of megakaryocytes and myeloid precursor cells, and typical cytogenetic abnormalities (see below).

Prognosis

The natural history of severe aplastic anemia is rapid deterioration and death. Provision first of red blood cell and later of platelet transfusions and effective antibiotics are of some benefit, but few patients show spontaneous recovery. The major prognostic determinant is the blood count; severe disease is defined by the presence of two of three parameters: absolute neutrophil count <500/μL, platelet count <20,000/μL, and corrected reticulocyte count <1% (or absolute reticulocyte count <60,000/μL). Survival of patients who fulfill these criteria is about 20% at 1 year after diagnosis with only supportive care; patients with very severe disease, defined by an absolute neutrophil count <200/μL, fare even more poorly. Treatment has markedly improved survival in this disease.

Aplastic Anemia: Treatment

Severe acquired aplastic anemia can be cured by replacement of the absent hematopoietic cells (and the immune system) by stem cell transplant, or it can be ameliorated by suppression of the immune system to allow recovery of the patient's residual bone marrow function. Hematopoietic growth factors have limited usefulness and glucocorticoids are of no value. Suspect exposures to drugs or chemicals should be discontinued; however, spontaneous recovery of severe blood count depression is rare, and a waiting period before beginning treatment may not be advisable unless the blood counts are only modestly depressed.

Hematopoietic Stem Cell Transplantation

This is the best therapy for the young patient with a fully histocompatible sibling donor (Chap. 108). Human leukocyte antigen (HLA) typing should be ordered as soon as the diagnosis of aplastic anemia is established in a child or younger adult. In transplant candidates, transfusion of blood from family members should be avoided so as to prevent sensitization to histocompatibility antigens; while transfusions in general should be minimized, limited numbers of blood products probably do not seriously affect outcome.

For allogeneic transplant from fully matched siblings, long-term survival rates for children are 80–90%. Transplant morbidity and mortality are increased among adults, due mainly to the higher risk of chronic GVHD and serious infections. Graft rejection was historically a major determinant of outcome in transplant for aplastic anemia, perhaps related to the underlying pathophysiology as well as to alloimmunization from transfusions (the latter now much improved by leukocyte depletion before blood product administration).

Most patients do not have a suitable sibling donor. Occasionally, a full phenotypic match can be found within the family and serve as well. Far more available are other alternative donors, either unrelated but histocompatible volunteers or closely but not perfectly matched family members. Survival using alternative donors is about half that of conventional sibling transplants but improving with higher-resolution HLA matching and more effective conditioning regimens and GVHD prophylaxis. Patients will be at risk for late complications, especially a higher rate of cancer, if radiation is used as a component of conditioning.

Immunosuppression

Used alone, ALG or antithymocyte globulin (ATG) induces hematologic recovery (independence from transfusion and a leukocyte count adequate to prevent infection) in about 50% of patients. The addition of cyclosporine to either ALG or ATG has further increased response rates to about 70% and especially improved outcomes for children and for severely neutropenic patients. Such combined treatment is now standard for patients with severe disease. An early robust hematologic response strongly correlates with long-term survival. Improvement in granulocyte number is generally apparent within 2 months of treatment. Most recovered patients continue to have some degree of blood count depression, the MCV remains elevated, and the bone marrow cellularity returns toward normal only very slowly, if at all. Relapse (recurrent pancytopenia) is frequent, often occurring as cyclosporine is discontinued; most, but not all, patients respond to reinstitution of immunosuppression, but some responders become dependent on continued cyclosporine administration. Development of MDS, with typical

marrow morphologic or cytogenetic abnormalities, occurs in about 15% of treated patients, usually but not invariably associated with a return of pancytopenia, and some patients develop leukemia. A laboratory diagnosis of PNH can generally be made at the time of presentation of aplastic anemia by flow cytometry; recovered patients may have frank hemolysis if the PNH clone expands. Bone marrow examinations should be performed if there is an unfavorable change in blood counts.

Horse ATG is given at 40 mg/kg per day for 4 days; rabbit ALG is administered at 3.5 mg/kg per day for 5 days. For ATG, anaphylaxis is a rare but occasionally fatal complication; allergy can be tested by a skin-prick test with an undiluted solution and immediate observation; desensitization is feasible. ATG binds to peripheral blood cells; therefore, platelet and granulocyte numbers may fall further during active treatment. Serum sickness, a flu-like illness with a characteristic cutaneous eruption and arthralgia, often develops about 10 days after initiating treatment. Methylprednisolone, 1 mg/kg per day for 2 weeks, can ameliorate the immune consequences of heterologous protein infusion. Excessive or extended glucocorticoid therapy is associated with avascular joint necrosis. Cyclosporine is administered orally at an initial dose of 12 mg/kg per day in adults (15 mg/kg per day in children), with subsequent adjustment according to blood levels obtained every 2 weeks. Trough levels should be between 150 and 200 ng/mL. The most important side effects of chronic cyclosporine treatment are nephrotoxicity, hypertension, seizures, and opportunistic infections, especially *Pneumocystis carinii* (prophylactic treatment with monthly inhaled pentamidine is recommended).

Most patients with aplastic anemia lack a suitable marrow donor, and immunosuppression is the treatment of choice. Overall survival is equivalent with transplantation and immunosuppression. However, successful transplant cures marrow failure, while patients who recover adequate blood counts after immunosuppression remain at risk of relapse and malignant evolution. Because of excellent results in children and younger adults, allogeneic transplant should be performed if a suitable sibling donor is available. Increasing age and the severity of neutropenia are the most important factors weighing in the decision between transplant and immunosuppression in adults who have a matched family donor: older patients do better with ATG and cyclosporine, whereas transplant is preferred if granulocytopenia is profound. Some patients may prefer immunosuppression; transplant is used for failure to recover blood counts or occurrence of late complications.

Outcomes following both transplant and immunosuppression have improved with time. High doses of cyclophosphamide, without stem cell rescue, have been reported to produce durable hematologic recovery, without relapse or evolution to MDS, but this treatment can produce sustained severe fatal neutropenia and response is often delayed. New immunosuppressive drugs in clinical trial may further improve outcome.

Other Therapies

The effectiveness of androgens has not been verified in controlled trials, but occasional patients will respond or even demonstrate blood count dependence on continued therapy. For patients with moderate disease or those with severe pancytopenia in whom immunosuppression has failed, a 3–4-month trial is appropriate. Hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), and interleukin 3 (IL-3) are not recommended as initial therapy for severe aplastic anemia, and even their role as adjuncts to immunosuppression is not well defined. Some patients may respond to combinations of growth factors after immunosuppression has failed.

Supportive Care

Meticulous medical attention is required so that the patient may survive to benefit from definitive therapy or, having failed treatment, to maintain a reasonable existence in the face of pancytopenia. First and most important, infection in the presence of severe neutropenia must be aggressively treated by prompt institution of parenteral, broad-spectrum antibiotics, usually ceftazidime or a combination of an aminoglycoside, cephalosporin, and semisynthetic penicillin. Therapy is empirical and must not await results of culture, although specific foci of infection such as oropharyngeal or anorectal abscesses, pneumonia, sinusitis, and typhlitis (necrotizing colitis) should be sought on physical examination and with radiographic studies. When indwelling plastic catheters become contaminated, vancomycin should be added. Persistent or recrudescent fever implies fungal disease: *Candida* and *Aspergillus* are common, especially after several courses of antibacterial antibiotics, and a progressive course may be averted by timely initiation of antifungal therapy. Granulocyte transfusions using G-CSF–mobilized peripheral blood have appeared to be effective in the treatment of overwhelming or refractory infections in a few patients. Hand washing, the single best method of preventing the spread of infection, remains a neglected practice. Nonabsorbed antibiotics for gut decontamination are poorly tolerated and not of proven value. Total reverse isolation does not reduce mortality from infections.

Both platelet and erythrocyte numbers can be maintained by transfusion. Alloimmunization historically limited the usefulness of platelet transfusions and is now minimized by several strategies, including use of single donors to reduce exposure and physical or chemical methods to diminish leukocytes in the product; HLA-matched platelets are often effective in patients refractory to random donor products. Inhibitors of fibrinolysis such as aminocaproic acid have not been shown to relieve mucosal oozing; the use of low-dose glucocorticoids to induce "vascular stability" is unproven and not recommended. Whether platelet transfusions are better used prophylactically or only as needed remains unclear. Any rational regimen of prophylaxis requires transfusions once or twice weekly in order to maintain the platelet count >10,000/μL (oozing from the gut, and presumably also from other vascular beds, increases precipitously at counts <5000/μL). Menstruation should be suppressed either by oral estrogens or nasal follicle-stimulating hormone/luteinizing hormone (FSH/LH) antagonists. Aspirin and other nonsteroidal anti-inflammatory agents inhibit platelet function and must be avoided.

Red blood cells should be transfused to maintain a normal level of activity, usually at a hemoglobin value of 70 g/L (90 g/L if there is underlying cardiac or pulmonary disease); a regimen of 2 units every 2 weeks will replace normal losses in a patient without a functioning bone marrow. In chronic anemia, the iron chelators deferoxamine and deferasirox should be added at around the fiftieth transfusion in order to avoid secondary hemochromatosis.

Pure Red Cell Aplasia

Other, more restricted forms of marrow failure occur, in which only a single circulating cell type is affected and the aregenerative marrow

shows corresponding absence or decreased numbers of specific precursor cells: aregenerative anemia as in PRCA (see below), thrombocytopenia with amegakaryocytosis (Chap. 109), and neutropenia without marrow myeloid cells in agranulocytosis (Chap. 61). In general, and in contrast to aplastic anemia and MDS, the unaffected lineages appear quantitatively and qualitatively normal. Agranulocytosis, the most frequent of these syndromes, is usually a complication of medical drug use (with agents similar to those related to aplastic anemia), either by a mechanism of direct chemical toxicity or by immune destruction. Agranulocytosis has an incidence similar to aplastic anemia but is especially frequent among the elderly and in women. The syndrome should resolve with discontinuation of exposure, but significant mortality is attached to neutropenia in the older and often previously unwell patient. Both pure white cell aplasia (agranulocytosis without incriminating drug exposure) and amegakaryocytic thrombocytopenia are exceedingly rare and, like PRCA, appear to be due to destructive antibodies or lymphocytes and can respond to immunosuppressive therapies. In all the single lineage failure syndromes, progression to pancytopenia or leukemia is unusual.

Definition and Differential Diagnosis

PRCA is characterized by anemia, reticulocytopenia, and absent or rare erythroid precursor cells in the bone marrow. The classification of PRCA is shown in Table 102-4. In adults, PRCA is acquired. An identical syndrome can occur constitutionally: Diamond-Blackfan anemia, or congenital PRCA, is diagnosed at birth or in early childhood and often responds to glucocorticoid treatment; a minority of patients have etiologic mutations in a ribosomal RNA processing gene called *RPS19*. Temporary red cell failure occurs in transient aplastic crisis of hemolytic anemias due to acute parvovirus infection (Chap. 177) and in transient erythroblastopenia of childhood, which affects normal children.

Table 102-4 Classification of Pure Red Cell Aplasia

Self-limited
Transient erythroblastopenia of childhood
Transient aplastic crisis of hemolysis (acute B19 parvovirus infection)
Fetal red blood cell aplasia
Nonimmune hydrops fetalis (in utero B19 parvovirus infection)
Hereditary pure red cell aplasia
Congenital pure red cell aplasia (Diamond-Blackfan syndrome)
Acquired pure red cell aplasia
Thymoma and malignancy
Thymoma
Lymphoid malignancies (and more rarely other hematologic diseases)
Paraneoplastic to solid tumors
Connective tissue disorders with immunologic abnormalities
Systemic lupus erythematosus, juvenile rheumatoid arthritis, rheumatoid arthritis
Multiple endocrine gland insufficiency
Virus
Persistent B19 parvovirus, hepatitis, adult T cell leukemia virus, Epstein-Barr virus
Pregnancy
Drugs
Especially phenytoin, azathioprine, chloramphenicol, procainamide, isoniazid
Erythropoietin
Idiopathic

Clinical Associations and Etiology

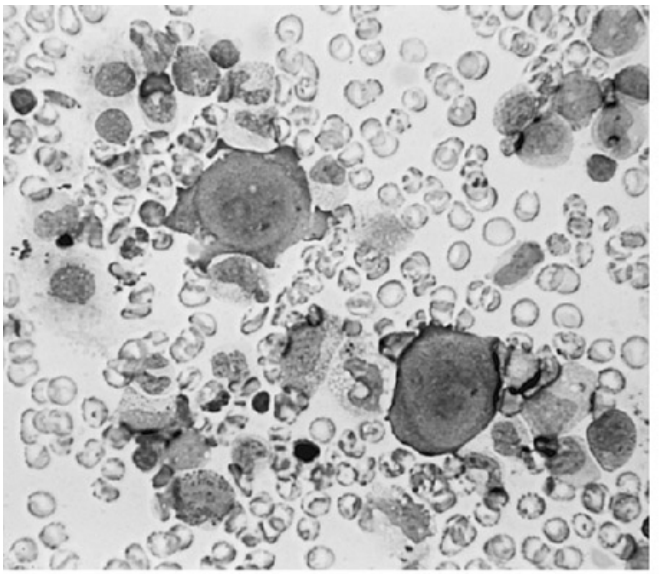
PRCA has important associations with immune system diseases. A small minority of cases occur with a thymoma. More frequently, red cell aplasia can be the major manifestation of large granular lymphocytosis or may occur in chronic lymphocytic leukemia. Some patients may be hypogammaglobulinemic. As with agranulocytosis, PRCA can be due to an idiosyncratic reaction to a drug. Subcutaneous administration of erythropoietin can lead to PRCA mediated by neutralizing antibodies.

Like aplastic anemia, PRCA results from diverse mechanisms. Antibodies to red blood cell precursors are frequently present in the blood, but T cell inhibition is probably the more common immune mechanism. Cytotoxic lymphocyte activity restricted by histocompatibility locus or specific for human T cell leukemia/lymphoma virus I–infected cells, as well as natural killer cell activity inhibitory of erythropoiesis, have been demonstrated in particularly well-studied individual cases.

Persistent Parvovirus B19 Infection

Chronic parvovirus infection is an important, treatable cause of PRCA. This common virus causes a benign exanthem of childhood (fifth disease) and a polyarthralgia/arthritis syndrome in adults. In patients with underlying hemolysis (or any condition that increases demand for red blood cell production), parvovirus infection can cause a transient aplastic crisis and an abrupt but temporary worsening of the anemia due to failed erythropoiesis. In normal individuals, acute infection is resolved by production of neutralizing antibodies to the virus, but in the setting of congenital, acquired, or iatrogenic immunodeficiency, persistent viral infection may occur. The bone marrow shows red cell aplasia and the presence of giant pronormoblasts (Fig. 102-2), which is the cytopathic sign of B19 parvovirus infection. Viral tropism for human erythroid progenitor cells is due to its use of erythrocyte P antigen as a cellular receptor for entry. Direct cytotoxicity of virus causes anemia if demands on erythrocyte production are high; in normal individuals, the temporary cessation of red cell production is not clinically apparent, and skin and joint symptoms are mediated by immune complex deposition.

Figure 102-2



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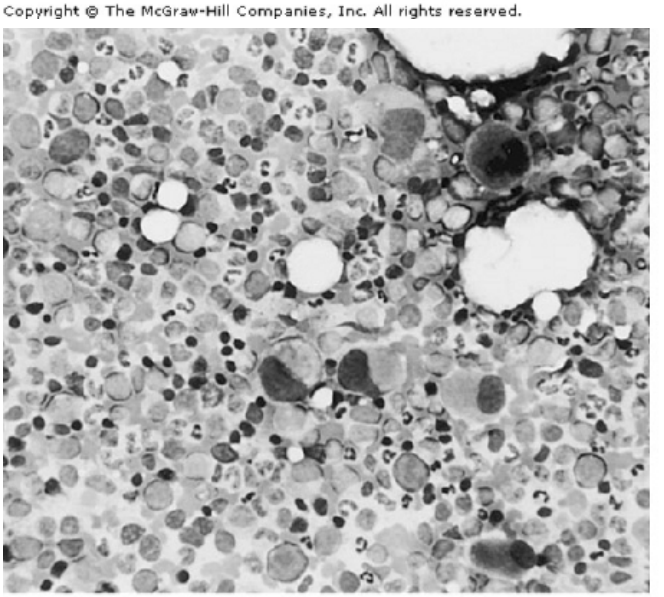
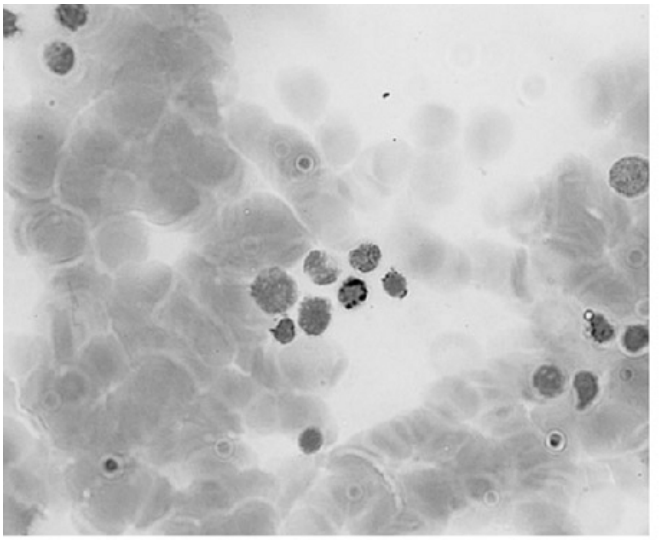
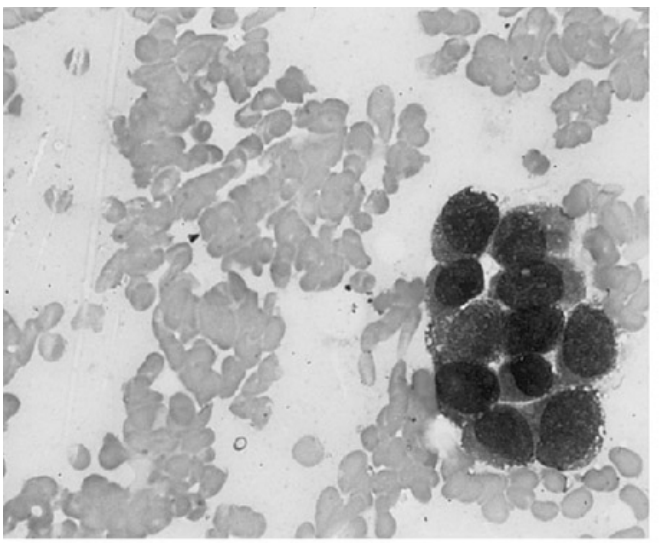


B



C



D

Pathognomonic cells in marrow failure syndromes. ***A.*** Giant pronormoblast, the cytopathic effect of B19 parvovirus infection of the erythroid progenitor cell. ***B.*** Uninuclear megakaryocyte and microblastic erythroid precursors typical of the 5q– myelodysplasia syndrome. ***C.*** Ringed sideroblast showing perinuclear iron granules. ***D.*** Tumor cells present on a touch preparation made from the marrow biopsy of a patient with metastatic carcinoma.

Pure Red Cell Aplasia: Treatment

History, physical examination, and routine laboratory studies may disclose an underlying disease or a suspect drug exposure. Thymoma should be sought by radiographic procedures. Tumor excision is indicated, but anemia does not necessarily improve with surgery. The diagnosis of parvovirus infection requires detection of viral DNA sequences in the blood (IgG and IgM antibodies are commonly absent). The presence of erythroid colonies has been considered predictive of response to immunosuppressive therapy in idiopathic PRCA.

Red cell aplasia is compatible with long survival with supportive care alone: a combination of erythrocyte transfusions and iron chelation. For persistent B19 parvovirus infection, almost all patients respond to intravenous immunoglobulin therapy (for example, 0.4 g/kg daily for 5 days), although relapse and retreatment may be expected, especially in patients with AIDS. The majority of patients with idiopathic PRCA respond favorably to immunosuppression. Most first receive a course of glucocorticoids. Also effective are cyclosporine, ATG, azathioprine, cyclophosphamide, and the monoclonal antibodydaclizumab, an antibody to the IL-2 receptor. PRCA developing on

erythropoietin therapy should be treated with immunosuppression and withdrawal of erythropoietin.

Myelodysplasia

Definition

The myelodysplasias (MDSs) are a heterogeneous group of hematologic disorders broadly characterized by cytopenias associated with a dysmorphic (or abnormal appearing) and usually cellular bone marrow, and by consequent ineffective blood cell production. A clinically useful nosology of these entities was first developed by the French-American-British Cooperative Group in 1983. Five entities were defined: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-t), and chronic myelomonocytic leukemia (CMML). The World Health Organization classification (2002) recognizes that the distinction between RAEB-t and acute myeloid leukemia is arbitrary and groups them together as acute leukemia, notes that CMML behaves as a myeloproliferative disease, and separates refractory anemias with dysmorphic change restricted to erythroid lineage from those with multilineage changes (Table 102-5).

Table 102-5 World Health Organization Classification of Myelodysplastic Syndromes

Disease	Frequency	Blood Findings	Bone Marrow Findings	Prognosis
Refractory anemia (RA)	5–10%	Anemia	Erythroid dysplasia only	Protracted course
		No or rare blasts	<5% blasts	Leukemic transformation in ~6%
			<15% ringed sideroblasts	
Refractory anemia with ringed sideroblasts (RARS)	10–12%	Anemia	Erythroid dysplasia only	Protracted course
		No blasts	≥15% ringed sideroblasts	Leukemia in ~1–2%
			<5% blasts	
Refractory cytopenia with multilineage dysplasia (RCMD)	24%	Cytopenias (2 or 3 lineages)	Dysplasia in ≥10% of cells in ≥2 lineages	Variable clinical course
		No or rare blasts	<5% blasts	Leukemia in ~11%
		No Auer rods	No Auer rods	
		$<1 \times 10^9$/L monocytes	<15% ringed sideroblasts	
RCMD with ringed sideroblasts (RCMD-RS)	15%	Cytopenias (2 or 3 lineages)	Dysplasia in ≥10% of cells in ≥2 lineages	
		No or rare blasts	≥15% ringed sideroblasts	
		No Auer rods	<5% blasts	
		$<1 \times 10^9$/L monocytes	No Auer rods	
Refractory anemia with excess blasts-1 (RAEB-1)	40%	Cytopenias	Unilineage or multilineage dysplasia	Progressive BM failure
	(RAEB-1 +2)	<5% blasts	5–9% blasts	Leukemia in ~25%
		No Auer rods	No Auer rods	
		$<1 \times 10^9$/L monocytes		
Refractory anemia with excess blasts-2 (RAEB-2)		Cytopenias	Unilineage or multilineage dysplasia	Progressive BM failure
		5–19% blasts	10–19% blasts	Leukemia in ~33%
		± Auer rods	± Auer rods	
		$<1 \times 10^9$/L monocytes		
Myelodysplastic syndrome, unclassified (MDS-U)	Unknown	Cytopenias	Dysplasia in myeloid or platelet lineage	Unknown

		No or rare blasts No Auer rods	<5% blasts No Auer rods	
MDS with isolated del(5q)	Unknown	Anemia <5% blasts Platelets nl or increased	Nl or increased megakaryocytes with hypolobated nuclei <5% blasts No Auer rods Isolated del(5q)	Long survival

Note: BM, bone marrow.

Source: Extracted from Jaffe ES et al (eds): *Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues*. Lyon, IARC Press, 2001.

Epidemiology

Idiopathic MDS is a disease of the elderly; the mean age at onset is 68 years. There is a slight male preponderance. MDS is a relatively common form of bone marrow failure, with reported incidence rates of 35 to >100 per million persons in the general population and 120 to >500 per million in the elderly. MDS is rare in children, but monocytic leukemia can be seen. Therapy-related MDS is not age-related and may occur in as many as 15% of patients within a decade following intensive combined modality treatment for cancer. Rates of MDS have increased over time, due to the recognition of the syndrome by physicians and the aging of the population.

Etiology and Pathophysiology

MDS is caused by environmental exposures such as radiation and benzene; other risk factors have been reported inconsistently. Secondary MDS occurs as a late toxicity of cancer treatment, usually with a combination of radiation and the radiomimetic alkylating agents such as busulfan, nitrosourea, or procarbazine (with a latent period of 5–7 years) or the DNA topoisomerase inhibitors (2 years). Both acquired aplastic anemia following immunosuppressive treatment and Fanconi's anemia can evolve into MDS.

MDS is a clonal hematopoietic stem cell disorder leading to impaired cell proliferation and differentiation. Cytogenetic abnormalities are found in about half of patients, and some of the same specific lesions are also seen in frank leukemia; aneuploidy is more frequent than translocations. Both presenting and evolving hematologic manifestations result from the accumulation of multiple genetic lesions: loss of tumor suppressor genes, activating oncogene mutations, or other harmful alterations. Cytogenetic abnormalities are not random (loss of all or part of 5, 7, and 20, trisomy of 8) and may be related to etiology (11q23 following topoisomerase II inhibitors); chronic myelomonocytic leukemia is often associated with t(5;12) that creates a chimeric *tel-PDGFβ* gene. The type and number of cytogenetic abnormalities strongly correlate with the probability of leukemic transformation and survival. Mutations of N-*ras* (an oncogene), *p53* and *IRF-1* (tumor suppressor genes), *Bcl-2* (an antiapoptotic gene), and others have been reported in some patients but likely occur late in the sequence leading to leukemic transformation. Apoptosis of marrow cells is increased in MDS, presumably due to these acquired genetic alterations or possibly to an overlaid immune response. An immune pathophysiology has been suggested for trisomy 8 MDS, which often responds clinically to immunosuppressive therapy. Such patients have T cell activity directed to the cytogenetically aberrant clone. Sideroblastic anemia may be related to mutations in mitochondrial genes; ineffective erythropoiesis and disordered iron metabolism are the functional consequences of the genetic alterations.

Clinical Features

Anemia dominates the early course. Most symptomatic patients complain of the gradual onset of fatigue and weakness, dyspnea, and pallor, but at least half the patients are asymptomatic and their MDS is discovered only incidentally on routine blood counts. Previous chemotherapy or radiation exposure is an important historic fact. Fever and weight loss should point to a myeloproliferative rather than myelodysplastic process. Children with Down syndrome are susceptible to MDS, and a family history may indicate a hereditary form of sideroblastic anemia or Fanconi's anemia.

The physical examination is remarkable for signs of anemia; about 20% of patients have splenomegaly. Some unusual skin lesions, including Sweet's syndrome (febrile neutrophilic dermatosis), occur with MDS. Autoimmune syndromes are not infrequent.

Laboratory Studies

Blood

Anemia is present in the majority of cases, either alone or as part of bi- or pancytopenia; isolated neutropenia or thrombocytopenia is more unusual. Macrocytosis is common, and the smear may be dimorphic with a distinctive population of large red blood cells. Platelets are also large and lack granules. In functional studies, they may show marked abnormalities, and patients may have bleeding symptoms despite seemingly adequate numbers. Neutrophils are hypogranulated; have hyposegmented, ringed, or abnormally segmented nuclei; contain Dohle bodies; and may be functionally deficient. Circulating myeloblasts usually correlate with marrow blast numbers, and their quantitation is important for classification and prognosis. The total white blood cell count is usually normal or low, except in chronic myelomonocytic leukemia. As in aplastic anemia, MDS can be associated with a clonal population of PNH cells.

Bone Marrow

The bone marrow is usually normal or hypercellular, but in 20% of cases it is sufficiently hypocellular to be confused with aplasia. No single characteristic feature of marrow morphology distinguishes MDS, but the following are commonly observed: dyserythropoietic changes (especially nuclear abnormalities) and ringed sideroblasts in the erythroid lineage; hypogranulation and hyposegmentation in granulocytic precursors, with an increase in myeloblasts; and megakaryocytes showing reduced numbers or disorganized nuclei. Megaloblastic nuclei associated with defective hemoglobinization in the erythroid lineage are common. Prognosis strongly correlates with the proportion of marrow blasts. Cytogenetic analysis and fluorescent in situ hybridization can identify chromosomal abnormalities.

Differential Diagnosis

Deficiencies of vitamin B_{12} or folate should be excluded by appropriate blood tests; vitamin B_6 deficiency can be assessed by a therapeutic trial of pyridoxine if the bone marrow shows ringed sideroblasts. Marrow dysplasia can be observed in acute viral infections, drug reactions, or chemical toxicity but should be transient. More difficult are the distinctions between hypocellular MDS and aplasia or between refractory anemia with excess blasts and early acute leukemia. The World Health Organization considers the presence of 20% blasts in the marrow as the criterion that separates acute myeloid leukemia from MDS.

Prognosis

The median survival varies greatly from years for patients with 5q– or sideroblastic anemia to a few months in refractory anemia with excess blasts or severe pancytopenia associated with monosomy 7; an International Prognostic Scoring System (Table 102-6) assists in making predictions. Most patients die as a result of complications of pancytopenia and not due to leukemic transformation; perhaps one-third will succumb to other diseases unrelated to their MDS. Precipitous worsening of pancytopenia, acquisition of new chromosomal abnormalities on serial cytogenetic determination, and increase in the number of blasts are all poor prognostic indicators. The outlook in therapy-related MDS, regardless of type, is very poor, and most patients will progress within a few months to refractory acute myeloid leukemia.

Table 102-6 International Prognostic Scoring System

	Score Value				
Prognostic Variable	**0**	**0.5**	**1.0**	**1.5**	**2.0**
Bone marrow blasts (%)	<5%	5–10%		11–20%	21–30%
Karyotype[a]	Good	Intermediate	Poor		
Cytopenia[b] (lineages affected)	0 or 1	2 or 3			
Risk Group Scores	**Score**				
Low	0				
Intermediate-1	0.5–1.0				
Intermediate-2	1.5–2.0				
High	≥2.5				

[a]Good, normal, -Y, del(5q), del (20q); poor, complex (≥3 abnormalities) or chromosome 7 abnormalities; intermediate, all other abnormalities.

[b]Cytopenias defined as Hb <100 g/L, platelet count < 100,000/μL, absolute neutrophil count <1500/μL.

Myelodysplasia: Treatment

The therapy of MDS has been unsatisfactory. Only stem cell transplantation offers cure: survival rates of 50% at 3 years have been reported, but older patients are particularly prone to develop treatment-related mortality and morbidity. Results of transplant using matched unrelated donors are comparable, although most series contain younger and more highly selected cases.

MDS has been regarded as particularly refractory to cytotoxic chemotherapy regimens but is probably no more resistant to effective treatment than acute myeloid leukemia in the elderly, in whom drug toxicity is often fatal and remissions, if achieved, are brief.

Low doses of cytotoxic drugs have been administered for their "differentiating" potential, and from this experience has emerged drug therapies based on pyrimidine analogues. Azacitidine is directly cytotoxic but also inhibits DNA methylation, thereby altering gene expression. Azacitidine improves blood counts and modestly improves survival in about 16% of MDS patients, compared to best supportive care. Azacitidine is administered subcutaneously at a dose of 75 mg/m^2, daily for 7 days, at 4-week intervals, for at least four cycles, although further cycles may be required to observe a response. Decitabine is closely related to azacitidine and more potent. Similar to azacitidine, about 20% of patients show responses in blood counts, with a duration of response of almost a year. Activity may be higher in more advanced MDS subtypes. Decitabine dose is 15 mg/m^2 by continuous intravenous infusion, every eight hours for three days, repeating the cycle every 6 weeks for at least four cycles. The major toxicity of both azacitidine and decitabine is myelosuppression,

leading to worsened blood counts. Other symptoms associated with cancer chemotherapy frequently occur. Ironically, it has been difficult to establish that either agent acts in patients by a mechanism of DNA demethylation.

Thalidomide, a drug with many activities including antiangiogenesis and immunomodulation, has modest biologic activity in MDS. Lenalidomide, a thalidomide derivative with a more favorable toxicity profile, is particularly effective in reversing anemia in MDS patients with 5q– syndrome; not only do a high proportion of these patients become transfusion-independent with normal or near-normal hemoglobin levels, but their cytogenetics also become normal. Lenalidomide is administered orally, 10 mg daily. Most patients will improve within 3 months of initiating therapy. Toxicities include myelosuppression (worsening thrombocytopenia and neutropenia, necessitating blood count monitoring) and an increased risk of deep vein thrombosis and pulmonary embolism.

Other treatments for MDS include amifostine, an organic thiophosphonate that blocks apoptosis; it can improve blood counts but has significant toxicities. ATG and cyclosporine, as employed in aplastic anemia, also may produce sustained independence from transfusion, especially in younger MDS patients with more favorable International Prognostic Scoring System (IPSS) scores.

Hematopoietic growth factors can improve blood counts but, as in most other marrow failure states, have been most beneficial to patients with the least severe pancytopenia. G-CSF treatment alone failed to improve survival in a controlled trial. Erythropoietin alone or in combination with G-CSF can improve hemoglobin levels, but mainly in those with low serum erythropoietin levels who have no or only a modest need for transfusions.

The same principles of supportive care described for aplastic anemia apply to MDS. Despite improvements in drug therapy, many patients will be anemic for years. RBC transfusion support should be accompanied by iron chelation in order to prevent secondary hemochromatosis.

Myelophthisic Anemias

Fibrosis of the bone marrow (see Fig. 103-2), usually accompanied by a characteristic blood smear picture called *leukoerythroblastosis*, can occur as a primary hematologic disease, called *myelofibrosis* or *myeloid metaplasia* (Chap. 103), and as a secondary process, called *myelophthisis*. Myelophthisis, or secondary myelofibrosis, is reactive. Fibrosis can be a response to invading tumor cells, usually an epithelial cancer of breast, lung, a prostate origin or neuroblastoma. Marrow fibrosis may occur with infection of mycobacteria (both *Mycobacterium tuberculosis* and *M. avium*), fungi, or HIV, and in sarcoidosis. Intracellular lipid deposition in Gaucher disease and obliteration of the marrow space related to absence of osteoclast remodeling in congenital osteopetrosis also can produce fibrosis. Secondary myelofibrosis is a late consequence of radiation therapy or treatment with radiomimetic drugs. Usually the infectious or malignant underlying processes are obvious. Marrow fibrosis can also be a feature of a variety of hematologic syndromes, especially chronic myeloid leukemia, multiple myeloma, lymphomas, myeloma, and hairy cell leukemia.

The pathophysiology has three distinct features: proliferation of fibroblasts in the marrow space (myelofibrosis); the extension of hematopoiesis into the long bones and into extramedullary sites, usually the spleen, liver, and lymph nodes (myeloid metaplasia); and ineffective erythropoiesis. The etiology of the fibrosis is unknown but most likely involves dysregulated production of growth factors: platelet-derived growth factor and transforming growth factor β have been implicated. Abnormal regulation of other hematopoietins would lead to localization of blood-producing cells in nonhematopoietic tissues and uncoupling of the usually balanced processes of stem cell proliferation and differentiation. Myelofibrosis is remarkable for pancytopenia despite very large numbers of circulating hematopoietic progenitor cells.

Anemia is dominant in secondary myelofibrosis, usually normocytic and normochromic. The diagnosis is suggested by the characteristic leukoerythroblastic smear (see Fig. 103-1). Erythrocyte morphology is highly abnormal, with circulating nucleated red blood cells, teardrops, and shape distortions. White blood cell numbers are often elevated, sometimes mimicking a leukemoid reaction, with circulating myelocytes, promyelocytes, and myeloblasts. Platelets may be abundant and are often of giant size. Inability to aspirate the bone marrow, the characteristic "dry tap," can allow a presumptive diagnosis in the appropriate setting before the biopsy is decalcified.

The course of secondary myelofibrosis is determined by its etiology, usually a metastatic tumor or an advanced hematologic malignancy. Treatable causes must be excluded, especially tuberculosis and fungus. Transfusion support can relieve symptoms.

Further Readings

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Polycythemia Vera and Other Myeloproliferative Diseases: Introduction

The World Health Organization (WHO) classification of the chronic myeloproliferative diseases includes seven disorders, some of which are rare or poorly characterized (Table 103-1) but all of which share an origin in a multipotent hematopoietic progenitor cell, overproduction of one or more of the formed elements of the blood without significant dysplasia, a predilection to extramedullary hematopoiesis, myelofibrosis, and transformation at varying rates to acute leukemia. Within this broad classification, however, significant phenotypic heterogeneity exists. Some diseases, such as chronic myelogenous leukemia (CML), chronic neutrophilic leukemia (CNL) and chronic eosinophilic leukemia (CEL) express primarily a myeloid phenotype, while in others, such as polycythemia vera (PV), idiopathic myelofibrosis (IMF), and essential thrombocytosis (ET), erythroid or megakaryocytic hyperplasia predominates. The latter three disorders, in contrast to the former three, also appear capable of transforming into each other.

Table 103-1 WHO Classification of Chronic Myeloproliferative Disorders
Chronic myelogenous leukemia, [Ph chromosome t(9;22)(q34;11), BCR/ABL-positive]
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia (and the hypereosinophilic syndrome)
Polycythemia vera
Chronic idiopathic myelofibrosis (with extramedullary hematopoiesis)
Essential thrombocythemia
Chronic myeloproliferative disease, unclassifiable

This phenotypic heterogeneity has a genetic basis; CML is the consequence of the balanced translocation between chromosomes 9 and 22 [t(9;22)(q34;11)]; CNL has been associated with a t(15:19) translocation, and CEL with a deletion or balanced translocations involving the PDGFRβ gene. By contrast, to a greater or lesser extent, PV, IMF, and ET are characterized by expression of a JAK2 mutation, V617F, which causes constitutive activation of this tyrosine kinase that is essential for the function of the erythropoietin and thrombopoietin receptors but not the granulocyte colony–stimulating factor receptor. This essential distinction is also reflected in the natural history of CML, CNL, and CEL, which is usually measured in years, and their high rate of transformation into acute leukemia. By contrast, the natural history of PV, IMF, and ET is usually measured in decades, and transformation to acute leukemia is uncommon in the absence of exposure to mutagenic agents. This chapter, therefore, will focus only on PV, IMF, and ET, because their clinical overlap is substantial and

their clinical courses are distinctly different. Other chronic myeloproliferative disorders will be discussed in Chapter 104.

Polycythemia Vera

Polycythemia vera (PV) is a clonal disorder involving a multipotent hematopoietic progenitor cell in which phenotypically normal red cells, granulocytes, and platelets accumulate in the absence of a recognizable physiologic stimulus. The most common of the chronic myeloproliferative disorders, PV occurs in 2 per 100,000 persons, sparing no adult age group and increasing with age to rates as high as 18/100,000. Familial transmission occurs but is infrequent. A slight overall male predominance has been observed, but women predominate within the reproductive age range.

Etiology

The etiology of PV is unknown. Although nonrandom chromosome abnormalities such as 20q, trisomy 8, and especially 9p, have been documented in up to 30% of untreated PV patients, unlike CML no consistent cytogenetic abnormality has been associated with the disorder. However, a mutation in the autoinhibitory, pseudokinase domain of the tyrosine kinase JAK2- which replaces valine with phenylalanine (V617F), causing constitutive activation of the kinase- appears to have a central role in the pathogenesis of PV.

JAK2 is a member of an evolutionarily well-conserved, nonreceptor tyrosine kinase family and serves as the cognate tyrosine kinase for the erythropoietin and thrombopoietin receptors. It also functions as an obligate chaperone for these receptors in the Golgi apparatus and is responsible for their cell-surface expression. The conformational change induced in the erythropoietin and thrombopoietin receptors following binding to erythropoietin or thrombopoietin leads to JAK2 autophosphorylation, receptor phosphorylation, and phosphorylation of proteins involved in cell proliferation, differentiation, and resistance to apoptosis. Transgenic animals lacking JAK2 die as embryos from severe anemia. Constitutive activation of JAK2 can explain the erythropoietin-independent erythroid colony formation, and the hypersensitivity of PV erythroid progenitor cells to erythropoietin and other hematopoietic growth factors, their resistance to apoptosis in vitro in the absence of erythropoietin, their rapid terminal differentiation, and their increase in Bcl-X_L expression, all of which are characteristic in PV.

Importantly, the JAK2 gene is located on the short arm of chromosome 9, and loss of heterozygosity on chromosome 9p, due to uniparental disomy is the most common cytogenetic abnormality in PV. The segment of 9p involved contains the JAK2 locus; loss of heterozygosity in this region leads to homozygosity for the mutant JAK2 V617F. Over 90% of PV patients express this mutation, as do approximately 45% of IMF and ET patients. Homozygosity for the mutation occurs in approximately 30% of PV patients and 60% of IMF patients; homozygosity is rare in ET. Over time, a portion of PV JAK2 V617F heterozygotes become homozygotes, but usually not after 10 years of the disease. PV patients who do not express JAK2 V617F are not clinically different than those who do, nor do JAK2 V617F heterozygotes differ clinically from homozygotes. In general, patients who express JAK2 V617F are older than those who do not, but they do not have a longer duration of disease.

JAK2 V617F is the basis for many of the phenotypic and biochemical characteristics of PV, such as elevation of the leukocyte alkaline phosphatase (LAP) score and increased expression of the mRNA of PVR-1, a glycosylphosphatidylinositol (GPI)-linked membrane protein; however, it cannot solely account for the entire PV phenotype. First, PV patients with the same phenotype and documented clonal disease lack this mutation. Second, IMF patients have the same mutation but a different clinical phenotype. Third, familial PV can occur without the mutation, even when other members of the same family express it. Fourth, not all the cells of the malignant clone express JAK2 V617F. Fifth, JAK2 V617F has been observed in patients with long-standing idiopathic erythrocytosis. However, while JAK2 V617F alone may not be sufficient to cause PV, it is essential for the transformation of ET to PV, though not for its transformation to IMF.

Clinical Features

Although splenomegaly may be the initial presenting sign in PV, most often the disorder is first recognized by the incidental discovery of a high hemoglobin or hematocrit. With the exception of aquagenic pruritus, no symptoms distinguish PV from other causes of erythrocytosis.

Uncontrolled erythrocytosis causes hyperviscosity, leading to neurologic symptoms such as vertigo, tinnitus, headache, visual disturbances, and transient ischemic attacks (TIAs). Systolic hypertension is also a feature of the red cell mass elevation. In some patients, venous or arterial thrombosis may be the presenting manifestation of PV. Any vessel can be affected, but cerebral, cardiac, or mesenteric vessels are most commonly involved. Intraabdominal venous thrombosis is particularly common in young women and may be catastrophic if a sudden and complete obstruction of the hepatic vein occurs. Indeed, PV should be suspected in any patient who develops hepatic vein thrombosis. Digital ischemia, easy bruising, epistaxis, acid-peptic disease, or gastrointestinal hemorrhage may occur due to vascular stasis or thrombocytosis. Erythema, burning, and pain in the extremities, a symptom complex known as erythromelalgia, is another complication of the thrombocytosis of PV. Given the large turnover of hematopoietic cells, hyperuricemia with secondary gout, uric acid stones, and symptoms due to hypermetabolism can also complicate the disorder.

The plasma erythropoietin level is a useful diagnostic test in patients with isolated erythrocytosis, because an elevated level excludes PV as the cause for the erythrocytosis.

Diagnosis

When PV presents with erythrocytosis in combination with leukocytosis, thrombocytosis, or both, the diagnosis is apparent. However, when patients present with an elevated hemoglobin or hematocrit alone, or with thrombocytosis alone, the diagnostic evaluation is more complex because of the many diagnostic possibilities (Table 103-2). Furthermore, unless the hemoglobin level is ≥20 gm% (hematocrit ≥60%), it is not possible to distinguish PV from disorders causing plasma volume contraction. Uniquely in PV, an expanded plasma volume can mask an elevated red cell mass; thus, red cell mass and plasma volume determinations are mandatory to establish the presence of an absolute erythrocytosis and to distinguish this from relative erythrocytosis due to a reduction in plasma volume alone (also known as

stress or *spurious erythrocytosis* or *Gaisböck's syndrome*). This is true even in with the discovery of the JAK2 V617F mutation, because not very patient with PV expresses this mutation, while patients without PV do. Figure 58-18 illustrates a diagnostic algorithm for the evaluation of suspected erythrocytosis.

Table 103-2 Causes of Erythrocytosis

Relative erythrocytosis: Hemoconcentration secondary to dehydration, androgens, or tobacco abuse
Absolute erythrocytosis
Hypoxia
Carbon monoxide intoxication
High affinity hemoglobin
High altitude
Pulmonary disease
Right-to-left shunts
Sleep-apnea syndrome
Neurologic disease
Renal disease
Renal artery stenosis
Focal sclerosing or membranous glomerulonephritis
Renal transplantation
Tumors
Hypernephroma
Hepatoma
Cerebellar hemangioblastoma
Uterine fibromyoma
Adrenal tumors
Meningioma
Pheochromocytoma
Drugs
Androgens
Recombinant erythropoietin
Familial (with normal hemoglobin function, Chuvash, erythropoietin receptor mutations)
Polycythemia vera

Once absolute erythrocytosis has been established, its cause must be determined. An elevated plasma erythropoietin level suggests either a hypoxic cause for erythrocytosis or autonomous erythropoietin production, in which case assessment of pulmonary function and an abdominal CT scan to evaluate renal and hepatic anatomy are appropriate. A normal erythropoietin level does not exclude a hypoxic cause for erythrocytosis. In PV, in contrast to hypoxic erythrocytosis, the arterial oxygen saturation is normal. However, a normal oxygen saturation does not exclude a high-affinity hemoglobin as a cause for erythrocytosis; documentation of previous hemoglobin levels and a

family study are important.

