HEMATOLOGY

Clinical Principles and Applications

Third Edition

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21 Bone Marrow Failure

Elaine M. Keohane

OUTLINE

OBJECTIVES

After completion of this chapter, the reader will be able to:

1. Describe the clinical consequences of bone marrow failure.
2. Describe the etiology of acquired and inherited aplastic anemias.
3. Discuss the pathophysiologic mechanisms of acquired and inherited aplastic anemias.
4. Describe the characteristic peripheral blood and bone marrow features in aplastic anemia.
5. Classify aplastic anemia as nonsevere, severe, or very severe based on laboratory tests.
6. Discuss treatment modalities for acquired and inherited aplastic anemia and the patients for whom each is most appropriate.
7. Differentiate among causes of pancytopenia based on laboratory tests and clinical findings.
8. Discuss the possible relationship between defects in the telomerase complex and bone marrow failure in acquired and inherited aplastic anemia.
9. Compare and contrast the pathophysiology, clinical picture, and laboratory findings in transient erythroblastopenia of childhood, Diamond-Blackfan anemia, and congenital dyserythropoietic anemia.
10. Describe the mechanisms causing cytopenia in myelophthisic anemia and anemia of chronic renal disease.

CASE STUDY

After studying the material in this chapter, the reader should be able to respond to the following case study:

A 52-year-old female data entry clerk complained of bilateral wrist pain. Her physician prescribed a nonsteroidal anti-inflammatory agent. Her wrist pain improved; however, over the next 3 months, she noted increasing fatigue and scattered bruises. Past medical history was unremarkable. She was on no other medications and had no recent chemical exposure. Physical examination revealed pallor and scattered ecchymoses with petechiae on chest and shoulders with no other abnormalities. Complete blood count results were as follows: hemoglobin, 8 g/dL; mean cell volume, 104 fL; reticulocytes, 0.6%; absolute reticulocytes 16×10^9/L; and WBC, 2×10^9/L, with absolute values of neutrophils 1.1×10^9/L and lymphocytes of 0.4×10^9/L. Platelet count was 27×10^9/L. Serum vitamin B_{12} and folate levels were normal. Bone marrow aspirate was normocellular with dyserythropoiesis, normal myelopoiesis, and normal megakaryopoiesis. Iron stain revealed normal stores. Bone marrow biopsy was moderately hypocellular (30%) with reduced activity in all three cell lines. There was no increase in reticulin or blasts. Cytogenetics were normal and Ham test was negative.

1. What term is used to describe a decrease in all cell lines?
2. Which anemia of bone marrow failure should be considered?
3. How would an increase in either reticulin or blasts alter the preliminary diagnosis?
4. How would the severity of this patient's condition be classified?
5. What treatment modality would be considered for this patient?

PATHOPHYSIOLOGY OF BONE MARROW FAILURE

Bone marrow failure is the reduction or cessation of blood cell production affecting one or more cell lines. Pancytopenia, or decreased numbers of circulating red blood cells (RBCs), white blood cells (WBCs), and platelets, is seen in most cases of bone marrow failure, particularly in severe or advanced stages.

The pathophysiology of bone marrow failure includes the following mechanisms: (1) destruction of hematopoietic stem cells due to injury by drugs, chemicals, radiation, viruses, or autoimmune mechanisms; (2) premature senescence and

apoptosis of stem cells due to inherited mutations; (3) ineffective hematopoiesis owing to stem cell mutations or vitamin B_{12} or folate deficiency; (4) disruption of the bone marrow microenvironment that supports hematopoiesis; (5) decreased production of hematopoietic growth factors or related hormones; or (6) loss of normal hematopoietic tissue due to infiltration of the marrow space with abnormal cells.

The clinical consequences of bone marrow failure vary depending on the extent and duration of the cytopenias. Severe pancytopenia can be rapidly fatal if untreated. Some patients may present initially with no symptoms and their cytopenia is inadvertently detected during a routine examination. Thrombocytopenia can result in clinically significant bleeding. The decrease in RBCs and hemoglobin (Hb) leads to symptoms of anemia, including fatigue, pallor, and cardiovascular complications. Sustained neutropenia increases the risk of bacterial or fungal infections that can be life-threatening.

This chapter focuses on aplastic anemia, a bone marrow failure syndrome resulting from damaged or defective stem cells (mechanisms 1 and 2). Bone marrow failure resulting from other mechanisms may have a presentation similar to aplastic anemia and differentiation is discussed later. Because there are many mechanisms involved in the various bone marrow failure syndromes, accurate diagnosis is essential so that the appropriate treatment can be instituted.

BOX 21-2 Etiologic Classification of Aplastic Anemia

Acquired

Idiopathic (70-80% of cases)
Secondary
- Dose dependent
  - Cytotoxic drugs
  - Benzene
  - Radiation
- Idiosyncratic
  - Drugs (see Box 21-3)
  - Chemicals
    - Pesticides
    - Lubricating agents/cutting oils
- Viruses
  - Epstein-Barr virus
  - Hepatitis (some types)
  - Human immunodeficiency virus (HIV)
- Miscellaneous conditions
  - Paroxysmal nocturnal hemoglobinuria
  - Autoimmune diseases
  - Pregnancy

Inherited

Fanconi anemia
Dyskeratosis congenita

APLASTIC ANEMIA

Aplastic anemia is a rare but potentially fatal bone marrow failure syndrome. The first reported case of aplastic anemia is attributed to Ehrlich[1] in 1888, who described a patient with severe anemia and neutropenia exhibiting a yellow hypocellular marrow on postmortem examination. The name *aplastic anemia* was given to the disease by Chauffard in 1904.[2] The characteristic features of aplastic anemia include pancytopenia, reticulocytopenia, bone marrow hypocellularity, and depletion of hematopoietic stem cells (Box 21-1). Aplastic anemia may be acquired or inherited. In adults, the acquired type constitutes most cases. In infants and children, approximately 70% of aplastic anemias are acquired, and 30% are inherited. Box 21-2 provides an etiologic classification of aplastic anemia.

Acquired Aplastic Anemia

Acquired aplastic anemia is classified as idiopathic when the cause is unknown and secondary when the etiology can be identified. The idiopathic type accounts for approximately 70% to 80% of aplastic anemia cases.[3] Clinical and laboratory findings are similar for idiopathic and secondary aplastic anemia. Patients with aplastic anemia initially may present with a normocytic or macrocytic anemia without reticulocytosis. Depending on the progression of the bone marrow failure, pancytopenia may develop slowly. More than half of aplastic anemias progress at a rapid rate, however, with complete cessation of erythropoiesis.

BOX 21-1 Characteristic Features of Aplastic Anemia

Peripheral blood pancytopenia
Reticulocytopenia
Bone marrow hypocellularity
Depletion of hematopoietic stem cells

Incidence

In North America and Europe, the incidence per year approximates 2 per 1 million.[4] In Asia and East Asia, the frequency is two to three times higher than in North America or Europe. Aplastic anemia can occur at any age but is more frequent in individuals 10 to 25 years old and individuals older than age 60.[4,5] There is no gender difference in incidence.[4]

Etiology

The cause of the bone marrow failure in idiopathic aplastic anemia is unknown. Secondary aplastic anemia is associated with exposure to certain drugs, chemicals, radiation, or infectious agents. Cytotoxic drugs, radiation, and chemicals, such as benzene, suppress the bone marrow in a predictable, dose-dependent manner.[6-8] Depending on the dose and exposure time, the bone marrow generally recovers after withdrawal of the agent. About 90% of secondary aplastic anemias occur secondary to idiosyncratic reactions to drugs or chemicals. In idiosyncratic reactions, the bone marrow failure is unpredictable and unrelated to dose, and the bone marrow does not usually recover when the agent is withdrawn.[6] Documentation of an identifiable factor or agent inducing aplastic anemia

in these cases is difficult because evidence is primarily circumstantial and symptoms may occur months or years after exposure. Some drugs associated with idiosyncratic, acquired aplastic anemia are listed in Box 21-3.[5,6]

Aplastic anemia as an idiosyncratic, adverse reaction to a drug, chemical, or other agent is a rare event and is likely due to a combination of genetic and environmental factors in susceptible individuals. Currently no tests are available to predict individual susceptibility to these idiosyncratic reactions; however, genetic differences affecting metabolic and immune response pathways may play a role. There is a twofold higher incidence of HLA-DR2 and its major serologic split, HLA-DR15, in aplastic anemia patients compared with the general population, but the relationship of this finding to the disease pathophysiology has not been elucidated.[9,10] A deficiency in the enzyme glutathione S-transferase resulting from *GSTT1* and *GSTM1/GSTT1* gene deletions was found in 30% and 22% of Caucasians with acquired aplastic anemia.[11] This deficiency and other factors yet to be discovered may affect the biometabolism of certain drugs and chemicals leading to the bone marrow suppression.