Other laboratory studies that may aid in diagnosis include the red cell count, mean corpuscular volume, and red cell distribution width (RDW). Only three situations cause microcytic erythrocytosis: β-thalassemia trait, hypoxic erythrocytosis, and PV. With β-thalassemia trait the RDW is normal, whereas with hypoxic erythrocytosis and PV, the RDW is usually elevated due to iron deficiency. In many patients, the LAP level is also increased, as is the uric acid level. Elevated serum vitamin B_{12} or B_{12}-binding capacity may be present. In patients with associated acid-peptic disease, occult gastrointestinal bleeding may lead to presentation with hypochromic, microcytic anemia.

A bone marrow aspirate and biopsy provide no specific diagnostic information since these may be normal or indistinguishable from ET or IMF, and unless there is a need to establish the presence of myelofibrosis or exclude some other disorder, these procedures need not be done. Although the presence of a cytogenetic abnormality such as trisomy 8 or 9 or 20q– in the setting of an expanded red cell mass supports a clonal etiology, no specific cytogenetic abnormality is associated with PV, and the absence of a cytogenetic marker does not exclude the diagnosis.

Complications

The major clinical complications of PV relate directly to the increase in blood viscosity associated with red cell mass elevation and indirectly to the increased turnover of red cells, leukocytes, and platelets with the attendant increase in uric acid and cytokine production. The latter appears to be responsible for the increase in peptic ulcer disease and for the pruritus associated with this disorder, although formal proof for this has not been obtained. A sudden massive increase in spleen size can be associated with splenic infarction or progressive cachexia. Myelofibrosis appears to be part of the natural history of the disease but is a reactive, reversible process that does not itself impede hematopoiesis and by itself has no prognostic significance. In some patients, however, the myelofibrosis is accompanied by significant extramedullary hematopoiesis, hepatosplenomegaly, and transfusion-dependent anemia. The organomegaly can cause significant mechanical discomfort, portal hypertension, and cachexia. Although the incidence of acute nonlymphocytic leukemia is increased in PV, the incidence of acute leukemia in patients not exposed to chemotherapy or radiation is low, and the development of leukemia is related to older age but not disease duration, suggesting that the treatment exposure may be a more important risk factor than the disease itself.

Erythromelalgia is a curious syndrome of unknown etiology associated with thrombocytosis, primarily involving the lower extremities and manifested usually by erythema, warmth, and pain of the affected appendage and occasionally digital infarction. It occurs with a variable frequency in myeloproliferative disorder patients and is usually responsive to salicylates. Some of the central nervous system symptoms observed in patients with PV, such as ocular migraine, may represent a variant of erythromelalgia.

If left uncontrolled, erythrocytosis can lead to thrombosis involving vital organs such as the liver, heart, brain, or lungs. Patients with massive splenomegaly are particularly prone to thrombotic events because the associated increase in plasma volume masks the true extent of the red cell mass elevation as measured by the hematocrit or hemoglobin level. A "normal" hematocrit or hemoglobin level in a PV patient with massive splenomegaly should be considered indicative of an elevated red cell mass until proven otherwise.

Polycythemia Vera: Treatment

PV is generally an indolent disorder whose clinical course is measured in decades, and its medical management should reflect its tempo. Thrombosis due to erythrocytosis is the most significant complication, and maintenance of the hemoglobin level at ≤140 g/L (14 g/dL; hematocrit <45%) in men and ≤120 g/L (12 g/dL; hematocrit <42%) in women is mandatory to avoid thrombotic complications. Phlebotomy serves initially to reduce hyperviscosity by bringing the red cell mass into the normal range. Periodic phlebotomies thereafter serve to maintain the red cell mass within the normal range and to induce a state of iron deficiency, which prevents an accelerated reexpansion of the red cell mass. In most PV patients, once an iron-deficient state is achieved, phlebotomy is usually only required at 3-month intervals. Neither phlebotomy nor iron deficiency increases the platelet count relative to the effect of the disease itself, and thrombocytosis is not correlated with thrombosis in PV, in contrast to the strong correlation between erythrocytosis and thrombosis in this disease. The use of salicylates as a tonic against thrombosis in PV patients is potentially harmful if the red cell mass is not controlled by phlebotomy. Anticoagulants are only indicated when a thrombosis has occurred and can be difficult to monitor owing to the artifactual imbalance between the test tube anticoagulant and plasma that occurs when blood from these patients is assayed for prothrombin or partial thromboplastin activity. Asymptomatic hyperuricemia (<10 mg%) requires no therapy, but allopurinol should be administered to avoid further elevation of the uric acid when chemotherapy is employed to reduce splenomegaly or leukocytosis or to treat pruritus. Generalized pruritus intractable to antihistamines or antidepressants such as doxepin can be a major problem in PV; hydroxyurea, interferon α (IFN-α), and psoralens with ultraviolet light in the A range (PUVA) therapy are other methods of palliation. Asymptomatic thrombocytosis requires no therapy unless the platelet count is sufficiently high to cause an acquired form of von Willebrand's disease due to proteolysis of high-molecular-weight vWF multimers. Symptomatic splenomegaly can be treated with IFN-α. Although the drug can be associated with significant side effects when used chronically, IFN-α reduces JAK2 V617F expression in PV patients, and its role in this disorder may be expanding. Anagrelide, a phosphodiesterase inhibitor, can reduce the platelet count and, if tolerated, is preferable to hydroxyurea because it lacks marrow toxicity. A reduction in platelet number may be necessary in the treatment of erythromelalgia or ocular migraine if salicylates are not effective or the platelet count is sufficiently high to cause an hemorrhagic diathesis. Alkylating agents and radioactive sodium phosphate (^{32}P) are leukemogenic in PV, and their use should be avoided. If a cytotoxic agent must be used, hydroxyurea is preferred, but this drug does not prevent either thrombosis or myelofibrosis in this disorder. Chemotherapy should be used for as short a time as possible. In some patients, massive splenomegaly unresponsive to reduction by hydroxyurea or IFN-α therapy and associated with intractable weight loss will require splenectomy. In some patients with end-stage disease, pulmonary hypertension may develop due to fibrosis and extramedullary hematopoiesis. Allogeneic bone marrow transplantation may be curative in young patients.

Most patients with PV can live long lives without functional impairment when their red cell mass is effectively managed with phlebotomy. Chemotherapy is never indicated to control the red cell mass unless venous access is inadequate.

Chronic Idiopathic Myelofibrosis

Chronic IMF (other designations include *agnogenic myeloid metaplasia* or *myelofibrosis with myeloid metaplasia*) is a clonal disorder of a multipotent hematopoietic progenitor cell of unknown etiology characterized by marrow fibrosis, extramedullary hematopoiesis, and splenomegaly. Chronic IMF is the least common chronic myeloproliferative disorder, and establishing this diagnosis in the absence of a specific clonal marker is difficult because myelofibrosis and splenomegaly are also features of both PV and CML. Furthermore, myelofibrosis and splenomegaly also occur in a variety of benign and malignant disorders (Table 103-3), many of which are amenable to specific therapies not effective in chronic IMF. In contrast to the other chronic myeloproliferative disorders and so-called acute or malignant myelofibrosis, which can occur at any age, chronic IMF primarily afflicts individuals in their sixth decade or later.

Table 103-3 Disorders Causing Myelofibrosis

Malignant	Nonmalignant
Acute leukemia (lymphocytic, myelogenous, megakaryocytic)	HIV infection
Chronic myelogenous leukemia	Hyperparathyroidism
Hairy cell leukemia	Renal osteodystrophy
Hodgkin disease	Systemic lupus erythematosus
Idiopathic myelofibrosis	Tuberculosis
Lymphoma	Vitamin D deficiency
Multiple myeloma	Thorium dioxide exposure
Myelodysplasia	Gray platelet syndrome
Polycythemia vera	
Systemic mastocytosis	

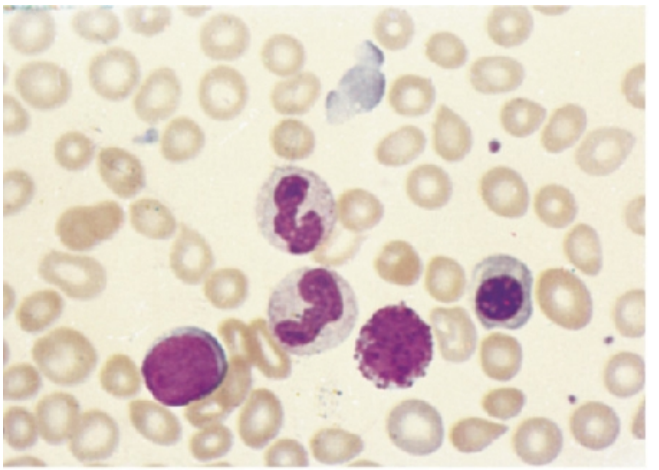
Etiology

The etiology of chronic IMF is unknown. Nonrandom chromosome abnormalities such as 9p, 20q–, 13q–, trisomy 8 or 9, or partial trisomy 1q are common, but no cytogenetic abnormality specific to the disease has been identified. The degree of myelofibrosis and the extent of extramedullary hematopoiesis are also not related. Fibrosis in this disorder is associated with overproduction of transforming growth factor β and tissue inhibitors of metalloproteinases, while osteosclerosis is associated with overproduction of osteoprotegerin, an osteoclast inhibitor. Marrow angiogenesis occurs due to increased production of vascular endothelial growth factor (VEGF). Importantly, fibroblasts in chronic IMF are polyclonal and not part of the neoplastic clone.

Clinical Features

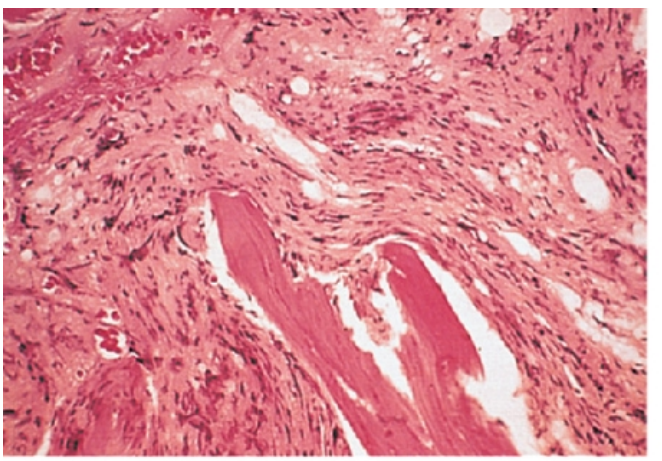
No signs or symptoms are specific for chronic IMF. Many patients are asymptomatic at presentation, and the disease is usually detected by the discovery of splenic enlargement and/or abnormal blood counts during a routine examination. However, in contrast to its companion myeloproliferative disorders, night sweats, fatigue, and weight loss may be presenting complaints. A blood smear shows the characteristic features of extramedullary hematopoiesis: teardrop-shaped red cells, nucleated red cells, myelocytes, and promyelocytes; myeloblasts may also be present (Fig. 103-1). Anemia, usually mild initially, is the rule, while the leukocyte and platelet counts are either normal or increased, but either can be depressed. Mild hepatomegaly may accompany the splenomegaly but is unusual in the absence of splenic enlargement; isolated lymphadenopathy should suggest another diagnosis. Both serum lactate dehydrogenase and alkaline phosphatase levels can be elevated. The LAP score can be low, normal, or high. Marrow is usually inaspirable due to the myelofibrosis (Fig. 103-2), and bone x-rays may reveal osteosclerosis. Exuberant extramedullary hematopoiesis can cause ascites, portal, pulmonary or intracranial hypertension, intestinal or ureteral obstruction, pericardial tamponade, spinal cord compression, or skin nodules. Splenic enlargement can be sufficiently rapid to cause splenic infarction with fever and pleuritic chest pain. Hyperuricemia and secondary gout may ensue.

Figure 103-1



Teardrop-shaped red blood cells indicative of membrane damage from passage through the spleen, a nucleated red blood cell, and immature myeloid cells indicative of extramedullary hematopoiesis are noted. This peripheral blood smear is related to any cause of extramedullary hematopoiesis.

Figure 103-2



This marrow section shows the marrow cavity replaced by fibrous tissue composed of reticulin fibers and collagen. When this fibrosis is due to a primary hematologic process, it is called *myelofibrosis*. When the fibrosis is secondary to a tumor or a granulomatous process, it is called *myelophthisis*.

Diagnosis

While the clinical picture described above is characteristic of chronic IMF, all of the clinical features described can also be observed in PV or CML. Massive splenomegaly commonly masks erythrocytosis in PV, and reports of intraabdominal thromboses in chronic IMF most likely represent instances of unrecognized PV. In some patients with chronic IMF, erythrocytosis has developed during the course of the disease. Furthermore, since many other disorders have features that overlap with chronic IMF but respond to distinctly different therapies, the diagnosis of chronic IMF is one of exclusion, which requires that the disorders listed in Table 103-3 be ruled out. A diagnostic algorithm has been proposed but does not distinguish one disease causing myeloid metaplasia from another.

The presence of teardrop-shaped red cells, nucleated red cells, myelocytes, and promyelocytes establishes the presence of extramedullary hematopoiesis, while the presence of leukocytosis, thrombocytosis with large and bizarre platelets, and circulating myelocytes suggests the presence of a myeloproliferative disorder as opposed to a secondary form of myelofibrosis (Table 103-3). Marrow is usually not aspirable due to increased marrow reticulin, but marrow biopsy will reveal a hypercellular marrow with trilineage hyperplasia and, in particular, increased numbers of megakaryocytes in clusters and with large, dysplastic nuclei However, there are no characteristic morphologic

abnormalities that distinguish IMF from the other chronic myeloproliferative disorders. Splenomegaly due to extramedullary hematopoiesis may be sufficiently massive to cause portal hypertension and variceal formation. In some patients, exuberant extramedullary hematopoiesis can dominate the clinical picture. An intriguing feature of chronic IMF is the occurrence of autoimmune abnormalities such as immune complexes, antinuclear antibodies, rheumatoid factor, or a positive Coombs' test. Whether these represent a host reaction to the disorder or are involved in its pathogenesis is unknown. Cytogenetic analysis of blood is useful both to exclude CML and for prognostic purposes, because complex karyotype abnormalities portend a poor prognosis in chronic IMF. For unknown reasons, the number of circulating CD34+ cells is markedly increased in chronic IMF (>15,000/μL) compared to the other chronic myeloproliferative disorders, unless they too develop myeloid metaplasia.

Importantly, approximately 45% of chronic IMF patients, like patients with its companion myeloproliferative disorders PV and ET, express the JAK2 V617F mutation, often as homozygotes. Such patients had a poorer survival in one retrospective study but not in another, where they were found to be older and to have higher hematocrits than those patients who were JAK2 V617F-negative.

Complications

Survival in chronic IMF varies according to specific clinical features (Table 103-4) but is shorter than in patients with PV or ET. The natural history of chronic IMF is one of increasing marrow failure with transfusion-dependent anemia and increasing organomegaly due to extramedullary hematopoiesis. As with CML, chronic IMF can evolve from a chronic phase to an accelerated phase with constitutional symptoms and increasing marrow failure. About 10% of patients develop an aggressive form of acute leukemia for which therapy is usually ineffective. Important prognostic factors for disease acceleration include anemia, leukocytosis, thrombocytopenia, the presence of circulating myeloblasts, older age, the presence of complex cytogenetic abnormalities, and constitutional symptoms such as unexplained fever, night sweats, or weight loss.

Table 103-4 Risk Stratification for Idiopathic Myelofibrosis

A. Prognostic factors[a]		
Hemoglobin <10 gm%		
White cell count < 4000/μL or > 30,000/μL		
Number of prognostic factors	**Risk group**	**Median survival (months)**
0	Low	93
1–2	High	17
B. Prognostic factors[b]		
Hemoglobin < 10 gm%		
Constitutional symptoms		
Blast cells > 1%		
Number of prognostic factors	**Risk group**	**Median survival (months)**
0–1	Low	99
2–3	High	21
C. Prognostic factors[c]	**Median survival (months)**	
Age <65 years		
Hemoglobin ≤10 gm%		
Karyotype: Normal	54	
Abnormal	22	
Age <65 years		
Hemoglobin >10 gm%		
Karyotype: Normal	180	
Abnormal	72	
Age >65 years		
Hemoglobin ≤10 gm%		
Karyotype: Normal	44	
Abnormal	16	
Age >65 years		
Hemoglobin >10 gm%		
Karyotype: Normal	70	
Abnormal	78	

[a]From B Dupriez et al. Blood 88:1013, 1996.

[b]From F Cervantes et al. Br J Haematol 102:684, 1998.

[c]From JT Reilly et al. Br J Haematol 98:96, 1997.

Chronic Idiopathic Myelofibrosis: Treatment

No specific therapy exists for chronic IMF. Anemia may be due to gastrointestinal blood loss and exacerbated by folic acid deficiency, and in rare instances, pyridoxine therapy has been effective. However, anemia is more often due to ineffective erythropoiesis uncompensated by extramedullary hematopoiesis in the spleen and liver. Neither recombinant erythropoietin nor androgens, such as Danazol, have proved consistently effective as therapy for anemia. Erythropoietin may worsen splenomegaly and will be ineffective if the serum erythropoietin level is >125 mU/L. A red cell splenic sequestration study can establish the presence of hypersplenism, for which splenectomy is indicated. Splenectomy may also be necessary if splenomegaly impairs alimentation and should be performed before cachexia sets in. In this situation, splenectomy should not be avoided because of concern over rebound thrombocytosis, loss of hematopoietic capacity, or compensatory hepatomegaly. However, for unexplained reasons, splenectomy increases the risk of blastic transformation. Splenic irradiation is, at best, temporarily palliative and associated with a significant risk of neutropenia and infection. Allopurinol can control significant hyperuricemia, and hydroxyurea has proved useful for controlling organomegaly in some patients. The role of IFN-α is still undefined and its side effects are more pronounced in the older individuals who are usually afflicted with this disorder. Glucocorticoids have been used to control constitutional symptoms and autoimmune complications and may ameliorate anemia alone or in combination with low dose thalidomide (50–100 mg/d). Allogeneic bone marrow transplantation is the only curative treatment and should be considered in younger patients; reduced-intensity conditioning regimens may permit hematopoietic cell transplantation to be extended to older individuals.

Essential Thrombocytosis

Essential thrombocytosis (other designations include *essential thrombocythemia*, *idiopathic thrombocytosis*, *primary thrombocytosis*, *hemorrhagic thrombocythemia*) is a clonal disorder of unknown etiology involving a multipotent hematopoietic progenitor cell manifested clinically by overproduction of platelets without a definable cause. ET is an uncommon disorder, with an incidence of 1–2/100,000 and a distinct female predominance, in contrast to the other chronic myeloproliferative disorders. No clonal marker is available to consistently distinguish ET from the more common nonclonal, reactive forms of thrombocytosis (Table 103-5), making its diagnosis difficult. Once considered a disease of the elderly and responsible for significant morbidity due to hemorrhage or thrombosis, with the widespread use of electronic cell counters, it is now clear that ET can occur at any age in adults and often without symptoms or disturbances of hemostasis. There is an unexplained female predominance in contrast to the other chronic myeloproliferative disorders or the reactive forms of thrombocytosis where no sex difference exists. Because no specific clonal marker is available, clinical criteria have been proposed to distinguish ET from the other chronic myeloproliferative disorders, which may also present with thrombocytosis but have differing prognoses and therapy (Table 103-5). These criteria do not establish clonality; therefore, they are truly useful only in identifying disorders such as CML, PV, or myelodysplasia, which can masquerade as ET, as opposed to actually establishing the presence of ET. Furthermore, as with "idiopathic" erythrocytosis, nonclonal benign forms of thrombocytosis exist (such as hereditary overproduction of thrombopoietin) that are not widely recognized because we currently lack adequate diagnostic tools.

Table 103-5 Causes of Thrombocytosis

Malignancy
Infection
Myeloproliferative disorders: polycythemia vera, idiopathic myelofibrosis, essential thrombocytosis, chronic myelogenous leukemia
Myelodysplastic disorders: 5q-syndrome, idiopathic refractory sideroblastic anemia
Postsplenectomy or hyposplenism
Hemorrhage
Iron deficiency anemia
Surgery
Rebound: Correction of vitamin B_{12} or folate deficiency, post-ethanol abuse
Hemolysis

Etiology

Megakaryocytopoiesis and platelet production depend upon thrombopoietin and its receptor, Mpl. As in the case of early erythroid and

myeloid progenitor cells, early megakaryocytic progenitors require the presence of interleukin 3 (IL-3) and stem cell factor for optimal proliferation in addition to thrombopoietin. Their subsequent development is also enhanced by the chemokine stromal cell–derived factor 1 (SDF-1). However, megakaryocyte maturation and differentiation require thrombopoietin.

Megakaryocytes are unique among hematopoietic progenitor cells because reduplication of their genome is endomitotic rather than mitotic. In the absence of thrombopoietin, endomitotic megakaryocytic reduplication and, by extension, the cytoplasmic development necessary for platelet production are impaired. Like erythropoietin, thrombopoietin is produced in both the liver and the kidneys, and an inverse correlation exists between the platelet count and plasma thrombopoietic activity. Like erythropoietin, plasma levels of thrombopoietin are controlled largely by the size of its progenitor cell pool. In contrast to erythropoietin, but like its myeloid counterparts, granulocyte- and granulocyte-macrophage colony-stimulating factors, thrombopoietin not only enhances the proliferation of its target cells but also enhances the reactivity of their end-stage product, the platelet. In addition to its role in thrombopoiesis, thrombopoietin also enhances the survival of multipotent hematopoietic stem cells.

The clonal nature of ET was established by analysis of glucose-6-phosphate dehydrogenase isoenzyme expression in patients hemizygous for this gene, by analysis of X-linked DNA polymorphisms in informative women patients, and by the expression in patients of nonrandom, though variable, cytogenetic abnormalities. Although thrombocytosis is its principal manifestation, like the other chronic myeloproliferative disorders, a multipotent hematopoietic progenitor cell is involved in ET. Furthermore, a number of families have been described in which ET was inherited, in one instance as an autosomal dominant trait. In addition to ET, IMF and PV have also been observed in some kindreds.

Clinical Features

Clinically, ET is most often identified incidentally when a platelet count is obtained during the course of a routine medical evaluation. Occasionally, review of previous blood counts will reveal that an elevated platelet count was present but overlooked for many years. No symptoms or signs are specific for ET, but these patients can have hemorrhagic and thrombotic tendencies expressed as easy bruising for the former and microvascular occlusions for the latter, such as erythromelalgia, ocular migraine, or TIAs. Physical examination is generally unremarkable except occasionally for mild splenomegaly. Massive splenomegaly is more indicative of another myeloproliferative disorder, in particular PV, IMF, or CML.

Anemia is unusual, but a mild neutrophilic leukocytosis is not. The blood smear is most remarkable for the number of platelets present, some of which may be very large. The LAP score is either normal or elevated. The large mass of circulating platelets may prevent the accurate measurement of serum potassium due to release of platelet potassium upon blood clotting. This type of hyperkalemia is a laboratory artifact and not associated with electrocardiographic abnormalities. Similarly, arterial oxygen measurements can be inaccurate unless thrombocythemic blood is collected on ice. The prothrombin and partial thromboplastin times are normal, while abnormalities of platelet function such as a prolonged bleeding time and impaired platelet aggregation can be present. However, in spite of much study, no platelet function abnormalities are characteristic of ET, and no platelet function test predicts the risk of clinically significant bleeding or thrombosis.

The elevated platelet count may hinder marrow aspiration, but marrow biopsy usually reveals megakaryocyte hyperplasia and hypertrophy, as well as an overall increase in marrow cellularity. If marrow reticulin is increased, another diagnosis should be considered. The absence of stainable iron demands an explanation because iron deficiency alone can cause thrombocytosis, and absent marrow iron in the presence of marrow hypercellularity is a feature of PV.

Nonrandom cytogenetic abnormalities occur in ET but are uncommon, and no specific or consistent abnormality is notable, even those involving chromosomes 3 and 1, where the genes for thrombopoietin and its receptor Mpl, respectively, are located.

Diagnosis

Thrombocytosis is encountered in a broad variety of clinical disorders (Table 103-5) in many of which production of cytokines is increased. The absolute level of the platelet count is not a useful diagnostic aid for distinguishing between benign and clonal causes of thrombocytosis. About 50% of ET patients express the JAK2 V617F mutation. When JAK2 V617F is absent, cytogenetic evaluation is mandatory to determine if the thrombocytosis is due to CML or a myelodysplastic disorder such as the 5q– syndrome. Because the bcr-abl translocation can be present in the absence of the Ph chromosome, and because bcr-abl RT-PCR is associated with false-positive results, fluorescence in situ hybridization (FISH) analysis for bcr-abl is the preferred assay in patients with thrombocytosis in whom a cytogenetic study for the Ph chromosome is negative. Anemia and ringed sideroblasts are not features of ET, but they are features of idiopathic refractory sideroblastic anemia, and in some of these patients the thrombocytosis occurs in association with JAK2 V617F expression. Massive splenomegaly should suggest the presence of another myeloproliferative disorder, and in this setting a red cell mass determination should be performed because splenomegaly can mask the presence of erythrocytosis. Importantly, what appears to be ET can evolve into PV or IMF after a period of many years, revealing the true nature of the underlying myeloproliferative disorder.

Complications

Perhaps no other condition in clinical medicine has caused otherwise astute physicians to intervene inappropriately more often than thrombocytosis, particularly if the platelet count is $>1 \times 10^6/\mu L$. It is commonly believed that a high platelet count causes intravascular stasis and thrombosis; however, no controlled clinical study has ever established this association, and in patients younger than age 60, the incidence of thrombosis was not greater in patients with thrombocytosis than in age-matched controls.

To the contrary, very high platelet counts are associated primarily with hemorrhage due to acquired von Willebrand disease. This is not meant to imply that an elevated platelet count cannot cause symptoms in a patient with ET, but rather that the focus should be on the

patient, not the platelet count. For example, some of the most dramatic neurologic problems in ET are migraine-related and respond only to lowering of the platelet count, while other symptoms such as erythromelalgia respond simply to platelet cyclooxygenase 1 inhibitors such as aspirin or ibuprofen, without a reduction in platelet number. Still others may represent an interaction between an atherosclerotic vascular system and a high platelet count, and others may have no relationship to the platelet count whatsoever. Recognition that PV can present with thrombocytosis as well as the discovery of previously unrecognized causes of hypercoagulability (Chap. 111) make the older literature on the complications of thrombocytosis unreliable.

Essential Thrombocytosis: Treatment

Survival of patients with ET is not different than for the general population. An elevated platelet count in an asymptomatic patient without cardiovascular risk factors requires no therapy. Indeed, before any therapy is initiated in a patient with thrombocytosis, the cause of symptoms must be clearly identified as due to the elevated platelet count. When the platelet count rises above $1 \times 10^6/\mu L$, a substantial quantity of high-molecular-weight von Willebrand multimers are removed from the circulation and destroyed by the platelets, resulting in an acquired form of von Willebrand disease. This can be identified by a reduction in ristocetin cofactor activity. In this situation, aspirin could promote hemorrhage. Bleeding in this situation usually responds to ε-aminocaproic acid, which can be given prophylactically before and after elective surgery. Plateletpheresis is at best a temporary and inefficient remedy that is rarely required. Importantly, ET patients treated with ^{32}P or alkylating agents are at risk of developing acute leukemia without any proof of benefit; combining either therapy with hydroxyurea increases this risk. If platelet reduction is deemed necessary on the basis of symptoms refractory to salicylates alone, IFN-α, the quinazoline derivative, anagrelide, or hydroxyurea can be used to reduce the platelet count, but none of these is uniformly effective nor without significant side effects. Hydroxyurea and aspirin are more effective than anagrelide and aspirin for prevention of TIAs but not more effective for the prevention of other types of arterial thrombosis and actually less effective for venous thrombosis. Normalizing the platelet count does not prevent either arterial or venous thrombosis. Risk of gastrointestinal bleeding is also higher when aspirin is combined with anagrelide.

As more clinical experience is acquired, ET is more benign than previously thought. Evolution to acute leukemia is more likely to be a consequence of therapy than of the disease itself. In managing patients with thrombocytosis, the physician's first obligation is to do no harm.

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Note: Large images and tables on this page may necessitate printing in landscape mode.

Harrison's Internal Medicine > Chapter 104. Acute and Chronic Myeloid Leukemia >

Acute and Chronic Myeloid Leukemia: Introduction

The myeloid leukemias are a heterogeneous group of diseases characterized by infiltration of the blood, bone marrow, and other tissues by neoplastic cells of the hematopoietic system. In 2006 the estimated number of new myeloid leukemia cases in the United States was 16,430. These leukemias comprise a spectrum of malignancies that, untreated, range from rapidly fatal to slowly growing. Based on their untreated course, the myeloid leukemias have traditionally been designated acute or chronic.

Acute Myeloid Leukemia

Incidence

The incidence of acute myeloid leukemia (AML) is ~3.7 per 100,000 people per year, and the age-adjusted incidence is higher in men than in women (4.6 versus 3.0). AML incidence increases with age; it is 1.9 in individuals <65 years and 18.6 in those >65. A significant increase in AML incidence has occurred over the past 10 years.

Etiology

Heredity, radiation, chemical and other occupational exposures, and drugs have been implicated in the development of AML. No direct evidence suggests a viral etiology.

Heredity

Certain syndromes with somatic cell chromosome aneuploidy, such as trisomy 21 noted in Down syndrome, are associated with an increased incidence of AML. Inherited diseases with defective DNA repair, e.g., Fanconi anemia, Bloom syndrome, and ataxia telangiectasia, are also associated with AML. Congenital neutropenia (Kostmann syndrome) is a disease with mutations in the granulocyte colony-stimulating factor (G-CSF) receptor and, often, neutrophil elastase that may evolve into AML. Myeloproliferative syndromes may also evolve into AML (Chap. 103). Germ-line mutations of CCAAT/enhancer-binding protein α (C/EBPα), runt-related transcription factor 1 (RUNX1), and tumor protein p53 (TP53) have also been associated with a higher predisposition to AML in some series.

Radiation

Survivors of the atomic bomb explosions in Japan had an increased incidence of myeloid leukemias that peaked 5–7 years after exposure. Therapeutic radiation alone seems to add little risk of AML but can increase the risk in people also exposed to alkylating agents.

Chemical and Other Exposures

Exposure to benzene, a solvent used in the chemical, plastic, rubber, and pharmaceutical industries, is associated with an increased incidence of AML. Smoking and exposure to petroleum products, paint, embalming fluids, ethylene oxide, herbicides, and pesticides, have also been associated with an increased risk of AML.

Drugs

Anticancer drugs are the leading cause of therapy-associated AML. Alkylating agent–associated leukemias occur on average 4–6 years after exposure, and affected individuals have aberrations in chromosomes 5 and 7. Topoisomerase II inhibitor–associated leukemias occur 1–3 years after exposure, and affected individuals often have aberrations involving chromosome 11q23. Chloramphenicol, phenylbutazone, and, less commonly, chloroquine and methoxypsoralen can result in bone marrow failure that may evolve into AML.

Classification

The World Health Organization (WHO) classification (Table 104-1) includes different biologically distinct groups based on immunophenotype, clinical features, and cytogenetic and molecular abnormalities in addition to morphology. In contrast to the previously used French-American-British (FAB) schema, the WHO classification places limited reliance on cytochemistry. Since much of the recent literature and some ongoing studies use the FAB classification, a description of this system is also provided in Table 104-1. A major difference between the WHO and FAB systems is the blast cutoff for a diagnosis of AML as opposed to myelodysplastic syndrome (MDS); it is 20% in the WHO classification and 30% in the FAB. AML with 20–30% blasts as defined by the WHO classification can benefit from approved therapies for MDS (such as decitabine or 5-azacytidine) that were approved in the past by the Food and Drug Administration (FDA) for marketing based on trials using the FAB criteria.

Table 104-1 Acute Myeloid Leukemia (AML) Classification Systems

World Health Organization Classification[a]

- I. AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22);*RUNX1/RUNX1T1*[b]
  - AML with abnormal bone marrow eosinophils [inv(16)(p13q22) or t(16;16)(p13;q22);*CBFB/MYH11*][b]
  - Acute promyelocytic leukemia [AML with t(15;17)(q22;q12) (*PML/RARα*) and variants][b]
  - AML with 11q23 (*MLL*) abnormalities
- II. AML with multilineage dysplasia
  - Following a myelodysplastic syndrome or myelodysplastic syndrome/myeloproliferative disorder
  - Without antecedent myelodysplastic syndrome
- III. AML and myelodysplastic syndromes, therapy-related
  - Alkylating agent–related
  - Topoisomerase type II inhibitor–related
  - Other types
- IV. AML not otherwise categorized
  - AML minimally differentiated
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic and monocytic leukemia
  - Acute erythroid leukemia
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
  - Myeloid sarcoma

Incidence

French-American-British (FAB) Classification[c]	
M0: Minimally differentiated leukemia	5%
M1: Myeloblastic leukemia without maturation	20%
M2: Myeloblastic leukemia with maturation	30%
M3: Hypergranular promyelocytic leukemia	10%
M4: Myelomonocytic leukemia	20%
M4Eo: Variant: Increase in abnormal marrow eosinophils	
M5: Monocytic leukemia	10%
M6: Erythroleukemia (DiGuglielmo's disease)	4%
M7: Megakaryoblastic leukemia	1%

[a]ES Jaffe et al: *World Health Organization Classification of Tumours*. Lyon, IARC Press, 2001.

[b]Diagnosis is AML regardless of blast count.

[c]JM Bennett et al: Ann Intern Med 103:620, 1985.

Importantly, the WHO schema is the first leukemia classification system to consider genetic along with morphologic features to define different subsets of AML.

Immunophenotype and Relevance to the WHO Classification

The immunophenotype of human leukemia cells can be studied by multiparameter flow cytometry after the cells are labeled with monoclonal antibodies to cell-surface antigens. This can be important for separating AML from acute lymphoblastic leukemia (ALL) and identifying some types of AML. For example, AML that is minimally differentiated (immature morphology and no lineage-specific cytochemical reactions) is diagnosed by flow-cytometric demonstration of the myeloid-specific antigens cluster designation (CD) 13 or 33. Similarly, acute megakaryoblastic leukemia can often be diagnosed only by expression of the platelet-specific antigens CD41 and/or CD61. While flow cytometry is useful, widely used, and, in some cases, essential for the diagnosis of AML, it is only supportive in establishing the different subtypes of AML through the WHO classification.

Clinical Features and Relevance to the WHO Classification

The WHO classification considers clinical features in subdividing AML. For example, it identifies therapy-related AML as a separate entity and subclassifies this group based on the specific types of prior chemotherapy received. It also divides AML with multilineage dysplasia based upon the presence or absence of an antecedent MDS. These clinical features contribute to the prognosis of the specific type of AML.

Genetic Findings and Relevance to the WHO Classification

The WHO classification is the first AML classification to incorporate genetic (chromosomal and molecular) information. Indeed, AML is first subclassified based on the presence or absence of specific recurrent genetic abnormalities. For example, AML FAB M3 is now designated *acute promyelocytic leukemia* (APL), based on the presence of either the t(15;17)(q22;q12) cytogenetic rearrangement or the *PML/RAR*$_{\alpha}$ product of the translocation. Thus, the WHO classification separates APL from all other types of AML as a first step and forces the clinician to correctly identify the entity and tailor treatment(s) accordingly.

Chromosomal Analyses

Chromosomal analysis of the leukemic cell provides the most important pretreatment prognostic information in AML. Two cytogenetic abnormalities have been invariably associated with specific morphologic features: t(l5;17)(q22;q12) with APL and inv(16)(p13q22) with AML with abnormal bone marrow eosinophils. Many other chromosomal abnormalities have been associated primarily with one morphologic/immunophenotypic group, including t(8;21)(q22;q22) with slender Auer rods, expression of CD19, and abundance of normal eosinophils, and t(9;11)(p22;q23), as well as other translocations involving 11q23, with monocytic features. Many of the recurring chromosomal abnormalities in AML have been associated with specific clinical characteristics. More commonly associated with younger age are t(8;21) and t(l5;17); with older age, del(5q) and del(7q). Myeloid sarcomas (see below) are associated with t(8;21) and disseminated intravascular coagulation (DIC) with t(15;17).

Molecular Classification

Molecular study of many recurring cytogenetic abnormalities has revealed genes that may be involved in leukemogenesis; this information is increasingly being incorporated into the WHO classification. For instance, the t(15;17) encodes a chimeric protein, promyelocytic leukemia (Pml)/retinoic acid receptor $_{\alpha}$ (Rar$_{\alpha}$), which is formed by the fusion of the retinoic acid receptor $_{\alpha}$ (*RAR*$_{\alpha}$) gene from chromosome 17 and the promyelocytic leukemia (*PML*) gene from chromosome 15. The *RAR*$_{\alpha}$ gene encodes a member of the nuclear hormone receptor family of transcription factors. After binding retinoic acid, *RAR*$_{\alpha}$ can promote expression of a variety of genes. The 15;17 translocation juxtaposes *PML* with *RAR*$_{\alpha}$ in a head-to-tail configuration that is under the transcriptional control of *PML*. Three

different breakpoints in the *PML* gene lead to various fusion proteins. The Pml-Rarα fusion protein tends to suppress gene transcription and blocks differentiation of the cells. Pharmacologic doses of the Rarα ligand, all-*trans*-retinoic acid (tretinoin), relieve the block and promote differentiation (see below). Similar examples exist with a variety of other balanced translocations and inversions, including the t(8;21), t(9;11), t(6;9), and inv(16).

Molecular aberrations are also being identified that are useful for classifying risk of relapse in patients without cytogenetic abnormalities. A partial tandem duplication (PTD) of the *MLL* gene is found in 5–10% of patients with normal cytogenetics and results in short remission duration. FMS-like tyrosine kinase 3 (Flt3) is a tyrosine kinase receptor important in the development of myeloid and lymphoid lineages. Activating mutations of the gene *FLT3* are present in ~30% of adult AML patients due to internal tandem duplications (ITDs) in the juxtamembrane domain or mutations of the activating loop of the kinase. These occur more commonly in patients with normal karyotype. Continuous activation of Flt3 and downstream target kinases, including signal transducer and activator of transcription protein 5, Ras/mitogen-activated protein kinase, and phosphatidylinositol 3-kinase/Akt, provides increased proliferation and antiapoptotic signals to the myeloid progenitor cell. Presence of *FLT3* ITD in patients with normal cytogenetics predicts for short remission duration and inferior survival. Other molecular prognostic factors in patients with normal karyotype AML include mutations of the nucleophosmin gene (*NPM1*) and *C/EBP*α that are associated with improved treatment outcome. In contrast, overexpression of genes such as brain and acute leukemia, cytoplasmic (*BAALC*) predicts for poor outcome. Gene expression profiles to predict outcome in normal karyotype AML patients are under active investigation.

Clinical Presentation

Symptoms

Patients with AML most often present with nonspecific symptoms that begin gradually or abruptly and are the consequence of anemia, leukocytosis, leukopenia or leukocyte dysfunction, or thrombocytopenia. Nearly half have had symptoms for $\geq$3 months before the leukemia was diagnosed.

Half mention fatigue as the first symptom, but most complain of fatigue or weakness at the time of diagnosis. Anorexia and weight loss are common. Fever with or without an identifiable infection is the initial symptom in ~10% of patients. Signs of abnormal hemostasis (bleeding, easy bruising) are noted first in 5% of patients. On occasion, bone pain, lymphadenopathy, nonspecific cough, headache, or diaphoresis is the presenting symptom.

Rarely patients may present with symptoms from a mass lesion located in the soft tissues, breast, uterus, ovary, cranial or spinal dura, gastrointestinal tract, lung, mediastinum, prostate, bone, or other organs. The mass lesion represents a tumor of leukemic cells and is called a *granulocytic sarcoma*, or *chloroma*. Typical AML may occur simultaneously, later, or not at all in these patients. This rare presentation is more common in patients with t(8;21).

Physical Findings

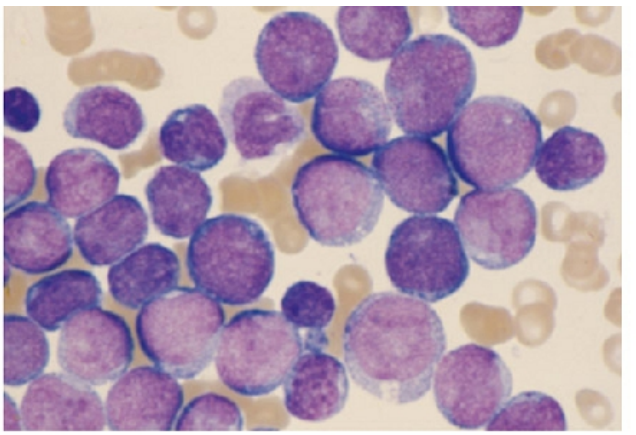
Fever, splenomegaly, hepatomegaly, lymphadenopathy, sternal tenderness, and evidence of infection and hemorrhage are often found at diagnosis. Significant gastrointestinal bleeding, intrapulmonary hemorrhage, or intracranial hemorrhage occur most often in APL. Bleeding associated with coagulopathy may also occur in monocytic AML and with extreme degrees of leukocytosis or thrombocytopenia in other morphologic subtypes. Retinal hemorrhages are detected in 15% of patients. Infiltration of the gingivae, skin, soft tissues, or the meninges with leukemic blasts at diagnosis is characteristic of the monocytic subtypes and those with 11q23 chromosomal abnormalities.

Hematologic Findings

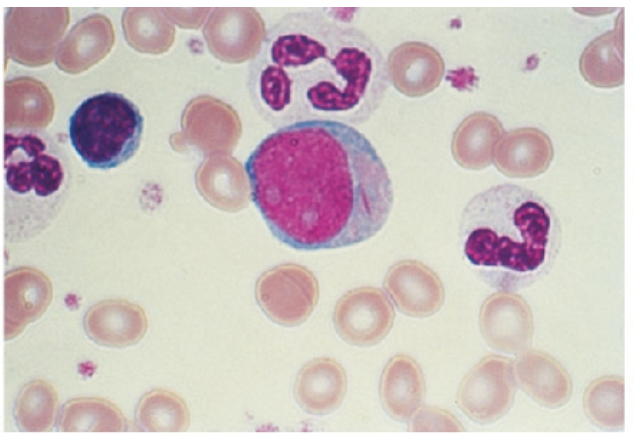
Anemia is usually present at diagnosis and can be severe. The degree varies considerably, irrespective of other hematologic findings, splenomegaly, or duration of symptoms. The anemia is usually normocytic normochromic. Decreased erythropoiesis often results in a reduced reticulocyte count, and red blood cell (RBC) survival is decreased by accelerated destruction. Active blood loss also contributes to the anemia.

The median presenting leukocyte count is about 15,000/μL. Between 25 and 40% of patients have counts <5000/μL, and 20% have counts >100,000/μL. Fewer than 5% have no detectable leukemic cells in the blood. The morphology of the malignant cell varies in difference subsets. In AML the cytoplasm often contains primary (nonspecific) granules, and the nucleus shows fine, lacy chromatin with one or more nucleoli characteristic of immature cells. Abnormal rod-shaped granules called Auer rods are not uniformly present, but when they are, myeloid lineage is virtually certain (Fig 104-1). Poor neutrophil function may be noted by impaired phagocytosis and migration and morphologically by abnormal lobulation and deficient granulation.

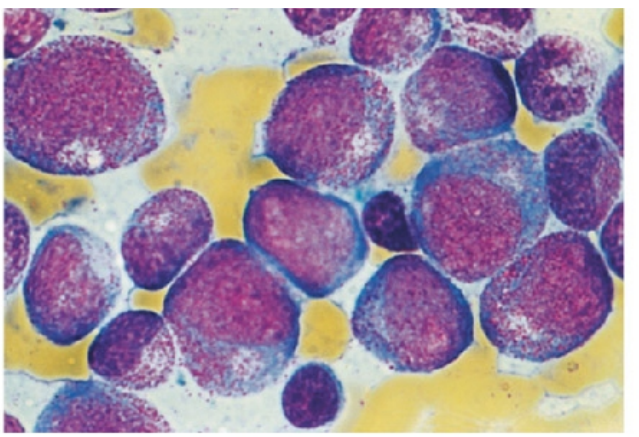
Figure 104-1



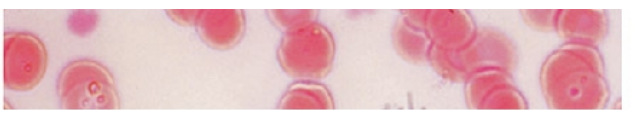
A



B



C



Morphology of AML cells. *A*. Uniform population of primitive myeloblasts with immature chromatin, nucleoli in some cells, and primary cytoplasmic granules. *B*. Leukemic myeloblast containing an Auer rod. *C*. Promyelocytic leukemia cells with prominent cytoplasmic primary granules. *D*. Peroxidase stain shows dark blue color characteristic of peroxidase in granules in AML.

Platelet counts <100,000/μL are found at diagnosis in ~75% of patients, and about 25% have counts <25,000/μL. Both morphologic and functional platelet abnormalities can be observed, including large and bizarre shapes with abnormal granulation and inability of platelets to aggregate or adhere normally to one another.

Pretreatment Evaluation

Once the diagnosis of AML is suspected, a rapid evaluation and initiation of appropriate therapy should follow (Table 104-2). In addition to clarifying the subtype of leukemia, initial studies should evaluate the overall functional integrity of the major organ systems, including the cardiovascular, pulmonary, hepatic, and renal systems. Factors that have prognostic significance, either for achieving complete remission (CR) or for predicting the duration of CR, should also be assessed before initiating treatment. Leukemic cells should be obtained from all patients and cryopreserved for future use as new tests and therapeutics become available. All patients should be evaluated for infection.

Table 104-2 Initial Diagnostic Evaluation and Management of Adult Patients with Acute Myeloid Leukemia

History

Increasing fatigue or decreased exercise tolerance (anemia)

Excess bleeding or bleeding from unusual sites (DIC, thrombocytopenia)

Fevers or recurrent infections (granulocytopenia)

Headache, vision changes, nonfocal neurologic abnormalities (CNS leukemia or bleed)

Early satiety (splenomegaly)

Family history of AML (Fanconi, Bloom, or Kostmann syndromes or ataxia telangiectasia)

History of cancer (exposure to alkylating agents, radiation, topoisomerase II inhibitors)

Occupational exposures (radiation, benzene, petroleum products, paint, smoking, pesticides)

Physical Examination

Performance status (prognostic factor)

Ecchymosis and oozing from IV sites (DIC, possible acute promyelocytic leukemia)

Fever and tachycardia (signs of infection)

Papilledema, retinal infiltrates, cranial nerve abnormalities (CNS leukemia)

Poor dentition, dental abscesses

Gum hypertrophy (leukemic infiltration, most common in monocytic leukemia)

Skin infiltration or nodules (leukemia infiltration, most common in monocytic leukemia)

Lymphadenopathy, splenomegaly, hepatomegaly

Back pain, lower extremity weakness [spinal granulocytic sarcoma, most likely in t(8;21) patients]

Laboratory and Radiologic Studies

CBC with manual differential cell count

Chemistry tests (electrolytes, creatinine, BUN, calcium, phosphorus, uric acid, hepatic enzymes, bilirubin, LDH, amylase, lipase)

Clotting studies (prothrombin time, partial thromboplastin time, fibrinogen, D-dimer)

Viral serologies (CMV, HSV-1, varicella zoster)

RBC type and screen

HLA typing of patient, siblings, and parents for potential allogeneic SCT

Bone marrow aspirate and biopsy (morphology, cytogenetics, flow cytometry, molecular studies)

Cryopreservation of viable leukemia cells

Echocardiogram or heart scan

PA and lateral chest radiograph

Placement of central venous access device

Interventions for Specific Patients

Dental evaluation (for those with poor dentition)

Lumbar puncture (for those with symptoms of CNS involvement)

Screening spine MRI (for patients with back pain, lower extremity weakness, paresthesias)

Social work referral for patient and family psychosocial support

Counseling for All Patients

Provide patient with information regarding his/her disease, financial counseling, and support group contacts

Abbreviations: BUN, blood urea nitrogen; CBC, complete blood count; CMV, cytomegalovirus; CNS, central nervous system; DIC, disseminated intravascular coagulation; HLA, human leukocyte antigen; HSV, herpes simplex virus; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PA, posteroanterior; RBC, red blood (cell) count; SCT, stem cell transplant.

Most patients are anemic and thrombocytopenic at presentation. Replacement of the appropriate blood components, if necessary, should begin promptly. Because qualitative platelet dysfunction or the presence of an infection may increase the likelihood of bleeding, evidence of hemorrhage justifies the immediate use of platelet transfusion, even if the platelet count is only moderately decreased.

About 50% of patients have a mild to moderate elevation of serum uric acid at presentation. Only 10% have marked elevations, but renal precipitation of uric acid and the nephropathy that may result is a serious but uncommon complication. The initiation of chemotherapy may aggravate hyperuricemia, and patients are usually started immediately on allopurinol and hydration at diagnosis. Rasburicase (recombinant uric oxidase) is also useful for treating uric acid nephropathy and often can normalize the serum uric acid level within hours with a single dose of treatment. The presence of high concentrations of lysozyme, a marker for monocytic differentiation, may be etiologic in renal tubular dysfunction, which could worsen other renal problems that arise during the initial phases of therapy.

Prognostic Factors

Many factors influence the likelihood of entering CR, the length of CR, and the curability of AML. CR is defined after examination of both blood and bone marrow. The blood neutrophil count must be ≥1000/μL and the platelet count ≥100,000/μL. Hemoglobin concentration is not considered in determining CR. Circulating blasts should be absent. While rare blasts may be detected in the blood during marrow regeneration, they should disappear on successive studies. Bone marrow cellularity should be >20% with trilineage maturation. The bone marrow should contain <5% blasts, and Auer rods should be absent. Extramedullary leukemia should not be present. For patients in morphologic CR, reverse transcriptase polymerase chain reaction (RT-PCR) to detect AML-associated molecular abnormalities and either metaphase cytogenetics or interphase cytogenetics by fluorescence in situ hybridization (FISH) to detect AML-associated cytogenetic aberrations are currently used to detect residual disease. Such detection of minimal residual disease may become a reliable discriminator between patients in CR who do or do not require additional and/or alternative therapies.

Age at diagnosis is among the most important risk factors. Advancing age is associated with a poorer prognosis, in part because of its influence on the patient's ability to survive induction therapy. Age also influences outcome because AML in older patients differs biologically. The leukemic cells in elderly patients more commonly express CD34 and the multidrug resistance 1 (MDR1) efflux pump that conveys resistance to natural product–derived agents such as the anthracyclines (see below). With each successive decade of age, a greater proportion of patients have more resistant disease. Chronic and intercurrent diseases impair tolerance to rigorous therapy; acute medical problems at diagnosis reduce the likelihood of survival. Performance status, independent of age, also influences ability to survive induction

therapy and thus respond to treatment.

Chromosome findings at diagnosis are important independent prognostic factors. Patients with t(15;17) have a very good prognosis (approximately 85% cured), and those with t(8;21) and inv(16) a good prognosis (approximately 50% cured), while those with no cytogenetic abnormality have a moderately favorable outcome (approximately 40% cured). Patients with a complex karyotype, t(6;9), inv (3), or 7 have a very poor prognosis. This emphasizes the importance of cytogenetic as well as the previously discussed molecular assessment of the leukemia cells at diagnosis and relevance of storing samples for potential later use.

A prolonged symptomatic interval with cytopenias preceding diagnosis or a history of an antecedent hematologic disorder is another pretreatment clinical feature associated with a lower CR rate and shorter survival time. The CR rate is lower in patients who have had anemia, leukopenia, and/or thrombocytopenia for >3 months before the diagnosis of AML when compared to those without such a history. Responsiveness to chemotherapy declines as the duration of the antecedent disorder(s) increases. Secondary AML developing after treatment with cytotoxic agents for other malignancies is usually difficult to treat successfully.

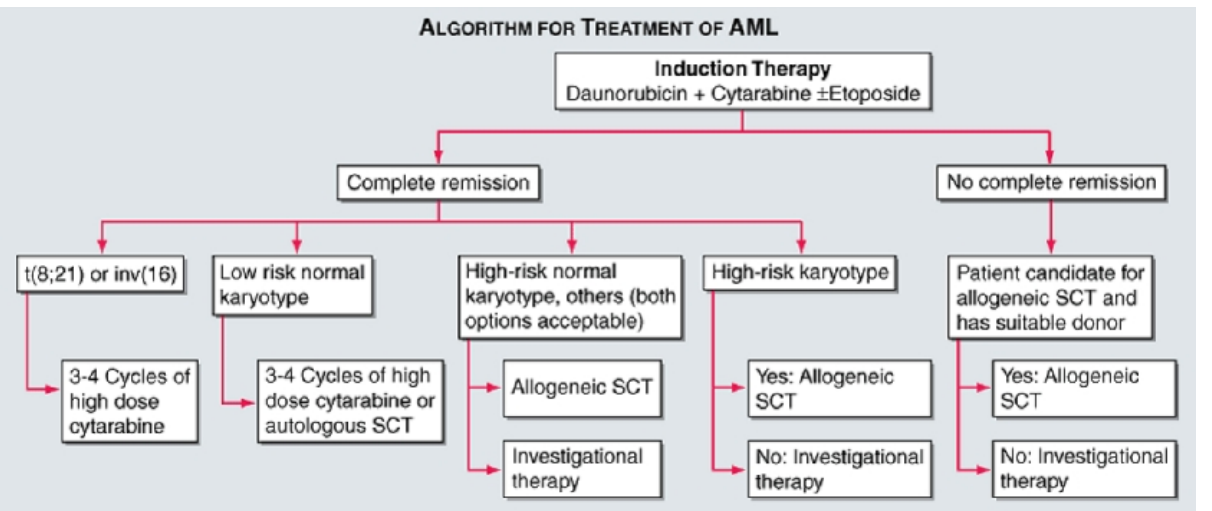
A high presenting leukocyte count is an independent prognostic factor for attaining a CR. Among patients with hyperleukocytosis (>100,000/μL), early central nervous system bleeding and pulmonary leukostasis contribute to poor outcome with initial therapy.

In addition to pretreatment variables such as age, cytogenetics, and leukocyte count, several treatment factors correlate with prognosis in AML, including, most importantly, achievement of CR. In addition, patients who achieve CR after one induction cycle have longer CR durations than those requiring multiple cycles.

Acute Myeloid Leukemia: Treatment

Treatment of the newly diagnosed patient with AML is usually divided into two phases, induction and postremission management (Fig. 104-2). The initial goal is to quickly induce CR. Once CR is obtained, further therapy must be used to prolong survival and achieve cure. The initial induction treatment and subsequent postremission therapy are often chosen based on the patient's age. The influence of intensifying therapy with traditional chemotherapy agents such as cytarabine and anthracyclines in younger patients (<60 years) appears to increase the cure rate of AML. In older patients the benefit of intensive therapy is controversial; novel therapies are being pursued.

Figure 104-2



Flow chart for the therapy of newly diagnosed acute myeloid leukemia. For all forms of AML except acute promyelocytic leukemia (APL), standard therapy includes a 7-day continuous infusion of cytarabine (100–200 mg/m^2 per day) and a 3-day course of daunorubicin (45–60 mg/m^2 per day) or idarubicin (12–13 mg/m^2 per day) with or without 3 days of etoposide. Patients who achieve complete remission undergo postremission consolidation therapy, including sequential courses of high-dose cytarabine, autologous stem cell transplant (SCT), high-dose combination chemotherapy with allogeneic SCT, or novel therapies, based on their predicted risk of relapse (i.e., risk-stratified therapy). Patients with APL usually receive tretinoin together with anthracycline chemotherapy for remission induction and then consolidation chemotherapy (daunorubicin) followed by maintenance tretinoin, with or without chemotherapy. The role of cytarabine in APL induction and consolidation is controversial.

Induction Chemotherapy

The most commonly used CR induction regimens (for patients other than those with APL) consist of combination chemotherapy with

cytarabine and an anthracycline. Cytarabine is a cell cycle S-phase–specific antimetabolite that becomes phosphorylated intracellularly to an active triphosphate form that interferes with DNA synthesis. Anthracyclines are DNA intercalaters. Their primary mode of action is thought to be inhibition of topoisomerase II, leading to DNA breaks. Cytarabine is usually administered as a continuous intravenous infusion for 7 days. Anthracycline therapy generally consists of daunorubicin intravenously on days 1, 2, and 3 (the 7 and 3 regimen). Treatment with idarubicin for 3 days in conjunction with cytarabine by 7-day continuous infusion is at least as effective and may be superior to daunorubicin in younger patients. The addition of etoposide may improve the CR duration.

After induction chemotherapy, the bone marrow is examined to determine if the leukemia has been eliminated. If ≥5% blasts exist with ≥20% cellularity, the patient is usually re-treated with cytarabine and an anthracycline in doses similar to those given initially, but for 5 and 2 days, respectively. Our recommendation, however, is to change therapy in this setting. Patients who fail to attain CR after two induction courses should immediately proceed to an allogeneic stem cell transplant (SCT) if an appropriate donor exists. This approach is only applied to patients under the age of 70 with acceptable end-organ function.

With the 7 and 3 cytarabine/daunorubicin regimen outlined above, 65–75% of adults with de novo AML under the age of 60 years achieve CR. Two-thirds achieve CR after a single course of therapy, and one-third require two courses. About 50% of patients who do not achieve CR have a drug-resistant leukemia, and 50% do not achieve CR because of fatal complications of bone marrow aplasia or impaired recovery of normal stem cells. Higher induction treatment–related mortality and frequency of resistant disease have been observed with increasing age and in patients with prior hematologic disorders (MDS or myeloproliferative syndromes) or chemotherapy treatment for another malignancy.

High-dose cytarabine-based regimens have very high CR rates after a single cycle of therapy. When given in high doses, more cytarabine may enter the cells, saturate the cytarabine-inactivating enzymes, and increase the intracellular levels of 1-β-D-arabinofuranylcytosine-triphosphate, the active metabolite incorporated into DNA. Thus, higher doses of cytarabine may increase the inhibition of DNA synthesis and thereby overcome resistance to standard-dose cytarabine. In two randomized studies, high-dose cytarabine with an anthracycline produced CR rates similar to those achieved with standard 7 and 3 regimens. However, the CR duration was longer after high-dose cytarabine than after standard-dose cytarabine.

The hematologic toxicity of high-dose cytarabine-based induction regimens has typically been greater than that associated with 7 and 3 regimens. Toxicity with high-dose cytarabine includes myelosuppression, pulmonary toxicity, and significant and occasionally irreversible cerebellar toxicity. All patients treated with high-dose cytarabine must be closely monitored for cerebellar toxicity. Full cerebellar testing should be performed before each dose, and further high-dose cytarabine should be withheld if evidence of cerebellar toxicity develops. This toxicity occurs more commonly in patients with renal impairment and in those over age 60. The increased toxicity observed with high-dose cytarabine has limited the use of this therapy in elderly AML patients.

Supportive Care

Measures geared to supporting patients through several weeks of granulocytopenia and thrombocytopenia are critical to the success of AML therapy. Patients with AML should be treated in centers expert in providing supportive measures.

Recombinant hematopoietic growth factors have been incorporated into clinical trials in AML. These trials have been designed to lower the infection rate after chemotherapy. Both G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) have reduced the median time to neutrophil recovery by an average of 5–7 days. This accelerated rate of neutrophil recovery, however, has not generally translated into significant reductions in infection rates or shortened hospitalizations. In most randomized studies, both G-CSF and GM-CSF have failed to improve the CR rate, disease-free survival, or overall survival. Although receptors for both G-CSF and GM-CSF are present on AML blasts, therapeutic efficacy is neither enhanced nor inhibited by these agents. The use of growth factors as supportive care for AML patients is controversial. We favor their use in elderly patients with complicated courses, those receiving intensive postremission regimens, patients with uncontrolled infections, or those participating in clinical trials.

Multilumen right atrial catheters should be inserted as soon as patients with newly diagnosed AML have been stabilized. They should be used thereafter for administration of intravenous medications and transfusions, as well as for blood drawing. Antibiotic-impregnated catheters should be considered if the risk of line-related infection is high.

Adequate and prompt blood bank support is critical to therapy of AML. Platelet transfusions should be given as needed to maintain a platelet count >10,000–20,000/μL. We believe that the platelet count should be kept at higher levels in febrile patients and during episodes of active bleeding or DIC. Patients with poor posttransfusion platelet count increments may benefit from administration of platelets from human leukocyte antigen (HLA)-matched donors. RBC transfusions should be administered to keep the hemoglobin level >80 g/L (8 g/dL) in the absence of active bleeding, DIC, or congestive heart failure. Blood products leukodepleted by filtration should be used to avert or delay alloimmunization as well as febrile reactions. Blood products should also be irradiated to prevent transfusion associated graft-versus-host disease (GVHD). Cytomegalovirus (CMV)-negative blood products should be used for CMV-seronegative patients who are potential candidates for allogeneic SCT. Leukodepleted products are also effective for these patients if CMV-negative products are not available.

Infectious complications remain the major cause of morbidity and death during induction and postremission chemotherapy for AML. Prophylactic administration of antibiotics in the absence of fever is controversial. Oral nystatin or clotrimazole is recommended to prevent localized candidiasis. For patients who are herpes simplex virus antibody titer–positive, acyclovir prophylaxis is effective in preventing reactivation of latent oral herpes infections.

Fever develops in most patients with AML, but infections are documented in only half of febrile patients. Early initiation of empirical broad-spectrum antibacterial and antifungal antibiotics has significantly reduced the number of patients dying of infectious complications (Chap. 82). An antibiotic regimen adequate to treat gram-negative organisms should be instituted at the onset of fever in a

granulocytopenic patient after clinical evaluation, including a detailed physical examination with inspection of the indwelling catheter exit site and a perirectal examination, as well as procurement of cultures and radiographs aimed at documenting the source of fever. Specific antibiotic regimens should be based on antibiotic sensitivity data obtained from the institution at which the patient is being treated. Acceptable regimens include imipenem-cilastin; an antipseudomonal semisynthetic penicillin (e.g., piperacillin) combined with an aminoglycoside; a third-generation cephalosporin with antipseudomonal activity (i.e., ceftazidime or cefepime); or double β-lactam combinations (ceftazidime and piperacillin). Aminoglycosides should be avoided if possible in patients with renal insufficiency. For patients with known immediate-type hypersensitivity reactions to penicillin, aztreonam may be substituted for β-lactams. Aztreonam should be combined with an aminoglycoside or a quinolone antibiotic rather than used alone.

Empirical vancomycin is not given initially in the absence of suspected gram-positive infection or mucositis but should be initiated in neutropenic patients who remain febrile for 3 days; empirical systemic antifungal therapy is added at 7 days if fever persists. Voriconazole has been shown to be equivalent in efficacy and less toxic than amphotericin-B. Caspofungin or liposomal amphotericin are also considered for fungal infections not responsive to first-line therapy or when such therapy is not tolerated. Antibacterial and antifungal antibiotics should be continued until patients are no longer neutropenic, regardless of whether a specific source has been found for the fever.

Treatment of Promyelocytic Leukemia

Tretinoin is an oral drug that induces the differentiation of leukemic cells bearing the t(15;17). APL is responsive to cytarabine and daunorubicin, but about 10% of patients treated with these drugs die from DIC induced by the release of granule components by dying tumor cells. Tretinoin does not produce DIC but produces another complication called the *retinoic acid syndrome*. Occurring within the first 3 weeks of treatment, it is characterized by fever, dyspnea, chest pain, pulmonary infiltrates, pleural and pericardial effusions, and hypoxia. The syndrome is related to adhesion of differentiated neoplastic cells to the pulmonary vasculature endothelium. Glucocorticoids, chemotherapy, and/or supportive measures can be effective for management of the retinoic acid syndrome. The mortality of this syndrome is about 10%.

Tretinoin (45 mg/m^2 per day orally until remission is documented) plus concurrent anthracycline chemotherapy appears to be among the safest and most effective treatments for APL. Unlike patients with other types of AML, patients with this subtype benefit from maintenance therapy with either tretinoin or chemotherapy.

Arsenic trioxide produces meaningful responses in up to 85% of patients refractory to tretinoin. The use of arsenic trioxide is being explored as part of initial treatment in clinical trials of APL. Additionally, studies combining arsenic trioxide with tretinoin in the absence of chemotherapy are ongoing.

The detection of minimal residual disease by RT-PCR amplification of the t(15;17) chimeric gene product appears to predict relapse. Disappearance of the signal is associated with long-term disease-free survival; its persistence predicts relapse. With increases in the sensitivity of the assay, some patients with persistent abnormal gene product have been found who do not suffer a relapse. Studies are underway to determine whether a critical threshold level of transcripts uniformly predicts for leukemia relapse.

Postremission Therapy

Induction of a durable first CR is critical to long-term disease-free survival in AML. However, without further therapy virtually all patients experience relapse. Once relapse has occurred, AML is generally curable only by SCT.

Postremission therapy is designed to eradicate residual leukemic cells to prevent relapse and prolong survival. Postremission therapy in AML is often based on age (younger than 55–65 and older than 55–65). For younger patients, most studies include intensive chemotherapy and allogeneic or autologous SCT. High-dose cytarabine is more effective than standard-dose cytarabine. The Cancer and Leukemia Group B (CALGB), for example, compared the duration of CR in patients randomly assigned postremission to four cycles of high (3 g/m^2, every 12 h on days 1, 3, and 5), intermediate (400 mg/m^2 for 5 days by continuous infusion), or standard (100 mg/m^2 per day for 5 days by continuous infusion) doses of cytarabine. A dose-response effect for cytarabine in patients with AML who were ≤60 years was demonstrated. High-dose cytarabine significantly prolonged CR and increased the fraction cured in patients with favorable [t(8;21) and inv (16)] and normal cytogenetics, but it had no significant effect on patients with other abnormal karyotypes. For older patients, exploration of attenuated intensive therapy that includes either chemotherapy or reduced intensity allogeneic SCT has been pursued. Postremission therapy is a setting for introduction of new agents (Table 104-3).

Table 104-3 Selected New Agents under Study for Treatment of Adults with AML

Class of Drugs	**Example Agent(s)**
MDR1 modulators	Cyclosporine, LY335979
Demethylating agents	Decitabine, 5-azacytidine, zebularine
Histone deacetylase inhibitors	Suberoylanilide hydroxamic acid (SAHA), MS275, LBH589, valproic acid
Heavy metals	Arsenic trioxide, antimony
Farnesyl transferase inhibitors	R115777, SCH66336
FLT3 inhibitors	SU11248, PKC412, MLN518, CHIR-258

HSP-90 antagonists	17-allylaminogeldanamycin (17-AAG) or derivatives
BCR-ABL PDGFR/KIT inhibitors	Imatinib (ST1571, Gleevec), dasatinib, nilotinib
Telomerase inhibitor	GRN163L
Cell cycle inhibitors	Flavopiridol, CYC202 (R-Roscovitine), SNS-032
Nucleoside analogues	Clofarabine, troxacitabine
Humanized antibodies	Anti-CD33 (SGN33), anti-DR4, anti-DR5, anti-KiR
Toxin-conjugated antibodies	Gemtuzumab ozogamicin (Mylotarg)
Radiolabeled antibodies	Yttrium-90-labeled human M195

Allogeneic SCT is used in patients <70 years old with an HLA-compatible donor who have high-risk cytogenetics. In the subset with normal cytogenetics and high-risk molecular features such as *FLT3* ITD, allogeneic SCT is best applied in the context of clinical trials, as the impact of aggressive therapy on outcome is unknown. Relapse following allogeneic SCT occurs in only a small fraction of patients, but toxicity is relatively high from treatment; complications include venoocclusive disease, GVHD, and infections. Autologous transplantation can be administered in young and older patients and uses the same preparative regimens. Patients subsequently receive their own stem cells collected while in remission. The toxicity is lower with autologous SCT (5% mortality rate), but the relapse rate is higher than with allogeneic SCT, and randomized studies have not demonstrated outcome superior to postremission conventional-dose chemotherapy. The increased relapse rate is due to the absence of the graft-versus-leukemia (GVL) effect seen with allogeneic SCT and possible contamination of the autologous stem cells with tumor cells. Purging tumor from the autologous stem cells has not lowered the relapse rate with autologous SCT.

Randomized trials comparing intensive chemotherapy and autologous and allogeneic SCT have shown improved duration of remission with allogeneic SCT compared to autologous SCT or chemotherapy alone. However, overall survival is generally not different; the improved disease control with allogeneic SCT is erased by the increase in fatal toxicity. While stem cells were previously harvested from the bone marrow, virtually all efforts currently collect these from the blood following mobilization regimens, including growth factors with or without chemotherapy. Prognostic factors may help select patients in first CR for whom transplant is most effective.

Our approach includes considering allogeneic SCT in first CR for patients with high-risk karyotypes. Patients with normal karyotypes who have other poor risk factors (e.g., an antecedent hematologic disorder, failure to attain remission with a single induction course, PTD of the *MLL* gene, ITD of the *FLT3* gene, overexpression of *BAALC*) are also potential candidates. If a suitable HLA donor does not exist, novel therapeutic approaches are considered. Other novel transplant strategies, including reduced-intensity SCT, are being explored for consolidation of high-risk AML patients. Patients with t(8;21) and inv(16) are treated with repetitive doses of high-dose cytarabine, which offers a high frequency of cure without the morbidity of transplant. In AML patients with t(8;21) and inv(16), those with *KIT* mutations may be considered for novel investigational studies.

Autologous SCT is generally applied to AML patients only in the context of a clinical trial or when the risk of repetitive intensive chemotherapy represents a higher risk than the autologous SCT (e.g., in patients with severe platelet alloimmunization).

Relapse

Once relapse occurs, patients are rarely cured with further standard-dose chemotherapy. Patients eligible for allogeneic SCT should receive transplants expeditiously at the first sign of relapse. Long-term disease-free survival is approximately the same (30–50%) with allogeneic SCT in first relapse or in second remission. Autologous SCT rescues about 20% of relapsed patients with AML who have chemosensitive disease. The most important factors predicting response at relapse are the length of the previous CR, whether initial CR was achieved with one or two courses of chemotherapy, and the type of postremission therapy.

Because of the poor outcome of patients in early first relapse (<12 months), it is justified (for patients without HLA-compatible donors) to explore innovative approaches, such as new drugs or immunotherapies (Table 104-3). Patients with longer first CR (>12 months) generally relapse with drug-sensitive disease and have a higher chance of attaining a CR. However, cure is uncommon, and treatment with novel approaches should be considered if SCT is not possible. One promising therapy is decitabine, a nucleoside analog that inhibits DNA methyltransferase and subsequently reverses aberrant methylation in AML cells. Interestingly, inhibiting DNA methyltransferase occurs at a much lower dose than previously used to produce a cytotoxic effect in AML. Low-dose decitabine yields CR in a small subset of patients with relapsed AML, including those with unfavorable karyotypes. New agents are needed.

For elderly patients (age >60) for whom clinical trials are not available, gemtuzumab ozogamicin (Mylotarg) is another alternative. This therapy is an antibody-targeted chemotherapy consisting of the humanized anti-CD33 antibody linked to calicheamicin, a potent antitumor antibiotic. The CR rate is ~30%. Its effectiveness in early relapsing (<6 months) or refractory AML patients is limited, possibly due to calicheamicin being a potent MDR1 substrate. Toxicity, including myelosuppression, infusion toxicity, and venoocclusive disease, can be observed with gemtuzumab ozogamicin. Pretreatment with glucocorticoids can diminish many of the infusion reactions associated with gemtuzumab ozogamicin. Studies are examining this treatment in combination with chemotherapy for both young and older patients with previously untreated AML.

Chronic Myelogenous Leukemia

Incidence

The incidence of chronic myelogenous leukemia (CML) is 1.5 per 100,000 people per year, and the age-adjusted incidence is higher in men

than in women (2.0 versus 1.2). The incidence of CML increases slowly with age until the middle forties, when it starts to rise rapidly. CML incidence for males decreased slightly (4.4%) between 1997 and 2003 as compared to 1977–1997.

Definition

The diagnosis of CML is established by identifying a clonal expansion of a hematopoietic stem cell possessing a reciprocal translocation between chromosomes 9 and 22. This translocation results in the head-to-tail fusion of the breakpoint cluster region (*BCR*) gene on chromosome 22q11 with the *ABL* (named after the abelson murine leukemia virus) gene located on chromosome 9q34. Untreated, the disease is characterized by the inevitable transition from a chronic phase to an accelerated phase and on to blast crisis in a median time of 4 years.

Etiology

No clear correlation with exposure to cytotoxic drugs has been found, and no evidence suggests a viral etiology. In the pre-imatinib era, cigarette smoking accelerated the progression to blast crisis and therefore adversely affected survival in CML. Atomic bomb survivors had an increased incidence; the development of a CML cell mass of 10,000/μL took 6.3 years. No increase in CML incidence was found in the survivors of the Chernobyl accident, suggesting that only large doses of radiation can induce CML.

Pathophysiology

The product of the fusion gene resulting from the t(9;22) plays a central role in the development of CML. This chimeric gene is transcribed into a hybrid *BCR/ABL* mRNA in which exon 1 of *ABL* is replaced by variable numbers of 5' *BCR* exons. Bcr/Abl fusion proteins, $p210^{BCR/ABL}$, are produced that contain NH_2-terminal domains of Bcr and the COOH-terminal domains of Abl. A rare breakpoint, occurring within the 3' region of the *BCR* gene, yields a fusion protein of 230 kDa, $p230^{BCR/ABL}$. Bcr/Abl fusion proteins can transform hematopoietic progenitor cells in vitro. Furthermore, reconstituting lethally irradiated mice with bone marrow cells infected with retrovirus carrying the gene encoding the $p210^{BCR/ABL}$ leads to the development of a myeloproliferative syndrome resembling CML in 50% of the mice. Specific antisense oligomers to the *BCR/ABL* junction inhibit the growth of t(9;22)-positive leukemic cells without affecting normal colony formation.

The mechanism(s) by which $p210^{BCR/ABL}$ promotes the transition from the benign state to the fully malignant one is still unclear. Messenger RNA for *BCR/ABL* can occasionally be detected in normal individuals. However, attachment of the *BCR* sequences to *ABL* results in three critical functional changes: (1) the Abl protein becomes constitutively active as a tyrosine kinase (TK) enzyme, activating downstream kinases that prevent apoptosis; (2) the DNA-protein-binding activity of Abl is attenuated; and (3) the binding of Abl to cytoskeletal actin microfilaments is enhanced.

Disease Progression

The events associated with transition to the acute phase, a common occurrence in the pre-imatinib era, were extensively studied. Chromosomal instability of the malignant clone, resulting, for example, in the acquisition of an additional t(9;22), trisomy 8, or 17p- (p53 loss), is a basic feature of CML. Acquisition of these additional genetic and/or molecular abnormalities is critical to the phenotypic transformation. Large deletions adjacent to the translocation breakpoint on the derivative 9 chromosome, detected by microsatellite polymerase chain reaction (PCR) or FISH, are associated with shorter survival times. Heterogeneous structural alterations of the p53 gene, as well as structural alterations and lack of protein production of the retinoblastoma gene and the catalytic component of telomerase, have been associated with disease progression in a subset of patients. Rare patients show alterations in the rat sarcoma viral oncogene homolog (*RAS*). Sporadic reports also document the presence of an altered *MYC* (named after the myelocytomatosis virus) gene. Progressive de novo DNA methylation at the *BCR/ABL* locus and hypomethylation of the *LINE-1* retrotransposon promoter herald blastic transformation. Further, interleukin 1β may be involved in the progression of CML to the blastic phase. In addition, functional inactivation of the tumor suppressor protein phosphatase A2 may be required for blastic transformation. Finally, CML that develops resistance to imatinib is at an increased risk of progressing to accelerated/blast crisis. Multiple pathways to disease transformation exist, but the exact timing and relevance of each remain unclear.

Clinical Presentation

Symptoms

The clinical onset of the chronic phase is generally insidious. Accordingly, some patients are diagnosed while still asymptomatic, during health-screening tests; other patients present with fatigue, malaise, and weight loss or have symptoms resulting from splenic enlargement, such as early satiety and left upper quadrant pain or mass. Less common are features related to granulocyte or platelet dysfunction, such as infections, thrombosis, or bleeding. Occasionally, patients present with leukostatic manifestations due to severe leukocytosis or thrombosis such as vasoocclusive disease, cerebrovascular accidents, myocardial infarction, venous thrombosis, priapism, visual disturbances, and pulmonary insufficiency. Patients with $p230^{BCR/ABL}$-positive CML have a more indolent course.

Progression of CML is associated with worsening symptoms. Unexplained fever, significant weight loss, increasing dose requirement of the drugs controlling the disease, bone and joint pain, bleeding, thrombosis, and infections suggest transformation into accelerated or blastic phases. Fewer than 10–15% of newly diagnosed patients present with accelerated disease or with de novo blastic phase CML.

Physical Findings

Minimal to moderate splenomegaly is the most common physical finding; mild hepatomegaly is found occasionally. Persistent splenomegaly despite continued therapy is a sign of disease acceleration. Lymphadenopathy and myeloid sarcomas are unusual except late in the course of the disease; when they are present, the prognosis is poor.

Hematologic Findings

Elevated white blood cell counts (WBCs), with increases in both immature and mature granulocytes, are present at diagnosis. Usually <5% circulating blasts and <10% blasts and promyelocytes are noted with the majority of cells being myelocytes, metamyelocytes and band forms. Cycling of the counts may be observed in patients followed without treatment. Platelet counts are almost always elevated at diagnosis, and a mild degree of normocytic normochromic anemia is present. Leukocyte alkaline phosphatase is low in CML cells. Serum levels of vitamin B_{12} and vitamin B_{12}–binding proteins are elevated. Phagocytic functions are usually normal at diagnosis and remain normal during the chronic phase. Histamine production secondary to basophilia is increased in later stages, causing pruritus, diarrhea, and flushing.

At diagnosis, bone marrow cellularity is increased, with an increased myeloid to erythroid ratio. The marrow blast percentage is generally normal or slightly elevated. Marrow or blood basophilia, eosinophilia, and monocytosis may be present. While collagen fibrosis in the marrow is unusual at presentation, significant degrees of reticulin stain–measured fibrosis are noted in about half of the patients.

Disease acceleration is defined by the development of increasing degrees of anemia unaccounted for by bleeding or therapy; cytogenetic clonal evolution; or blood or marrow blasts between 10 and 20%, blood or marrow basophils ≥20%, or platelet count <100,000/μL. *Blast crisis* is defined as acute leukemia, with blood or marrow blasts ≥20%. Hyposegmented neutrophils may appear (Pelger-Huet anomaly). Blast cells can be classified as myeloid, lymphoid, erythroid, or undifferentiated, based on morphologic, cytochemical, and immunologic features. Occurrence of de novo blast crisis or following imatinib therapy is rare.

Chromosomal Findings

The cytogenetic hallmark of CML, found in 90–95% of patients, is the t(9;22)(q34;q11.2). Originally, this was recognized by the presence of a shortened chromosome 22 (22q-), designated as the *Philadelphia chromosome*, that arises from the reciprocal t(9;22). Some patients may have complex translocations (designated as *variant translocations*) involving three, four, or five chromosomes (usually including chromosomes 9 and 22). However, the molecular consequences of these changes are similar to those resulting from the typical t(9;22). All patients should have evidence of the translocation molecularly or by cytogenetics or FISH to make a diagnosis of CML.

Prognostic Factors

The clinical outcome of patients with CML is variable. Before imatinib mesylate, death was expected in 10% of patients within 2 years and in about 20% yearly thereafter, and the median survival time was ~4 years. Therefore, several prognostic models that identify different risk groups in CML were developed. The most commonly used staging systems have been derived from multivariate analyses of prognostic factors. The *Sokal index* identified percentage of circulating blasts, spleen size, platelet count, age, and cytogenetic clonal evolution as the most important prognostic indicators. This system was developed based on chemotherapy-treated patients. The *Hasford system* was developed on interferon (IFN)-α–treated patients. It identified percentage of circulating blasts, spleen size, platelet count, age, and percentage of eosinophils and basophils as the most important prognostic indicators. This system differs from the Sokal index by ignoring clonal evolution and incorporating percentage of eosinophils and basophils. When applied to a data set of 272 patients treated with IFN-α, the Hasford system was better than the Sokal score for predicting survival time; it identified more low-risk patients but left only a small number of cases in the high-risk group. Preliminary results suggest that both the Sokal and the Hasford systems are applicable to imatinib-treated patients.

Chronic Myelogenous Leukemia: Treatment

The therapy of CML is changing rapidly because we have a proven curative treatment (allogeneic transplantation) that has significant toxicity and a new targeted treatment (imatinib) with excellent outcome based on 5-year follow-up data. Therefore, physician experience and patient preference must be factored into the treatment selection process. Discussion of both treatment options with a patient is indicated. The decision should focus on the outcomes, risks, and toxicities of the various approaches.

At present, the goal of therapy in CML is to achieve prolonged, durable, nonneoplastic, nonclonal hematopoiesis, which entails the eradication of any residual cells containing the *BCR/ABL* transcript. Hence the goal is complete molecular remission and cure. A proposed imatinib treatment algorithm for the newly diagnosed CML patient is presented in Table 104-4.

Table 104-4 Imatinib Treatment Milestones for Newly Diagnosed CML Patients

	Proposed Course of Action[a]	
	Transplantation from an HLA-compatible (related or unrelated) donor, dasatinib, new drugs	Continue same[b] or increase dose[c]
Time, months	**Milestones**	
3	No complete hematologic remission	Complete hematologic remission[b,d]

6	No cytogenetic remission	Any cytogenetic remission[c]
12	Minor[e] or no cytogenetic remission	Complete[b,f] or partial[c,g] cytogenetic remission
18	Partial, minor, or no cytogenetic remission	Complete cytogenetic remission[b]
Anytime	Loss of previously achieved hematologic, cytogenetic, or molecular remission	

[a]Nutritional Comprehensive Cancer Network, Chronic myelogenous leukemia.

[b]Denotes that at the indicated milestones, patients should stay on the same dose.

[c]Denotes that at the indicated milestones, for patients on 400 mg/d, one can either continue the same or increase the dose to a maximum of 600–800 mg, as tolerated.

[d]Complete hematologic remission, WBC <10,000/μL, normal blood morphology, hemoglobin and platelet counts, and disappearance of splenomegaly.

[e]Minor cytogenetic remission, 36–85% bone marrow metaphases with t(9;22).

[f]Complete cytogenetic remission, no bone marrow metaphases with t(9;22).

[g]Partial cytogenetic remission, 1-35% bone marrow metaphases with t(9;22).

Abbreviations: HLA, human leukocyte antigen; WBC, white blood cell count.

Allogeneic SCT

Allogeneic SCT is complicated by early mortality owing to the transplant procedure. Outcome of SCT depends on multiple factors including: (1) the patient (e.g., age and phase of disease); (2) the type of donor [e.g., syngeneic (monozygotic twins) or HLA-compatible allogeneic, related or unrelated]; (3) the preparative regimen (myeloblative or reduced intensity); (4) GVHD; and (5) posttransplantation treatment.

The Patient

Patients should have acceptable end-organ function, be <70 years, and have a healthy, histocompatible donor. Furthermore, survival after SCT in the accelerated and blastic phases of the disease is significantly diminished and is associated with high rates of relapse. Bone marrow transplantation (BMT) early in the chronic phase (1–2 years from diagnosis) is superior to later BMT. In the imatinib era, allogeneic transplantation should be used when possible for patients with accelerated/blastic phases of the disease or those whose disease fails to respond or progresses on imatinib.

The Donor

Transplantation from a family donor, who is either fully matched or mismatched at only one HLA locus, should be considered for any patient with CML who is a candidate for an HLA-related sibling transplant. Syngeneic BMT in patients with chronic-phase CML results in 7-year disease-free survival in 55% of patients, with a 30% relapse rate. BMT with an HLA-identical sibling in the chronic phase achieves 5-year disease-free survival in 40–70% of patients, with a 25% relapse rate. BMT from an HLA-matched unrelated donor in chronic phase <1 year from diagnosis and <30 years of age results in 5-year disease-free survival similar to matched-sibling donor transplantation. For all other groups, patients receiving BMT from unrelated donors have higher rates of graft failure and acute and chronic GVHD and prolonged convalescence after treatment, compared to those who receive allogeneic transplants from related donors.

Sex mismatch has an adverse effect on transplantation, with worse outcome associated with a female donor and male recipient. This has been attributed to GVHD against the male histocompatibility Y antigen.

Peripheral blood is now being studied as a source of hematopoietic progenitor cells; it may offer rapid engraftment and less risk for the donor. With unrelated donors, some studies demonstrated no difference in GVHD and improved disease-free survival when comparing peripheral blood to bone marrow stem cells. Using matched sibling donors in chronic-phase CML, marrow stem cells led to a higher cumulative incidence of relapse at 3 years, while peripheral blood stem cell recipients had a higher cumulative incidence of chronic GVHD. At the current time, some centers collect bone marrow and some peripheral blood from sibling donors for newly diagnosed chronic-phase CML patients. Patients with more advanced stages are offered peripheral blood SCT. Umbilical-cord blood may permit mismatched SCT with notably less GVHD; GVL effects do not appear to be impaired. A problem with cord blood is obtaining a sufficient number of progenitor cells to reconstitute hematopoiesis in an adult.

Preparative Regimens

Myeloablative regimens have been studied by several groups. Cyclophosphamide plus total-body irradiation is comparable to busulphan plus cyclophosphamide in the 3-year probabilities of survival, relapse, event-free survival, speed of engraftment, and incidence of

venoocclusive disease of the liver. Significantly more patients in the total-body irradiation arm experienced major elevations of creatinine, acute GVHD, longer periods of fever, positive blood cultures, hospital admissions, and longer inpatient hospital stays. However, increased chronic GVHD, obstructive bronchiolitis, and alopecia were noted with busulphan. Measurement of busulphan levels revealed no significant association between busulphan levels and regimen-related toxicity, but low levels were associated with an increased risk of relapse. Intravenous busulphan allows better control of serum levels.

Reduced-intensity transplants in which the preparative regimen is aimed at eliminating host lymphocytes rather than bone marrow have been reported by numerous groups. No randomized trials comparing the two approaches have been published. Retrospective comparisons reveal that reduced-intensity conditioning regimens produce equivalent or acceptable results (in toxicity as well as outcome). Reduced toxicity with preserved antitumor efficacy is the goal, and therefore reduced-intensity transplantation is our recommendation.

Development and Type of GVHD

Development of grade I GVHD (Chap. 108) decreases the risk of relapse compared to no GVHD. An even lower relapse rate was observed in patients with grade II GVHD but was accompanied by a substantially higher transplant-related mortality rate. The decreased relapse rate may be caused by a GVL effect. Depletion of T lymphocytes from donor marrow can prevent GVHD but results in an increased risk of relapse, which exceeds the relapse rate after syngeneic SCT. Thus, T lymphocytes from the donor marrow mediate a significant antileukemic or GVL effect, and even syngeneic marrow may exhibit limited GVL activity in CML.

Posttransplantation Treatment

BCR/ABL transcript levels have served as early predictors for hematologic relapse following transplantation. These should facilitate risk-adapted approaches with immunosuppression or TK inhibitor(s), or a combination of the two. Donor leukocyte infusions (without any preparative chemotherapy or GVHD prophylaxis) can induce hematologic and cytogenetic remissions in patients with CML who have relapsed after allogeneic SCT.

Imatinib can control CML that has recurred after allogeneic SCT but is sometimes associated with myelosuppression and recurrence of severe GVHD. Imatinib after allogeneic SCT is being studied for prevention of relapse in patients with advanced disease at the time of transplantation (i.e., patients at high risk for relapse), patients undergoing reduced-intensity transplants, or patients with slow reduction of *BCR/ABL* message following transplantation. Imatinib has also been combined with donor lymphocytes to induce rapid molecular remissions in CML patients with disease relapse after allogeneic SCT. Of interest are studies with newer TK inhibitors following transplantation for imatinib-resistant CML.

Imatinib Mesylate

Imatinib mesylate (Gleevec) functions through competitive inhibition at the ATP binding site of the Abl kinase in the inactive conformation, which leads to inhibition of tyrosine phosphorylation of proteins involved in Bcr/Abl signal transduction. It shows specificity for Bcr/Abl, the receptor for platelet-derived growth factor, and Kit tyrosine kinases. Imatinib induces apoptosis in cells expressing Bcr/Abl.

In newly diagnosed CML, imatinib (400 mg/d) is more effective than IFN-α and cytarabine. The complete hematologic remission rate, at 18 months, of patients treated with imatinib was 97% compared to 69% in patients treated with IFN-α and cytarabine. Similarly, the complete cytogenetic remission rate was 76% with imatinib compared to 14% with IFN-α and cytarabine.

All imatinib-treated patients who achieved major molecular remission (26%), defined as $\geq$3 log reduction in *BCR/ABL* transcript level at 18 months compared to pretreatment level, were progression-free at 5 years. The progression-free survival (PFS) at 5 years for patients achieving complete cytogenetic remission but less pronounced molecular remission is 98%. The 5-year PFS for patients not achieving complete cytogenetic remission at 18 months was 87%. These results have led to a consensus that molecular responses can be used as a treatment goal in CML. Specific milestones have been developed for chronic-phase CML patients (Table 104-4). For example, chronic-phase CML patients who do not achieve any cytogenetic remission following six months of imatinib are unlikely to achieve major molecular remission and should be offered other treatment approaches.

Progression to accelerated/blastic phases of the disease was noted in 3% of patients treated with imatinib as compared to 8.5% of patients treated with IFN-α and cytarabine during the first year. Over time, the annual incidence of disease progression on imatinib decreased gradually to <1% during the fourth and fifth years, and no patient who achieved complete cytogenetic remission during the first year of imatinib treatment progressed to the accelerated/blastic phases of the disease.

Imatinib is administered orally. The main side effects are fluid retention, nausea, muscle cramps, diarrhea, and skin rashes. The management of these side effects is usually supportive. Myelosuppression is the most common hematologic side effect. Myelosuppression, while rare, may require holding drug and/or growth factor support. Doses <300 mg/d seem ineffective and may lead to development of resistance.

Four mechanisms of resistance to imatinib have been described to date. These are (1) gene amplification, (2) mutations at the kinase site, (3) enhanced expression of multidrug exporter proteins, and (4) alternative signaling pathways functionally compensating for the imatinib-sensitive mechanisms. All four mechanisms are being targeted in clinical trials.

BCR/ABL gene amplification and decreased intracellular imatinib concentrations are addressed by intensifying the therapy with higher (up to 800 mg/d) imatinib doses. Response in some patients has led to early intensification of imatinib dosage in newly diagnosed CML patients, resulting in improved major molecular remissions when retrospectively compared to controls treated with 400 mg/d. Randomized

studies comparing 400 mg/d doses to 800 mg/d in newly diagnosed CML patients are ongoing.

Mutations at the kinase domain are being targeted by novel TK inhibitors that have a different conformation than imatinib, demonstrating activity against most imatinib-resistant mutations. Nilotinib (Tasigma), like imatinib, binds to the kinase domain in the inactive conformation. Dasatinib (Sprycel) binds to the kinase domain in the open conformation and also inhibits the SRC (sarcoma) family of kinases, addressing the last mechanism of resistance. CML with the T315I mutation is resistant to imatinib, nilotinib, and dasatinib.