BOX 21-3 Drugs Reported to Have a Rare Association with Idiosyncratic Aplastic Anemia

Antiarthritics
Gold compounds
Penicillamine

Antibiotics
Chloramphenicol
Sulfonamides

Anticonvulsants
Carbamazepine
Phenytoin

Antidepressants
Dothiepin
Phenothiazine

Antidiabetes Agents
Chlorpropamide
Tolbutamide

Anti-inflammatories (Nonsteroidal)
Diclofenac
Fenoprofen
Ibuprofen
Indomethacin
Naproxen
Phenylbutazone
Piroxicam
Sulindac

Antiprotozoals
Chloroquine
Quinacrine

Antithyroidals
Carbimazole
Thiouracil

Acquired aplastic anemia appears occasionally as a complication from infections. Viruses implicated in aplastic anemia include Epstein-Barr virus, human immunodeficiency virus (HIV), hepatitis virus, and human parvovirus B19. A history of acute non-A, non-B, or non-C hepatitis 1 to 3 months before onset is found in 2% to 10% of patients with acquired aplastic anemia.[12] Posthepatitis aplastic anemia syndrome has a poor prognosis.

Acquired aplastic anemia has been reported as a rare complication in pregnancy and autoimmune disorders.[6] Aplastic anemia also occurs as a complication in paroxysmal nocturnal hemoglobinuria. The distinction between these conditions is discussed later.

Pathophysiology

The primary lesion in acquired aplastic anemia is a quantitative and qualitative deficiency of hematopoietic stem cells, rather than a defect of the bone marrow stroma or a deficiency of growth factors. In culture, stem cells of patients with acquired aplastic anemia have diminished colony formation.[13] The hematopoietic stem and early progenitor cell compartment is identified by expression of CD34 surface antigens. When measured by flow cytometry, the $CD34^+$ cell population in the bone marrow of patients with aplastic anemia can be 10 times lower compared with normal individuals.[13] There is an increase in apoptotic $CD34^+$ cells in aplastic anemia, and they have an increased expression of Fas receptors that mediate apoptosis.[14,15] Bone marrow cells in aplastic anemia also have an increased expression of apoptotic genes determined by gene chip analysis.[16] The bone marrow stroma is functionally normal in acquired aplastic anemia. Stromal cells from patients with aplastic anemia produce normal or increased quantities of growth factors, and they are able to support the growth of $CD34^+$ cells from normal donors in culture and in vivo after transplantation.[6,17] Individuals with aplastic anemia have elevated levels of growth factors in their serum, such as erythropoietin.[18] Additionally, the serum level of flt3 ligand, a growth factor that stimulates proliferation of stem and progenitor cells, is 200 times higher in patients with severe aplastic anemia compared with normal individuals.[19] Despite elevated levels, growth factors are generally unsuccessful in correcting the cytopenias found in acquired aplastic anemia secondary to the damage and depletion of the stem cells.

The pathophysiology of acquired a plastic anemia involves the severe depletion of hematopoietic stem and progenitor cells from the bone marrow by a direct or indirect mechanism. In the direct mechanism, a cytotoxic drug, chemical, radiation, or virus damages the DNA of the stem and progenitor cells

causing apoptosis and cytolysis.[6] In the indirect method, exposure to certain drugs or chemicals in susceptible individuals results in an autoimmune T cell attack that destroys the stem and progenitor cells.[20] The autoimmune pathophysiology was first suggested in the 1970s when aplastic anemia patients undergoing immunosuppressive conditioning before bone marrow transplant experienced an improvement in cell counts.[21] Evidence that supports the autoimmune pathophysiology is that in acquired aplastic anemia (1) there is an increase in cytotoxic ($CD8^+$) T lymphocytes in the blood and bone marrow detected by flow cytometry; (2) these T cells produce increased amounts of cytokines that inhibit hematopoiesis and induce apoptosis, including interferon-γ and tumor necrosis factor-α[22-24]; (3) there is an increase in tumor necrosis factor-α receptors on $CD34^+$ cells[25]; and (4) there is improvement in the cytopenia in most patients after immunosuppression therapy.[6,20] Possible mechanisms by which a drug, chemical, or virus may elicit an autoimmune response may be by altering self-proteins, inducing expression of abnormal proteins, exposing hidden antigens, inducing an immune response that cross-reacts with self-antigens, or disrupting immune regulation networks.[20,23] The antigens responsible for triggering and sustaining the autoimmune attack on the stem cells are unknown. Autoantibodies to kinectin, an intracellular protein found in hematopoietic cells, have been detected in aplastic anemia patients but not in normal controls; the relationship of the autoantibody to the pathophysiology of the disease is unknown.[26]