Dasatinib is approved by the FDA for the treatment of all stages of CML with resistance or intolerance to prior therapy, including imatinib. Nilotinib will likely follow suit. Both are oral agents given twice daily, with toxicity profiles similar to imatinib with small but significant differences. Dasatinib was shown to cause pleural effusion in 22% of patients with 7% developing grade 3-4 toxicity. Nilotinib was associated with sudden death in six of approximately 550 CML patients. A suspected relationship to nilotinib was reported in two of these cases.

These new agents have changed the treatment algorithm of CML. For example, patients who do not achieve any cytogenetic remission at six months on imatinib will now be offered either dasatinib or SCT. IFN-α, though FDA-approved for CML, will only be offered if all other options have failed.

The encouraging results with imatinib have led clinicians to offer it as first-line therapy for newly diagnosed CML patients, including those who otherwise would have benefited from transplant (e.g., young patients with a matched sibling donor). Prior exposure to imatinib does not affect transplant outcome. However, delaying BMT for high-risk patients (Sokal/Hasford criteria) may result in disease progression. SCT after disease progression is associated with poorer outcome. Therefore, we recommend close monitoring of imatinib response, especially in these patients (Table 104-4).

Interferon

Before imatinib, when allogeneic SCT was not feasible, IFN-α therapy was the treatment of choice. Only longer follow-up of patients treated with imatinib will prove whether IFN-α will still have a role in the treatment of CML. Its mode(s) of action in CML is still unknown.

Chemotherapy

Initial management of patients with chemotherapy is currently reserved for rapid lowering of WBCs, reduction of symptoms, and reversal of symptomatic splenomegaly. Hydroxyurea, a ribonucleotide reductase inhibitor, induces rapid disease control. The initial dose is 1–4 g/d; the dose should be halved with each 50% reduction of the leukocyte count. Unfortunately, cytogenetic remissions with hydroxyurea are uncommon. Busulphan, an alkylating agent that acts on early progenitor cells, has a more prolonged effect. However, we do not recommend its use because of its serious side effects, which include unexpected, and occasionally fatal, myelosuppression in 5–10% of patients; pulmonary, endocardial, and marrow fibrosis; and an Addison-like wasting syndrome.

Autologous SCT

Autologous SCT could potentially cure CML if a means to select the residual normal progenitors, which coexist with their malignant counterparts, could be developed. As a source of autologous hematopoietic stem cells for transplantation, blood offers certain advantages over marrow (e.g., faster engraftment for the patient and no general anesthesia for the donor). Normal hematopoietic stem cells appear with increased frequency in the blood of patients with CML during the recovery phase after chemotherapy and G-CSF. A role for imatinib before stem cell collection to achieve minimal residual disease and following transplantation to maintain this status is currently being investigated. Specifically, several groups store peripheral blood stem cells from patients in major or complete molecular remissions. However, only a few cases have been transplanted following imatinib therapy. Therefore, such approaches should be performed only in clinical trials.

Leukapheresis and Splenectomy

Intensive leukapheresis may control the blood counts in chronic-phase CML; however, it is expensive and cumbersome. It is useful in emergencies where leukostasis-related complications such as pulmonary failure or cerebrovascular accidents are likely. It may also have a role in the treatment of pregnant women in whom it is important to avoid potentially teratogenic drugs.

Splenectomy was used in CML in the past because of the suggestion that evolution to the acute phase might occur in the spleen. However, this does not appear to be the case, and splenectomy is now reserved for symptomatic relief of painful splenomegaly unresponsive to imatinib or chemotherapy, or for significant anemia or thrombocytopenia associated with hypersplenism. Splenic radiation is used rarely to reduce the size of the spleen.

Minimal Residual Disease

The kinetics of *BCR/ABL* transcript elimination are currently replacing qualitative detection of the *BCR/ABL* message, in spite of a lack of standard acceptable methodology. A consensus panel has proposed ways to harmonize the different methods and to use a conversion factor so that individual laboratories will be able to express *BCR/ABL* transcript levels on an agreed upon scale.

Slow reduction of *BCR/ABL* transcripts following SCT correlates with the possibility of hematologic relapse. However, the definition of "slow reduction" depends on the preparative regimen (reduced-intensity versus fully myeloablative) and the selection of time-points to measure the transcript levels. While persistent RT-PCR positivity at 6 months was regarded as an indication for additional therapy in the

past, current studies utilize periods between engraftment and day 100 for evaluating the clearance rate of *BCR/ABL* transcripts and recommending additional therapies. Large trials with longer follow-up are needed to establish consensus guidelines.

The randomized trial of imatinib versus IFN-α and cytarabine was the first to establish the concept of $\log_{10}$ reduction of *BCR/ABL* transcript from a standardized baseline for untreated patients. This measurement unit was developed instead of either the transcript numbers expressed per μg of leukocyte RNA or the ratio of *BCR/ABL* to a housekeeping gene on a log scale. In this randomized trial, patients who achieved ≥3 log reduction of BCR/ABL message had an extremely low probability of relapse, with a median follow-up of 60 months. It is unclear whether achieving complete molecular remission should still be the goal of treatment in this disease.

These studies also established the value and convenience of using peripheral blood instead of bone marrow testing as a means to assess disease status in patients who achieve complete cytogenetic responses. However, one still needs to consider following CML patients in complete cytogenetic remission and at least major molecular remission with annual cytogenetic bone marrow testing, as these patients are at risk of developing cytogenetic aberrations in t(9;22)-negative cells and secondary MDS/AML. These aberrations in the t(9;22)-negative cells are frequently transient, and their clinical significance is unclear. Such aberrations may occur in 7–10% of imatinib-treated patients. Development of MDS/AML is rare.

Treatment of Blast Crisis

Treatments for primary blast crisis, including imatinib, are generally ineffective. Only 52% of patients treated with imatinib achieved hematologic remission (21% complete hematologic remission), and the median overall survival was 6.6 months. Patients who achieve complete hematologic remission or whose disease returns to a second chronic phase should be considered for allogeneic SCT. Other approaches include induction chemotherapy tailored to the phenotype of the blast cell followed by imatinib, with or without additional chemotherapy and SCT. Blast crisis following initial therapy with imatinib carries a dismal prognosis even if treated with dasatinib or nilotinib.

Further Readings

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Malignancies of Lymphoid Cells: Introduction

Malignancies of lymphoid cells range from the most indolent to the most aggressive human malignancies. These cancers arise from cells of the immune system at different stages of differentiation, resulting in a wide range of morphologic, immunologic, and clinical findings. Insights on the normal immune system have allowed a better understanding of these sometimes confusing disorders.

Some malignancies of lymphoid cells almost always present as leukemia (i.e., primary involvement of bone marrow and blood), while others almost always present as lymphomas (i.e., solid tumors of the immune system). However, other malignancies of lymphoid cells can present as either leukemia or lymphoma. In addition, the clinical pattern can change over the course of the illness. This change is more often seen in a patient who seems to have a lymphoma and then develops the manifestations of leukemia over the course of the illness.

Biology of Lymphoid Malignancies: Concepts of the WHO Classification of Lymphoid Malignancies

The classification of lymphoid cancers evolved steadily throughout the twentieth century. The distinction between leukemia and lymphoma was made early, and separate classification systems were developed for each. Leukemias were first divided into acute and chronic subtypes based on average survival. Chronic leukemias were easily subdivided into those of lymphoid or myeloid origin based on morphologic characteristics. However, a spectrum of diseases that were formerly all called *chronic lymphoid leukemia* has become apparent (Table 105-1). The acute leukemias were usually malignancies of blast cells with few identifying characteristics. When cytochemical stains became available, it was possible to divide these objectively into myeloid malignancies and acute leukemias of lymphoid cells. Acute leukemias of lymphoid cells have been subdivided based on morphologic characteristics by the French-American-British (FAB) group (Table 105-2). Using this system, lymphoid malignancies of small uniform blasts (e.g., typical childhood acute lymphoblastic leukemia) were called L1, lymphoid malignancies with larger and more variable size cells were called L2, and lymphoid malignancies of uniform cells with basophilic and sometimes vacuolated cytoplasm were called L3 (e.g., typical Burkitt's lymphoma cells). Acute leukemias of lymphoid cells have also been subdivided based on immunologic (i.e., T cell vs. B cell) and cytogenetic abnormalities (Table 105-2). Major cytogenetic subgroups include the t(9;22) (e.g., Philadelphia chromosome–positive acute lymphoblastic leukemia) and the t(8;14) found in the L3 or Burkitt's leukemia.

Table 105-1 Lymphoid Disorders that Can Present as "Chronic Leukemia" and Be Confused with Typical B Cell Chronic Lymphoid Leukemia

Follicular lymphoma	Prolymphocytic leukemia (B cell or T cell)
Splenic marginal zone lymphoma	Lymphoplasmacytic lymphoma
Nodal marginal zone lymphoma	Sézary syndrome
Mantle cell lymphoma	Smoldering adult T cell leukemia/ lymphoma
Hairy cell leukemia	

Table 105-2 Classification of Acute Lymphoid Leukemia (ALL)

Immunologic Subtype	% of Cases	FAB Subtype	Cytogenetic Abnormalities
Pre-B ALL	75	L1, L2	t(9;22), t(4;11), t(1;19)
T cell ALL	20	L1, L2	14q11 or 7q34
B cell ALL	5	L3	t(8;14), t(8;22), t(2;8)

Note: FAB, French-American-British classification.

Non-Hodgkin's lymphomas were separated from Hodgkin's disease by recognition of the Sternberg-Reed cells early in the twentieth century. The histologic classification for non-Hodgkin's lymphomas has been one of the most contentious issues in oncology. Imperfect morphologic systems were supplanted by imperfect immunologic systems, and poor reproducibility of diagnosis has hampered progress. In 1999, the World Health Organization (WHO) classification of lymphoid malignancies was devised through a process of consensus development among international leaders in hematopathology and clinical oncology. The WHO classification takes into account morphologic, clinical, immunologic, and genetic information and attempts to divide non-Hodgkin's lymphomas and other lymphoid malignancies into clinical/pathologic entities that have clinical and therapeutic relevance. This system is presented in Table 105-3. This system is clinically relevant and has a higher degree of diagnostic accuracy than those used previously. The possibilities for subdividing lymphoid malignancies are extensive. However, Table 105-3 presents in bold those malignancies that occur in at least 1% of patients. Specific lymphoma subtypes will be dealt with in more detail below. Lymphomas associated with HIV infection are discussed in Chap. 182.

Table 105-3 WHO Classification of Lymphoid Malignancies

B Cell	T Cell	Hodgkin's Disease
Precursor B cell neoplasm	Precursor T cell neoplasm	Nodular lymphocyte-

		predominant Hodgkin's disease
Precursor B lymphoblastic leukemia/lymphoma (precursor B cell acute lymphoblastic leukemia)	**Precursor T lymphoblastic lymphoma/leukemia (precursor T cell acute lymphoblastic leukemia)**	
Mature (peripheral) B cell neoplasms	Mature (peripheral) T cell neoplasms	Classical Hodgkin's disease
B cell chronic lymphocytic leukemia/small lymphocytic lymphoma	T cell prolymphocytic leukemia	Nodular sclerosis Hodgkin's disease
B cell prolymphocytic leukemia	T cell granular lymphocytic leukemia	Lymphocyte-rich classic Hodgkin's disease
Lymphoplasmacytic lymphoma	Aggressive NK cell leukemia	Mixed-cellularity Hodgkin's disease
Splenic marginal zone B cell lymphoma (± villous lymphocytes)	Adult T cell lymphoma/leukemia (HTLV-I+)	Lymphocyte-depletion Hodgkin's disease
Hairy cell leukemia	Extranodal NK/T cell lymphoma, nasal type	
Plasma cell myeloma/plasmacytoma	Enteropathy-type T cell lymphoma	
Extranodal marginal zone B cell lymphoma of MALT type	Hepatosplenic γd T cell lymphoma	
Mantle cell lymphoma	Subcutaneous panniculitis-like T cell lymphoma	
Follicular lymphoma	**Mycosis fungoides/Sézary syndrome**	
Nodal marginal zone B cell lymphoma (± monocytoid B cells)	Anaplastic large cell lymphoma, primary cutaneous type	
Diffuse large B cell lymphoma	**Peripheral T cell lymphoma, not otherwise specified (NOS)**	
Burkitt's lymphoma/Burkitt cell leukemia	**Angioimmunoblastic T cell lymphoma**	
	Anaplastic large cell lymphoma, primary systemic type	

Note: HTLV, human T cell lymphotropic virus; MALT, mucosa-associated lymphoid tissue; NK, natural killer; WHO, World Health Organization.

Malignancies in bold occur in at least 1% of patients.

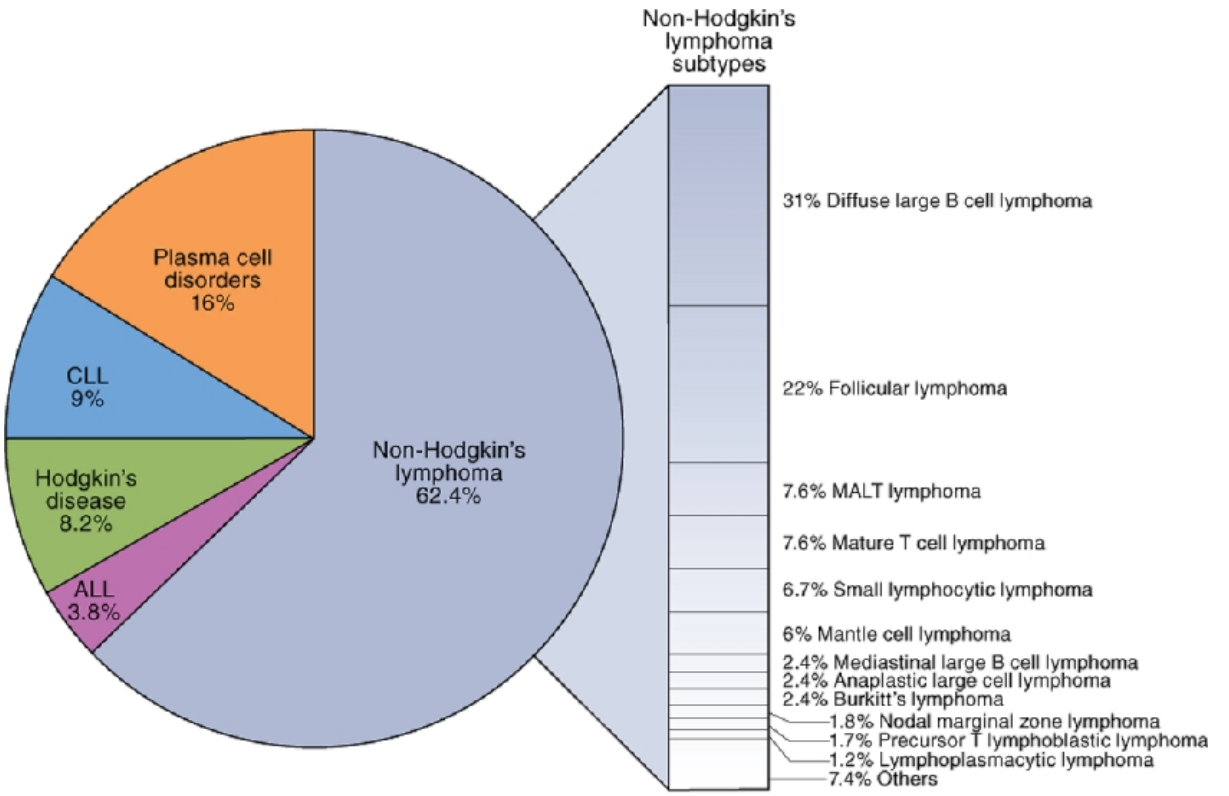
Source: Adapted from Harris et al.

General Aspects of Lymphoid Malignancies

Etiology and Epidemiology

The relative frequency of the various lymphoid malignancies is shown in Fig. 105-1. Chronic lymphoid leukemia (CLL) is the most prevalent form of leukemia in western countries. It occurs most frequently in older adults and is exceedingly rare in children. In 2007, 15,340 new cases were diagnosed in the United States, but because of the prolonged survival associated with this disorder, the total prevalence is many times higher. CLL is more common in men than in women and more common in whites than in blacks. This is an uncommon malignancy in Asia. The etiologic factors for typical CLL are unknown.

Figure 105-1



Relative frequency of lymphoid malignancies.

In contrast to CLL, acute lymphoid leukemias (ALLs) are predominantly cancers of children and young adults. The L3 or Burkitt's leukemia occurring in children in developing countries seems to be associated with infection by the Epstein-Barr virus (EBV) in infancy. However, the explanation for the etiology of more common subtypes of ALL is much less certain. Childhood ALL occurs more often in higher socioeconomic subgroups. Children with trisomy 21 (Down's syndrome) have an increased risk for childhood acute lymphoblastic leukemia as well as acute myeloid leukemia. Exposure to high-energy radiation in early childhood increases the risk of developing T cell acute lymphoblastic leukemia.

The etiology of ALL in adults is also uncertain. ALL is unusual in middle-aged adults but increases in incidence in the elderly. However, acute myeloid leukemia is still much more common in older patients. Environmental exposures including certain industrial exposures, exposure to agricultural chemicals, and smoking might increase the risk of developing ALL as an adult. ALL was diagnosed in 5200 persons and AML in 13,410 persons in the United States in 2007.

The preponderance of evidence suggests that Hodgkin's disease is of B cell origin. The incidence of Hodgkin's disease appears fairly stable, with 8190 new cases diagnosed in 2007 in the United States. Hodgkin's disease is more common in whites than in blacks and more common in males than in females. A bimodal distribution of age at diagnosis has been observed, with one peak incidence occurring in patients in their twenties and the other in those in their eighties. Some of the late age peak may be attributed to confusion among entities with similar appearance such as anaplastic large cell lymphoma and T cell–rich B cell lymphoma. Patients in the younger age groups diagnosed in the United States largely have the nodular sclerosing subtype of Hodgkin's disease. Elderly patients, patients infected with HIV, and patients in third world countries more commonly have mixed-cellularity Hodgkin's disease or lymphocyte-depleted Hodgkin's disease. Infection by HIV is a risk factor for developing Hodgkin's disease. In addition, an association between infection by EBV and Hodgkin's disease has been suggested. A monoclonal or oligoclonal proliferation of EBV-infected cells in 20–40% of the patients with Hodgkin's disease has led to proposals for this virus having an etiologic role in Hodgkin's disease. However, the matter is not settled definitively.

For unknown reasons, non-Hodgkin's lymphomas increased in frequency in the United States at the rate of 4% per year between 1950 and the late 1990s. The rate of increase in the past few years seems to be decreasing. About 63,190 new cases of non-Hodgkin's lymphoma were diagnosed in the United States in 2007. Non-Hodgkin's lymphomas are more frequent in the elderly and more frequent in men. Patients with both primary and secondary immunodeficiency states are predisposed to developing non-Hodgkin's lymphomas. These include patients with HIV infection; patients who have undergone organ transplantation; and patients with inherited immune deficiencies, the sicca syndrome, and rheumatoid arthritis.

The incidence of non-Hodgkin's lymphomas and the patterns of expression of the various subtypes differ geographically. T cell lymphomas

are more common in Asia than in western countries, while certain subtypes of B cell lymphomas such as follicular lymphoma are more common in western countries. A specific subtype of non-Hodgkin's lymphoma known as the angiocentric nasal T/natural killer (NK) cell lymphoma has a striking geographic occurrence, being most frequent in Southern Asia and parts of Latin America. Another subtype of non-Hodgkin's lymphoma associated with infection by human T cell lymphotropic virus (HTLV) I is seen particularly in southern Japan and the Caribbean (Chap. 181).

A number of environmental factors have been implicated in the occurrence of non-Hodgkin's lymphoma, including infectious agents, chemical exposures, and medical treatments. Several studies have demonstrated an association between exposure to agricultural chemicals and an increased incidence in non-Hodgkin's lymphoma. Patients treated for Hodgkin's disease can develop non-Hodgkin's lymphoma; it is unclear whether this is a consequence of the Hodgkin's disease or its treatment. However, a number of non-Hodgkin's lymphomas are associated with infectious agents (Table 105-4). HTLV-I infects T cells and leads directly to the development of adult T cell lymphoma (ATL) in a small percentage of infected patients. The cumulative lifetime risk of developing lymphoma in an infected patient is 2.5%. The virus is transmitted by infected lymphocytes ingested by nursing babies of infected mothers, blood-borne transmission, or sexually. The median age of patients with ATL is ~56 years, emphasizing the long latency. HTLV-I is also the cause of tropical spastic paraparesis– a neurologic disorder that occurs somewhat more frequently than lymphoma and with shorter latency and usually from transfusion-transmitted virus (Chap. 181).

Table 105-4 Infectious Agents Associated with the Development of Lymphoid Malignancies

Infectious Agent	**Lymphoid Malignancy**
Epstein-Barr virus	Burkitt's lymphoma
	Post–organ transplant lymphoma
	Primary CNS diffuse large B cell lymphoma
	Hodgkin's disease
	Extranodal NK/T cell lymphoma, nasal type
HTLV-I	Adult T cell leukemia/lymphoma
HIV	Diffuse large B cell lymphoma
	Burkitt's lymphoma
Hepatitis C virus	Lymphoplasmacytic lymphoma
Helicobacter pylori	Gastric MALT lymphoma
Human herpesvirus 8	Primary effusion lymphoma
	Multicentric Castleman's disease

Note: CNS, central nervous system; HTLV, human T cell lymphotropic virus; MALT, mucosa-associated lymphoid tissue; NK, natural killer.

EBV is associated with the development of Burkitt's lymphoma in Central Africa and the occurrence of aggressive non-Hodgkin's lymphomas in immunosuppressed patients in western countries. The majority of primary central nervous system (CNS) lymphomas are associated with EBV. EBV infection is strongly associated with the occurrence of extranodal nasal T/NK cell lymphomas in Asia and South America. Infection with HIV predisposes to the development of aggressive, B cell non-Hodgkin's lymphoma. This may be through overexpression of interleukin 6 by infected macrophages. Infection of the stomach by the bacterium *Helicobacter pylori* induces the development of gastric MALT (mucosa-associated lymphoid tissue) lymphomas. This association is supported by evidence that patients treated with antibiotics to eradicate *H. pylori* have regression of their MALT lymphoma. The bacterium does not transform lymphocytes to produce the lymphoma; instead, a vigorous immune response is made to the bacterium, and the chronic antigenic stimulation leads to the neoplasia. MALT lymphomas of the skin may be related to *Borrelia* sp. infections, those of the eyes to *Chlamydophila psittaci*, and those of the small intestine to *Campylobacter jejuni*.

Chronic hepatitis C virus infection has been associated with the development of lymphoplasmacytic lymphoma. Human herpesvirus 8 is associated with primary effusion lymphoma in HIV-infected persons and multicentric Castleman's disease, a diffuse lymphadenopathy associated with systemic symptoms of fever, malaise, and weight loss.

In addition to infectious agents, a number of other diseases or exposures may predispose to developing lymphoma (Table 105-5).

Table 105-5 Diseases or Exposures Associated with Increased Risk of Development of Malignant Lymphoma

Inherited immunodeficiency disease
Klinefelter's syndrome

- Chédiak-Higashi syndrome
- Ataxia telangiectasia syndrome
- Wiscott-Aldrich syndrome
- Common variable immunodeficiency disease

Acquired immunodeficiency diseases

- Iatrogenic immunosuppression
- HIV-1 infection
- Acquired hypogammaglobulinemia

Autoimmune disease

- Sjögren's syndrome
- Celiac sprue
- Rheumatoid arthritis and systemic lupus erythematosus

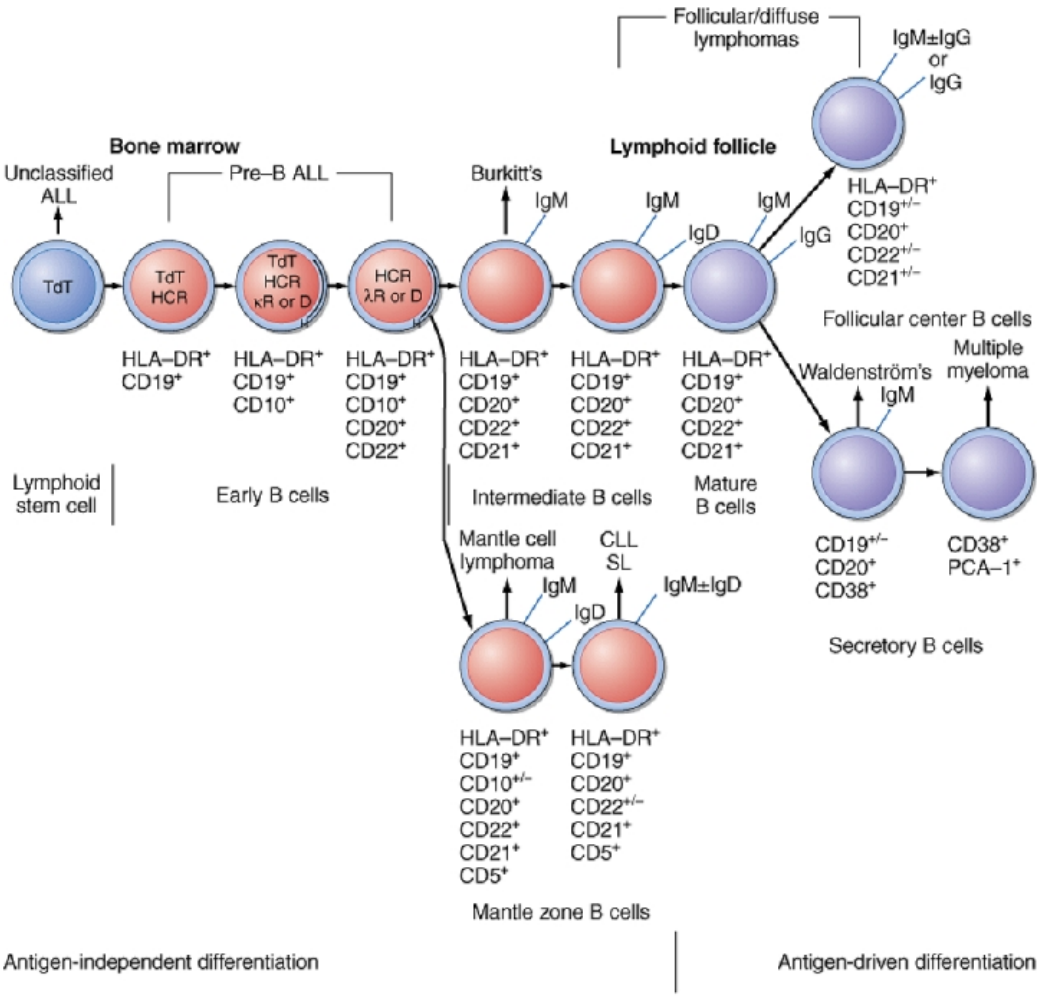
Chemical or drug exposures

- Phenytoin
- Dioxin, phenoxyherbicides
- Radiation
- Prior chemotherapy and radiation therapy

Immunology

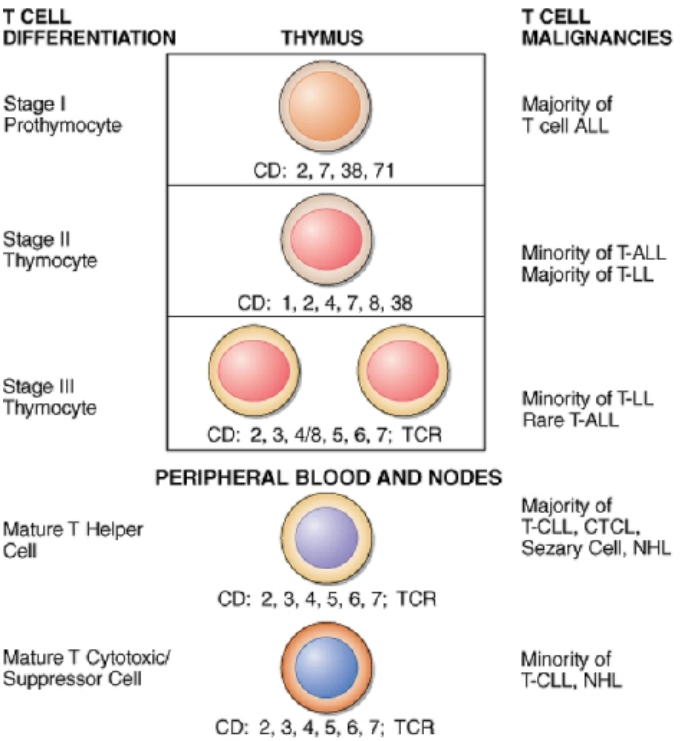
All lymphoid cells are derived from a common hematopoietic progenitor that gives rise to lymphoid, myeloid, erythroid, monocyte, and megakaryocyte lineages. Through the ordered and sequential activation of a series of transcription factors, the cell first becomes committed to the lymphoid lineage and then gives rise to B and T cells. About 75% of all lymphoid leukemias and 90% of all lymphomas are of B cell origin. A cell becomes committed to B cell development when it begins to rearrange its immunoglobulin genes. The sequence of cellular changes, including changes in cell-surface phenotype, that characterizes normal B cell development is shown in Fig. 105-2. A cell becomes committed to T cell differentiation upon migration to the thymus and rearrangement of T cell antigen receptor genes. The sequence of the events that characterize T cell development is depicted in Fig. 105-3.

Figure 105-2



Pathway of normal B cell differentiation and relationship to B cell lymphomas. HLA-DR, CD10, CD19, CD20, CD21, CD22, CD5, and CD38 are cell markers used to distinguish stages of development. Terminal transferase (TdT) is a cellular enzyme. Immunoglobulin heavy chain gene rearrangement (HCR) and light chain gene rearrangement or deletion (κR or D, λR or D) occur early in B cell development. The approximate normal stage of differentiation associated with particular lymphomas is shown. ALL, acute lymphoid leukemia; CLL, chronic lymphoid leukemia; SL, small lymphocytic lymphoma.

Figure 105-3



Pathway of normal T cell differentiation and relationship to T cell lymphomas. CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD38, and CD71 are cell markers used to distinguish stages of development. T cell antigen receptors (TCR) rearrange in the thymus, and mature T cells emigrate to nodes and peripheral blood. ALL, acute lymphoid leukemia; T-ALL, T cell ALL; T-LL, T cell lymphoblastic lymphoma; T-CLL, T cell chronic lymphoid leukemia; CTCL, cutaneous T cell lymphoma; NHL, non-Hodgkin's lymphoma.

Although lymphoid malignancies often retain the cell-surface phenotype of lymphoid cells at particular stages of differentiation, this information is of little consequence. The so-called stage of differentiation of a malignant lymphoma does not predict its natural history. For example, the clinically most aggressive lymphoid leukemia is Burkitt's leukemia, which has the phenotype of a mature follicle center IgM-bearing B cell. Leukemias bearing the immunologic cell-surface phenotype of more primitive cells (e.g., pre-B ALL, CD10+) are less aggressive and more amenable to curative therapy than the "more mature" appearing Burkitt's leukemia cells. Furthermore, the apparent stage of differentiation of the malignant cell does not reflect the stage at which the genetic lesions that gave rise to the malignancy developed. For example, follicular lymphoma has the cell-surface phenotype of a follicle center cell, but its characteristic chromosomal translocation, the t(14;18), which involves juxtaposition of the antiapoptotic *bcl-2* gene next to the immunoglobulin heavy chain gene (see below), had to develop early in ontogeny as an error in the process of immunoglobulin gene rearrangement. Why the subsequent steps that led to transformation became manifest in a cell of follicle center differentiation is not clear.

The major value of cell-surface phenotyping is to aid in the differential diagnosis of lymphoid tumors that appear similar by light microscopy. For example, benign follicular hyperplasia may resemble follicular lymphoma; however, the demonstration that all the cells bear the same immunoglobulin light chain isotype strongly suggests the mass is a clonal proliferation rather than a polyclonal response to an exogenous stimulus.

Malignancies of lymphoid cells are associated with recurring genetic abnormalities. While specific genetic abnormalities have not been identified for all subtypes of lymphoid malignancies, it is presumed that they exist. Genetic abnormalities can be identified at a variety of levels including gross chromosomal changes (i.e., translocations, additions, or deletions); rearrangement of specific genes that may or may not be apparent from cytogenetic studies; and overexpression, underexpression, or mutation of specific oncogenes. Altered expression or mutation of specific proteins is particularly important. Many lymphomas contain balanced chromosomal translocations involving the antigen receptor genes; immunoglobulin genes on chromosomes 2, 14, and 22 in B cells; and T cell antigen receptor genes on chromosomes 7 and 14 in T cells. The rearrangement of chromosome segments to generate mature antigen receptors must create a site of vulnerability to aberrant recombination. B cells are even more susceptible to acquiring mutations during their maturation in germinal centers; the generation of antibody of higher affinity requires the introduction of mutations into the variable region genes in the germinal centers. Other nonimmunoglobulin genes, e.g., *bcl-6*, may acquire mutations as well.

In the case of diffuse large B cell lymphoma, the translocation t(14;18) occurs in ~30% of patients and leads to overexpression of the *bcl-2* gene found on chromosome 18. Some other patients without the translocation also overexpress the BCL-2 protein. This protein is involved in suppressing apoptosis– i.e., the mechanism of cell death most often induced by cytotoxic chemotherapeutic agents. A higher relapse rate has been observed in patients whose tumors overexpress the BCL-2 protein, but not in those patients whose lymphoma cells show only the

translocation. Thus, particular genetic mechanisms have clinical ramifications.

Table 105-6 presents the best documented translocations and associated oncogenes for various subtypes of lymphoid malignancies. In some cases, such as the association of the t(14;18) in follicular lymphoma, the t(2;5) in anaplastic large T/null cell lymphoma, the t(8;14) in Burkitt's lymphoma, and the t(11;14) in mantle cell lymphoma, the great majority of tumors in patients with these diagnoses display these abnormalities. In other types of lymphoma where a minority of the patients have tumors expressing specific genetic abnormalities, the defects may have prognostic significance. No specific genetic abnormalities have been identified in Hodgkin's disease other than aneuploidy.

Table 105-6 Cytogenetic Translocation and Associated Oncogenes Often Seen in Lymphoid Malignancies

Disease	Cytogenetic Abnormality	Oncogene
CLL/small lymphocytic lymphoma	t(14;15)(q32;q13)	-
MALT lymphoma	t(11;18)(q21;q21)	API2/MALT, BCL-10
Precursor B cell acute lymphoid leukemia	t(9;22)(q34;q11) or variant	BCR/ABL
	t(4;11)(q21;q23)	AF4, ALLI
Precursor acute lymphoid leukemia	t(9;22)	BCR, ABL
	t(1;19)	E2A, PBX
	t(17;19)	HLF, E2A
	t(5;14)	HOX11L2, CTIP2
Mantle cell lymphoma	t(11;14)(q13;q32)	BCL-1, IgH
Follicular lymphoma	t(14;18)(q32;q21)	BCL-2, IgH
Diffuse large cell lymphoma	t(3;-)(q27;-)[a]	BCL-6
	t(17;-)(p13;-)	p53
Burkitt's lymphoma, Burkitt's leukemia	t(8;-)(q24;-)[a]	C-MYC
CD30+ Anaplastic large cell lymphoma	t(2;5)(p23;q35)	ALK
Lymphoplasmacytoid lymphoma	t(9;14)(p13;q32)	PAX5, IgH

[a]Numerous sites of translocation may be involved with these genes.

Note: CLL, chronic lymphoid leukemia; MALT, mucosa-associated lymphoid tissue; IgH, immunoglobulin heavy chain.

In typical B cell CLL, trisomy 12 conveys a poorer prognosis. In ALL in both adults and children, genetic abnormalities have important prognostic significance. Patients whose tumor cells display the t(9;22) have a much poorer outlook than patients who do not have this translocation. Other genetic abnormalities that occur frequently in adults with ALL include the t(4;11) and the t(8;14). The t(4;11) is associated with younger age, female predominance, high white cell counts, and L1 morphology. The t(8;14) is associated with older age, male predominance, frequent CNS involvement, and L3 morphology. Both are associated with a poor prognosis. In childhood ALL, hyperdiploidy has been shown to have a favorable prognosis.

Gene profiling using array technology allows the simultaneous assessment of the expression of thousands of genes. This technology provides the possibility to identify new genes with pathologic importance in lymphomas, the identification of patterns of gene expression with diagnostic and/or prognostic significance, and the identification of new therapeutic targets. Recognition of patterns of gene expression is complicated and requires sophisticated mathematical techniques. Early successes using this technology in lymphoma include the identification of previously unrecognized subtypes of diffuse large B cell lymphoma whose gene expression patterns resemble either those of follicular center B cells or activated peripheral blood B cells. Patients whose lymphomas have a germinal center B cell pattern of gene expression have a considerably better prognosis than those whose lymphomas have a pattern resembling activated peripheral blood B cells. This improved prognosis is independent of other known prognostic factors. Similar information is being generated in follicular lymphoma and mantle cell lymphoma. The challenge remains to provide information from such techniques in a clinically useful time frame.

Approach to the Patient: Lymphoid Cell Malignancies

Regardless of the type of lymphoid malignancy, the initial evaluation of the patient should include performance of a careful history and physical examination. These will help confirm the diagnosis, identify those manifestations of the disease that might require prompt attention, and aid in the selection of further studies to optimally characterize the patient's status to allow the best choice of therapy. It is difficult to overemphasize the importance of a carefully done history and physical examination. They might provide observations that lead to reconsidering the diagnosis, provide hints at etiology, clarify the stage, and allow the physician to establish rapport with the patient that will make it possible to develop and carry out a therapeutic plan.

For patients with ALL, evaluation is usually completed after a complete blood count, chemistry studies reflecting major organ function, a bone marrow biopsy with genetic and immunologic studies, and a lumbar puncture. The latter is necessary to rule out occult CNS involvement. At this point, most patients would be ready to begin therapy. In ALL, prognosis is dependent upon the genetic characteristics of the tumor, the patient's age, the white cell count, and the patient's overall clinical status and major organ function.

In CLL, the patient evaluation should include a complete blood count, chemistry tests to measure major organ function, serum protein electrophoresis, and a bone marrow biopsy. However, some physicians believe that the diagnosis would not always require a bone marrow biopsy. Patients often have imaging studies of the chest and abdomen looking for pathologic lymphadenopathy. Patients with typical B cell CLL can be subdivided into three major prognostic groups. Those patients with only blood and bone marrow involvement by leukemia but no lymphadenopathy, organomegaly, or signs of bone marrow failure have the best prognosis. Those with lymphadenopathy and organomegaly have an intermediate prognosis, and patients with bone marrow failure, defined as hemoglobin <100 g/L (10 g/dL) or platelet count <100,000/μL, have the worst prognosis. The pathogenesis of the anemia or thrombocytopenia is important to discern. The prognosis is adversely affected when either or both of these abnormalities are due to progressive marrow infiltration and loss of productive marrow. However, either or both may be due to autoimmune phenomena or to hypersplenism that can develop during the course of the disease. These destructive mechanisms are usually completely reversible (glucocorticoids for autoimmune disease; splenectomy for hypersplenism) and do not influence disease prognosis.

Two popular staging systems have been developed to reflect these prognostic groupings (Table 105-7). Patients with typical B cell CLL can have their course complicated by immunologic abnormalities including autoimmune hemolytic anemia, autoimmune thrombocytopenia, and hypogammaglobulinemia. Patients with hypogammaglobulinemia benefit from regular (monthly) γ globulin administration. Because of expense, γ globulin is often withheld until the patient experiences a significant infection. These abnormalities do not have a clear prognostic significance and should not be used to assign a higher stage.

Table 105-7 Staging of Typical B Cell Lymphoid Leukemia

Stage	Clinical Features	Median Survival, Years
RAI System		
0: Low risk	Lymphocytosis only in blood and marrow	>10
I: Intermediate risk	Lymphocytosis + lymphadenopathy + splenomegaly ± hepatomegaly	7
II		
III: High risk	Lymphocytosis + anemia	1.5
IV	Lymphocytosis + thrombocytopenia	
Binet System		
A	Fewer than three areas of clinical lymphadenopathy; no anemia or thrombocytopenia	>10
B	Three or more involved node areas; no anemia or thrombocytopenia	7
C	Hemoglobin ≤10 g/dL and/or platelets <100,000/μL	2

Two other features may be used to assess prognosis in B cell CLL, but neither has yet been incorporated into a staging classification. At least two subsets of CLL have been identified based on the cytoplasmic expression of ZAP-70; expression of this protein, which is usually expressed in T cells, identifies a subgroup with poorer prognosis. A less powerful subsetting tool is CD38 expression. CD38+ tumors tend to have a poorer prognosis than CD38– tumors.

The initial evaluation of a patient with Hodgkin's disease or non-Hodgkin's lymphoma is similar. In both situations, the determination of an accurate anatomic stage is an important part of the evaluation. The staging system is the Ann Arbor staging system originally developed for Hodgkin's disease (Table 105-8).

Table 105-8 The Ann Arbor Staging System for Hodgkin's Disease

Stage	Definition
I	Involvement of a single lymph node region or lymphoid structure (e.g., spleen, thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes should be considered "lateralized" and, when involved on both sides, constitute stage II disease)
III	Involvement of lymph node regions or lymphoid structures on both sides of the diaphragm
III_1	Subdiaphragmatic involvement limited to spleen, splenic hilar nodes, celiac nodes, or portal nodes
III_2	Subdiaphragmatic involvement includes paraaortic, iliac, or mesenteric nodes plus structures in III_1
IV	Involvement of extranodal site(s) beyond that designated as "E"

More than one extranodal deposit at any location
Any involvement of liver or bone marrow

A No symptoms

B Unexplained weight loss of >10% of the body weight during the 6 months before staging investigation

Unexplained, persistent, or recurrent fever with temperatures >38°C during the previous month

Recurrent drenching night sweats during the previous month

E Localized, solitary involvement of extralymphatic tissue, excluding liver and bone marrow

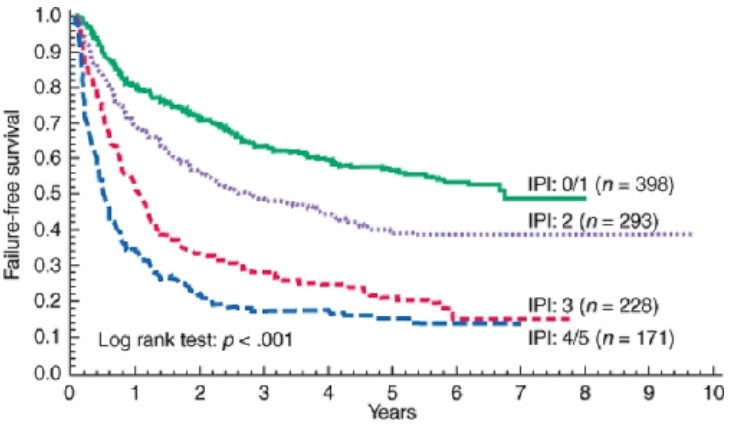
Evaluation of patients with Hodgkin's disease will typically include a complete blood count; erythrocyte sedimentation rate; chemistry studies reflecting major organ function; CT scans of the chest, abdomen, and pelvis; and a bone marrow biopsy. Neither a positron emission tomography (PET) scan nor a gallium scan is absolutely necessary for primary staging, but one performed at the completion of therapy allows evaluation of persisting radiographic abnormalities, particularly the mediastinum. Knowing that the PET scan or gallium scan is abnormal before treatment can help in this assessment. In most cases, these studies will allow assignment of anatomic stage and the development of a therapeutic plan.

In patients with non-Hodgkin's lymphoma, the same evaluation described for patients with Hodgkin's disease is usually carried out. In addition, serum levels of lactate dehydrogenase (LDH) and β_2-microglobulin and serum protein electrophoresis are often included in the evaluation. Anatomic stage is assigned in the same manner as used for Hodgkin's disease. However, the prognosis of patients with non-Hodgkin's lymphoma is best assigned using the International Prognostic Index (IPI) (Table 105-9). This is a powerful predictor of outcome in all subtypes of non-Hodgkin's lymphoma. Patients are assigned an IPI score based on the presence or absence of five adverse prognostic factors and may have none or all five of these adverse prognostic factors. Figure 105-4 shows the prognostic significance of this score in 1300 patients with all types of non-Hodgkin's lymphoma. With the addition of rituximab to CHOP, treatment outcomes have improved and the original IPI has lost some of its discrimination power. A revised IPI has been proposed that better predicts outcome of rituximab plus chemotherapy-based programs (Table 105-9). CT scans are routinely used in the evaluation of patients with all subtypes of non-Hodgkin's lymphoma, but PET and gallium scans are much more useful in aggressive subtypes such as diffuse large B cell lymphoma than in more indolent subtypes such a follicular lymphoma or small lymphocytic lymphoma. While the IPI does divide patients with follicular lymphoma into subsets with distinct prognoses, the distribution of such patients is skewed toward lower-risk categories. A follicular lymphoma–specific IPI (FLIPI) has been proposed that replaces performance status with hemoglobin level [<120 g/L (<12 g/dL)] and number of extranodal sites with number of nodal sites (more than four). Low risk (zero or one factor) was assigned to 36% of patients, intermediate risk (two factors) to 37%, and poor risk (more than two factors) to 27% of patients.

Table 105-9 International Prognostic Index for NHL

Five clinical risk factors:	
Age ≥ 60 years	
Serum lactate dehydrogenase levels elevated	
Performance status ≥2 (ECOG) or ≤ 70 (Karnofsky)	
Ann Arbor stage III or IV	
>1 site of extranodal involvement	
Patients are assigned a number for each risk factor they have	
Patients are grouped differently based upon the type of lymphoma	
For diffuse large B cell lymphoma:	
0, 1 factor = low risk:	35% of cases; 5-year survival, 73%
2 factors = low-intermediate risk:	27% of cases; 5-year survival, 51%
3 factors = high-intermediate risk:	22% of cases; 5-year survival, 43%
4, 5 factors = high risk:	16% of cases; 5-year survival, 26%
For diffuse large B cell lymphoma treated with R-CHOP:	
0 factor = very good:	10% of cases; 5-year survival, 94%
1, 2 factors = good:	45% of cases; 5-year survival, 79%
3, 4, 5 factors = poor:	45% of cases; 5-year survival, 55%

Figure 105-4



Relationship of International Prognostic Index (IPI) to survival. Kaplan-Meier survival curves for 1300 patients with various kinds of lymphoma stratified according to the IPI.

Clinical Features, Treatment, and Prognosis of Specific Lymphoid Malignancies

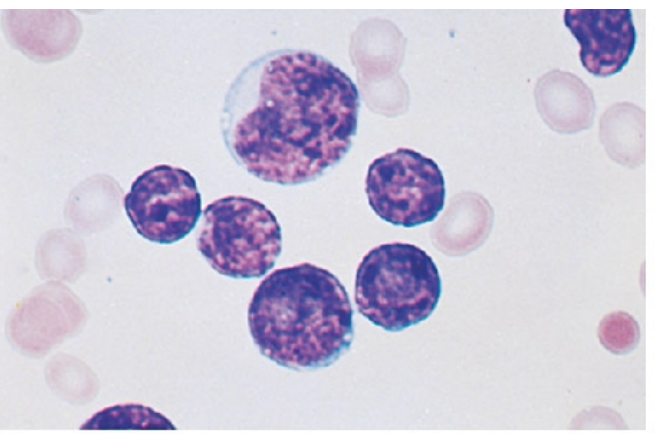
Precursor Cell B Cell Neoplasms

Precursor B Cell Lymphoblastic Leukemia/Lymphoma

The most common cancer in childhood is B cell ALL. Although this disorder can also present as a lymphoma in either adults or children, presentation as lymphoma is rare.

The malignant cells in patients with precursor B cell lymphoblastic leukemia are most commonly of pre-B cell origin. Patients typically present with signs of bone marrow failure such as pallor, fatigue, bleeding, fever, and infection related to peripheral blood cytopenias. Peripheral blood counts regularly show anemia and thrombocytopenia but might show leukopenia, a normal leukocyte count, or leukocytosis based largely on the number of circulating malignant cells (Fig. 105-5). Extramedullary sites of disease are frequently involved in patients who present with leukemia, including lymphadenopathy, hepato- or splenomegaly, CNS disease, testicular enlargement, and/or cutaneous infiltration.

Figure 105-5



Acute lymphoblastic leukemia. The cells are heterogeneous in size, have round or convoluted nuclei, high nuclear/cytoplasmic ratio, and absence of cytoplasmic granules.

The diagnosis is usually made by bone marrow biopsy, which shows infiltration by malignant lymphoblasts. Demonstration of a pre-B cell immunophenotype (Fig. 105-2) and, often, characteristic cytogenetic abnormalities (Table 105-6) confirm the diagnosis. An adverse prognosis in patients with precursor B cell ALL is predicted by a very high white cell count, the presence of symptomatic CNS disease, and

unfavorable cytogenetic abnormalities. For example, t(9;22), frequently found in adults with B cell ALL, has been associated with a very poor outlook. The bcr/abl kinase inhibitors have improved the prognosis.

Precursor B Cell Lymphoblastic Leukemia: Treatment

The treatment of patients with precursor B cell ALL involves remission induction with combination chemotherapy, a consolidation phase that includes administration of high-dose systemic therapy and treatment to eliminate disease in the CNS, and a period of continuing therapy to prevent relapse and effect cure. The overall cure rate in children is 90%, while ~50% of adults are long-term disease-free survivors. This reflects the high proportion of adverse cytogenetic abnormalities seen in adults with precursor B cell ALL.

Precursor B cell lymphoblastic lymphoma is a rare presentation of precursor B cell lymphoblastic malignancy. These patients often have a rapid transformation to leukemia and should be treated as though they had presented with leukemia. The few patients who present with the disease confined to lymph nodes have a high cure rate.

Mature (Peripheral) B Cell Neoplasms

B Cell Chronic Lymphoid Leukemia/Small Lymphocytic Lymphoma

B cell CLL/small lymphocytic lymphoma represents the most common lymphoid leukemia, and when presenting as a lymphoma, it accounts for ~7% of non-Hodgkin's lymphomas. Presentation can be as either leukemia or lymphoma. The major clinical characteristics of B cell CLL/small lymphocytic lymphoma are presented in Table 105-10.

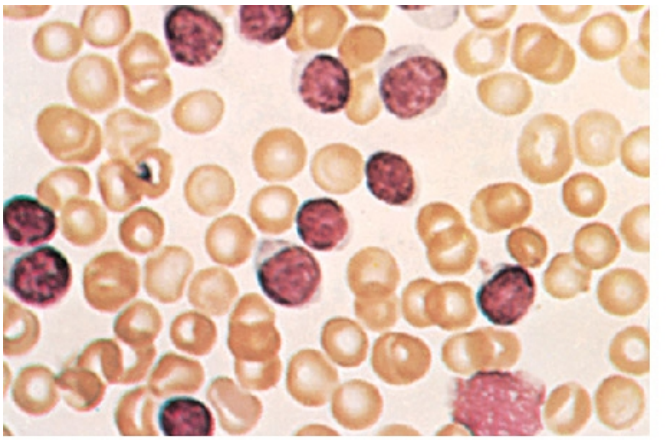
Table 105-10 Clinical Characteristics of Patients with Common Types of Non-Hodgkin's Lymphomas (NHL)

Disease	Median Age, years	Frequency in Children	% Male	Stage I/II vs III/IV, %	B Symptoms, %	Bone Marrow Involvement, %	Gastrointestinal Tract Involvement, %	% Surviving 5 years
B cell chronic lymphocytic leukemia/small lymphocytic lymphoma	65	Rare	53	9 vs 91	33	72	3	51
Mantle cell lymphoma	63	Rare	74	20 vs 80	28	64	9	27
Extranodal marginal zone B cell lymphoma of MALT type	60	Rare	48	67 vs 33	19	14	50	74
Follicular lymphoma	59	Rare	42	33 vs 67	28	42	4	72
Diffuse large B cell lymphoma	64	~25% of childhood NHL	55	54 vs 46	33	16	18	46
Burkitt's lymphoma	31	~30% of childhood NHL	89	62 vs 38	22	33	11	45
Precursor T cell lymphoblastic lymphoma	28	~40% of childhood NHL	64	11 vs 89	21	50	4	26
Anaplastic large T/null cell lymphoma	34	Common	69	51 vs 49	53	13	9	77
Peripheral T cell non-Hodgkin's lymphoma	61	~5% of childhood NHL	55	20 vs 80	50	36	15	25

Note: MALT, mucosa-associated lymphoid tissue.

The diagnosis of typical B cell CLL is made when an increased number of circulating lymphocytes (i.e., >4 x 10^9/L and usually >10 x 10^9/L) is found (Fig. 105-6) that are monoclonal B cells expressing the CD5 antigen. Finding bone marrow infiltration by the same cells confirms the diagnosis. The peripheral blood smear in such patients typically shows many "smudge" or "basket" cells, nuclear remnants of cells damaged by the physical shear stress of making the blood smear. If cytogenetic studies are performed, trisomy 12 is found in 25–30% of patients. Abnormalities in chromosome 13 are also seen.

Figure 105-6



Chronic lymphocytic leukemia. The peripheral white blood cell count is high due to increased numbers of small, well-differentiated, normal-appearing lymphocytes. The leukemia lymphocytes are fragile, and substantial numbers of broken, smudged cells are usually also present on the blood smear.

If the primary presentation is lymphadenopathy and a lymph node biopsy is performed, pathologists usually have little difficulty in making the diagnosis of small lymphocytic lymphoma based on morphologic findings and immunophenotype. However, even in these patients, 70–75% will be found to have bone marrow involvement and circulating monoclonal B lymphocytes are often present.

The differential diagnosis of typical B cell CLL is extensive (Table 105-1). Immunophenotyping will eliminate the T cell disorders and can often help sort out other B cell malignancies. For example, only mantle cell lymphoma and typical B cell CLL are usually CD5 positive. Typical B cell small lymphocytic lymphoma can be confused with other B cell disorders including lymphoplasmacytic lymphoma (i.e., the tissue manifestation of Waldenström's macroglobulinemia), nodal marginal zone B cell lymphoma, and mantle cell lymphoma. In addition, some small lymphocytic lymphomas have areas of large cells that can lead to confusion with diffuse large B cell lymphoma. An expert hematopathologist is vital for making this distinction.

Typical B cell CLL is often found incidentally when a complete blood count is done for another reason. However, complaints that might lead to the diagnosis include fatigue, frequent infections, and new lymphadenopathy. The diagnosis of typical B cell CLL should be considered in a patient presenting with an autoimmune hemolytic anemia or autoimmune thrombocytopenia. B cell CLL has also been associated with red cell aplasia. When this disorder presents as lymphoma, the most common abnormality is asymptomatic lymphadenopathy, with or without splenomegaly. The staging systems predict prognosis in patients with typical B cell CLL (Table 105-7). The evaluation of a new patient with typical B cell CLL/small lymphocytic lymphoma will include many of the studies (Table 105-11) that are used in patients with other non-Hodgkin's lymphomas. In addition, particular attention needs to be given to detecting immune abnormalities such as autoimmune hemolytic anemia, autoimmune thrombocytopenia, hypogammaglobulinemia, and red cell aplasia. Molecular analysis of immunoglobulin gene sequences in CLL has demonstrated that about half the patients have tumors expressing mutated immunoglobulin genes and half have tumors expressing unmutated or germ-line immunoglobulin sequences. Patients with unmutated immunoglobulins tend to have a more aggressive clinical course and are less responsive to therapy. Unfortunately, immunoglobulin gene sequencing is not routinely available. CD38 expression is said to be low in the better-prognosis patients expressing mutated immunoglobulin and high in poorer-prognosis patients expressing unmutated immunoglobulin, but this test has not been confirmed as a reliable means of distinguishing the two groups. ZAP-70 expression correlates with the presence of unmutated immunoglobulin genes, but the assay is not yet standardized and widely available.

Table 105-11 Staging Evaluation for Non-Hodgkin's Lymphoma
Physical examination
Documentation of B symptoms
Laboratory evaluation
Complete blood counts
Liver function tests
Uric acid
Calcium
Serum protein electrophoresis
Serum β_2-microglobulin

Chest radiograph

CT scan of abdomen, pelvis, and usually chest

Bone marrow biopsy

Lumbar puncture in lymphoblastic, Burkitt's, and diffuse large B cell lymphoma with positive marrow biopsy

Gallium scan (SPECT) or PET scan in large cell lymphoma

Note: SPECT, single photon emission CT; PET, positron emission tomography.

B Cell Chronic Lymphoid Leukemia/Small Lymphocytic Lymphoma: Treatment

Patients whose presentation is typical B cell CLL with no manifestations of the disease other than bone marrow involvement and lymphocytosis (i.e., Rai stage O and Binet stage A; Table 105-7) can be followed without specific therapy for their malignancy. These patients have a median survival >10 years, and some will never require therapy for this disorder. If the patient has an adequate number of circulating normal blood cells and is asymptomatic, many physicians would not initiate therapy for patients in the intermediate stage of the disease manifested by lymphadenopathy and/or hepatosplenomegaly. However, the median survival for these patients is ~7 years, and most will require treatment in the first few years of follow-up. Patients who present with bone marrow failure (i.e., Rai stage III or IV or Binet stage C) will require initial therapy in almost all cases. These patients have a serious disorder with a median survival of only 1.5 years. It must be remembered that immune manifestations of typical B cell CLL should be managed independently of specific antileukemia therapy. For example, glucocorticoid therapy for autoimmune cytopenias and γ globulin replacement for patients with hypogammaglobulinemia should be used whether or not antileukemia therapy is given.

Patients who present primarily with lymphoma and have a low IPI score have a 5-year survival of ~75%, but those with a high IPI score have a 5-year survival of <40% and are more likely to require early therapy.

The most common treatments for patients with typical B cell CLL/small lymphocytic lymphoma have been chlorambucil or fludarabine, alone or in combination. Chlorambucil can be administered orally with few immediate side effects, while fludarabine is administered IV and is associated with significant immune suppression. However, fludarabine is by far the more active agent and is the only drug associated with a significant incidence of complete remission. The combination of rituximab (375–500 mg/m^2 day 1), fludarabine (25 mg/m^2 days 2–4 on cycle 1 and 1–3 in subsequent cycles), and cyclophosphamide (250 mg/m^2 with fludarabine) achieves complete responses in 69% of patients, and those responses are associated with molecular remissions in half of the cases. Half the patients experience grade III or IV neutropenia. For young patients presenting with leukemia requiring therapy, regimens containing fludarabine are the treatment of choice. Because fludarabine is an effective second-line agent in patients with tumors unresponsive to chlorambucil, the latter agent is often chosen in elderly patients who require therapy. Many patients who present with lymphoma will receive a combination chemotherapy regimen used in other lymphomas such as CVP (cyclophosphamide, vincristine, and prednisone) or CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), although fludarabine-containing regimens may be preferable. Alemtuzimab (anti-CD52) is an antibody with activity in the disease, but it kills both B and T cells and is associated with more immune compromise than rituximab. Young patients with this disease can be candidates for bone marrow transplantation. Allogeneic bone marrow transplantation can be curative but is associated with a significant treatment-related mortality. Mini-transplants using immunosuppressive rather than myeloablative doses of preparative drugs are being studied (Chap. 108). The use of autologous transplantation in patients with this disorder has been discouraging.

Extranodal Marginal Zone B Cell Lymphoma of Malt Type

Extranodal marginal zone B cell lymphoma of MALT type (MALT lymphoma) makes up ~8% of non-Hodgkin's lymphomas. This small cell lymphoma presents in extranodal sites. It was previously considered a small lymphocytic lymphoma or sometimes a pseudolymphoma. The recognition that the gastric presentation of this lymphoma was associated with *H. pylori* infection was an important step in recognizing it as a separate entity. The clinical characteristics of MALT lymphoma are presented in Table 105-10.

The diagnosis of MALT lymphoma can be made accurately by an expert hematopathologist based on a characteristic pattern of infiltration of small lymphocytes that are monoclonal B cells and CD5 negative. In some cases, transformation to diffuse large B cell lymphoma occurs, and both diagnoses may be made in the same biopsy. The differential diagnosis includes benign lymphocytic infiltration of extranodal organs and other small cell B cell lymphomas.

MALT lymphoma may occur in the stomach, orbit, intestine, lung, thyroid, salivary gland, skin, soft tissues, bladder, kidney, and CNS. It may present as a new mass, be found on routine imaging studies, or be associated with local symptoms such as upper abdominal discomfort in gastric lymphoma. Most MALT lymphomas are gastric in origin. At least two genetic forms of gastric MALT exist: one (accounting for ~50% of cases) characterized by t(11;18)(q21;q21) that juxtaposes the amino terminal of the *API2* gene with the carboxy terminal of the *MALT1* gene creating an API2/MALT1 fusion product, and the other characterized by multiple sites of genetic instability including trisomies of chromosomes 3, 7, 12, and 18. About 95% of gastric MALT lymphomas are associated with *H. pylori* infection, and those that are do not usually express t(11;18). The t(11;18) usually results in activation of NF-κB, which acts a survival factor for the cells. Lymphomas with t(11;18) translocations are genetically stable and do not evolve to diffuse large B cell lymphoma. By contrast, t(11;18)-negative MALT lymphomas often acquire *BCL6* mutations and progress to aggressive histology lymphoma. MALT lymphomas are localized to the organ of origin in ~40% of cases and to the organ and regional lymph nodes in ~30% of patients. However, distant metastasis can occur– particularly with transformation to diffuse large B cell lymphoma. Many patients who develop this lymphoma will have an autoimmune or inflammatory process such as Sjögren's syndrome (salivary gland MALT), Hashimoto's thyroiditis (thyroid MALT), *Helicobacter* gastritis (gastric MALT), *C. psittaci* conjunctivitis (ocular MALT), or *Borelia* skin infections (cutaneous MALT).

Evaluation of patients with MALT lymphoma follows the pattern (Table 105-11) for staging a patient with non-Hodgkin's lymphoma. In

particular, patients with gastric lymphoma need to have studies performed to document the presence or absence of *H. pylori* infection. Endoscopic studies including ultrasound can help define the extent of gastric involvement. Most patients with MALT lymphoma have a good prognosis, with a 5-year survival of ~75%. In patients with a low IPI score, the 5-year survival is ~90%, while it drops to ~40% in patients with a high IPI score.

Mucosa-Associated Lymphoid Tissue Lymphoma: Treatment

MALT lymphoma is often localized. Local therapy such as radiation or surgery can effect cure, and this is one of the few times where surgery might be a reasonable primary therapy for a patient with non-Hodgkin's lymphoma. Patients with gastric MALT lymphomas who are infected with *H. pylori* can achieve remission in the majority of cases with eradication of the infection. These remissions can be durable, but molecular evidence of persisting neoplasia is frequent and the long-term outcome is uncertain. Patients who present with more extensive disease are most often treated with single-agent chemotherapy such as chlorambucil. Data on combination regimens that include rituximab are being generated, but its efficacy in other B cell tumors and low toxicity support its use. Coexistent diffuse large B cell lymphoma must be treated with combination chemotherapy (see below). The additional acquired mutations that mediate the histologic progression also convey *Helicobacter* independence to the growth.

Mantle Cell Lymphoma

Mantle cell lymphoma makes up ~6% of all non-Hodgkin's lymphomas. This lymphoma was previously placed in a number of other subtypes. Its existence was confirmed by the recognition that these lymphomas have a characteristic chromosomal translocation, t(11;14), between the immunoglobulin heavy chain gene on chromosome 14 and the *bcl-1* gene on chromosome 11, and regularly overexpress the BCL-1 protein, also known as cyclin D1. Table 105-10 shows the clinical characteristics of mantle cell lymphoma.

The diagnosis of mantle cell lymphoma can be made accurately by an expert hematopathologist. As with all subtypes of lymphoma, an adequate biopsy is important. The differential diagnosis of mantle cell lymphoma includes other small cell B cell lymphomas. In particular, mantle cell lymphoma and small lymphocytic lymphoma share a characteristic expression of CD5. Mantle cell lymphoma usually has a slightly indented nucleus.

The most common presentation of mantle cell lymphoma is with palpable lymphadenopathy, frequently accompanied by systemic symptoms. Approximately 70% of patients will be stage IV at the time of diagnosis, with frequent bone marrow and peripheral blood involvement. Of the extranodal organs that can be involved, gastrointestinal involvement is particularly important to recognize. Patients who present with lymphomatosis polyposis in the large intestine usually have mantle cell lymphoma. Table 105-11 outlines the evaluation of patients with mantle cell lymphoma. Patients who present with gastrointestinal tract involvement often have Waldeyer's ring involvement, and vice versa. The 5-year survival for all patients with mantle cell lymphoma is ~25%, with only occasional patients who present with a high IPI score surviving 5 years and ~50% of patients with a low IPI score surviving 5 years.

Mantle Cell Lymphoma: Treatment

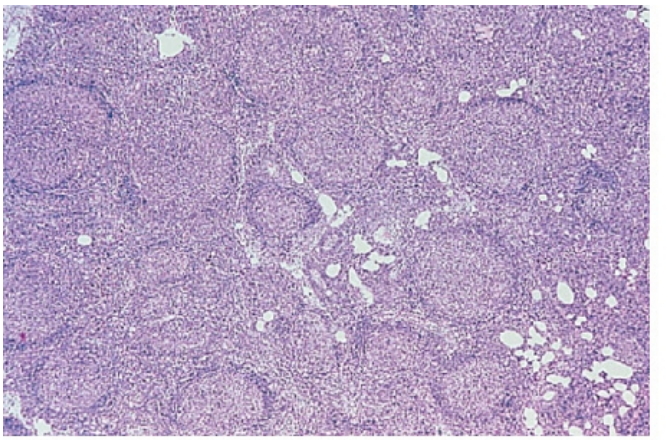
Current therapies for mantle cell lymphoma are unsatisfactory. Patients with localized disease might be treated with combination chemotherapy followed by radiotherapy; however, these patients are exceedingly rare. For the usual presentation with disseminated disease, treatments have been unsatisfactory, with the minority of patients achieving complete remission. Aggressive combination chemotherapy regimens followed by autologous or allogeneic bone marrow transplantation are frequently offered to younger patients. For the occasional elderly, asymptomatic patient, observation followed by single-agent chemotherapy might be the most practical approach. An intensive combination chemotherapy regimen originally used in the treatment of acute leukemia, HyperC-VAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, and methotrexate), in combination with rituximab seems to be associated with better response rates- particularly in younger patients. CHOP plus rituximab has shown better response rates than CHOP alone, but long-term follow-up is lacking. Bortezomib induces transient partial responses in a minority of patients.

Follicular Lymphoma

Follicular lymphomas make up 22% of non-Hodgkin's lymphomas worldwide and at least 30% of non-Hodgkin's lymphomas diagnosed in the United States. This type of lymphoma can be diagnosed accurately on morphologic findings alone and has been the diagnosis in the majority of patients in therapeutic trials for "low-grade" lymphoma in the past. The clinical characteristics of follicular lymphoma are presented in Table 105-10.

Evaluation of an adequate biopsy by an expert hematopathologist is sufficient to make a diagnosis of follicular lymphoma. The tumor is composed of small cleaved and large cells in varying proportions organized in a follicular pattern of growth (Fig. 105-7). Confirmation of B cell immunophenotype and the existence of the t(14;18) and abnormal expression of BCL-2 protein are confirmatory. The major differential diagnosis is between lymphoma and reactive follicular hyperplasia. The coexistence of diffuse large B cell lymphoma must be considered. Patients with follicular lymphoma are often subclassified into those with predominantly small cells, those with a mixture of small and large cells, and those with predominantly large cells. While this distinction cannot be made simply or very accurately, these subdivisions do have prognostic significance. Patients with follicular lymphoma with predominantly large cells have a higher proliferative fraction, progress more rapidly, and have a shorter overall survival with simple chemotherapy regimens.

Figure 105-7



Follicular lymphoma. The normal nodal architecture is effaced by nodular expansions of tumor cells. Nodules vary in size and contain predominantly small lymphocytes with cleaved nuclei along with variable numbers of larger cells with vesicular chromatin and prominent nucleoli.

The most common presentation for follicular lymphoma is with new, painless lymphadenopathy. Multiple sites of lymphoid involvement are typical, and unusual sites such as epitrochlear nodes are sometimes seen. However, essentially any organ can be involved, and extranodal presentations do occur. Most patients do not have fevers, sweats, or weight loss, and an IPI score of 0 or 1 is found in ~50% of patients. Fewer than 10% of patients have a high (i.e., 4 or 5) IPI score. The staging evaluation for patients with follicular lymphoma should include the studies included in Table 105-11.

Follicular Lymphoma: Treatment

Follicular lymphoma is one of the malignancies most responsive to chemotherapy and radiotherapy. In addition, tumors in as many as 25% of the patients undergo spontaneous regression– usually transient– without therapy. In an asymptomatic patient, no initial treatment and watchful waiting can be an appropriate management strategy and is particularly likely to be adopted for older patients with advanced stage disease. For patients who do require treatment, single-agent chlorambucil or cyclophosphamide or combination chemotherapy with CVP or CHOP are most frequently used. With adequate treatment, 50–75% of patients will achieve a complete remission. While most patients relapse (median response duration is ~2 years), at least 20% of complete responders will remain in remission for >10 years. For the rare patient (15%) with localized follicular lymphoma, involved field radiotherapy produces long-term disease-free survival in the majority.

A number of therapies have been shown to be active in the treatment of patients with follicular lymphoma. These include cytotoxic agents such as fludarabine, and biologic agents such as interferon α, monoclonal antibodies with or without radionuclides, and lymphoma vaccines. In patients treated with a doxorubicin-containing combination chemotherapy regimen, interferon α given to patients in complete remission seems to prolong survival. The monoclonal antibody rituximab can cause objective responses in 35–50% of patients with relapsed follicular lymphoma, and radiolabeled antibodies appear to have response rates well in excess of 50%. The addition of rituximab to CHOP and other effective combination chemotherapy programs is beginning to show prolonged overall survival and a decreased risk of histologic progression. Trials with tumor vaccines have been encouraging. Both autologous and allogeneic hematopoietic stem cell transplantation yield high complete response rates in patients with relapsed follicular lymphoma, and long-term remissions can occur.

Patients with follicular lymphoma with a predominance of large cells have a shorter survival when treated with single-agent chemotherapy but seem to benefit from receiving an anthracycline-containing combination chemotherapy regimen plus rituximab. When their disease is treated aggressively, the overall survival for such patients is no lower than for patients with other follicular lymphomas, and the failure-free survival is superior.

Patients with follicular lymphoma have a high rate of histologic transformation to diffuse large B cell lymphoma (5–7% per year). This is recognized ~40% of the time during the course of the illness by repeat biopsy and is present in almost all patients at autopsy. This transformation is usually heralded by rapid growth of lymph nodes– often localized– and the development of systemic symptoms such as fevers, sweats, and weight loss. Although these patients have a poor prognosis, aggressive combination chemotherapy regimens can sometimes cause a complete remission in the diffuse large B cell lymphoma, at times leaving the patient with persisting follicular lymphoma.

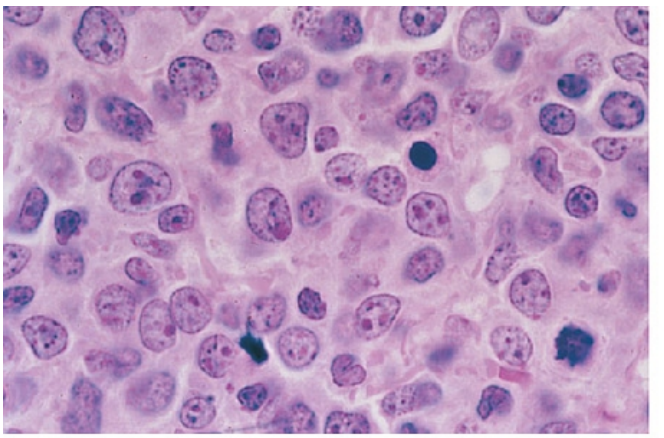
Diffuse Large B Cell Lymphoma

Diffuse large B cell lymphoma is the most common type of non-Hodgkin's lymphoma, representing approximately one-third of all cases. This lymphoma makes up the majority of cases in previous clinical trials of "aggressive" or "intermediate-grade" lymphoma. Table 105-10 shows the clinical characteristics of diffuse large B cell lymphoma.

The diagnosis of diffuse large B cell lymphoma can be made accurately by an expert hematopathologist (Fig. 105-8). Cytogenetic and

molecular genetic studies are not necessary for diagnosis, but some evidence has accumulated that patients whose tumors overexpress the BCL-2 protein might be more likely to relapse than others. Patients with prominent mediastinal involvement are sometimes diagnosed as a separate subgroup having primary mediastinal diffuse large B cell lymphoma. This latter group of patients has a younger median age (i.e., 37 years) and a female predominance (66%). Subtypes of diffuse large B cell lymphoma, including those with an immunoblastic subtype and tumors with extensive fibrosis, are recognized by pathologists but do not appear to have important independent prognostic significance.

Figure 105-8



Diffuse large B cell lymphoma. The neoplastic cells are heterogeneous but predominantly large cells with vesicular chromatin and prominent nucleoli.

Diffuse large B cell lymphoma can present as either primary lymph node disease or at extranodal sites. More than 50% of patients will have some site of extranodal involvement at diagnosis, with the most common sites being the gastrointestinal tract and bone marrow, each being involved in 15–20% of patients. Essentially any organ can be involved, making a diagnostic biopsy imperative. For example, diffuse large B cell lymphoma of the pancreas has a much better prognosis than pancreatic carcinoma but would be missed without biopsy. Primary diffuse large B cell lymphoma of the brain is being diagnosed with increasing frequency. Other unusual subtypes of diffuse large B cell lymphoma such as pleural effusion lymphoma and intravascular lymphoma have been difficult to diagnose and associated with a very poor prognosis.

Table 105-11 shows the initial evaluation of patients with diffuse large B cell lymphoma. After a careful staging evaluation, ~50% of patients will be found to have stage I or II disease and ~50% will have widely disseminated lymphoma. Bone marrow biopsy shows involvement by lymphoma in ~15% of cases, with marrow involvement by small cells more frequent than by large cells.

Diffuse Large B Cell Lymphoma: Treatment

The initial treatment of all patients with diffuse large B cell lymphoma should be with a combination chemotherapy regimen. The most popular regimen in the United States is CHOP plus rituximab, although a variety of other anthracycline-containing combination chemotherapy regimens appear to be equally efficacious. Patients with stage I or nonbulky stage II can be effectively treated with three to four cycles of combination chemotherapy followed by involved field radiotherapy. The need for radiation therapy is unclear. Cure rates of 70–80% in stage II disease and 85–90% in stage I disease can be expected.

For patients with bulky stage II, stage III, or stage IV disease, six to eight cycles of CHOP plus rituximab are usually administered. A large randomized trial showed the superiority of CHOP combined with rituximab over CHOP alone in elderly patients. A frequent approach would be to administer four cycles of therapy and then reevaluate. If the patient has achieved a complete remission after four cycles, two more cycles of treatment might be given and then therapy discontinued. Using this approach, 70–80% of patients can be expected to achieve a complete remission, and 50–70% of complete responders will be cured. The chances for a favorable response to treatment are predicted by the IPI. In fact, the IPI was developed based on the outcome of patients with diffuse large B cell lymphoma treated with CHOP-like regimens. For the 35% of patients with a low IPI score of 0–1, the 5-year survival is >70%, while for the 20% of patients with a high IPI score of 4–5, the 5-year survival is ~20%. The addition of rituximab to CHOP has improved each of those numbers by ~15%. A number of other factors, including molecular features of the tumor, levels of circulating cytokines and soluble receptors, and other surrogate markers, have been shown to influence prognosis. However, they have not been validated as rigorously as the IPI and have not been uniformly applied clinically.

Because a number of patients with diffuse large B cell lymphoma are either initially refractory to therapy or relapse after apparently effective chemotherapy, 30–40% of patients will be candidates for salvage treatment at some point. Alternative combination chemotherapy regimens can induce complete remission in as many as 50% of these patients, but long-term disease-free survival is seen in ≤10%. Autologous bone marrow transplantation is superior to salvage chemotherapy at usual doses and leads to long-term disease-free survival in

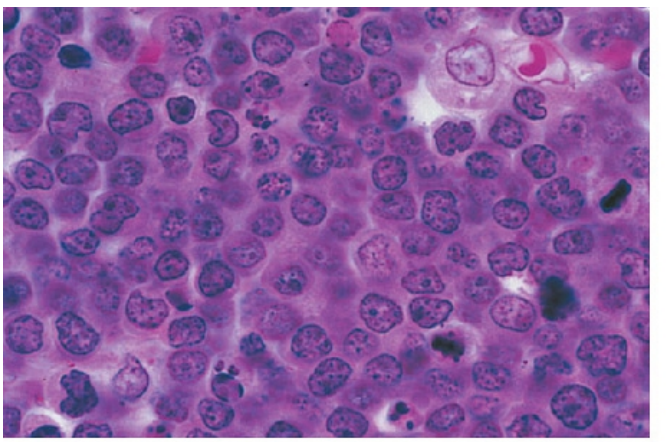
~40% of patients whose lymphomas remain chemotherapy-sensitive after relapse.

Burkitt's Lymphoma/Leukemia

Burkitt's lymphoma/leukemia is a rare disease in adults in the United States, making up <1% of non-Hodgkin's lymphomas, but it makes up ~30% of childhood non-Hodgkin's lymphoma. Burkitt's leukemia, or L3 ALL, makes up a small proportion of childhood and adult acute leukemias. Table 105-10 shows the clinical features of Burkitt's lymphoma.

Burkitt's lymphoma can be diagnosed morphologically by an expert hematopathologist with a high degree of accuracy. The cells are homogeneous in size and shape (Fig. 105-9). Demonstration of a very high proliferative fraction and the presence of the t(8;14) or one of its variants, t(2;8) (*c-myc* and the λ light chain gene) or t(8;22) (*c-myc* and the κ light chain gene), can be confirmatory. Burkitt's cell leukemia is recognized by the typical monotonous mass of medium-sized cells with round nuclei, multiple nucleoli, and basophilic cytoplasm with cytoplasmic vacuoles. Demonstration of surface expression of immunoglobulin and one of the above-noted cytogenetic abnormalities is confirmatory.

Figure 105-9



Burkitt's lymphoma. The neoplastic cells are homogenous, medium-sized B cells with frequent mitotic figures, a morphologic correlate of high growth fraction. Reactive macrophages are scattered through the tumor and their pale cytoplasm in a background of blue-staining tumor cells give the tumor a so-called starry sky appearance.

Three distinct clinical forms of Burkitt's lymphoma are recognized; endemic, sporadic, and immunodeficiency-associated. Endemic and sporadic Burkitt's lymphomas occur frequently in children in Africa, and the sporadic form in western countries. Immunodeficiency-associated Burkitt's lymphoma is seen in patients with HIV infection.

Pathologists sometimes have difficulty distinguishing between Burkitt's lymphoma and diffuse large B cell lymphoma. In the past, a separate subgroup of non-Hodgkin's lymphoma intermediate between the two was recognized. When tested, this subgroup could not be diagnosed accurately. Distinction between the two major types of B cell aggressive non-Hodgkin's lymphoma can sometimes be made based on the extremely high proliferative fraction seen in patients with Burkitt's lymphoma (i.e., essentially 100% of tumor cells are in cycle) caused by *c-myc* deregulation.

Most patients in the United States with Burkitt's lymphoma present with peripheral lymphadenopathy or an intraabdominal mass. The disease is rapidly progressive and has a propensity to metastasize to the CNS. Initial evaluation should always include an examination of cerebral spinal fluid to rule out metastasis in addition to the other staging evaluations noted in Table 105-11. Once the diagnosis of Burkitt's lymphoma is suspected, a diagnosis must be made promptly and staging evaluation must be accomplished expeditiously. This is the most rapidly progressive human tumor, and any delay in initiating therapy can adversely affect the patient's prognosis.

Burkitt's Lymphoma: Treatment

Treatment of Burkitt's lymphoma in both children and adults should begin within 48 h of diagnosis and involves the use of intensive combination chemotherapy regimens incorporating high doses of cyclophosphamide. Prophylactic therapy to the CNS is mandatory. Burkitt's lymphoma was one of the first cancers shown to be curable by chemotherapy. Today, cure can be expected in 70–80% of both children and young adults when effective therapy is administered precisely. Salvage therapy has been generally ineffective in patients failing the initial treatment, emphasizing the importance of the initial treatment approach.

Other B Cell Lymphoid Malignancies

B cell prolymphocytic leukemia involves blood and marrow infiltration by large lymphocytes with prominent nucleoli. Patients typically have a high white cell count, splenomegaly, and minimal lymphadenopathy. The chances for a complete response to therapy are poor.

Hairy cell leukemia is a rare disease that presents predominantly in older males. Typical presentation involves pancytopenia, although occasional patients will have a leukemic presentation. Splenomegaly is usual. The malignant cells appear to have "hairy" projections on light and electron microscopy and show a characteristic staining pattern with tartrate-resistant acid phosphatase. Bone marrow is typically not able to be aspirated, and biopsy shows a pattern of fibrosis with diffuse infiltration by the malignant cells. Patients with this disorder are prone to unusual infections, including infection by *Mycobacterium avium intracellulare*, and to vasculitic syndromes. Hairy cell leukemia is responsive to chemotherapy with interferon α, pentostatin, or cladribine, with the latter being the usually preferred treatment. Clinical complete remissions with cladribine occur in the majority of patients, and long-term disease-free survival is frequent.

Splenic marginal zone lymphoma involves infiltration of the splenic white pulp by small, monoclonal B cells. This is a rare disorder that can present as leukemia as well as lymphoma. Definitive diagnosis is often made at splenectomy, which is also an effective therapy. This is an extremely indolent disorder, but when chemotherapy is required, the most usual treatment has been chlorambucil.

Lymphoplasmacytic lymphoma is the tissue manifestation of Waldenström's macroglobulinemia (Chap. 106). This type of lymphoma has been associated with chronic hepatitis C virus infection, and an etiologic association has been proposed. Patients typically present with lymphadenopathy, splenomegaly, bone marrow involvement, and occasionally peripheral blood involvement. The tumor cells do not express CD5. Patients often have a monoclonal IgM protein, high levels of which can dominate the clinical picture with the symptoms of hyperviscosity. Treatment of lymphoplasmacytic lymphoma can be aimed primarily at reducing the abnormal protein, if present, but will usually also involve chemotherapy. Chlorambucil, fludarabine, and cladribine have been utilized. The median 5-year survival for patients with this disorder is ~60%.

Nodal marginal zone lymphoma, also known as *monocytoid B cell lymphoma*, represents ~1% of non-Hodgkin's lymphomas. This lymphoma has a slight female predominance and presents with disseminated disease (i.e., stage III or IV) in 75% of patients. Approximately one-third of patients have bone marrow involvement, and a leukemic presentation occasionally occurs. The staging evaluation and therapy should use the same approach as used for patients with follicular lymphoma. Approximately 60% of the patients with nodal marginal zone lymphoma will survive 5 years after diagnosis.

Precursor Cell T Cell Malignancies

Precursor T Cell Lymphoblastic Leukemia/Lymphoma

Precursor T cell malignancies can present either as ALL or as an aggressive lymphoma. These malignancies are more common in children and young adults, with males more frequently affected than females.

Precursor T cell ALL can present with bone marrow failure, although the severity of anemia, neutropenia, and thrombocytopenia is often less than in precursor B cell ALL. These patients sometimes have very high white cell counts, a mediastinal mass, lymphadenopathy, and hepatosplenomegaly. Precursor T cell lymphoblastic lymphoma is most often found in young men presenting with a large mediastinal mass and pleural effusions. Both presentations have a propensity to metastasize to the CNS, and CNS involvement is often present at diagnosis.

Precursor T Cell Lymphoblastic Leukemia/Lymphoma: Treatment

Children with precursor T cell ALL seem to benefit from very intensive remission induction and consolidation regimens. The majority of patients treated in this manner can be cured. Older children and young adults with precursor T cell lymphoblastic lymphoma are also often treated with "leukemia-like" regimens. Patients who present with localized disease have an excellent prognosis. However, advanced age is an adverse prognostic factor. Adults with precursor T cell lymphoblastic lymphoma who present with high LDH levels or bone marrow or CNS involvement are often offered bone marrow transplantation as part of their primary therapy.

Mature (Peripheral) T Cell Disorders

Mycosis Fungoides

Mycosis fungoides is also known as *cutaneous T cell lymphoma*. This lymphoma is more often seen by dermatologists than internists. The median age of onset is in the mid-fifties, and the disease is more common in males and in blacks.

Mycosis fungoides is an indolent lymphoma with patients often having several years of eczematous or dermatitic skin lesions before the diagnosis is finally established. The skin lesions progress from patch stage to plaque stage to cutaneous tumors. Early in the disease, biopsies are often difficult to interpret, and the diagnosis may only become apparent by observing the patient over time. In advanced stages, the lymphoma can spread to lymph nodes and visceral organs. Patients with this lymphoma may develop generalized erythroderma and circulating tumor cells, called *Sézary's syndrome*.

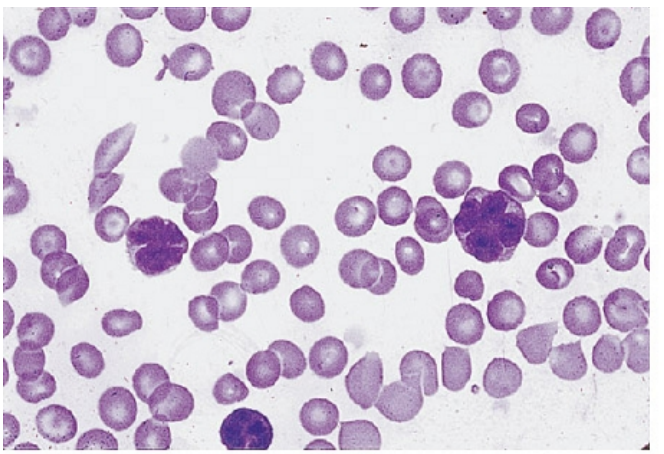
Rare patients with localized early stage mycosis fungoides can be cured with radiotherapy, often total-skin electron beam irradiation. More advanced disease has been treated with topical glucocorticoids, topical nitrogen mustard, phototherapy, psoralen with ultraviolet A (PUVA), electron beam radiation, interferon, antibodies, fusion toxins, and systemic cytotoxic therapy. Unfortunately, these treatments are palliative.

Adult T Cell Lymphoma/Leukemia

Adult T cell lymphoma/leukemia is one manifestation of infection by the HTLV-I retrovirus. Patients can be infected through transplacental transmission, mother's milk, blood transfusion, and by sexual transmission of the virus. Patients who acquire the virus from their mother through breast milk are most likely to develop lymphoma, but the risk is still only 2.5% and the latency averages 55 years. Nationwide testing for HTLV-I antibodies and the aggressive implementation of public health measures could theoretically lead to the disappearance of adult T cell lymphoma/leukemia. Tropical spastic paraparesis, another manifestation of HTLV-I infection (Chap. 181), occurs after a shorter latency (1–3 years) and is most common in individuals who acquire the virus during adulthood from transfusion or sex.

The diagnosis of adult T cell lymphoma/leukemia is made when an expert hematopathologist recognizes the typical morphologic picture, a T cell immunophenotype (i.e., CD4 positive), and the presence in serum of antibodies to HTLV-I. Examination of the peripheral blood will usually reveal characteristic, pleomorphic abnormal CD4-positive cells with indented nuclei, which have been called "flower" cells (Fig. 105-10).

Figure 105-10



Adult T cell leukemia/lymphoma. Peripheral blood smear showing leukemia cells with typical "flower-shaped" nucleus.

A subset of patients have a smoldering clinical course and long survival, but most patients present with an aggressive disease manifested by lymphadenopathy, hepatosplenomegaly, skin infiltration, pulmonary infiltrates, hypercalcemia, lytic bone lesions, and elevated LDH levels. The skin lesions can be papules, plaques, tumors, and ulcerations. Lung lesions can be either tumor or opportunistic infection in light of the underlying immunodeficiency in the disease. Bone marrow involvement is not usually extensive, and anemia and thrombocytopenia are not usually prominent. Although treatment by combination chemotherapy regimens can result in objective responses, true complete remissions are unusual, and the median survival of patients is ~7 months.

Anaplastic Large T/Null Cell Lymphoma

Anaplastic large T/null cell lymphoma was previously usually diagnosed as undifferentiated carcinoma or malignant histiocytosis. Discovery of the CD30 (Ki-1) antigen and the recognition that some patients with previously unclassified malignancies displayed this antigen led to the identification of a new type of lymphoma. Subsequently, discovery of the t(2;5) and the resultant frequent overexpression of the anaplastic lymphoma kinase (ALK) protein confirmed the existence of this entity. This lymphoma accounts for ~2% of all non-Hodgkin's lymphomas. Table 105-10 shows the clinical characteristics of patients with anaplastic large T/null cell lymphoma.

The diagnosis of anaplastic large T/null cell lymphoma is made when an expert hematopathologist recognizes the typical morphologic picture and a T cell or null cell immunophenotype with CD30 positivity. Documentation of the t(2;5) and/or overexpression of ALK protein confirm the diagnosis. Some diffuse large B cell lymphomas can also have an anaplastic appearance but have the same clinical course or response to therapy as other diffuse large B cell lymphomas.

Patients with anaplastic large T/cell null cell lymphoma are typically young (median age, 33 years) and male (~70%). Some 50% of patients present in stage I/II, and the remainder with more extensive disease. Systemic symptoms and elevated LDH levels are seen in about one-half of patients. Bone marrow and the gastrointestinal tract are rarely involved, but skin involvement is frequent. Some patients with disease confined to the skin have a different and more indolent disorder that has been termed *cutaneous anaplastic large T/null cell lymphoma* and might be related to lymphomatoid papulosis.

Anaplastic Large T/Null Cell Lymphoma: Treatment

Treatment regimens appropriate for other aggressive lymphomas, such as diffuse large B cell lymphoma, should be utilized in patients with

anaplastic large T/null cell lymphoma, with the exception that the B cell–specific antibody, rituximab, is omitted. Surprisingly, given the anaplastic appearance, this disorder has the best survival rate of any aggressive lymphoma. The 5-year survival is >75%. While traditional prognostic factors such as the IPI predict treatment outcome, overexpression of the ALK protein is an important prognostic factor, with patients overexpressing this protein having a superior treatment outcome.

Peripheral T Cell Lymphoma

The peripheral T cell lymphomas make up a heterogeneous morphologic group of aggressive neoplasms that share a mature T cell immunophenotype. They represent ~7% of all cases of non-Hodgkin's lymphoma. A number of distinct clinical syndromes are included in this group of disorders. Table 105-10 shows the clinical characteristics of patients with peripheral T cell lymphoma.

The diagnosis of peripheral T cell lymphoma, or any of its specific subtypes, requires an expert hematopathologist, an adequate biopsy, and immunophenotyping. Most peripheral T cell lymphomas are CD4+, but a few will be CD8+, both CD4+ and CD8+, or have an NK cell immunophenotype. No characteristic genetic abnormalities have yet been identified, but translocations involving the T cell antigen receptor genes on chromosomes 7 or 14 may be detected. The differential diagnosis of patients suspected of having peripheral T cell lymphoma includes reactive T cell infiltrative processes. In some cases, demonstration of a monoclonal T cell population using T cell receptor gene rearrangement studies will be required to make a diagnosis.

The initial evaluation of a patient with a peripheral T cell lymphoma should include the studies in Table 105-11 for staging patients with non-Hodgkin's lymphoma. Unfortunately, patients with peripheral T cell lymphoma usually present with adverse prognostic factors, with >80% of patients having an IPI score ≥2 and >30% having an IPI score ≥4. As this would predict, peripheral T cell lymphomas are associated with a poor outcome, and only 25% of the patients survive 5 years after diagnosis. Treatment regimens are the same as those used for diffuse large B cell lymphoma (omitting rituximab), but patients with peripheral T cell lymphoma have a poorer response to treatment. Because of this poor treatment outcome, hematopoietic stem cell transplantation is often considered early in the care of young patients.

A number of specific clinical syndromes are seen in the peripheral T cell lymphomas. *Angioimmunoblastic T cell lymphoma* is one of the more common subtypes, making up ~20% of T cell lymphomas. These patients typically present with generalized lymphadenopathy, fever, weight loss, skin rash, and polyclonal hypergammaglobulinemia. In some cases, it is difficult to separate patients with a reactive disorder from those with true lymphoma.

Extranodal T/NK cell lymphoma of nasal type has also been called *angiocentric lymphoma* and was previously termed *lethal midline granuloma*. This disorder is more frequent in Asia and South America than in the United States and Europe. EBV is thought to play an etiologic role. Although most frequent in the upper airway, it can involve other organs. The course is aggressive, and patients frequently have the hemophagocytic syndrome. When marrow and blood involvement occur, distinction between this disease and leukemia might be difficult. Some patients will respond to aggressive combination chemotherapy regimens, but the overall outlook is poor.

Enteropathy-type intestinal T cell lymphoma is a rare disorder that occurs in patients with untreated gluten-sensitive enteropathy. Patients are frequently wasted and sometimes present with intestinal perforation. The prognosis is poor. *Hepatosplenic T cell lymphoma* is a systemic illness that presents with sinusoidal infiltration of the liver, spleen, and bone marrow by malignant T cells. Tumor masses generally do not occur. The disease is associated with systemic symptoms and is often difficult to diagnosis. Treatment outcome is poor. *Subcutaneous panniculitis-like T cell lymphoma* is a rare disorder that is often confused with panniculitis. Patients present with multiple subcutaneous nodules, which progress and can ulcerate. Hemophagocytic syndrome is common. Response to therapy is poor. The development of the hemophagocytic syndrome (profound anemia, ingestion of erythrocytes by monocytes and macrophages) in the course of any peripheral T cell lymphoma is generally associated with a fatal outcome.

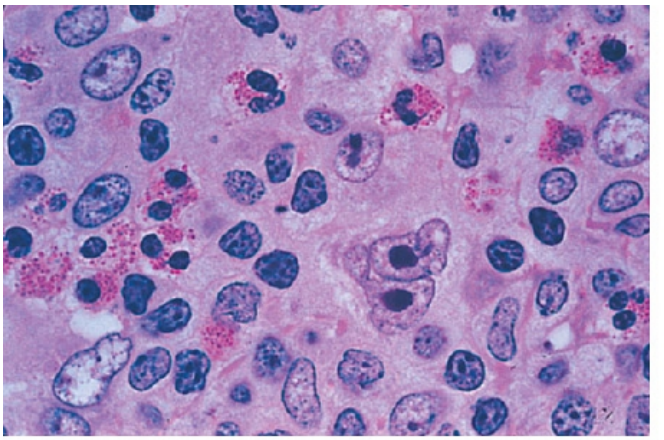
Hodgkin's Disease

Classical Hodgkin's Disease

Hodgkin's disease occurs in 8000 patients in the United States each year, and the disease does not appear to be increasing in frequency. Most patients present with palpable lymphadenopathy that is nontender; in most patients, these lymph nodes are in the neck, supraclavicular area, and axilla. More than half the patients will have mediastinal adenopathy at diagnosis, and this is sometimes the initial manifestation. Subdiaphragmatic presentation of Hodgkin's disease is unusual and more common in older males. One-third of patients present with fevers, night sweats, and/or weight loss- B symptoms in the Ann Arbor staging classification (Table 105-8). Occasionally, Hodgkin's disease can present as a fever of unknown origin. This is more common in older patients who are found to have mixed-cellularity Hodgkin's disease in an abdominal site. Rarely, the fevers persist for days to weeks, followed by afebrile intervals and then recurrence of the fever. This pattern is known as *Pel-Ebstein fever*. Hodgkin's disease can occasionally present with unusual manifestations. These include severe and unexplained itching, cutaneous disorders such as erythema nodosum and ichthyosiform atrophy, paraneoplastic cerebellar degeneration and other distant effects on the CNS, nephrotic syndrome, immune hemolytic anemia and thrombocytopenia, hypercalcemia, and pain in lymph nodes on alcohol ingestion.

The diagnosis of Hodgkin's disease is established by review of an adequate biopsy specimen by an expert hematopathologist. In the United States, most patients have nodular sclerosing Hodgkin's disease, with a minority of patients having mixed-cellularity Hodgkin's disease. Lymphocyte-predominant and lymphocyte-depleted Hodgkin's disease are rare. Mixed-cellularity Hodgkin's disease or lymphocyte-depletion Hodgkin's disease are seen more frequently in patients infected by HIV (Fig. 105-11). The differential diagnosis of a lymph node biopsy suspicious for Hodgkin's disease includes inflammatory processes, mononucleosis, non-Hodgkin's lymphoma, phenytoin-induced adenopathy, and nonlymphomatous malignancies.

Figure 105-11



Mixed cellularity Hodgkin's disease. A Reed-Sternberg cell is present near the center of the field; a large cell with a bilobed nucleus and prominent nucleoli giving an "owl's eyes" appearance. The majority of the cells are normal lymphocytes, neutrophils, and eosinophils that form a pleiomorphic cellular infiltrate.

The staging evaluation for a patient with Hodgkin's disease would typically include a careful history and physical examination; complete blood count; erythrocyte sedimentation rate; serum chemistry studies including LDH; chest radiograph; CT scan of the chest, abdomen, and pelvis; and bone marrow biopsy. Many patients would also have a PET scan or a gallium scan. Although rarely utilized, a bipedal lymphangiogram can be helpful. PET and gallium scans are most useful to document remission. Staging laparotomies were once popular for most patients with Hodgkin's disease but are now done rarely because of an increased reliance on systemic rather than local therapy.

Classical Hodgkin's Disease: Treatment

Patients with localized Hodgkin's disease are cured >90% of the time. In patients with good prognostic factors, extended-field radiotherapy has a high cure rate. Increasingly, patients with all stages of Hodgkin's disease are treated initially with chemotherapy. Patients with localized or good-prognosis disease receive a brief course of chemotherapy followed by radiotherapy to sites of node involvement. Patients with more extensive disease or those with B symptoms receive a complete course of chemotherapy. The most popular chemotherapy regimens used in Hodgkin's disease include doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) and mechlorethamine, vincristine, procarbazine, and prednisone (MOPP), or combinations of the drugs in these two regimens. Today, most patients in the United States receive ABVD, but a weekly chemotherapy regimen administered for 12 weeks called *Stanford V* is becoming increasingly popular, but includes radiation therapy, which has been associated with life-threatening late toxicities such as premature coronary artery disease and second solid tumors. In Europe a high-dose regimen called *BEACOPP* incorporating alkylating agents has become popular and might have a better response rate in very high risk patients. Long-term disease-free survival in patients with advanced disease can be achieved in >75% of patients who lack systemic symptoms and in 60–70% of patients with systemic symptoms.

Patients who relapse after primary therapy of Hodgkin's disease can frequently still be cured. Patients who relapse after initial treatment only with radiotherapy have an excellent outcome when treated with chemotherapy. Patients who relapse after an effective chemotherapy regimen are usually not curable with subsequent chemotherapy administered at standard doses. However, patients with a long initial remission can be an exception to this rule. Autologous bone marrow transplantation can cure half of patients who fail effective chemotherapy regimens.

Because of the very high cure rate in patients with Hodgkin's disease, long-term complications have become a major focus for clinical research. In fact, in some series of patients with early-stage disease, more patients died from late complications of therapy than from Hodgkin's disease itself. This is particularly true in patients with localized disease. The most serious late side effects include second malignancies and cardiac injury. Patients are at risk for the development of acute leukemia in the first 10 years after treatment with combination chemotherapy regimens that contain alkylating agents plus radiation therapy. The risk for development of acute leukemia appears to be greater after MOPP-like regimens than with ABVD. The risk of development of acute leukemia after treatment for Hodgkin's disease is also related to the number of exposures to potentially leukemogenic agents (i.e., multiple treatments after relapse) and the age of the patient being treated, with those >60 years at particularly high risk. The development of carcinomas as a complication of treatment for Hodgkin's disease has become a major problem. These tumors usually occur ≥10 years after treatment and are associated with use of radiotherapy. For this reason, young women treated with thoracic radiotherapy for Hodgkin's disease should institute screening mammograms 5–10 years after treatment, and all patients who receive thoracic radiotherapy for Hodgkin's disease should be discouraged from smoking. Thoracic radiation also accelerates coronary artery disease, and patients should be encouraged to minimize risk factors for coronary artery disease such as smoking and elevated cholesterol levels.

A number of other late side effects from the treatment of Hodgkin's disease are well known. Patients who receive thoracic radiotherapy are at very high risk for the eventual development of hypothyroidism and should be observed for this complication; intermittent measurement of thyrotropin should be made to identify the condition before it becomes symptomatic. Lhermitte's syndrome occurs in ~15% of patients who receive thoracic radiotherapy. This syndrome is manifested by an "electric shock" sensation into the lower extremities on flexion of the neck. Infertility is a concern for all patients undergoing treatment for Hodgkin's disease. In both women and men, the risk of permanent infertility is age-related, with younger patients more likely to recover fertility. In addition, treatment with ABVD rather than MOPP increases the chances to retain fertility.

Nodular Lymphocyte-Predominant Hodgkin's Disease

Nodular lymphocyte-predominant Hodgkin's disease is now recognized as an entity distinct from classical Hodgkin's disease. Previous classification systems recognized that biopsies from a subset of patients diagnosed as having Hodgkin's disease contained a predominance of small lymphocytes and rare Reed-Sternberg cells. A subset of these patients have tumors with nodular growth pattern and a clinical course that varied from that of patients with classical Hodgkin's disease. This is an unusual clinical entity and represents <5% of cases of Hodgkin's disease.

Nodular lymphocyte-predominant Hodgkin's disease has a number of characteristics that suggest its relationship to non-Hodgkin's lymphoma. These include a clonal proliferation of B cells and a distinctive immunophenotype; tumor cells express J chain and display CD45 and epithelial membrane antigen (ema) and do not express two markers normally found on Sternberg-Reed cells, CD30 and CD15. This lymphoma tends to have a chronic, relapsing course and sometimes transforms to diffuse large B cell lymphoma.

The treatment of patients with nodular lymphocyte-predominant Hodgkin's disease is controversial. Some clinicians favor no treatment and merely close follow-up. In the United States, most physicians will treat localized disease with radiotherapy and disseminated disease with regimens utilized for patients with classical Hodgkin's disease. Regardless of the therapy utilized, most series report a long-term survival of >80%.

Lymphoma-Like Disorders

The most common condition that pathologists and clinicians might confuse with lymphoma is reactive, atypical lymphoid hyperplasia. Patients might have localized or disseminated lymphadenopathy and might have the systemic symptoms characteristic of lymphoma. Underlying causes include a drug reaction to phenytoin or carbamazepine. Immune disorders such as rheumatoid arthritis and lupus erythematosus, viral infections such as cytomegalovirus and EBV, and bacterial infections such as cat-scratch disease may cause adenopathy (Chap. 60). In the absence of a definitive diagnosis after initial biopsy, continued follow-up, further testing, and repeated biopsies, if necessary, are the appropriate approach rather than instituting therapy.

Specific conditions that can be confused with lymphoma include *Castleman's disease*, which can present with localized or disseminated lymphadenopathy; some patients have systemic symptoms. The disseminated form is often accompanied by anemia and polyclonal hypergammaglobulinemia, and the condition has been associated with overproduction of interleukin 6, possibly produced by human herpesvirus 8. Patients with localized disease can be treated effectively with local therapy, while the initial treatment for patients with disseminated disease is usually with systemic glucocorticoids.

Sinus histiocytosis with massive lymphadenopathy (*Rosai-Dorfman's disease*) usually presents with bulky lymphadenopathy in children or young adults. The disease is usually nonprogressive and self-limited, but patients can manifest autoimmune hemolytic anemia.

Lymphomatoid papulosis is a cutaneous lymphoproliferative disorder that is often confused with anaplastic large cell lymphoma involving the skin. The cells of lymphomatoid papulosis are similar to those seen in lymphoma and stain for CD30, and T cell receptor gene rearrangements are sometimes seen. However, the condition is characterized by waxing and waning skin lesions that usually heal, leaving small scars. In the absence of effective communication between the clinician and the pathologist regarding the clinical course in the patient, this disease will be misdiagnosed. Since the clinical picture is usually benign, misdiagnosis is a serious mistake.

Acknowledgment

James Armitage was a coauthor of this chapter in prior editions, and substantial material from those editions has been included here.

Further Readings

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Note: Large images and tables on this page may necessitate printing in landscape mode.

Harrison's Internal Medicine > Chapter 106. Plasma Cell Disorders >

Plasma Cell Disorders: Introduction

The *plasma cell disorders* are monoclonal neoplasms related to each other by virtue of their development from common progenitors in the B lymphocyte lineage. Multiple myeloma, Waldenström's macroglobulinemia, primary amyloidosis (Chap. 324), and the heavy chain diseases comprise this group and may be designated by a variety of synonyms such as *monoclonal gammopathies*, *paraproteinemias*, *plasma cell dyscrasias*, and *dysproteinemias*. Mature B lymphocytes destined to produce IgG bear surface immunoglobulin molecules of both M and G heavy chain isotypes with both isotypes having identical idiotypes (variable regions). Under normal circumstances, maturation to antibody-secreting plasma cells is stimulated by exposure to the antigen for which the surface immunoglobulin is specific; however, in the plasma cell disorders the control over this process is lost. The clinical manifestations of all the plasma cell disorders relate to the expansion of the neoplastic cells, to the secretion of cell products (immunoglobulin molecules or subunits, lymphokines), and to some extent to the host's response to the tumor. Normal development of B lymphocytes is discussed in Chap. 308.

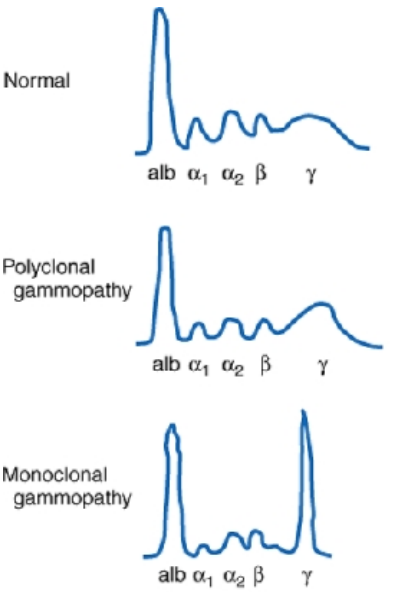
There are three categories of structural variation among immunoglobulin molecules that form antigenic determinants, and these are used to classify immunoglobulins (Chap. 308). *Isotypes* are those determinants that distinguish among the main classes of antibodies of a given species and are the same in all normal individuals of that species. Therefore, isotypic determinants are, by definition, recognized by antibodies from a distinct species (heterologous sera) but not by antibodies from the same species (homologous sera). There are five heavy chain isotypes (M, G, A, D, E) and two light chain isotypes (,). *Allotypes* are distinct determinants that reflect regular small differences between individuals of the same species in the amino acid sequences of otherwise similar immunoglobulins. These differences are determined by allelic genes; by definition, they are detected by antibodies made in the same species. *Idiotypes* are the third category of antigenic determinants. They are unique to the molecules produced by a given clone of antibody-producing cells. Idiotypes are formed by the unique structure of the antigen-binding portion of the molecule.

Antibody molecules (Fig. 308-9) are composed of two heavy chains (mol wt ~ 50,000) and two light chains (mol wt ~ 25,000). Each chain has a constant portion (limited amino acid sequence variability) and a variable region (extensive sequence variability). The light and heavy chains are linked by disulfide bonds and are aligned so that their variable regions are adjacent to one another. This variable region forms the antigen recognition site of the antibody molecule; its unique structural features form a particular set of determinants, or idiotypes, that are reliable markers for a particular clone of cells because each antibody is formed and secreted by a single clone. Each chain is specified by distinct genes, synthesized separately, and assembled into an intact antibody molecule after translation. Because of the mechanics of the gene rearrangements necessary to specify the immunoglobulin variable regions (VDJ joining for the heavy chain, VJ joining for the light chain), a particular clone rearranges only one of the two chromosomes to produce an immunoglobulin molecule of only one light chain isotype and only one allotype (allelic exclusion). After exposure to antigen, the variable region may become associated with a new heavy chain isotype (class switch). Each clone of cells performs these sequential gene arrangements in a unique way. This results in each clone producing a unique immunoglobulin molecule. In most cells, light chains are synthesized in slight excess, are secreted as free light chains by plasma cells, and are cleared by the kidney, but <10 mg of such light chains is excreted per day.

Electrophoretic analysis of components of the serum proteins permits determination of the amount of immunoglobulin in the serum (Fig. 106-1). The immunoglobulins move heterogeneously in an electric field and form a broad peak in the gamma region. The globulin region of the electrophoretic pattern is usually increased in the sera of patients with plasma cell tumors. There is a sharp spike in this region called an *M component* (M for monoclonal). Less commonly, the M component may appear in the β_2 or α_2 globulin region. The antibody must be present at a concentration of at least 5 g/L (0.5 g/dL) to be detectable by this method. This corresponds to ~10^9 cells producing the antibody. Confirmation that such an M component is truly monoclonal relies on the use of immunoelectrophoresis that shows a single light and heavy chain type. Hence immunoelectrophoresis and electrophoresis provide qualitative and quantitative assessment of the M component, respectively. Once the presence of an M component has been confirmed, electrophoresis provides the more practical information for managing patients with monoclonal gammopathies. In a given patient, the amount of M component in the serum is a reliable measure of the tumor burden. This makes the M component an excellent tumor marker, yet it is not specific enough to be used to screen asymptomatic patients. In addition to the plasma cell disorders, M components may be detected in other lymphoid neoplasms such as chronic lymphocytic leukemia and lymphomas of B or T cell origin; nonlymphoid neoplasms such as chronic myeloid leukemia, breast cancer, and colon cancer; a variety of nonneoplastic conditions such as cirrhosis, sarcoidosis, parasitic diseases, Gaucher disease, and pyoderma gangrenosum; and a number of autoimmune conditions, including rheumatoid arthritis, myasthenia gravis, and cold agglutinin disease. At least two very rare skin diseases– lichen myxedematosus, or papular mucinosis, and necrobiotic xanthogranuloma– are associated with a monoclonal gammopathy. In papular mucinosis, highly cationic IgG is deposited in the dermis of patients. This organ specificity may reflect the specificity of the antibody for some antigenic component of the dermis. Necrobiotic xanthogranuloma is a histiocytic infiltration of the skin, usually of the face, that produces red or yellow nodules that can enlarge to plaques. Some 10% progress

to myeloma.

Figure 106-1



Representative patterns of serum electrophoresis. The upper panel illustrates the normal pattern of serum protein on electrophoresis. Since there are many different immunoglobulins in the serum, their differing mobilities in an electric field produce a broad peak. In conditions associated with increases in polyclonal immunoglobulin, the broad peak is more prominent (middle panel). In monoclonal gammopathies, the predominance of a product of a single cell produces a "church spire" sharp peak, usually in the γ globulin region (bottom panel).

The nature of the M component is variable in plasma cell disorders. It may be an intact antibody molecule of any heavy chain subclass, or it may be an altered antibody or fragment. Isolated light or heavy chains may be produced. In some plasma cell tumors such as extramedullary or solitary bone plasmacytomas, <⅓ of patients will have an M component. In ~20% of myelomas, only light chains are produced and in most cases are secreted in the urine as Bence Jones proteins. The frequency of myelomas of a particular heavy chain class is roughly proportional to the serum concentration, and therefore IgG myelomas are more common than IgA and IgD myelomas.

Multiple Myeloma

Definition

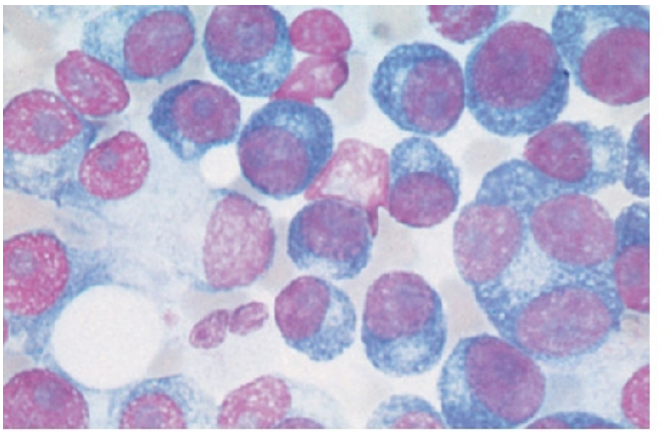
Multiple myeloma represents a malignant proliferation of plasma cells derived from a single clone. The terms *multiple myeloma* and *myeloma* may be used interchangeably. The tumor, its products, and the host response to it result in a number of organ dysfunctions and symptoms of bone pain or fracture, renal failure, susceptibility to infection, anemia, hypercalcemia, and occasionally clotting abnormalities, neurologic symptoms, and manifestations of hyperviscosity.

Etiology

The cause of myeloma is not known. Myeloma occurred with increased frequency in those exposed to the radiation of nuclear warheads in World War II after a 20-year latency. A variety of chromosomal alterations have been found in patients with myeloma; 13q14 deletions, 17p13 deletions, and 11q abnormalities predominate. The most common translocations are t(11;14)(q13;q32) and t(4;14)(p16;q32), and evidence is strong that errors in switch recombination– the genetic mechanism to change antibody heavy chain isotype– participate in the transformation pathway. Overexpression of *myc* or *ras* genes has been noted in some cases. Mutations in p53 and Rb-1 have also been described, but no common molecular pathogenesis has yet emerged.

Myeloma has been seen more commonly than expected among farmers, wood workers, leather workers, and those exposed to petroleum products. The neoplastic event in myeloma may involve cells earlier in B cell differentiation than the plasma cell. Circulating B cells bearing surface immunoglobulin that share the idiotype of the M component are present in myeloma patients. Interleukin (IL) 6 may play a role in driving myeloma cell proliferation; a large fraction of myeloma cells exposed to IL-6 in vitro respond by proliferating. The IL-6 dependency of myeloma is controversial. It remains difficult to distinguish benign from malignant plasma cells on the basis of morphologic criteria in all but a few cases (Fig. 106-2).

Figure 106-2



Multiple myeloma (marrow). The cells bear characteristic morphologic features of plasma cells, round or oval cells with an eccentric nucleus composed of coarsely clumped chromatin, a densely basophilic cytoplasm, and a perinuclear clear zone (hof) containing the Golgi apparatus. Binucleate and multinucleate malignant plasma cells can be seen.

Incidence and Prevalence

About 19,900 cases of myeloma were diagnosed in 2007, and 10,790 people died from the disease in the United States. Myeloma increases in incidence with age. The median age at diagnosis is 68 years; it is uncommon under age 40. The yearly incidence is around 4 per 100,000 and remarkably similar throughout the world. Males are more commonly affected than females, and blacks have nearly twice the incidence of whites. Myeloma accounts for ~1% of all malignancies in whites and 2% in blacks; 13% of all hematologic cancers in whites and 33% in blacks.

The incidence of myeloma is highest in African-American and Pacific islanders; intermediate in Europeans and North American Caucasians; and lowest in developing countries including Asia. The higher incidence in more developed countries may result from the combination of a longer life expectancy and more frequent medical surveillance. Incidence of multiple myeloma in other ethnic groups including native Hawaiians, female Hispanics, American Indians from New Mexico, and Alaskan natives is higher relative to U.S. Caucasians in the same geographic area. Chinese and Japanese populations have a lower incidence than Caucasians. Immunoproliferative small intestinal disease with alpha heavy chain disease is most prevalent in the Mediterranean area. Despite these differences in prevalence, the characteristics, response to therapy, and prognosis of myeloma are similar worldwide.

Pathogenesis and Clinical Manifestations

(Table 106-1) Multiple myeloma (MM) cells bind via cell-surface adhesion molecules to bone marrow stromal cells (BMSCs) and extracellular matrix (ECM), which triggers MM cell growth, survival, drug resistance, and migration in the bone marrow milieu (Fig. 106-3). These effects are due both to direct MM cell–BMSC binding and to induction of various cytokines including IL-6, insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and stromal cell–derived growth factor (SDF)-1. Growth, drug resistance, and migration are mediated via Ras/Raf/mitogen-activated protein kinase, PI3-K/Akt, and protein kinase C signaling cascades, respectively.

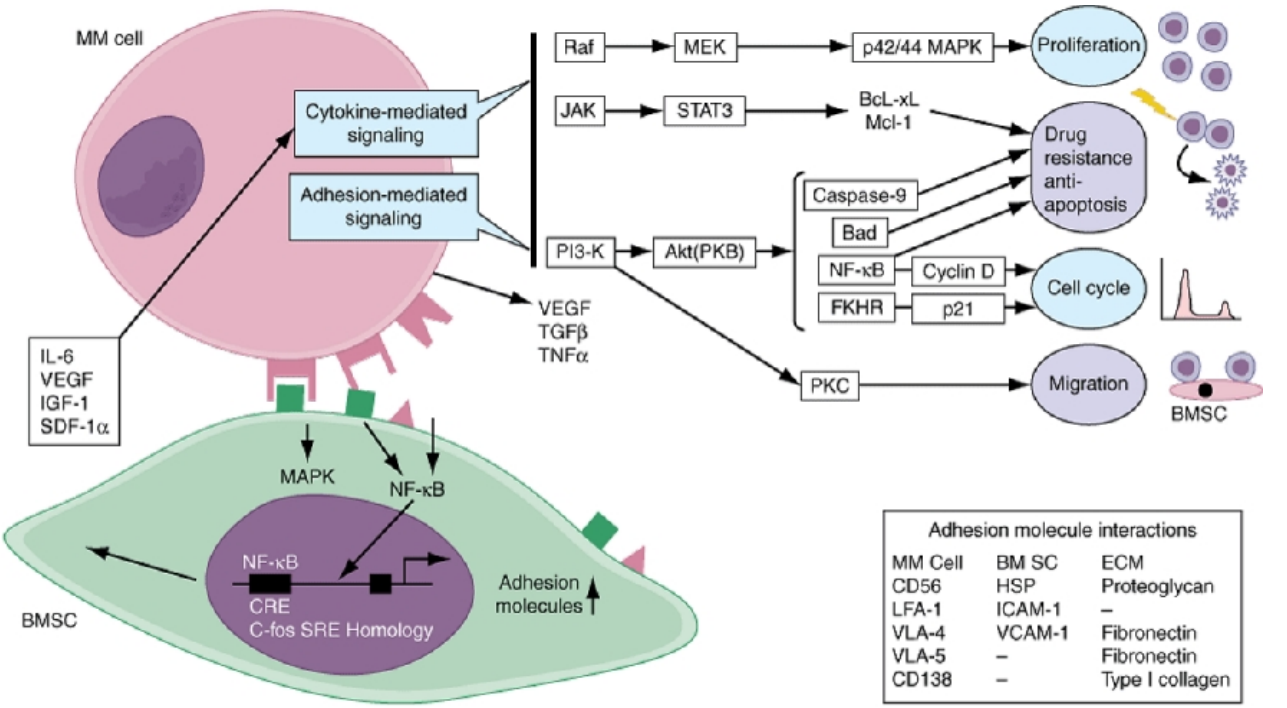
Table 106-1 Clinical Features of Multiple Myeloma

Clinical Finding	Underlying Cause and Pathogenetic Mechanism
Hypercalcemia, osteoporosis, pathologic fractures, lytic bone lesions, bone pain	Tumor expansion, production of osteoclast activating factor by tumor cells, osteoblast inhibitory factors
Renal failure	Hypercalcemia, light chain deposition, amyloidosis, urate nephropathy, drug toxicity (nonsteroidal anti-inflammatory agents, bisphosphonates), contrast dye
Easy fatigue- anemia	Bone marrow infiltration, production of inhibitory factors, hemolysis, decreased red cell production, decreased erythropoietin levels
Recurrent infections	Hypogammaglobulinemia, low CD4 count, decreased neutrophil migration
Neurologic symptoms	Hyperviscosity, cryoglobulinemia, amyloid deposits, hypercalcemia, nerve compression, anti-neuronal antibody, POEMS syndrome, therapy-related toxicity

Nausea and vomiting	Renal failure, hypercalcemia
Bleeding/clotting disorder	Interference with clotting factors, antibody to clotting factors, amyloid damage of endothelium, platelet dysfunction, antibody coating of platelet, therapy-related hypercoagulable defects

Note: POEMS, polyneuropathy, organomegaly, endocrinopathy, multiple myeloma, and skin changes.

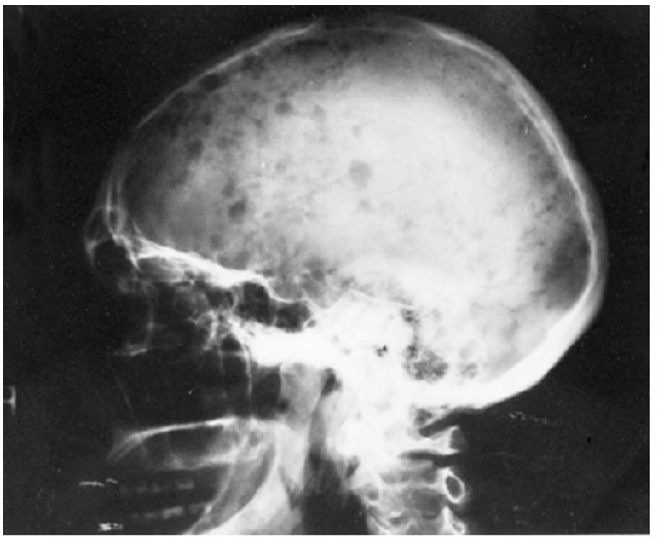
Figure 106-3



Pathogenesis of multiple myeloma. Multiple myeloma cells interact with bone marrow stromal cells and extracellular matrix proteins via adhesion molecules, triggering adhesion-mediated signaling as well as cytokine production. This triggers cytokine-mediated signaling that provides growth, survival, and anti-apoptotic effects as well as development of drug resistance. HSP, heparin sulfate proteoglycan.

Bone pain is the most common symptom in myeloma, affecting nearly 70% of patients. The pain usually involves the back and ribs, and unlike the pain of metastatic carcinoma, which often is worse at night, the pain of myeloma is precipitated by movement. Persistent localized pain in a patient with myeloma usually signifies a pathologic fracture. The bone lesions of myeloma are caused by the proliferation of tumor cells, activation of osteoclasts that destroy bone, and suppression of osteoblasts that form new bone. The osteoclasts respond to osteoclast activating factors (OAF) made by the myeloma cells [OAF activity can be mediated by several cytokines, including IL-1, lymphotoxin, VEGF, receptor activator of NF-κB (RANK) ligand, macrophage inhibitory factor (MIP)-1α, and tumor necrosis factor (TNF)]. However, production of these factors decreases following administration of glucocorticoids or interferon (IFN) α. The bone lesions are lytic in nature and are rarely associated with osteoblastic new bone formation. Therefore, radioisotopic bone scanning is less useful in diagnosis than is plain radiography. The bony lysis results in substantial mobilization of calcium from bone, and serious acute and chronic complications of hypercalcemia may dominate the clinical picture (see below). Localized bone lesions may expand to the point that mass lesions may be palpated, especially on the skull (Fig. 106-4), clavicles, and sternum, and the collapse of vertebrae may lead to spinal cord compression.

Figure 106-4



Bony lesions in multiple myeloma. The skull demonstrates the typical "punched out" lesions characteristic of multiple myeloma. The lesion represents a purely osteolytic lesion with little or no osteoblastic activity. *(Courtesy of Dr. Geraldine Schechter; with permission.)*

The next most common clinical problem in patients with myeloma is susceptibility to bacterial infections. The most common infections are pneumonias and pyelonephritis, and the most frequent pathogens are *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* in the lungs and *Escherichia coli* and other gram-negative organisms in the urinary tract. In ~25% of patients, recurrent infections are the presenting features, and >75% of patients will have a serious infection at some time in their course. The susceptibility to infection has several contributing causes. First, patients with myeloma have diffuse hypogammaglobulinemia if the M component is excluded. The hypogammaglobulinemia is related to both decreased production and increased destruction of normal antibodies. Moreover, some patients generate a population of circulating regulatory cells in response to their myeloma that can suppress normal antibody synthesis. In the case of IgG myeloma, normal IgG antibodies are broken down more rapidly than normal because the catabolic rate for IgG antibodies varies directly with the serum concentration. The large M component results in fractional catabolic rates of 8–16% instead of the normal 2%. These patients have very poor antibody responses, especially to polysaccharide antigens such as those on bacterial cell walls. Most measures of T cell function in myeloma are normal, but a subset of CD4+ cells may be decreased. Granulocyte lysozyme content is low, and granulocyte migration is not as rapid as normal in patients with myeloma, probably the result of a tumor product. There are also a variety of abnormalities in complement functions in myeloma patients. All these factors contribute to the immune deficiency of these patients. Some commonly used therapeutic agents, e.g., dexamethasone, suppress immune responses and increase susceptibility to infection.

Renal failure occurs in nearly 25% of myeloma patients, and some renal pathology is noted in over half. Many factors contribute to this. Hypercalcemia is the most common cause of renal failure. Glomerular deposits of amyloid, hyperuricemia, recurrent infections, frequent use of nonsteroidal anti-inflammatory agents for pain control, use of iodinated contrast dye for imaging, bisphosphonate use, and occasional infiltration of the kidney by myeloma cells all may contribute to renal dysfunction. However, tubular damage associated with the excretion of light chains is almost always present. Normally, light chains are filtered, reabsorbed in the tubules, and catabolized. With the increase in the amount of light chains presented to the tubule, the tubular cells become overloaded with these proteins, and tubular damage results either directly from light chain toxic effects or indirectly from the release of intracellular lysosomal enzymes. The earliest manifestation of this tubular damage is the adult Fanconi syndrome (a type 2 proximal renal tubular acidosis), with loss of glucose and amino acids, as well as defects in the ability of the kidney to acidify and concentrate the urine. The proteinuria is not accompanied by hypertension, and the protein is nearly all light chains. Generally, very little albumin is in the urine because glomerular function is usually normal. When the glomeruli are involved, nonselective proteinuria is also observed. Patients with myeloma also have a decreased anion gap [i.e., $Na^+ - (Cl^- + HCO_3^-)$] because the M component is cationic, resulting in retention of chloride. This is often accompanied by hyponatremia that is felt to be artificial (pseudohyponatremia) because each volume of serum has less water as a result of the increased protein. Renal dysfunction due to light chain deposition disease, light chain cast nephropathy, and amyloidosis is partially reversible with effective therapy. Myeloma patients are susceptible to developing acute renal failure if they become dehydrated.

Anemia occurs in ~80% of myeloma patients. It is usually normocytic and normochromic and related both to the replacement of normal marrow by expanding tumor cells and to the inhibition of hematopoiesis by factors made by the tumor. In addition, mild hemolysis may contribute to the anemia. A larger than expected fraction of patients may have megaloblastic anemia due to either folate or vitamin B_{12} deficiency. Granulocytopenia and thrombocytopenia are very rare. Clotting abnormalities may be seen due to the failure of antibody-coated platelets to function properly or to the interaction of the M component with clotting factors I, II, V, VII, or VIII. Deep venous thrombosis is also observed with use of thalidomide or lenalidomide in combination with dexamethasone. Raynaud's phenomenon and impaired circulation may result if the M component forms cryoglobulins, and hyperviscosity syndromes may develop depending on the physical properties of the M component (most common with IgM, IgG3, and IgA paraproteins). Hyperviscosity is defined on the basis of the

relative viscosity of serum as compared with water. Normal relative serum viscosity is 1.8 (i.e., serum is normally almost twice as viscous as water). Symptoms of hyperviscosity occur at a level of 5–6, a level usually reached at paraprotein concentrations of ~40 g/L (4 g/dL) for IgM, 50 g/L (5 g/dL) for IgG3, and 70 g/L (7 g/dL) for IgA.

Although neurologic symptoms occur in a minority of patients, they may have many causes. Hypercalcemia may produce lethargy, weakness, depression, and confusion. Hyperviscosity may lead to headache, fatigue, visual disturbances, and retinopathy. Bony damage and collapse may lead to cord compression, radicular pain, and loss of bowel and bladder control. Infiltration of peripheral nerves by amyloid can be a cause of carpal tunnel syndrome and other sensorimotor mono- and polyneuropathies. Sensory neuropathy is also a side effect of thalidomide and bortezomib therapy.

Many of the clinical features of myeloma, e.g., cord compression, pathologic fractures, hyperviscosity, sepsis, and hypercalcemia, can present as medical emergencies. Despite the widespread distribution of plasma cells in the body, tumor expansion is dominantly within bone and bone marrow and, for reasons unknown, rarely causes enlargement of spleen, lymph nodes, or gut-associated lymphatic tissue.

Diagnosis and Staging

The classic triad of myeloma is marrow plasmacytosis (>10%), lytic bone lesions, and a serum and/or urine M component. Bone marrow plasma cells are CD138+ and monoclonal. The most important differential diagnosis in patients with myeloma involves their separation from individuals with monoclonal gammopathies of uncertain significance (MGUS). MGUS are vastly more common than myeloma, occurring in 1% of the population over age 50 and in up to 10% individuals over age 75. The diagnostic criteria for MGUS, smoldering myeloma, and myeloma are described in Table 106-2. When bone marrow cells are exposed to radioactive thymidine in order to quantitate dividing cells, patients with MGUS always have a labeling index < 1%; patients with myeloma always have a labeling index > 1%. With long-term follow-up, ~1% per year of patients with MGUS go on to develop myeloma. Non-IgG subtype, abnormal kappa/lambda free light chain ratio, and serum M protein > 15 g/L (1.5 g/dL) are associated with higher incidence of progression of MGUS to myeloma. Typically, patients with MGUS require no therapy. Their survival is ~2 years shorter than age-matched controls without MGUS. There are two important variants of myeloma, solitary bone plasmacytoma and extramedullary plasmacytoma. These lesions are associated with an M component in <30% of the cases, they may affect younger individuals, and both are associated with median survivals of ≥10 years. Solitary bone plasmacytoma is a single lytic bone lesion without marrow plasmacytosis. Extramedullary plasmacytomas usually involve the submucosal lymphoid tissue of the nasopharynx or paranasal sinuses without marrow plasmacytosis. Both tumors are highly responsive to local radiation therapy. If an M component is present, it should disappear after treatment. Solitary bone plasmacytomas may recur in other bony sites or evolve into myeloma. Extramedullary plasmacytomas rarely recur or progress.

Table 106-2 Diagnostic Criteria for Multiple Myeloma, Myeloma Variants, and Monoclonal Gammopathy of Unknown Significance

Monoclonal gammopathy of undetermined significance (MGUS)
M protein in serum < 30 g/L
Bone marrow clonal plasma cells < 10%
No evidence of other B cell proliferative disorders
No myeloma-related organ or tissue impairment (no end organ damage, including bone lesions)[a]
Asymptomatic myeloma (smouldering myeloma)
M protein in serum ≥ 30 g/L *and/or*
Bone marrow clonal plasma cells ≥ 10%
No myeloma-related organ or tissue impairment (no end organ damage, including bone lesions)[a] or symptoms
Symptomatic multiple myeloma
M protein in serum and/or urine
Bone marrow (clonal) plasma cells[b] or plasmacytoma
Myeloma-related organ or tissue impairment (end organ damage, including bone lesions)
Nonsecretory myeloma
No M protein in serum and/or urine with immunofixation
Bone marrow clonal plasmacytosis ≥ 10% or plasmacytoma
Myeloma-related organ or tissue impairment (end organ damage, including bone lesions)[a]
Solitary plasmacytoma of bone
No M protein in serum and/or urine[c]
Single area of bone destruction due to clonal plasma cells

Bone marrow not consistent with multiple myeloma

Normal skeletal survey (and MRI of spine and pelvis if done)

No related organ or tissue impairment (no end organ damage other than solitary bone lesion)[a]

[a]Myeloma-related organ or tissue impairment (end organ damage) (ROTI): Calcium levels increased: serum calcium > 0.25 mmol/L above the upper limit of normal or > 2.75 mmol/L; renal insufficiency: creatinine > 173 mmol/L; anemia: hemoglobin 2 g/dL below the lower limit of normal or hemoglobin < 10 g/dL; bone lesions: lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify); other: symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (>2 episodes in 12 months).

[b]If flow cytometry is performed, most plasma cells (>90%) will show a "neoplastic" phenotype.

[c]A small M component may sometimes be present.

The clinical evaluation of patients with myeloma includes a careful physical examination searching for tender bones and masses. Only a small minority of patients has an enlargement of the spleen and lymph nodes, the physiologic sites of antibody production. Chest and bone radiographs may reveal lytic lesions or diffuse osteopenia. MRI offers a sensitive means to document extent of bone marrow infiltration and cord or root compression in patients with pain syndromes. A complete blood count with differential may reveal anemia. Erythrocyte sedimentation rate is elevated. Rare patients (~2%) may have plasma cell leukemia with >2000 plasma cells/μL. This may be seen in disproportionate frequency in IgD (12%) and IgE (25%) myelomas. Serum calcium, urea nitrogen, creatinine, and uric acid levels may be elevated. Protein electrophoresis and measurement of serum immunoglobulins and free light chains are useful for detecting and characterizing M spikes, supplemented by immunoelectrophoresis, which is especially sensitive for identifying low concentrations of M components not detectable by protein electrophoresis. A 24-h urine specimen is necessary to quantitate protein excretion, and a concentrated aliquot is used for electrophoresis and immunologic typing of any M component. Serum alkaline phosphatase is usually normal even with extensive bone involvement because of the absence of osteoblastic activity. It is also important to quantitate serum β_2-microglobulin (see below). Serum soluble IL-6 receptor levels and C-reactive protein may reflect physiologic IL-6 levels in the patient.

The serum M component will be IgG in 53% of patients, IgA in 25%, and IgD in 1%; 20% of patients will have only light chains in serum and urine. Dipsticks for detecting proteinuria are not reliable at identifying light chains, and the heat test for detecting Bence Jones protein is falsely negative in ~50% of patients with light chain myeloma. Fewer than 1% of patients have no identifiable M component; these patients usually have light chain myeloma in which renal catabolism has made the light chains undetectable in the urine. IgD myeloma may also present as light chain myeloma. About two-thirds of patients with serum M components also have urinary light chains. The light chain isotype may have an impact on survival. Patients secreting lambda light chains have a significantly shorter overall survival than those secreting kappa light chains. It is not clear whether this is due to some genetically important determinant of cell proliferation or because lambda light chains are more likely to cause renal damage and form amyloid than are kappa light chains. The heavy chain isotype may have an impact on patient management as well. About half of patients with IgM paraproteins develop hyperviscosity compared with only 2–4% of patients with IgA and IgG M components. Among IgG myelomas, it is the IgG3 subclass that has the highest tendency to form both concentration- and temperature-dependent aggregates, leading to hyperviscosity and cold agglutination at lower serum concentrations.

The various staging systems for patients with myeloma (Table 106-3) are functional systems for predicting survival and are based on a variety of clinical and laboratory tests, unlike the anatomic staging systems for solid tumors. The Durie-Salmon staging system is based on the hemoglobin, calcium, M component, and degree of skeletal involvement; the total-body tumor burden is estimated to be low (stage I), intermediate (stage II), or high (stage III), and the stages are further subdivided on the basis of renal function [A if serum creatinine <177 mol/L (<2 mg/dL), B if >177 (>2)]. Patients in stage IA have a median survival of >5 years and those in stage IIIB about 15 months. This staging system has been found not to predict prognosis after treatment with high-dose therapy or the novel targeted therapies that have emerged.

Table 106-3 Myeloma Staging Systems

Durie-Salmon Staging System

Stage	Criteria	Estimated Tumor Burden, $\times 10^{12}$ cells/m^2
I	All of the following: 1. Hemoglobin >100 g/L (>10 g/dL) 2. Serum calcium <3 mmol/L (<12 mg/dL) 3. Normal bone x-ray or solitary lesion 4. Low M-component production a. IgG level <50 g/L (<5 g/dL) b. IgA level <30 g/L (<3 g/dL) c. Urine light chain <4 g/24 h	<0.6 (low)
II	Fitting neither I nor III	0.6–1.20 (intermediate)

III	One or more of the following: 1. Hemoglobin <85 g/L (<8.5 g/dL) 2. Serum calcium >3 mmol/L (>12 mg/dL) 3. Advanced lytic bone lesions 4. High M-component production a. IgG level >70 g/L (>7 g/dL) b. IgA level >50 g/L (>5 g/dL) c. Urine light chains >12 g/24 h	>1.20 (high)
Level	**Stage**	**Median Survival, Months**
Subclassification based on serum creatinine levels		
A < 177 μmol/L (<2 mg/dL)	IA	61
B > 177 μmol/L (>2 mg/dL)	IIA, B	55
	IIIA	30
	IIIB	15
International Staging System		
β_2M < 3.5, alb ≥ 3.5	I (28%)	62
β_2M < 3.5, alb < 3.5 *or* β_2M = 3.5–5.5	II (39%)	44
β_2M > 5.5	III (33%)	29

Note: β_2M, serum β_2-microglobulin in mg/L; alb, serum albumin in g/dL; (#), % patients presenting at each stage.

Serum β_2-microglobulin is a protein of 11,000 mol wt with homologies with the constant region of immunoglobulins that is the light chain of the class I major histocompatibility antigens (HLA-A, -B, -C) on the surface of every cell. Serum β_2-microglobulin is the single most powerful predictor of survival and can substitute for staging. Patients with β_2-microglobulin levels <0.004 g/L have a median survival of 43 months and those with levels >0.004 g/L only 12 months. Serum β_2-microglobulin and albumin levels are the basis for a three-stage International Staging System (ISS). It is also felt that once the diagnosis of myeloma is firm, histologic features of atypia may also exert an influence on prognosis. IL-6 may be an autocrine and/or paracrine growth factor for myeloma cells; elevated levels are associated with more aggressive disease. High labeling index and high levels of lactate dehydrogenase are also associated with poor prognosis.

Other factors that may influence prognosis are the number of cytogenetic abnormalities including hyperploidy, chromosome 13q and 17p deletion, t(4;14) and t(11;14); % plasma cells in the marrow; circulating plasma cells; performance status; as well as serum levels of soluble IL-6 receptor, C-reactive protein, hepatocyte growth factor, C-terminal cross-linked telopeptide of collagen I, transforming growth factor (TGF) β, and syndecan-1. Microarray profiling and comparative genomic hybridization have formed the basis for RNA- and DNA-based prognostic staging systems, respectively. The ISS system is the most widely used method of assessing prognosis (Table 106-3).

Multiple Myeloma: Treatment

About 10% of patients with myeloma will have an indolent course demonstrating only very slow progression of disease over many years. Such patients only require antitumor therapy when the disease becomes symptomatic with development of anemia, hypercalcemia, progressive lytic bone lesions (including vertebral compression fractures), progressive rise in serum myeloma protein levels and/or Bence Jones proteinuria, or recurrent infections. Patients with solitary bone plasmacytomas and extramedullary plasmacytomas may be expected to enjoy prolonged disease-free survival after local radiation therapy to a dose of around 40 Gy. There is a low incidence of occult marrow involvement in patients with solitary bone plasmacytoma. Such patients are usually detected because their serum M component falls slowly or disappears initially only to return after a few months. These patients respond well to systemic chemotherapy.

Patients with symptomatic and/or progressive myeloma require therapeutic intervention. In general such therapy is of two sorts: systemic therapy to control the progression of myeloma, and symptomatic supportive care to prevent serious morbidity from the complications of the disease. Therapy can significantly prolong survival and improve the quality of life for myeloma patients.

The initial standard treatment for newly diagnosed myeloma is dependent on whether or not the patient is a candidate for high-dose chemotherapy with autologous stem cell transplant.

In patients who are transplant candidates, alkylating agents such as melphalan should be avoided since they damage stem cells, leading to decreased ability to collect stem cells for autologous transplant. High-dose pulsed glucocorticoids have been used either alone (dexamethasone 40 mg for 4 days every 2 weeks) or in combination VAD chemotherapy (vincristine, 0.4 mg/d in a 4-day continuous infusion; doxorubicin, 9 mg/m^2 per day in a 4-day continuous infusion; dexamethasone, 40 mg/d for 4 days per week for 3 weeks) for initial cytoreduction. However, two studies have combined thalidomide with dexamethasone as initial therapy for newly diagnosed multiple myeloma in transplant candidates and reported rapid responses in two-thirds of patients, while allowing for successful harvesting of peripheral blood stem cells for transplantation. A randomized phase III trial showed statistically significantly higher response rates for

thalidomide (200 mg PO qhs) plus dexamethasone (40 mg for 4 days every 2 weeks) compared to dexamethasone alone, setting the stage for use of this combination as standard therapy in newly diagnosed patients. Initial therapy is continued until maximal cytoreduction. Importantly, novel agents bortezomib, a proteasome inhibitor, and lenalidomide, an immunomodulatory derivative of thalidomide, have similarly been combined with dexamethasone and obtained high response rates without compromising collection of stem cells for transplantation.

In patients who are not transplant candidates, therapy has consisted of intermittent pulses of an alkylating agent, L-phenylalanine mustard (L-PAM, melphalan) and prednisone administered for 4–7 days every 4–6 weeks. The usual doses of melphalan/prednisone (MP) are melphalan, 8 mg/m^2 per day, and prednisone, 25–60 mg/m^2 per day for 4 days. Doses may need adjustment due to unpredictable absorption and based on marrow tolerance. Patients responding to therapy generally have a prompt and gratifying reduction in bone pain, hypercalcemia, and anemia, and often have fewer infections. The serum M component lags substantially behind the symptomatic improvement, often taking 4–6 weeks to fall. This fall depends on the rate of tumor kill and the fractional catabolic rate of immunoglobulin, which in turn depends on the serum concentration (for IgG). Light chain excretion, with a functional half-life of ~6 h, may fall within the first week of treatment. However, since urine light chain levels may relate to renal tubular function, they are not a reliable measure of tumor cell kill. Calculations of tumor cell kill are made by extrapolation of the serum M component level and rely heavily on the assumption that every tumor cell produces immunoglobulin at a constant rate. About 60% of patients will achieve at least a 75% reduction in serum M component level and tumor cell mass in response to melphalan and prednisone. Although this is a tumor reduction of <1 log, clinical responses may last many months. The important feature of the level of the M protein is not how far or how fast it falls, but the rate of its increase after therapy. Efforts to improve the fraction of patients responding and the degree of response have involved adding other active agents to the treatment program. In patients >65 years, combining thalidomide with MP (MPT) obtains higher response rates and overall survival than MP alone, and MPT is the standard therapy for patients who are not transplant candidates.

Randomized studies comparing standard-dose therapy to high-dose melphalan therapy (HDT) with hematopoietic stem cell support have shown that HDT can achieve high overall response rates and prolonged progression-free and overall survival; however, few, if any, patients are cured. Although complete responses are rare (<5%) with standard-dose chemotherapy, HDT achieves 25–40% complete responses. In randomized studies, HDT produced better median event-free survival in four of five studies, higher complete response rate in four of five trials, and better overall survival in three of five studies. Two successive HDTs (tandem transplants) are more effective than single HDT in the subset of patients who do not achieve a complete or very good partial response to the first transplant. Allogeneic transplants may also produce high response rates, but treatment-related mortality may be as high as 40%. Non-myeloablative allogeneic transplantation is now under evaluation to reduce toxicity, while permitting an immune graft-vs.-myeloma effect.

There is no standard maintenance therapy to prolong time to progression. IFN-α has allowed modest benefit but has significant side effects. Oral prednisone maintenance therapy was effective in a single trial. Ongoing studies are evaluating maintenance thalidomide and lenalidomide to prolong progression-free survival post-transplant.

Relapsed myeloma can be treated with novel agents including lenalidomide and/or bortezomib. These agents target not only the tumor cell but also the tumor cell–bone marrow interaction and the bone marrow milieu. These agents in combination with dexamethasone can achieve up to 60% partial responses and 10–15% complete responses in patients with relapsed disease. The combination of bortezomib and liposomal doxorubicin is active in relapsed myeloma. Thalidomide, if not used as initial therapy, can achieve responses in refractory cases. High-dose melphalan and stem cell transplant, if not used earlier, also have activity in patients with refractory disease.

The median overall survival of patients with myeloma is 5–6 years, with subsets of patients surviving over 10 years. The major causes of death are progressive myeloma, renal failure, sepsis, or therapy-related acute leukemia or myelodysplasia. Nearly a quarter of patients die of myocardial infarction, chronic lung disease, diabetes, or stroke, all intercurrent illnesses related more to the age of the patient group than to the tumor.

Supportive care directed at the anticipated complications of the disease may be as important as primary antitumor therapy. The hypercalcemia generally responds well to bisphosphonates, glucocorticoid therapy, hydration, and natriuresis. Calcitonin may add to the inhibitory effects of glucocorticoids on bone resorption. Bisphosphonates (e.g., pamidronate 90 mg or zoledronate 4 mg once a month) reduce osteoclastic bone resorption and preserve performance status and quality of life, decrease bone-related complications, and may also have antitumor effects. Treatments aimed at strengthening the skeleton, such as fluorides, calcium, and vitamin D, with or without androgens, have been suggested but are not of proven efficacy. Iatrogenic worsening of renal function may be prevented by maintaining a high fluid intake to prevent dehydration and to help excrete light chains and calcium. In the event of acute renal failure, plasmapheresis is ~10 times more effective at clearing light chains than peritoneal dialysis; however, its role in reversing renal failure remains controversial. Importantly, reducing the protein load by effective antitumor therapy with agents such as bortezomib may result in functional improvement. Urinary tract infections should be watched for and treated early. Plasmapheresis may be the treatment of choice for hyperviscosity syndromes. Although the pneumococcus is a dreaded pathogen in myeloma patients, pneumococcal polysaccharide vaccines may not elicit an antibody response. Prophylactic administration of IV γ-globulin preparations is used in the setting of recurrent serious infections. Chronic oral antibiotic prophylaxis is probably not warranted. Patients developing neurologic symptoms in the lower extremities, severe localized back pain, or problems with bowel and bladder control may need emergency MRI and radiation therapy for palliation. Most bone lesions respond to analgesics and chemotherapy, but certain painful lesions may respond most promptly to localized radiation. The anemia associated with myeloma may respond to erythropoietin along with hematinics (iron, folate, cobalamin). The pathogenesis of the anemia should be established and specific therapy instituted, where possible.

Waldenström's Macroglobulinemia

In 1948, Waldenström described a malignancy of lymphoplasmacytoid cells that secreted IgM. In contrast to myeloma, the disease was associated with lymphadenopathy and hepatosplenomegaly, but the major clinical manifestation was the hyperviscosity syndrome. The disease resembles the related diseases chronic lymphocytic leukemia, myeloma, and lymphocytic lymphoma. It originates from a post–germinal center B cell that has undergone somatic mutations and antigenic selection in the lymphoid follicle and has the characteristics of

an IgM-bearing memory B cell. Waldenström's macroglobulinemia and IgM myeloma follow a similar clinical course, but therapeutic options are different. The diagnosis of IgM myeloma is usually reserved for patients with lytic bone lesions and predominant infiltration with CD138+ plasma cells in the bone marrow. Such patients are at greater risk of pathologic fractures than patients with Waldenström's macroglobulinemia.

The cause of macroglobulinemia is unknown. The disease is similar to myeloma in being slightly more common in men and occurring with increased incidence with age (median 64 years). There have been reports that the IgM in some patients with macroglobulinemia may have specificity for myelin-associated glycoprotein (MAG), a protein that has been associated with demyelinating disease of the peripheral nervous system and may be lost earlier and to a greater extent than the better known myelin basic protein in patients with multiple sclerosis. Sometimes patients with macroglobulinemia develop a peripheral neuropathy before the appearance of the neoplasm. There is speculation that the whole process begins with a viral infection that may elicit an antibody response that cross-reacts with a normal tissue component.

Like myeloma, the disease involves the bone marrow, but unlike myeloma, it does not cause bone lesions or hypercalcemia. Like myeloma, a serum M component is present in the serum in excess of 30 g/L (3 g/dL), but unlike myeloma, the size of the IgM paraprotein results in little renal excretion, and only ~20% of patients excrete light chains. Therefore, renal disease is not common. The light chain isotype is kappa in 80% of the cases. Patients present with weakness, fatigue, and recurrent infections, similar to myeloma patients, but epistaxis, visual disturbances, and neurologic symptoms such as peripheral neuropathy, dizziness, headache, and transient paresis are much more common in macroglobulinemia. Physical examination reveals adenopathy and hepatosplenomegaly, and ophthalmoscopic examination may reveal vascular segmentation and dilatation of the retinal veins characteristic of hyperviscosity states. Patients may have a normocytic, normochromic anemia, but rouleaux formation and a positive Coombs' test are much more common than in myeloma. Malignant lymphocytes are usually present in the peripheral blood. About 10% of macroglobulins are cryoglobulins. These are pure M components and are not the mixed cryoglobulins seen in rheumatoid arthritis and other autoimmune diseases. Mixed cryoglobulins are composed of IgM or IgA complexed with IgG, for which they are specific. In both cases, Raynaud's phenomenon and serious vascular symptoms precipitated by the cold may occur, but mixed cryoglobulins are not commonly associated with malignancy. Patients suspected of having a cryoglobulin based on history and physical examination should have their blood drawn into a warm syringe and delivered to the laboratory in a container of warm water to avoid errors in quantitating the cryoglobulin.

Waldenström's Macroglobulinemia: Treatment

Control of serious hyperviscosity symptoms such as an altered state of consciousness or paresis can be achieved acutely by plasmapheresis because 80% of the IgM paraprotein is intravascular. The median survival is ~50 months, similar to that of multiple myeloma. However, many individuals with Waldenström's macroglobulinemia have indolent disease that does not require therapy. Pretreatment parameters including older age, male sex, general symptoms, and cytopenias define a high-risk population. Fludarabine (25 mg/m^2 per day for 5 days every 4 weeks) or cladribine (0.1 mg/kg per day for 7 days every 4 weeks) are highly effective single agents. About 80% of patients respond to chemotherapy, and their median survival is >3 years. Rituximab (anti-CD20) can produce responses alone or combined with chemotherapy. As in multiple myeloma, bortezomib and lenalidomide also have activity.

POEMS Syndrome

The features of this syndrome are *p*olyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *m*ultiple myeloma, and *s*kin changes (POEMS). Patients usually have a severe, progressive sensorimotor polyneuropathy associated with sclerotic bone lesions from myeloma. Polyneuropathy occurs in ~1.4% of myelomas, but the POEMS syndrome is only a rare subset of that group. Unlike typical myeloma, hepatomegaly and lymphadenopathy occur in about two-thirds of patients, and splenomegaly is seen in one-third. The lymphadenopathy frequently resembles Castleman's disease histologically, a condition that has been linked to IL-6 overproduction. The endocrine manifestations include amenorrhea in women and impotence and gynecomastia in men. Hyperprolactinemia due to loss of normal inhibitory control by the hypothalamus may be associated with other central nervous system manifestations such as papilledema and elevated cerebrospinal fluid pressure and protein. Type 2 diabetes mellitus occurs in about one-third of patients. Hypothyroidism and adrenal insufficiency are occasionally noted. Skin changes are diverse: hyperpigmentation, hypertrichosis, skin thickening, and digital clubbing. Other manifestations include peripheral edema, ascites, pleural effusions, fever, and thrombocytosis.

The pathogenesis of the disease is unclear, but high circulating levels of the proinflammatory cytokines IL-1, IL-6, VEGF, and TNF have been documented and levels of the inhibitory cytokine TGF-β are lower than expected. Treatment of the myeloma may result in an improvement in the other disease manifestations.

Patients are often treated similarly to those with myeloma. Plasmapheresis does not appear to be of benefit in POEMS syndrome. Patients presenting with isolated sclerotic lesions may have resolution of neuropathic symptoms after local therapy for plasmacytoma with radiotherapy. Similar to multiple myeloma, novel agents as well as high-dose therapy with autologous stem cell transplant have been pursued in selected patients and have been associated with prolonged progression-free survival.

Heavy Chain Diseases

The heavy chain diseases are rare lymphoplasmacytic malignancies. Their clinical manifestations vary with the heavy chain isotype. Patients secrete a defective heavy chain that usually has an intact Fc fragment and a deletion in the Fd region. Gamma, alpha, and mu heavy chain diseases have been described, but no reports of delta or epsilon heavy chain diseases have appeared. Molecular biologic analysis of these tumors has revealed structural genetic defects that may account for the aberrant chain secreted.

Gamma Heavy Chain Disease (Franklin's Disease)

This disease affects individuals of widely different age groups and countries of origin. It is characterized by lymphadenopathy, fever, anemia, malaise, hepatosplenomegaly, and weakness. Its most distinctive symptom is palatal edema, resulting from involvement of nodes

in Waldeyer's ring, and this may progress to produce respiratory compromise. The diagnosis depends on the demonstration of an anomalous serum M component [often <20 g/L (<2 g/dL)] that reacts with anti-IgG but not anti-light chain reagents. *The M component is typically present in both serum and urine*. Most of the paraproteins have been of the gamma$_1$ subclass, but other subclasses have been seen. The patients may have thrombocytopenia, eosinophilia, and nondiagnostic bone marrow. Patients usually have a rapid downhill course and die of infection; however, some patients have survived 5 years with chemotherapy.

Alpha Heavy Chain Disease (Seligmann's Disease)

This is the most common of the heavy chain diseases. It is closely related to a malignancy known as *Mediterranean lymphoma*, a disease that affects young persons in parts of the world where intestinal parasites are common, such as the Mediterranean, Asia, and South America. The disease is characterized by an infiltration of the lamina propria of the small intestine with lymphoplasmacytoid cells that secrete truncated alpha chains. Demonstrating alpha heavy chains is difficult because the alpha chains tend to polymerize and appear as a smear instead of a sharp peak on electrophoretic profiles. Despite the polymerization, hyperviscosity is not a common problem in alpha heavy chain disease. Without J chain–facilitated dimerization, viscosity does not increase dramatically. Light chains are absent from serum and urine. The patients present with chronic diarrhea, weight loss, and malabsorption and have extensive mesenteric and paraaortic adenopathy. Respiratory tract involvement occurs rarely. Patients may vary widely in their clinical course. Some may develop diffuse aggressive histologies of malignant lymphoma. Chemotherapy may produce long-term remissions. Rare patients appear to have responded to antibiotic therapy, raising the question of the etiologic role of antigenic stimulation, perhaps by some chronic intestinal infection. Chemotherapy plus antibiotics may be more effective than chemotherapy alone. Immunoproliferative small intestinal disease (IPSID) is recognized as an infectious pathogen–associated human lymphoma that has association with *Campylobacter jejuni*. It involves mainly the proximal small intestine resulting in malabsorption, diarrhea, and abdominal pain. IPSID is associated with excessive plasma cell differentiation and produces truncated alpha heavy chain proteins lacking the light chains as well as the first constant domain. Early-stage IPSID responds to antibiotics (30–70% complete remission). Most untreated IPSID patients progress to lymphoplasmacytic and immunoblastic lymphoma.

Mu Heavy Chain Disease

The secretion of isolated mu heavy chains into the serum appears to occur in a very rare subset of patients with chronic lymphocytic leukemia. The only features that may distinguish patients with mu heavy chain disease are the presence of vacuoles in the malignant lymphocytes and the excretion of kappa light chains in the urine. The diagnosis requires ultracentrifugation or gel filtration to confirm the nonreactivity of the paraprotein with the light chain reagents, because some intact macroglobulins fail to interact with these serums. The tumor cells seem to have a defect in the assembly of light and heavy chains, because they appear to contain both in their cytoplasm. There is no evidence that such patients should be treated differently from other patients with chronic lymphocytic leukemia (Chap. 105).
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Note: Large images and tables on this page may necessitate printing in landscape mode.

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Blood Group Antigens and Antibodies

The study of red blood cell (RBC) antigens and antibodies forms the foundation of transfusion medicine. Serologic studies initially characterized these antigens, but now the molecular composition and structure of many are known. Antigens, either carbohydrate or protein, are assigned to a blood group system based on the structure and similarity of the determinant epitopes. Other cellular blood elements and plasma proteins are also antigenic and can result in *alloimmunization*, the production of antibodies directed against the blood group antigens of another individual. These antibodies are called *alloantibodies*.

Antibodies directed against RBC antigens may result from "natural" exposure, particularly to carbohydrates that mimic some blood group antigens. Those antibodies that occur via natural stimuli are usually produced by a T cell–independent response (thus, generating no memory) and are IgM isotype. *Autoantibodies* (antibodies against autologous blood group antigens) arise spontaneously or as the result of

infectious sequelae (e.g., from *Mycoplasma pneumoniae*) and are also often IgM. These antibodies are often clinically insignificant due to their low affinity for antigen at body temperature. However, IgM antibodies can activate the complement cascade and result in hemolysis. Antibodies that result from allogeneic exposure, such as transfusion or pregnancy, are usually IgG. IgG antibodies commonly bind to antigen at warmer temperatures and may hemolyze RBCs. Unlike IgM antibodies, IgG antibodies can cross the placenta and bind fetal erythrocytes bearing the corresponding antigen, resulting in hemolytic disease of the newborn, or *hydrops fetalis*.

Alloimmunization to leukocytes, platelets, and plasma proteins may also result in transfusion complications such as fevers and urticaria but generally does not cause hemolysis. Assay for these other alloantibodies is not routinely performed; however, they may be detected using special assays.

ABO Antigens and Antibodies

The first blood group antigen system, recognized in 1900, was ABO, the most important in transfusion medicine. The major blood groups of this system are A, B, AB, and O. O type RBCs lack A or B antigens. These antigens are carbohydrates attached to a precursor backbone, may be found on the cellular membrane either as glycosphingolipids or glycoproteins, and are secreted into plasma and body fluids as glycoproteins. H substance is the immediate precursor on which the A and B antigens are added. This H substance is formed by the addition of fucose to the glycolipid or glycoprotein backbone. The subsequent addition of *N*-acetylgalactosamine creates the A antigen, while the addition of galactose produces the B antigen.

The genes that determine the A and B phenotypes are found on chromosome 9p and are expressed in a Mendelian codominant manner. The gene products are glycosyl transferases, which confer the enzymatic capability of attaching the specific antigenic carbohydrate. Individuals who lack the "A" and "B" transferases are phenotypically type "O," while those who inherit both transferases are type "AB." Rare individuals lack the H gene, which codes for fucose transferase, and cannot form H substance. These individuals are homozygous for the silent h allele (hh) and have Bombay phenotype (O_h).

The ABO blood group system is important because essentially all individuals produce antibodies to the ABH carbohydrate antigen that they lack. The naturally occurring anti-A and anti-B antibodies are termed *isoagglutinins*. Thus, type A individuals produce anti-B, while type B individuals make anti-A. Neither isoagglutinin is found in type AB individuals, while type O individuals produce both anti-A and anti-B. Thus, persons with type AB are "universal recipients" because they do not have antibodies against any ABO phenotype, while persons with type O blood can donate to essentially all recipients because their cells are not recognized by any ABO isoagglutinins. The rare individuals with Bombay phenotype produce antibodies to H substance (which is present on all red cells except those of hh phenotype) as well as to both A and B antigens and are therefore compatible only with other hh donors.

In most people, A and B antigens are secreted by the cells and are present in the circulation. Nonsecretors are susceptible to a variety of infections (e.g., *Candida albicans*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*) as many organisms may bind to polysaccharides on cells. Soluble blood group antigens may block this binding.

Rh System

The Rh system is the second most important blood group system in pretransfusion testing. The Rh antigens are found on a 30- to 32-kDa RBC membrane protein that has no defined function. Although >40 different antigens in the Rh system have been described, five determinants account for the vast majority of phenotypes. The presence of the D antigen confers Rh "positivity," while persons who lack the D antigen are Rh negative. Two allelic antigen pairs, E/e and C/c, are also found on the Rh protein. The three Rh genes, E/e, D, and C/c, are arranged in tandem on chromosome 1 and inherited as a haplotype, i.e., cDE or Cde. Two haplotypes can result in the phenotypic expression of two to five Rh antigens.

The D antigen is a potent alloantigen. About 15% of individuals lack this antigen. Exposure of these Rh-negative people to even small amounts of Rh-positive cells, by either transfusion or pregnancy, can result in the production of anti-D alloantibody.

Other Blood Group Systems and Alloantibodies

More than 100 blood group systems are recognized, composed of more than 500 antigens. The presence or absence of certain antigens has been associated with various diseases and anomalies; antigens also act as receptors for infectious agents. Alloantibodies of importance in routine clinical practice are listed in Table 107-1.

Table 107-1 RBC Blood Group Systems and Alloantigens

Blood Group System	Antigen	Alloantibody	Clinical Significance
Rh (D, C/c, E/e)	RBC protein	IgG	HTR, HDN
Lewis (Le^a, Le^b)	Oligosaccharide	IgM/IgG	Rare HTR
Kell (K/k)	RBC protein	IgG	HTR, HDN
Duffy (Fy^a/Fy^b)	RBC protein	IgG	HTR, HDN
Kidd (Jk^a/Jk^b)	RBC protein	IgG	HTR (often delayed), HDN (mild)
I/i	Carbohydrate	IgM	None

MNSsU	RBC protein	IgM/IgG	Anti-M rare HDN, anti-S, -s, and -U HDN, HTR

Note: RBC, red blood cell; HDN, hemolytic disease of the newborn; HTR, hemolytic transfusion reaction.

Antibodies to *Lewis system* carbohydrate antigens are the most common cause of incompatibility during pretransfusion screening. The Lewis gene product is a fucosyl transferase and maps to chromosome 19. The antigen is not an integral membrane structure but is adsorbed to the RBC membrane from the plasma. Antibodies to Lewis antigens are usually IgM and cannot cross the placenta. Lewis antigens may be adsorbed onto tumor cells and may be targets of therapy.

I system antigens are also oligosaccharides related to H, A, B, and Le. I and i are not allelic pairs but are carbohydrate antigens that differ only in the extent of branching. The i antigen is an unbranched chain that is converted by the I gene product, a glycosyltransferase, into a branched chain. The branching process affects all the ABH antigens, which become progressively more branched in the first 2 years of life. Some patients with cold agglutinin disease or lymphomas can produce anti-I autoantibodies that cause RBC destruction. Occasional patients with mononucleosis or *Mycoplasma* pneumonia may develop cold agglutinins of either anti-I or anti-i specificity. Most adults lack i expression; thus, finding a donor for patients with anti-i is not difficult. Even though most adults express I antigen, binding is generally low at body temperature. Thus, administration of warm blood prevents isoagglutination.

The *P system* is another group of carbohydrate antigens controlled by specific glycosyltransferases. Its clinical significance is in rare cases of syphilis and viral infection that lead to paroxysmal cold hemoglobinuria. In these cases, an unusual autoantibody to P is produced that binds to RBCs in the cold and fixes complement upon warming. Antibodies with these biphasic properties are called *Donath-Landsteiner antibodies*. The P antigen is the cellular receptor of parvovirus B19 and also may be a receptor for *Escherichia coli* binding to urothelial cells.

The *MNSsU system* is regulated by genes on chromosome 4. M and N are determinants on glycophorin A, an RBC membrane protein, and S and s are determinants on glycophorin B. Anti-S and anti-s IgG antibodies may develop after pregnancy or transfusion and lead to hemolysis. Anti-U antibodies are rare but problematic; virtually every donor is incompatible because nearly all persons express U.

The *Kell* protein is very large (720 amino acids), and its secondary structure contains many different antigenic epitopes. The immunogenicity of Kell is third behind the ABO and Rh systems. The absence of the Kell precursor protein (controlled by a gene on X) is associated with acanthocytosis, shortened RBC survival, and a progressive form of muscular dystrophy that includes cardiac defects. This rare condition is called the *McLeod phenotype*. The K_x gene is linked to the 91-kDa component of the NADPH-oxidase on the X chromosome, deletion or mutation of which accounts for about 60% of cases of chronic granulomatous disease.

The *Duffy* antigens are codominant alleles, Fy^a and Fy^b, that also serve as receptors for *Plasmodium vivax*. More than 70% of persons in malaria-endemic areas lack these antigens, probably from selective influences of the infection on the population.

The *Kidd* antigens, Jk^a and Jk^b, may elicit antibodies transiently. A delayed hemolytic transfusion reaction that occurs with blood tested as compatible is often related to delayed appearance of anti-Jk^a.

Pretransfusion Testing

Pretransfusion testing of a potential recipient consists of the "type and screen." The "forward type" determines the ABO and Rh phenotype of the recipient's RBC by using antisera directed against the A, B, and D antigens. The "reverse type" detects isoagglutinins in the patient's serum and should correlate with the ABO phenotype, or forward type.

The alloantibody screen identifies antibodies directed against other RBC antigens. The alloantibody screen is performed by mixing patient serum with type O RBCs that contain the major antigens of most blood group systems and whose extended phenotype is known. The specificity of the alloantibody is identified by correlating the presence or absence of antigen with the results of the agglutination.

Cross-matching is ordered when there is a high probability that the patient will require a packed RBC (PRBC) transfusion. Blood selected for cross-matching must be ABO compatible and lack antigens for which the patient has alloantibodies. Nonreactive cross-matching confirms the absence of any major incompatibility and reserves that unit for the patient.

In the case of Rh-negative patients, every attempt must be made to provide Rh-negative blood components to prevent alloimmunization to the D antigen. In an emergency, Rh-positive blood can be safely transfused to an Rh-negative patient who lacks anti-D; however, the recipient is likely to become alloimmunized and produce anti-D. Rh-negative women of childbearing age who are transfused with products containing Rh-positive RBCs should receive passive immunization with anti-D (RhoGam or WinRho) to reduce or prevent sensitization.

Blood Components

Blood products intended for transfusion are routinely collected as whole blood (450 mL) in various anticoagulants. Most donated blood is processed into components: PRBCs, platelets, and fresh-frozen plasma (FFP) or cryoprecipitate (Table 107-2). Whole blood is first separated into PRBCs and platelet-rich plasma by slow centrifugation. The platelet-rich plasma is then centrifuged at high speed to yield one unit of random donor (RD) platelets and one unit of FFP. Cryoprecipitate is produced by thawing FFP to precipitate the plasma proteins, then separated by centrifugation.

Table 107-2 Characteristics of Selected Blood Components

Component	Volume, mL	Content	Clinical Response
PRBC	180–200	RBCs with variable leukocyte content and small amount of plasma	Increase hemoglobin 10 g/L and hematocrit 3%
Platelets	50–70	5.5×10^{10}/RD unit	Increase platelet count 5000–10,000/μL
	200–400	≥3.0×10^{11}/SDAP product	CCI ≥ 10×10^9/L within 1 h and ≥7.5×10^9/L within 24 h posttransfusion
FFP	200–250	Plasma proteins- coagulation factors, proteins C and S, antithrombin	Increases coagulation factors about 2%
Cryoprecipitate	10–15	Cold-insoluble plasma proteins, fibrinogen, factor VIII, vWF	Topical fibrin glue, also 80 IU factor VIII

Note: PRBC, packed red blood cells; RBC, red blood cell; RD, random donor; SDAP, single-donor apheresis platelets; CCI, corrected count increment; FFP, fresh-frozen plasma; vWF, von Willebrand factor.

Apheresis technology is used for the collection of multiple units of platelets from a single donor. These single-donor apheresis platelets (SDAP) contain the equivalent of at least six units of RD platelets and have fewer contaminating leukocytes than pooled RD platelets.

Plasma may also be collected by apheresis. Plasma derivatives such as albumin, intravenous immunoglobulin, antithrombin, and coagulation factor concentrates are prepared from pooled plasma from many donors and are treated to eliminate infectious agents.

Whole Blood

Whole blood provides both oxygen-carrying capacity and volume expansion. It is the ideal component for patients who have sustained acute hemorrhage of ≥25% total blood volume loss. Whole blood is stored at 4°C to maintain erythrocyte viability, but platelet dysfunction and degradation of some coagulation factors occurs. In addition, 2,3-bisphosphoglycerate levels fall over time, leading to an increase in the oxygen affinity of the hemoglobin and a decreased capacity to deliver oxygen to the tissues, a problem with all red cell storage. Whole blood is not readily available since it is routinely processed into components.

Packed Red Blood Cells

This product increases oxygen-carrying capacity in the anemic patient. Adequate oxygenation can be maintained with a hemoglobin content of 70 g/L in the normovolemic patient without cardiac disease; however, comorbid factors often necessitate transfusion at a higher threshold. The decision to transfuse should be guided by the clinical situation and not by an arbitrary laboratory value. In the critical care setting, liberal use of transfusions to maintain near-normal levels of hemoglobin may have unexpected negative effects on survival. In most patients requiring transfusion, levels of hemoglobin of 100 g/L are sufficient to keep oxygen supply from being critically low.

PRBCs may be modified to prevent certain adverse reactions. Leukocyte reduction of cellular blood products is increasingly common, and universal prestorage leukocyte reduction has been recommended. Prestorage filtration appears superior to bedside filtration as smaller amounts of cytokines are generated in the stored product. These PRBC units contain $<5 \times 10^6$ donor white blood cells (WBCs), and their use lowers the incidence of posttransfusion fever, cytomegalovirus (CMV) infections, and alloimmunization. Other theoretical benefits include less immunosuppression in the recipient and lower risk of infections. Plasma, which may cause allergic reactions, can be removed from cellular blood components by washing.

Platelets

Thrombocytopenia is a risk factor for hemorrhage, and platelet transfusion reduces the incidence of bleeding. The threshold for prophylactic platelet transfusion is 10,000/μL. In patients without fever or infections, a threshold of 5000/μL may be sufficient to prevent spontaneous hemorrhage. For invasive procedures, 50,000/μL platelets is the usual target level.

Platelets are given either as pools prepared from five to eight RDs or as SDAPs from a single donor. In an unsensitized patient without increased platelet consumption [splenomegaly, fever, disseminated intravascular coagulation (DIC)], six to eight units of RD platelets (about 1 unit per 10 kg body weight) are transfused, and each unit is anticipated to increase the platelet count 5000–10,000/μL. Patients who have received multiple transfusions may be alloimmunized to many HLA- and platelet-specific antigens and have little or no increase in their posttransfusion platelet counts. Patients who may require multiple transfusions are best served by receiving SDAP and leukocyte-reduced components to lower the risk of alloimmunization.

Refractoriness to platelet transfusion may be evaluated using the corrected count increment (CCI):

$$\text{CCI} = \frac{\text{posttransfusion count} - \text{pretransfusion count}}{\text{number of platelets transfused} \times 10^{11}} \times \text{BSA}$$

where BSA is body surface area measured in square meters. The platelet count performed 1 h after the transfusion is acceptable if the CCI is 10×10^9/mL, and after 18–24 h an increment of 7.5×10^9/mL is expected. Patients who have suboptimal responses are likely to have

received multiple transfusions and have antibodies directed against class I HLA antigens. Refractoriness can be investigated by detecting anti-HLA antibodies in the recipient's serum. Patients who are sensitized will often react with 100% of the lymphocytes used for the HLA-antibody screen, and HLA-matched SDAPs should be considered for those patients who require transfusion. Although ABO-identical HLA-matched SDAPs provide the best chance for increasing the platelet count, locating these products is difficult. Platelet cross-matching is available in some centers. Additional clinical causes for a low platelet CCI include fever, bleeding, splenomegaly, DIC, or medications in the recipient.

Fresh-Frozen Plasma

FFP contains stable coagulation factors and plasma proteins: fibrinogen, antithrombin, albumin, as well as proteins C and S. Indications for FFP include correction of coagulopathies, including the rapid reversal of warfarin; supplying deficient plasma proteins; and treatment of thrombotic thrombocytopenic purpura. FFP should not be routinely used to expand blood volume. FFP is an acellular component and does not transmit intracellular infections, e.g., CMV. Patients who are IgA-deficient and require plasma support should receive FFP from IgA-deficient donors to prevent anaphylaxis (see below).

Cryoprecipitate

Cryoprecipitate is a source of fibrinogen, factor VIII, and von Willebrand factor (vWF). It is ideal for supplying fibrinogen to the volume-sensitive patient. When factor VIII concentrates are not available, cryoprecipitate may be used since each unit contains approximately 80 units of factor VIII. Cryoprecipitate may also supply vWF to patients with dysfunctional (type II) or absent (type III) von Willebrand disease.

Plasma Derivatives

Plasma from thousands of donors may be pooled to derive specific protein concentrates, including albumin, intravenous immunoglobulin, antithrombin, and coagulation factors. In addition, donors who have high-titer antibodies to specific agents or antigens provide hyperimmune globulins, such as anti-D (RhoGam, WinRho), and antisera to hepatitis B virus (HBV), varicella-zoster virus, CMV, and other infectious agents.

Adverse Reactions to Blood Transfusion

Adverse reactions to transfused blood components occur despite multiple tests, inspections, and checks. Fortunately, the most common reactions are not life-threatening, although serious reactions can present with mild symptoms and signs. Some reactions can be reduced or prevented by modified (filtered, washed, or irradiated) blood components. When an adverse reaction is suspected, the transfusion should be stopped and reported to the blood bank for investigation.

Transfusion reactions may result from immune and nonimmune mechanisms. Immune-mediated reactions are often due to preformed donor or recipient antibody; however, cellular elements may also cause adverse effects. Nonimmune causes of reactions are due to the chemical and physical properties of the stored blood component and its additives.

Transfusion-transmitted viral infections are increasingly rare due to improved screening and testing. As the risk of viral infection is reduced, the relative risk of other reactions increases, such as hemolytic transfusion reactions and sepsis from bacterially contaminated components. More effort is being directed at improving pretransfusion quality assurance to further increase the safety of transfusion therapy. Infections, like any adverse transfusion reaction, must be brought to the attention of the blood bank for appropriate studies (Table 107-3).

Table 107-3 Risks of Transfusion Complications

	Frequency, Episodes:Unit
Reactions	
Febrile (FNHTR)	1–4:100
Allergic	1–4:100
Delayed hemolytic	1:1000
TRALI	1:5000
Acute hemolytic	1:12,000
Fatal hemolytic	1:100,000
Anaphylactic	1:150,000
Infections[a]	
Hepatitis B	1:63,000

Hepatitis C	1:1,600,000
HIV-1	1:1,960,000
HIV-2	None reported
HTLV-I and -II	1:641,000
Malaria	1:4,000,000
Other complications	
RBC allosensitization	1:100
HLA allosensitization	1:10
Graft-versus-host disease	Rare

[a]Infectious agents rarely associated with transfusion, theoretically possible or of unknown risk include: Hepatitis A virus, parvovirus B-19, *Babesia microti* (babesiosis), *Borrelia burgdorferi* (Lyme disease), *Trypanosoma cruzi* (Chagas disease), and *Treponema pallidum* , human herpesvirus-8 and hepatitis G virus.

Note: FNHTR, febrile nonhemolytic transfusion reaction; TRALI, transfusion-related acute lung injury; HTLV, human T lymphotropic virus; RBC, red blood cell.

Immune-Mediated Reactions

Acute Hemolytic Transfusion Reactions

Immune-mediated hemolysis occurs when the recipient has preformed antibodies that lyse donor erythrocytes. The ABO isoagglutinins are responsible for the majority of these reactions, although alloantibodies directed against other RBC antigens, i.e., Rh, Kell, and Duffy, may result in hemolysis.

Acute hemolytic reactions may present with hypotension, tachypnea, tachycardia, fever, chills, hemoglobinemia, hemoglobinuria, chest and/or flank pain, and discomfort at the infusion site. Monitoring the patient's vital signs before and during the transfusion is important to identify reactions promptly. When acute hemolysis is suspected, the transfusion must be stopped immediately, intravenous access maintained, and the reaction reported to the blood bank. A correctly labeled posttransfusion blood sample and any untransfused blood should be sent to the blood bank for analysis. The laboratory evaluation for hemolysis includes the measurement of serum haptoglobin, lactate dehydrogenase (LDH), and indirect bilirubin levels.

The immune complexes that result in RBC lysis can cause renal dysfunction and failure. Diuresis should be induced with intravenous fluids and furosemide or mannitol. Tissue factor released from the lysed erythrocytes may initiate DIC. Coagulation studies including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and platelet count should be monitored in patients with hemolytic reactions.

Errors at the patient's bedside, such as mislabeling the sample or transfusing the wrong patient, are responsible for the majority of these reactions. The blood bank investigation of these reactions includes examination of the pre- and posttransfusion samples for hemolysis and repeat typing of the patient samples; direct antiglobulin test (DAT), sometimes called the *direct Coombs test*, of the posttransfusion sample; repeating the cross-matching of the blood component; and checking all clerical records for errors. DAT detects the presence of antibody or complement bound to RBCs in vivo.

Delayed Hemolytic and Serologic Transfusion Reactions

Delayed hemolytic transfusion reactions (DHTRs) are not completely preventable. These reactions occur in patients previously sensitized to RBC alloantigens who have a negative alloantibody screen due to low antibody levels. When the patient is transfused with antigen-positive blood, an anamnestic response results in the early production of alloantibody that binds donor RBCs. The alloantibody is detectable 1–2 weeks following the transfusion, and the posttransfusion DAT may become positive due to circulating donor RBCs coated with antibody or complement. The transfused, alloantibody-coated erythrocytes are cleared by the reticuloendothelial system. These reactions are detected most commonly in the blood bank when a subsequent patient sample reveals a positive alloantibody screen or a new alloantibody in a recently transfused recipient.

No specific therapy is usually required, although additional RBC transfusions may be necessary. Delayed serologic transfusion reactions are similar to DHTR, as the DAT is positive and alloantibody is detected; however, RBC clearance is not increased.

Febrile Nonhemolytic Transfusion Reaction

The most frequent reaction associated with the transfusion of cellular blood components is a febrile nonhemolytic transfusion reaction (FNHTR). These reactions are characterized by chills and rigors and a ≥1°C rise in temperature. FNHTR is diagnosed when other causes of fever in the transfused patient are ruled out. Antibodies directed against donor leukocyte and HLA antigens may mediate these reactions;

thus, multiply transfused patients and multiparous women are felt to be at increased risk. Although antibodies may be demonstrated in the recipient's serum, investigation is not routinely done because of the mild nature of most FNHTR. The use of leukocyte-reduced blood products may prevent or delay sensitization to leukocyte antigens and thereby reduce the incidence of these febrile episodes. Cytokines released from cells within stored blood components may mediate FNHTR; thus, leukoreduction before storage may prevent these reactions. The incidence and severity of these reactions can be decreased in patients with recurrent reactions by premedicating with acetaminophen or other antipyretic agents.

Allergic Reactions

Urticarial reactions are related to plasma proteins found in transfused components. Mild reactions may be treated symptomatically by temporarily stopping the transfusion and administering antihistamines (diphenhydramine, 50 mg orally or intramuscularly). The transfusion may be completed after the signs and/or symptoms resolve. Patients with a history of allergic transfusion reaction should be premedicated with an antihistamine. Cellular components can be washed to remove residual plasma for the extremely sensitized patient.

Anaphylactic Reaction

This severe reaction presents after transfusion of only a few milliliters of the blood component. Symptoms and signs include difficulty breathing, coughing, nausea and vomiting, hypotension, bronchospasm, loss of consciousness, respiratory arrest, and shock. Treatment includes stopping the transfusion, maintaining vascular access, and administering epinephrine (0.5–1.0 mL of 1:1000 dilution subcutaneously). Glucocorticoids may be required in severe cases.

Patients who are IgA-deficient, <1% of the population, may be sensitized to this Ig class and are at risk for anaphylactic reactions associated with plasma transfusion. Individuals with severe IgA deficiency should therefore receive only IgA-deficient plasma and washed cellular blood components. Patients who have anaphylactic or repeated allergic reactions to blood components should be tested for IgA deficiency.

Graft-versus-Host Disease

Graft-versus-host disease (GVHD) is a frequent complication of allogeneic stem cell transplantation, in which lymphocytes from the donor attack and cannot be eliminated by an immunodeficient host. Transfusion-related GVHD is mediated by donor T lymphocytes that recognize host HLA antigens as foreign and mount an immune response, which is manifested clinically by the development of fever, a characteristic cutaneous eruption, diarrhea, and liver function abnormalities. GVHD can also occur when blood components that contain viable T lymphocytes are transfused to immunodeficient recipients or to immunocompetent recipients who share HLA antigens with the donor (e.g., a family donor). In addition to the aforementioned clinical features of GVHD, transfusion-associated GVHD (TA-GVHD) is characterized by marrow aplasia and pancytopenia. TA-GVHD is highly resistant to treatment with immunosuppressive therapies, including glucocorticoids, cyclosporine, antithymocyte globulin, and ablative therapy followed by allogeneic bone marrow transplantation. Clinical manifestations appear at 8–10 days, and death occurs at 3–4 weeks posttransfusion.

TA-GVHD can be prevented by irradiation of cellular components (minimum of 2500 cGy) before transfusion to patients at risk. Patients at risk for TA-GVHD include fetuses receiving intrauterine transfusions, selected immunocompetent (e.g., lymphoma patients) or immunocompromised recipients, recipients of donor units known to be from a blood relative, and recipients who have undergone marrow transplantation. Directed donations by family members should be discouraged (they are not less likely to transmit infection); lacking other options, the blood products from family members should always be irradiated.

Transfusion-Related Acute Lung Injury

This uncommon reaction results from the transfusion of donor plasma that contains high-titer anti-HLA antibodies that bind recipient leukocytes. The leukocytes aggregate in the pulmonary vasculature and release mediators that increase capillary permeability. The recipient develops symptoms of respiratory compromise and signs of noncardiogenic pulmonary edema, including bilateral interstitial infiltrates on chest x-ray. Treatment is supportive, and patients usually recover without sequelae. Testing the donor's plasma for anti-HLA antibodies can support this diagnosis. The implicated donors are frequently multiparous women, and transfusion of their plasma component should be avoided.

Posttransfusion Purpura

This reaction presents as thrombocytopenia 7–10 days after platelet transfusion and occurs predominantly in women. Platelet-specific antibodies are found in the recipient's serum, and the most frequently recognized antigen is HPA-1a found on the platelet glycoprotein IIIa receptor. The delayed thrombocytopenia is due to the production of antibodies that react to both donor and recipient platelets. Additional platelet transfusions can worsen the thrombocytopenia and should be avoided. Treatment with intravenous immunoglobulin may neutralize the effector antibodies, or plasmapheresis can be used to remove the antibodies.

Alloimmunization

A recipient may become alloimmunized to a number of antigens on cellular blood elements and plasma proteins. Alloantibodies to RBC antigens are detected during pretransfusion testing, and their presence may delay finding antigen-negative cross-match-compatible products for transfusion. Women of childbearing age who are sensitized to certain RBC antigens (i.e., D, c, E, Kell, or Duffy) are at risk for bearing a fetus with hemolytic disease of the newborn. Matching for D antigen is the only pretransfusion selection test to prevent RBC alloimmunization.

Alloimmunization to antigens on leukocytes and platelets can result in refractoriness to platelet transfusions. Once alloimmunization has developed, HLA-compatible platelets from donors who share similar antigens with the recipient may be difficult to find. Hence, prudent transfusion practice is directed at preventing sensitization through the use of leukocyte-reduced cellular components, as well as limiting antigenic exposure by the judicious use of transfusions and use of SDAPs.

Nonimmunologic Reactions

Fluid Overload

Blood components are excellent volume expanders, and transfusion may quickly lead to volume overload. Monitoring the rate and volume of the transfusion and using a diuretic can minimize this problem.

Hypothermia

Refrigerated (4°C) or frozen (–18°C or below) blood components can result in hypothermia when rapidly infused. Cardiac dysrhythmias can result from exposing the sinoatrial node to cold fluid. Use of an in-line warmer will prevent this complication.

Electrolyte Toxicity

RBC leakage during storage increases the concentration of potassium in the unit. Neonates and patients in renal failure are at risk for hyperkalemia. Preventive measures, such as using fresh or washed RBCs, are warranted for neonatal transfusions because this complication can be fatal.

Citrate, commonly used to anticoagulate blood components, chelates calcium and thereby inhibits the coagulation cascade. Hypocalcemia, manifested by circumoral numbness and/or tingling sensation of the fingers and toes, may result from multiple rapid transfusions. Because citrate is quickly metabolized to bicarbonate, calcium infusion is seldom required in this setting. If calcium or any other intravenous infusion is necessary, it must be given through a separate line.

Iron Overload

Each unit of RBCs contains 200–250 mg of iron. Symptoms and signs of iron overload affecting endocrine, hepatic, and cardiac function are common after 100 units of RBCs have been transfused (total-body iron load of 20 g). Preventing this complication by using alternative therapies (e.g., erythropoietin) and judicious transfusion is preferable and cost effective. Deferoxamine and other chelating agents are available, but the response is often suboptimal.

Hypotensive Reactions

Transient hypotension may be noted among transfused patients who take angiotensin-converting enzyme (ACE) inhibitors. Since blood products contain bradykinin that is normally degraded by ACE, patients on ACE inhibitors may have increased bradykinin levels that cause hypotension. The blood pressure typically returns to normal without intervention.

Immunomodulation

Transfusion of allogeneic blood is immunosuppressive. Multiply transfused renal transplant recipients are less likely to reject the graft, and transfusion may result in poorer outcomes in cancer patients and increase the risk of infections. Transfusion-related immunomodulation is thought to be mediated by transfused leukocytes. Leukocyte-depleted cellular products may cause less immunosuppression, though controlled data have not been obtained and are unlikely to be obtained as the blood supply becomes universally leukocyte-depleted.

Infectious Complications

Nucleic acid amplification testing (NAT) began in 1999 to screen donated blood for the presence of HIV and hepatitis C virus (HCV) RNA. Since 2003 NAT has been used to detect West Nile virus (WNV) RNA in donated blood.

Viral Infections

Hepatitis C Virus

Blood donations are tested for antibodies to HCV and HCV RNA. Fewer than 200 HCV RNA-positive, antibody-negative donors have been found. The risk of acquiring HCV through transfusion is now calculated to be approximately 1 in 2,000,000 units. Infection with HCV may be asymptomatic or lead to chronic active hepatitis, cirrhosis, and liver failure.

Human Immunodeficiency Virus Type 1

Donated blood is tested for antibodies to HIV-1, HIV-1 p24 antigen, and HIV RNA using NAT. Approximately a dozen seronegative donors have been shown to harbor HIV RNA. The risk of HIV-1 infection per transfusion episode is 1 in 2 million. Antibodies to HIV-2 are also measured in donated blood. No cases of HIV-2 infection have been reported in the United States since 1992.

Hepatitis B Virus

Donated blood is screened for HBV using assays for hepatitis B surface antigen (HbsAg). NAT testing is not practical because of slow viral replication and lower levels of viremia. The risk of transfusion-associated HBV infection is 1 in 63,000 units, twentyfold greater than for HCV. Vaccination of individuals who require long-term transfusion therapy can prevent this complication.

Other Hepatitis Viruses

Hepatitis A virus is rarely transmitted by transfusion; infection is typically asymptomatic and does not lead to chronic disease. Other transfusion-transmitted viruses– TTV, SEN-V, and GBV-C– do not cause chronic hepatitis or other disease states. Routine testing does not appear to be warranted.

West Nile Virus

Transfusion-transmitted WNV infections were documented in 2002. This RNA virus can be detected using NAT; routine screening began in 2003, and more than 1000 blood donors have tested positive. WNV infections range in severity from asymptomatic to fatal, with the older population at greater risk.

Cytomegalovirus

This ubiquitous virus infects ≥50% of the general population and is transmitted by the infected "passenger" WBCs found in transfused PRBCs or platelet components. Cellular components that are leukocyte-reduced have a decreased risk of transmitting CMV, regardless of the serologic status of the donor. Groups at risk for CMV infections include immunosuppressed patients, CMV-seronegative transplant recipients, and neonates; these patients should receive leukocyte-depleted components or CMV seronegative products.

Human T Lymphotropic Virus (HTLV) Type I

Assays to detect HTLV-I and -II are used to screen all donated blood. HTLV-I is associated with adult T cell leukemia/lymphoma and tropical spastic paraparesis in a small percentage of infected persons (Chap. 181). The risk of HTLV-I infection via transfusion is 1 in 641,000 transfusion episodes. HTLV-II is not clearly associated with any disease.

Parvovirus B-19

Blood components and pooled plasma products can transmit this virus, the etiologic agent of erythema infectiosum, or fifth disease, in children. Parvovirus B-19 shows tropism for erythroid precursors and inhibits both erythrocyte production and maturation. Pure red cell aplasia, presenting either as acute aplastic crisis or chronic anemia with shortened RBC survival, may occur in individuals with an underlying hematologic disease, such as sickle cell disease or thalassemia (Chap. 102). The fetus of a seronegative woman is at risk for developing hydrops from this virus.

Bacterial Contamination

The relative risk of transfusion-transmitted bacterial infection has increased as the absolute risk of viral infections has dramatically decreased.

Most bacteria do not grow well at cold temperatures; thus, PRBCs and FFP are not common sources of bacterial contamination. However, some gram-negative bacteria can grow at 1° to 6°C. *Yersinia, Pseudomonas, Serratia, Acinetobacter* and *Escherichia* species have all been implicated in infections related to PRBC transfusion. Platelet concentrates, which are stored at room temperature, are more likely to contain skin contaminants such as gram-positive organisms, including coagulase-negative staphylococci. It is estimated that 1 in 1000–2000 platelet components is contaminated with bacteria. The risk of death due to transfusion-associated sepsis has been calculated at 1 in 17,000 for single-unit platelets derived from whole blood donation and 1 in 61,000 for apheresis product. Since 2004, blood banks have instituted methods to detect contaminated platelet components.

Recipients of transfusion contaminated with bacteria may develop fever and chills, which can progress to septic shock and DIC. These reactions may occur abruptly, within minutes of initiating the transfusion, or after several hours. The onset of symptoms and signs is often sudden and fulminant, which distinguishes bacterial contamination from an FNHTR. The reactions, particularly those related to gram-negative contaminants, are the result of infused endotoxins formed within the contaminated stored component.

When these reactions are suspected, the transfusion must be stopped immediately. Therapy is directed at reversing any signs of shock, and broad-spectrum antibiotics should be given. The blood bank should be notified to identify any clerical or serologic error. The blood component bag should be sent for culture and Gram stain.

Other Infectious Agents

Various parasites, including those causing malaria, babesiosis, and Chagas disease, can be transmitted by blood transfusion. Geographic migration and travel of donors shift the incidence of these rare infections. Other agents implicated in transfusion transmission include Lyme disease and variant Creutzfeldt-Jakob disease. These infections should be considered in the transfused patient in the appropriate clinical setting.

Alternatives to Transfusion

Alternatives to allogeneic blood transfusions that avoid homologous donor exposures with attendant immunologic and infectious risks remain attractive. Autologous blood is the best option when transfusion is anticipated. However, the cost:benefit ratio of autologous

transfusion remains high. No transfusion is a zero-risk event; clerical errors and bacterial contamination remain potential complications even with autologous transfusions. Additional methods of autologous transfusion in the surgical patient include preoperative hemodilution, recovery of shed blood from sterile surgical sites, and postoperative drainage collection. Directed or designated donation from friends and family of the potential recipient has not been safer than volunteer donor component transfusions. Such directed donations may in fact place the recipient at higher risk for complications such as GVHD and alloimmunization.

Granulocyte and granulocyte-macrophage colony-stimulating factor are clinically useful to hasten leukocyte recovery in patients with leukopenia related to high-dose chemotherapy. Erythropoietin stimulates erythrocyte production in patients with anemia of chronic renal failure and other conditions, thus avoiding or reducing the need for transfusion. This hormone can also stimulate erythropoiesis in the autologous donor to enable additional donation.

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Hematopoietic Cell Transplantation: Introduction

Bone marrow transplantation was the original term used to describe the collection and transplantation of hematopoietic stem cells, but with the demonstration that the peripheral blood and umbilical cord blood are also useful sources of stem cells, *hematopoietic cell transplantation* has become the preferred generic term for this process. The procedure is usually carried out for one of two purposes: (1) to replace an abnormal but nonmalignant lymphohematopoietic system with one from a normal donor, or (2) to treat malignancy by allowing the administration of higher doses of myelosuppressive therapy than would otherwise be possible. The use of hematopoietic cell transplantation has been increasing, both because of its efficacy in selected diseases and because of increasing availability of donors. The International Bone Marrow Transplant Registry (http://www.ibmtr.org) estimates that about 50,000 transplants are performed each year.

The Hematopoietic Stem Cell

Several features of the hematopoietic stem cell make transplantation clinically feasible, including its remarkable regenerative capacity, its ability to home to the marrow space following intravenous injection, and the ability of the stem cell to be cryopreserved. Transplantation of a single stem cell can replace the entire lymphohematopoietic system of an adult mouse. In humans, transplantation of a few percent of a donor's bone marrow volume regularly results in complete and sustained replacement of the recipient's entire lymphohematopoietic system, including all red cells, granulocytes, B and T lymphocytes, and platelets, as well as cells comprising the fixed macrophage population, including Kupffer cells of the liver, pulmonary alveolar macrophages, osteoclasts, Langerhans cells of the skin, and brain microglial cells. The ability of the hematopoietic stem cell to home to the marrow following intravenous injection is mediated, at least in part, by the interaction of cell-surface molecules, termed *selectins*, on bone marrow endothelial cells with ligands, termed *integrins*, on early hematopoietic cells. Human hematopoietic stem cells can survive freezing and thawing with little, if any, damage, making it possible to remove and store a portion of the patient's own bone marrow for later reinfusion following treatment of the patient with high-dose myelotoxic therapy.

Categories of Hematopoietic Cell Transplantation

Hematopoietic cell transplantation can be described according to the relationship between the patient and the donor and by the anatomic source of stem cells. In ~1% of cases, patients have identical twins who can serve as donors. With the use of syngeneic donors, there is no risk of graft-versus-host disease (GVHD) that often complicates allogeneic transplantation, and unlike the use of autologous marrow, there is no risk that the stem cells are contaminated with tumor cells.

Allogeneic transplantation involves a donor and recipient who are not immunologically identical. Following allogeneic transplantation, immune cells transplanted with the stem cells or developing from them can react against the patient, causing GVHD. Alternatively, if the immunosuppressive preparative regimen used to treat the patient before transplant is inadequate, immunocompetent cells of the patient can cause graft rejection. The risks of these complications are greatly influenced by the degree of matching between donor and recipient for antigens encoded by genes of the major histocompatibility complex.

The human leukocyte antigen (HLA) molecules are responsible for binding antigenic proteins and presenting them to T cells. The antigens presented by HLA molecules may derive from exogenous sources (e.g., during active infections) or may be endogenous proteins. If individuals are not HLA-matched, T cells from one individual will react strongly to the mismatched HLA, or "major antigens," of the second. Even if the individuals are HLA-matched, the T cells of the donor may react to differing endogenous, or "minor antigens," presented by the HLA of the recipient. Reactions to minor antigens tend to be less vigorous. The genes of major relevance to transplantation include HLA-A, -B, -C, and -D; they are closely linked and therefore tend to be inherited as haplotypes, with only rare crossovers between them. Thus, the odds that any one full sibling will match a patient are one in four, and the probability that the patient has an HLA-identical sibling is $1 - (0.75)^n$, where n equals the number of siblings.

With current techniques, the risk of graft rejection is 1–3%, and the risk of severe, life-threatening acute GVHD is ~15% following transplantation between HLA-identical siblings. The incidence of graft rejection and GVHD increases progressively with the use of family member donors mismatched for one, two, or three antigens. While survival following a one-antigen mismatched transplant is not markedly altered, survival following two- or three-antigen mismatched transplants is significantly reduced, and such transplants should be performed only as part of clinical trials.

Since the formation of the National Marrow Donor Program, it has become possible to identify HLA-matched unrelated donors for many patients. The genes encoding HLA antigens are highly polymorphic, and thus the odds of any two unrelated individuals being HLA-identical are extremely low, somewhat less than 1 in 10,000. However, by identifying and typing >7 million volunteer donors, HLA-matched donors can now be found for ~50% of patients for whom a search is initiated. It takes, on average, 3–4 months to complete a search and schedule and initiate an unrelated donor transplant. Results so far suggest that GVHD is somewhat increased and survival somewhat poorer with such donors than with HLA-matched siblings.

Autologous transplantation involves the removal and storage of the patient's own stem cells with subsequent reinfusion after the patient

receives high-dose myeloablative therapy. Unlike allogeneic transplantation, there is no risk of GVHD or graft rejection with autologous transplantation. On the other hand, autologous transplantation lacks a graft-versus-tumor (GVT) effect, and the autologous stem cell product can be contaminated with tumor cells that could lead to relapse. A variety of techniques have been developed to "purge" autologous products of tumor cells. Some use antibodies directed at tumor-associated antigens plus complement, antibodies linked to toxins, or antibodies conjugated to immunomagnetic beads. In vitro incubation with certain chemotherapeutic agents such as 4-hydroperoxycyclophosphamide and long-term culture of bone marrow have also been shown to diminish tumor cell numbers in stem cell products. Another technique is positive selection of stem cells using antibodies to CD34, with subsequent column adherence or flow techniques to select normal stem cells while leaving tumor cells behind. All these approaches can reduce the number of tumor cells from 1000- to 10,000-fold and are clinically feasible; however, no prospective randomized trials have yet shown that any of these approaches results in a decrease in relapse rates or improvements in disease-free or overall survival.

Bone marrow aspirated from the posterior and anterior iliac crests has traditionally been the source of hematopoietic stem cells for transplantation. Typically, anywhere from 1.5 to 5×10^8 nucleated marrow cells per kilogram are collected for allogeneic transplantation. Several studies have found improved survival in the settings of both matched sibling and unrelated transplantation by transplanting higher numbers of bone marrow cells.

Hematopoietic stem cells circulate in the peripheral blood but in very low concentrations. Following the administration of certain hematopoietic growth factors, including granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), and during recovery from intensive chemotherapy, the concentration of hematopoietic progenitor cells in blood, as measured either by colony-forming units or expression of the CD34 antigen, increases markedly. This has made it possible to harvest adequate numbers of stem cells from the peripheral blood for transplantation. Donors are typically treated with 4 or 5 days of hematopoietic growth factor, following which stem cells are collected in one or two 4-h pheresis sessions. In the autologous setting, transplantation of $>2.5 \times 10^6$ CD34 cells per kilogram, a number easily collected in most circumstances, leads to rapid and sustained engraftment in virtually all cases. Compared to the use of autologous marrow, use of peripheral blood stem cells results in more rapid hematopoietic recovery, with granulocytes recovering to 500/μL by day 12 and platelets recovering to 20,000/μL by day 14. While this more rapid recovery diminishes the morbidity of transplantation, no studies show improved survival.

Hesitation in studying the use of peripheral blood stem cells for allogeneic transplantation was because peripheral blood stem cell products contain as much as one log more T cells than are contained in the typical marrow harvest; in animal models, the incidence of GVHD is related to the number of T cells transplanted. Nonetheless, clinical trials have shown that the use of growth factor–mobilized peripheral blood stem cells from HLA-matched family members leads to faster engraftment without an increase in acute GVHD. Chronic GVHD may be increased with peripheral blood stem cells, but in trials conducted so far, this has been more than balanced by reductions in relapse rates and nonrelapse mortality, with the use of peripheral blood stem cells resulting in improved overall survival.

Umbilical cord blood contains a high concentration of hematopoietic progenitor cells, allowing for its use as a source of stem cells for transplantation. Cord blood transplantation from family members has been explored in the setting where the immediate need for transplantation precludes waiting the 9 or so months generally required for the baby to mature to the point of donating marrow. Use of cord blood results in slower engraftment and peripheral count recovery than seen with marrow but a low incidence of GVHD, perhaps reflecting the low number of T cells in cord blood. Several banks have been developed to harvest and store cord blood for possible transplantation to unrelated patients from material that would otherwise be discarded. A summary of the first 562 unrelated cord blood transplants, facilitated by the New York Blood Center, reported engraftment in ~85% of patients but at a slower pace than seen with marrow. Severe GVHD was seen in 23% of patients. The risk of graft failure was related to the dose of cord blood cells per kilogram infused. The low cell content of most cord blood collections has limited the use of this approach for adult patients.

The Transplant Preparative Regimen

The treatment regimen administered to patients immediately preceding transplantation is designed to eradicate the patient's underlying disease and, in the setting of allogeneic transplantation, immunosuppress the patient adequately to prevent rejection of the transplanted marrow. The appropriate regimen therefore depends on the disease setting and source of marrow. For example, when transplantation is performed to treat severe combined immunodeficiency and the donor is a histocompatible sibling, no treatment is required because no host cells require eradication and the patient is already too immunoincompetent to reject the transplanted marrow. For aplastic anemia, there is no large population of cells to eradicate, and high-dose cyclophosphamide plus antithymocyte globulin are sufficient to immunosuppress the patient adequately to accept the marrow graft. In the setting of thalassemia and sickle cell anemia, high-dose busulfan is frequently added to cyclophosphamide in order to eradicate hyperplastic host hematopoiesis. A variety of different regimens have been developed to treat malignant diseases. Most of these regimens include agents that have high activity against the tumor in question at conventional doses and have myelosuppression as their predominant dose-limiting toxicity. Therefore, these regimens commonly include busulfan, cyclophosphamide, melphalan, thiotepa, carmustine, etoposide, and total-body irradiation in various combinations.

Although high-dose treatment regimens have typically been used in transplantation, the understanding that much of the antitumor effect of transplantation derives from an immunologically mediated GVT response has led investigators to ask if less-intensive "nonmyeloablative" regimens might be effective and more tolerable. Evidence for a GVT effect comes from studies showing that posttransplant relapse rates are lowest in patients who develop acute and chronic GVHD, higher in those without GVHD, and higher still in recipients of T cell–depleted allogeneic or syngeneic marrow. The demonstration that complete remissions can be obtained in many patients who have relapsed posttransplant by simply administering viable lymphocytes from the original donor further strengthens the argument for a potent GVT effect. Accordingly, a variety of less-intensive nonmyeloablative regimens have been studied, ranging in intensity from the very minimum required to achieve engraftment (e.g., fludarabine plus 200 cGy total-body irradiation) to regimens of more immediate intensity (e.g., fludarabine plus melphalan). Studies to date document that engraftment can be readily achieved with less toxicity than seen with conventional transplantation. Furthermore, the severity of GVHD appears to be decreased because less tissue damage is done by the lower doses of drugs in the preparative regimen. Complete sustained responses have been documented in many patients, particularly those with more indolent hematologic malignancies. The role of nonmyeloablative transplants in any disease, however, has not been fully defined.

The Transplant Procedure

Marrow is usually collected from the donor's posterior and sometimes anterior iliac crests with the donor under general or spinal anesthesia. Typically, 10–15 mL/kg of marrow is aspirated, placed in heparinized media, and filtered through 0.3- and 0.2-mm screens to remove fat and bony spicules. The collected marrow may undergo further processing depending on the clinical situation, such as the removal of red cells to prevent hemolysis in ABO-incompatible transplants, the removal of donor T cells to prevent GVHD, or attempts to remove possible contaminating tumor cells in autologous transplantation. Marrow donation is safe, with only very rare complications reported.

Peripheral blood stem cells are collected by leukophoresis after the donor has been treated with hematopoietic growth factors or, in the setting of autologous transplantation, sometimes after treatment with a combination of chemotherapy and growth factors. Stem cells for transplantation are generally infused through a large-bore central venous catheter. Such infusions are usually well tolerated, although occasionally patients develop fever, cough, or shortness of breath. These symptoms usually resolve with slowing of the infusion. When the stem cell product has been cryopreserved using dimethyl sulfoxide, patients more often experience short-lived nausea or vomiting due to the odor and taste of the cryoprotectant.

Engraftment

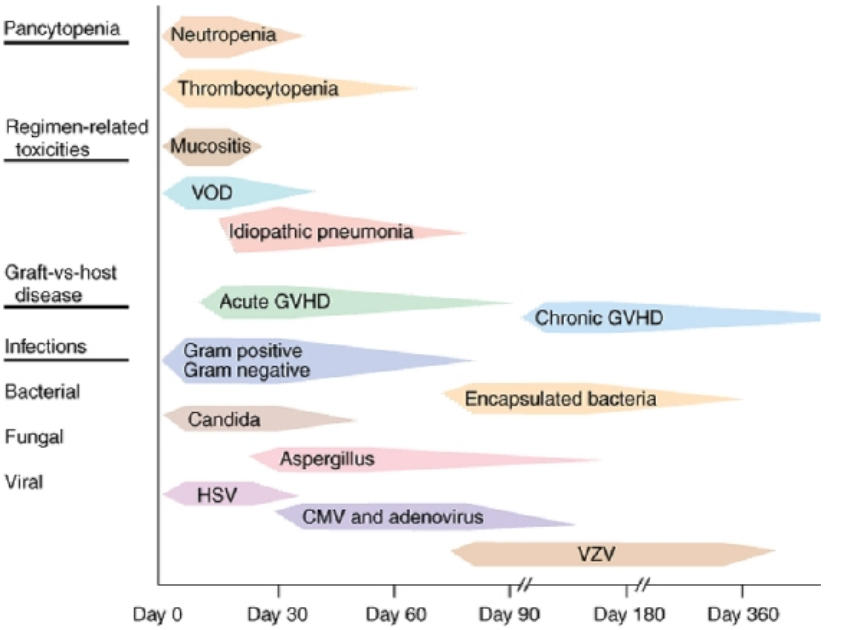
Peripheral blood counts usually reach their nadir several days to a week posttransplant as a consequence of the preparative regimen, then cells produced by the transplanted stem cells begin to appear in the peripheral blood. The rate of recovery depends on the source of stem cells, the use of posttransplant growth factors, and the form of GVHD prophylaxis employed. If marrow is the source of stem cells, recovery to 100 granulocytes/μL occurs by day 16 and to 500/μL by day 22. Use of G-CSF–mobilized peripheral blood stem cells speeds the rate of recovery by ~1 week when compared to marrow. Use of a myeloid growth factor (G-CSF or GM-CSF) posttransplant can further accelerate recovery by 3–5 days, while use of methotrexate to prevent GVHD delays engraftment by a similar period. Following allogeneic transplantation, engraftment can be documented using fluorescence in situ hybridization of sex chromosomes if donor and recipient are sex-mismatched, HLA-typing if HLA-mismatched, or restriction fragment length polymorphism analysis if sex- and HLA-matched.

Complications Following Hematopoietic Cell Transplant

Early Direct Chemoradiotoxicities

The transplant preparative regimens commonly used cause a spectrum of acute toxicities that vary according to the specific regimen but frequently result in nausea, vomiting, and mild skin erythema (Fig. 108-1). Regimens that include high-dose cyclophosphamide can result in hemorrhagic cystitis, which can usually be prevented by bladder irrigation or with the sulfhydryl compound mercaptoethanesulfonate (MESNA); rarely, acute hemorrhagic carditis is seen. Most preparative regimens will result in oral mucositis, which typically develops 5–7 days posttransplant and often requires narcotic analgesia. Use of a patient-controlled analgesic pump provides the greatest patient satisfaction and results in a lower cumulative dose of narcotic. Patients begin losing their hair 5–6 days posttransplant and by 1 week are usually profoundly pancytopenic.

Figure 108-1



Major syndromes complicating marrow transplantation. VOD, venoocclusive disease; GVHD, graft-versus-host disease; HSV, herpes simplex virus; CMV, cytomegalovirus; VZV, varicella-zoster virus. The size of the shaded area roughly reflects the risk of the complication.

Approximately 10% of patients will develop venoocclusive disease of the liver, a syndrome resulting from direct cytotoxic injury to hepatic-venular and sinusoidal endothelium, with subsequent deposition of fibrin and the development of a local hypercoagulable state. This chain of events results in the clinical symptoms of tender hepatomegaly, ascites, jaundice, and fluid retention. These symptoms can develop any time during the first month posttransplant, with the peak incidence at day 16. Predisposing factors include prior exposure to intensive chemotherapy, pretransplant hepatitis of any cause, and use of more intense conditioning regimens. The mortality of venoocclusive disease is ~30%, with progressive hepatic failure culminating in a terminal hepatorenal syndrome. Both thrombolytic and antithrombotic agents, such as tissue plasminogen activator, heparin, and prostaglandin E, have been studied as therapy, but none has proven of consistent major benefit in controlled trials, and all have significant toxicity. Early studies with defibrotide, a polydeoxyribonucleotide, seem encouraging.

Although most pneumonias developing posttransplant are caused by infectious agents, in ~5% of patients a diffuse interstitial pneumonia will develop that is thought to be the result of direct toxicity of the preparative regimen. Bronchoalveolar lavage typically shows alveolar hemorrhage, and biopsies are typically characterized by diffuse alveolar damage, although some cases may have a more clearly interstitial pattern. High-dose glucocorticoids are often used as treatment, although randomized trials testing their utility have not been reported.

Late Direct Chemoradiotoxicities

Late complications of the preparative regimen include decreased growth velocity in children and delayed development of secondary sex characteristics. These complications can be partly ameliorated with the use of appropriate growth and sex hormone replacement. Most men become azoospermic, and most postpubertal women will develop ovarian failure, which should be treated. Thyroid dysfunction, usually well compensated, is sometimes seen. Cataracts develop in 10–20% of patients and are most common in patients treated with total-body irradiation and those who receive glucocorticoid therapy posttransplant for treatment of GVHD. Aseptic necrosis of the femoral head is seen in 10% of patients and is particularly frequent in those receiving chronic glucocorticoid therapy.

Graft-versus-Host Disease

GVHD is the result of allogeneic T cells that were either transferred with the donor's stem cell inoculum or develop from it, reacting with antigenic targets on host cells. GVHD developing within the first 3 months posttransplant is termed *acute GVHD*, while GVHD developing or persisting beyond 3 months posttransplant is termed *chronic GVHD*. Acute GVHD most often first becomes apparent 2–4 weeks posttransplant and is characterized by an erythematous maculopapular rash; persistent anorexia or diarrhea, or both; and by liver disease with increased serum levels of bilirubin, alanine and aspartate aminotransferase, and alkaline phosphatase. Since many conditions can mimic acute GVHD, diagnosis usually requires skin, liver, or endoscopic biopsy for confirmation. In all these organs, endothelial damage and lymphocytic infiltrates are seen. In skin, the epidermis and hair follicles are damaged; in liver, the small bile ducts show segmental disruption; and in intestines, destruction of the crypts and mucosal ulceration may be noted. A commonly used rating system for acute GVHD is shown in Table 108-1. Grade I acute GVHD is of little clinical significance, does not affect the likelihood of survival, and does not require treatment. In contrast, grades II to IV GVHD are associated with significant symptoms and a poorer probability of survival, and they require aggressive therapy. The incidence of acute GVHD is higher in recipients of stem cells from mismatched or unrelated donors,

in older patients, and in patients unable to receive full doses of drugs used to prevent the disease.

Table 108-1 Clinical Staging and Grading of Acute Graft-versus-Host Disease

Clinical Stage	Skin	Liver- Bilirubin, μmol/L (mg/dL)	Gut
1	Rash <25% body surface	34–51 (2–3)	Diarrhea 500–1000 mL/d
2	Rash 25–50% body surface	51–103 (3–6)	Diarrhea 1000–1500 mL/d
3	Generalized erythroderma	103–257 (6–15)	Diarrhea >1500 mL/d
4	Desquamation and bullae	>257 (> 15)	Ileus
Overall Clinical Grade	**Skin Stage**	**Liver Stage**	**Gut Stage**
I	1–2	0	0
II	1–3	1	1
III	1–3	2–3	2–3
IV	2–4	2–4	2–4

One general approach to the prevention of GVHD is the administration of immunosuppressive drugs early after transplant. Combinations of methotrexate and either cyclosporine or tacrolimus are among the most effective and widely used regimens. Prednisone, anti–T cell antibodies, mycophenolate mofetil, and other immunosuppressive agents have also been or are being studied in various combinations. A second general approach to GVHD prevention is removal of T cells from the stem cell inoculum. While effective in preventing GVHD, T cell depletion is associated with an increased incidence of graft failure and of tumor recurrence posttransplant; as yet, little evidence suggests that T-cell depletion improves cure rates in any specific setting.

Despite prophylaxis, significant acute GVHD will develop in ~30% of recipients of stem cells from matched siblings and in as many as 60% of those receiving stem cells from unrelated donors. The disease is usually treated with glucocorticoids, antithymocyte globulin, or monoclonal antibodies targeted against T cells or T cell subsets.

Between 20 and 50% of patients surviving >6 months after allogeneic transplantation will develop chronic GVHD. The disease is more common in older patients, in recipients of mismatched or unrelated stem cells, and in those with a preceding episode of acute GVHD. The disease resembles an autoimmune disorder with malar rash, sicca syndrome, arthritis, obliterative bronchiolitis, and bile duct degeneration and cholestasis. Single-agent prednisone or cyclosporine is standard treatment at present, although trials of other agents are under way. In most patients, chronic GVHD resolves, but it may require 1–3 years of immunosuppressive treatment before these agents can be withdrawn without the disease recurring. Because patients with chronic GVHD are susceptible to significant infection, they should receive prophylactic trimethoprim-sulfamethoxazole, and all suspected infections should be investigated and treated aggressively.

Graft Failure

While complete and sustained engraftment is usually seen posttransplant, occasionally marrow function either does not return or, after a brief period of engraftment, is lost. Graft failure after autologous transplantation can be the result of inadequate numbers of stem cells being transplanted, damage during ex vivo treatment or storage, or exposure of the patient to myelotoxic agents posttransplant. Infections with cytomegalovirus (CMV) or human herpes virus type 6 have also been associated with loss of marrow function. Graft failure after allogeneic transplantation can also be due to immunologic rejection of the graft by immunocompetent host cells. Immunologically based graft rejection is more common following use of less-immunosuppressive preparative regimens, in recipients of T cell–depleted stem cell products, and in patients receiving grafts from HLA-mismatched donors.

Treatment of graft failure usually involves removing all potentially myelotoxic agents from the patient's regimen and attempting a short trial of a myeloid growth factor. Persistence of lymphocytes of host origin in allogeneic transplant recipients with graft failure indicates immunologic rejection. Reinfusion of donor stem cells in such patients is usually unsuccessful unless preceded by a second immunosuppressive preparative regimen. Standard preparative regimens are generally tolerated poorly if administered within 100 days of a first transplant because of cumulative toxicities. However, use of regimens combining, for example, anti-CD3 antibodies with high-dose glucocorticoids, fludarabine plus low-dose total-body irradiation, or cyclophosphamide plus antithymocyte globulin have been effective in some cases.

Infection

Posttransplant patients, particularly recipients of allogeneic transplantation, require unique approaches to the problem of infection. Early after transplantation, patients are profoundly neutropenic, and because the risk of bacterial infection is so great, most centers initiate antibiotic treatment once the granulocyte count falls to <500/μL. Fluconazole prophylaxis at a dose of 200–400 mg/kg per day reduces the risk of candidal infections. Patients seropositive for herpes simplex should receive acyclovir prophylaxis. One approach to infection prophylaxis is shown in Table 108-2. Despite these prophylactic measures, most patients will develop fever and signs of infection posttransplant. The management of patients who become febrile despite bacterial and fungal prophylaxis is a difficult challenge and is guided by individual aspects of the patient and by the institution's experience. The general problem of infection in the immunocompromised host is discussed in Chap. 126.

Table 108-2 Approach to Infection Prophylaxis in Allogeneic Transplant Recipients

Organism		Approach
Bacterial	Ceftazidime	2 g IV q8h while neutropenic
Fungal	Fluconazole	400 mg PO qd to day 75 posttransplant
Pneumocystis carinii	Trimethoprim-sulfamethoxazole	1 double-strength tablet PO bid 2 days/week until day 180 or off immunosuppression
Viral		
Herpes simplex	Acyclovir	800 mg PO bid to day 30
Varicella zoster	Acyclovir	800 mg PO bid to day 365
Cytomegalovirus	Ganciclovir	5 mg/kg IV bid for 7 days, then 5 (mg/kg)/d 5 days/week to day 100

Once patients engraft, the incidence of bacterial infection diminishes; however, patients, particularly allogeneic transplant recipients, remain at significant risk of infection. During the period from engraftment until about 3 months posttransplant, the most common causes of infection are gram-positive bacteria, fungi (particularly *Aspergillus*) and viruses including CMV. CMV infection, which in the past was frequently seen and often fatal, can be prevented in seronegative patients by the use of seronegative blood products. The use of ganciclovir, either as prophylaxis beginning at the time of engraftment or initiated when CMV first reactivates as evidenced by development of antigenemia, can significantly reduce the risk of CMV disease in seropositive patients. Elimination of white blood cells from transfused blood products is another method to prevent CMV transmission. Foscarnet is effective for some patients who develop CMV antigenemia or infection despite the use of ganciclovir or who cannot tolerate the drug.

Pneumocystis jiroveci pneumonia, once seen in 5–10% of patients, can be prevented by treating patients with oral trimethoprim-sulfamethoxazole for 1 week pretransplant and resuming the treatment once patients have engrafted.

The risk of infection diminishes considerably beyond 3 months after transplant unless chronic GVHD develops, requiring continuous immunosuppression. Most transplant centers recommend continuing trimethoprim-sulfamethoxazole prophylaxis while patients are receiving any immunosuppressive drugs and also recommend careful monitoring for late CMV reactivation. In addition, many centers recommend prophylaxis against varicella zoster, using acyclovir for 1 year posttransplant.

Treatment of Specific Diseases Using Hematopoietic Cell Transplantation

Nonmalignant Diseases: Treatment

Immunodeficiency Disorders

By replacing abnormal stem cells with cells from a normal donor, hematopoietic cell transplantation can cure patients of a variety of immunodeficiency disorders including severe combined immunodeficiency, Wiskott-Aldrich syndrome, and Chédiak-Higashi syndrome. The widest experience has been with severe combined immunodeficiency disease, where cure rates of 90% can be expected with HLA-identical donors and success rates of 50–70% have been reported using haplotype-mismatched parents as donors (Table 108-3).

Table 108-3 Estimated 5-Year Survival Rates Following Transplantation[a]

Disease	Allogeneic, %	Autologous, %
Severe combined immunodeficiency	90	N/A
Aplastic anemia	90	N/A
Thalassemia	90	N/A
Acute myeloid leukemia		
First remission	55–60	50
Second remission	40	30
Acute lymphocytic leukemia		
First remission	50	40
Second remission	40	30
Chronic myeloid leukemia		
Chronic phase	70	ID
Accelerated phase	40	ID
Blast crisis	15	ID
Chronic lymphocytic leukemia	50	ID

Myelodysplasia	45	ID
Multiple myeloma	30	35
Non-Hodgkin's lymphoma		
First relapse/second remission	40	40
Hodgkin's disease		
First relapse/second remission	40	50
Breast cancer		
High-risk stage II	N/A	70
Stage IV	N/A	15

[a]These estimates are generally based on data reported by the International Bone Marrow Transplant Registry. The analysis has not been reviewed by their Advisory Committee.

Note: N/A, not applicable; ID, insufficient data.

Aplastic Anemia

Transplantation from matched siblings after a preparative regimen of high-dose cyclophosphamide and antithymocyte globulin can cure up to 90% of patients <40 years with severe aplastic anemia. Results in older patients and in recipients of mismatched family member or unrelated marrow are less favorable; therefore, a trial of immunosuppressive therapy is generally recommended for such patients before considering transplantation. Transplantation is effective in all forms of aplastic anemia including, for example, the syndromes associated with paroxysmal nocturnal hemoglobinuria and Fanconi's anemia. Patients with Fanconi's anemia are abnormally sensitive to the toxic effects of alkylating agents and so less intensive preparative regimens must be used in their treatment (Chap. 102).

Hemoglobinopathies

Marrow transplantation from an HLA-identical sibling following a preparative regimen of busulfan and cyclophosphamide can cure 70–90% of patients with thalassemia major. The best outcomes can be expected if patients are transplanted before they develop hepatomegaly or portal fibrosis and if they have been given adequate iron chelation therapy. Among such patients, the probabilities of 5-year survival and disease-free survival are 95 and 90%, respectively. Although prolonged survival can be achieved with aggressive chelation therapy, transplantation is the only curative treatment for thalassemia. Transplantation is being studied as a curative approach to patients with sickle cell anemia. Two-year survival and disease-free survival rates of 90 and 80%, respectively, have been reported following matched sibling transplantation. Decisions about patient selection and the timing of transplantation remain difficult, but transplantation represents a reasonable option for younger patients who suffer repeated crises or other significant complications and who have not responded to other interventions (Chap. 99).

Other Nonmalignant Diseases

Theoretically, hematopoietic cell transplantation should be able to cure any disease that results from an inborn error of the lymphohematopoietic system. Transplantation has been used successfully to treat congenital disorders of white blood cells such as Kostmann's syndrome, chronic granulomatous disease, and leukocyte adhesion deficiency. Congenital anemias such as Blackfan-Diamond anemia can also be cured with transplantation. Infantile malignant osteopetrosis is due to an inability of the osteoclast to resorb bone, and since osteoclasts derive from the marrow, transplantation can cure this rare inherited disorder.

Hematopoietic cell transplantation has been used as treatment for a number of storage diseases caused by enzymatic deficiencies, such as Gaucher's disease, Hurler's syndrome, Hunter's syndrome, and infantile metachromatic leukodystrophy. Transplantation for these diseases has not been uniformly successful, but treatment early in the course of these diseases, before irreversible damage to extramedullary organs has occurred, increases the chance for success.

Transplantation is being explored as a treatment for severe acquired autoimmune disorders. These trials are based on studies demonstrating that transplantation can reverse autoimmune disorders in animal models and on the observation that occasional patients with coexisting autoimmune disorders and hematologic malignancies have been cured of both with transplantation.

Malignant Diseases: Treatment

Acute Leukemia

Allogeneic hematopoietic cell transplantation cures 15–20% of patients who do not achieve complete response from induction chemotherapy for acute myeloid leukemia (AML) and is the only form of therapy that can cure such patients. Cure rates of 30–35% are seen when patients are transplanted in second remission or in first relapse. The best results with allogeneic transplantation are achieved when applied during first remission, with disease-free survival rates averaging 55–60%. Chemotherapy alone can cure a portion of AML patients, and so the relative merits of transplanting all patients during first remission versus only transplanting very-high-risk patients and those who relapse continue to be discussed. Autologous transplantation is also able to cure a portion of patients with AML. The rates of disease recurrence with autologous transplantation are higher than those seen after allogeneic transplantation, and cure rates are somewhat less.

Similar to patients with AML, adults with acute lymphocytic leukemia who do not achieve a complete response to induction chemotherapy can be cured in 15–20% of cases with immediate transplantation. Cure rates improve to 30–50% in second remission, and therefore transplantation can be recommended for adults who have persistent disease after induction chemotherapy or who have subsequently relapsed. Transplantation in first remission results in cure rates around 55%. While transplantation appears to offer a clear advantage over chemotherapy for patients with high-risk disease, such as those with Philadelphia chromosome–positive disease, debate continues about whether adults with standard-risk disease should be transplanted in first remission or whether transplantation should be reserved until relapse. Autologous transplantation is associated with a higher relapse rate but a somewhat lower risk of nonrelapse mortality when compared to allogeneic transplantation. On balance, most experts recommend use of allogeneic stem cells if an appropriate donor is available.

Chronic Leukemia

Allogeneic hematopoietic cell transplantation is the only therapy shown to cure a substantial portion of patients with chronic myeloid leukemia (CML). Five-year disease-free survival rates are 15–20% for patients transplanted for blast crisis, 25–50% for accelerated-phase patients, and 60–70% for chronic phase patients, with cure rates as high as 80% at selected centers. Use of unrelated donors results in more GVHD and slightly worse survival than seen with matched siblings, although 3-year disease-free survival rates of 70% have been reported at some large centers. The timing of transplantation in CML has become more complicated with the introduction of imatinib mesylate, a remarkably effective, relatively nontoxic oral agent. Even though imatinib is not generally regarded as curative, given its favorable toxicity profile, most physicians favor its use as initial therapy for CML, with transplantation being reserved for those who fail to achieve a complete cytogenetic response with imatinib, relapse after an initial response, or are intolerant of the drug (Chap. 104).

Allogeneic transplantation has been used to only a limited extent for chronic lymphocytic leukemia, in large part because of the chronic nature of the disease and because of the age profile of patients. With allogeneic transplantation, complete remissions have been achieved in the majority of patients so far reported, with disease-free survival rates of ~50% at 3 years. However, treatment-related mortality has been substantial, and further follow-up is needed. Encouraging results have been seen using reduced intensity preparative regimens before allogeneic transplantation.

Myelodysplasia

Between 40 and 50% of patients with myelodysplasia appear to be cured with allogeneic transplantation. Results are better among younger patients and those with less-advanced disease. However, some patients with myelodysplasia can live for extended periods without intervention, and so transplantation is generally recommended only for patients with disease categorized as intermediate risk I or greater according to the International Prognostic Scoring System (Chap. 102).

Lymphoma

Patients with disseminated intermediate- or high-grade non-Hodgkin's lymphoma who have not been cured by first-line chemotherapy and are transplanted in first relapse or second remission can still be cured in 40–50% of cases. This represents a clear advantage over results obtained with conventional-dose salvage chemotherapy. It is unsettled whether patients with high-risk disease benefit from transplantation in first remission. Most experts favor the use of autologous rather than allogeneic transplantation for patients with intermediate or high grade non-Hodgkin's lymphoma, because fewer complications occur with this approach and survival appears equivalent. For patients with recurrent disseminated indolent non-Hodgkin's lymphoma, autologous transplantation results in high response rates and improved progression-free survival compared to salvage chemotherapy. However, late relapses are seen after transplantation. The role of autologous transplantation in the initial treatment of patients is under study. Nonmyeloablative preparative regimens followed by allogeneic transplantation result in high response rates in patients with indolent lymphomas, but the exact role of this approach remains to be defined.

The role of transplantation in Hodgkin's disease is similar to that in intermediate- and high-grade non-Hodgkin's lymphoma. With transplantation, 5-year disease-free survival is 20–30% in patients who never achieve a first remission with standard chemotherapy and up to 70% for those transplanted in second remission. Transplantation has no defined role in first remission in Hodgkin's disease.

Myeloma

Patients with myeloma who have progressed on first-line therapy can sometimes benefit from allogeneic or autologous transplantation. Autologous transplantation has been studied as part of the initial therapy of patients, and both disease-free survival as well as overall survival were improved with this approach in randomized trials. The use of autologous transplantation followed by nonmyeloablative allogeneic transplantation has shown encouraging results.

Solid Tumors

Among women with metastatic breast cancer, 15–20% disease-free survival rates at 3 years have been reported, with better results seen in younger patients who have responded completely to standard-dose therapy before undergoing transplantation. Randomized trials have not shown superior survival for patients treated for metastatic disease with high-dose chemotherapy plus stem cell support. Randomized trials evaluating transplantation as treatment for primary breast cancer have yielded mixed results. No role for autologous transplantation has been established in the treatment of breast cancer.

Patients with testicular cancer who have failed first-line chemotherapy have been treated with autologous transplantation; ~10–20% of such patients apparently have been cured with this approach.

The use of high-dose chemotherapy with autologous stem cell support is being studied for several other solid tumors, including ovarian

cancer, small cell lung cancer, neuroblastoma, and pediatric sarcomas. As in most other settings, the best results have been obtained in patients with limited amounts of disease and where the remaining tumor remains sensitive to conventional-dose chemotherapy. Few randomized trials of transplantation in these diseases have been completed.

Partial and complete responses have been reported following nonmyeloablative allogeneic transplantation for some solid tumors, most notably renal cell cancers. The GVT effect, well documented in the treatment of hematologic malignancies, may apply to selected solid tumors under certain circumstances.

Posttransplant Relapse

Patients who relapse following autologous transplantation sometimes respond to further chemotherapy, particularly if the remission following transplantation was long. More options are available for patients who relapse following allogeneic transplantation. Of particular interest are the response rates seen with infusion of unirradiated donor lymphocytes. Complete responses in as many as 75% of patients with chronic myeloid leukemia, 40% in myelodysplasia, 25% in AML, and 15% in myeloma have been reported. Major complications of donor lymphocyte infusions include transient myelosuppression and the development of GVHD. These complications depend on the number of donor lymphocytes given and the schedule of infusions, with less GVHD seen with lower dose, fractionated schedules.

Further Readings

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