Blood cells in patients with aplastic anemia who do not respond to immunosuppression therapy have a progressive shortening of their telomeres and a decrease in telomerase activity.[27] In addition, mutations in the RNA component of the telomerase gene (*TERC*) and the telomerase reverse transcriptase gene (*TERT*) have been found in subsets of patients with aplastic anemia.[28,29] Telomerase is an enzyme that is important for the repair and maintenance of telomeres at the end of chromosomes. Cells with abnormally short telomeres are unable to proceed through the cell cycle and become senescent and apoptotic. The mechanism of stem cell depletion in a subset of patients with aplastic anemia may be linked to defective telomere maintenance imparting a susceptibility to bone marrow failure after environmental insult.[29]

Clinical Findings

Symptoms vary in acquired aplastic anemia from very severe to mild or asymptomatic. Patients usually present with symptoms typical of insidious-onset anemia: pallor, fatigue, and weakness. Severe anemia can result in serious cardiac complications or even cardiac failure and death. Petechiae, bruising, epistaxis, bleeding gums, menorrhagia, retinal hemorrhages, intestinal bleeding, and sometimes intracranial bleeding may occur secondary to thrombocytopenia. Fever and bacterial or fungal infections are unusual at initial presentation but may occur after prolonged periods of neutropenia. Splenomegaly and hepatomegaly are absent.

Laboratory Findings

Pancytopenia is typical, although initially only one or two cell lines may be decreased. The absolute neutrophil count is decreased, and the absolute lymphocyte count may be normal or decreased. The Hb is usually less than 10 g/dL, the mean cell volume (MCV) is normal or increased, and the percent and absolute reticulocytes and immature reticulocyte fraction are decreased. Table 21-1 contains the diagnostic criteria for aplastic anemia by degree of severity.[4,5,30,31] This classification is helpful in guiding treatment decisions.

On a peripheral blood film, neutrophils, monocytes, and platelets are decreased, and the RBCs are normocytic or macrocytic. Toxic granulation may be observed in the neutrophils, but the RBCs and platelets are normal in appearance. Blasts and other immature blood cells are characteristically absent.

Serum iron and percent transferrin saturation are increased reflecting the decreased use of iron for erythropoiesis. Liver function tests may be abnormal if the pancytopenia was preceded by hepatitis. Approximately one third of aplastic anemia patients develop paroxysmal nocturnal hemoglobinuria.[32] In paroxysmal nocturnal hemoglobinuria, an acquired stem cell mutation results in circulating blood cells that lack glyco-

TABLE 21-1 Diagnostic Criteria for Aplastic Anemia

	NSAA	SAA	VSAA
Bone marrow	Hypocellular bone marrow plus at least two of the following:	Bone marrow cellularity <25%* plus at least two of the following:	Same as SAA
Neutrophils ($\times 10^9$/L)	0.5-1.5	0.2-0.5	<0.2
Platelets ($\times 10^9$/L)	20-50	<20	Same as SAA
Other	Hb ≤10 g/dL plus reticulocytes <30 × 10^9/L	Reticulocytes <20 × 10^9/L or <1% corrected for Hct	Same as SAA

*Or 25% to 50% cellularity with <30% residual hematopoietic cells.
NSAA, nonsevere aplastic anemia; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; Hct, hematocrit.

sylphosphatidylinositol-linked proteins, such as CD55 and CD59. The absence of CD55 and CD59 on the surface of the RBCs renders them more susceptible to lysis by complement. The Ham test for complement-mediated hemolysis is positive if there are a sufficient number of paroxysmal nocturnal hemoglobinuria cells; however, flow cytometry is a more sensitive method to detect small numbers of circulating CD55 and CD59 negative paroxysmal nocturnal hemoglobinuria cells compared with the Ham test[5] (see Chapter 33). Flow cytometry has been able to detect small numbers of circulating paroxysmal nocturnal hemoglobinuria granulocytes in almost 90% of newly diagnosed aplastic anemia patients by concentrating the cells in the granulocyte gate.[33] The significance of this finding is unclear, but it may be related to the ability of glycosylphosphatidylinositol-deficient stem cells to escape the immune destruction in aplastic anemia.[33]

Bone marrow aspirates and biopsy specimens have prominent fat cells with areas of patchy cellularity. Biopsy samples are required for an accurate quantitative assessment of the marrow cellularity, and severe hypocellularity is a characteristic feature (Fig. 21-1). Erythroid, granulocytic, and megakaryocytic cells are decreased or absent. Dyserythropoiesis may be present, but there is no dysplasia of the granulocyte or platelet cell lines. Blasts and abnormal cell infiltrates are characteristically absent. Reticulin staining is normal.

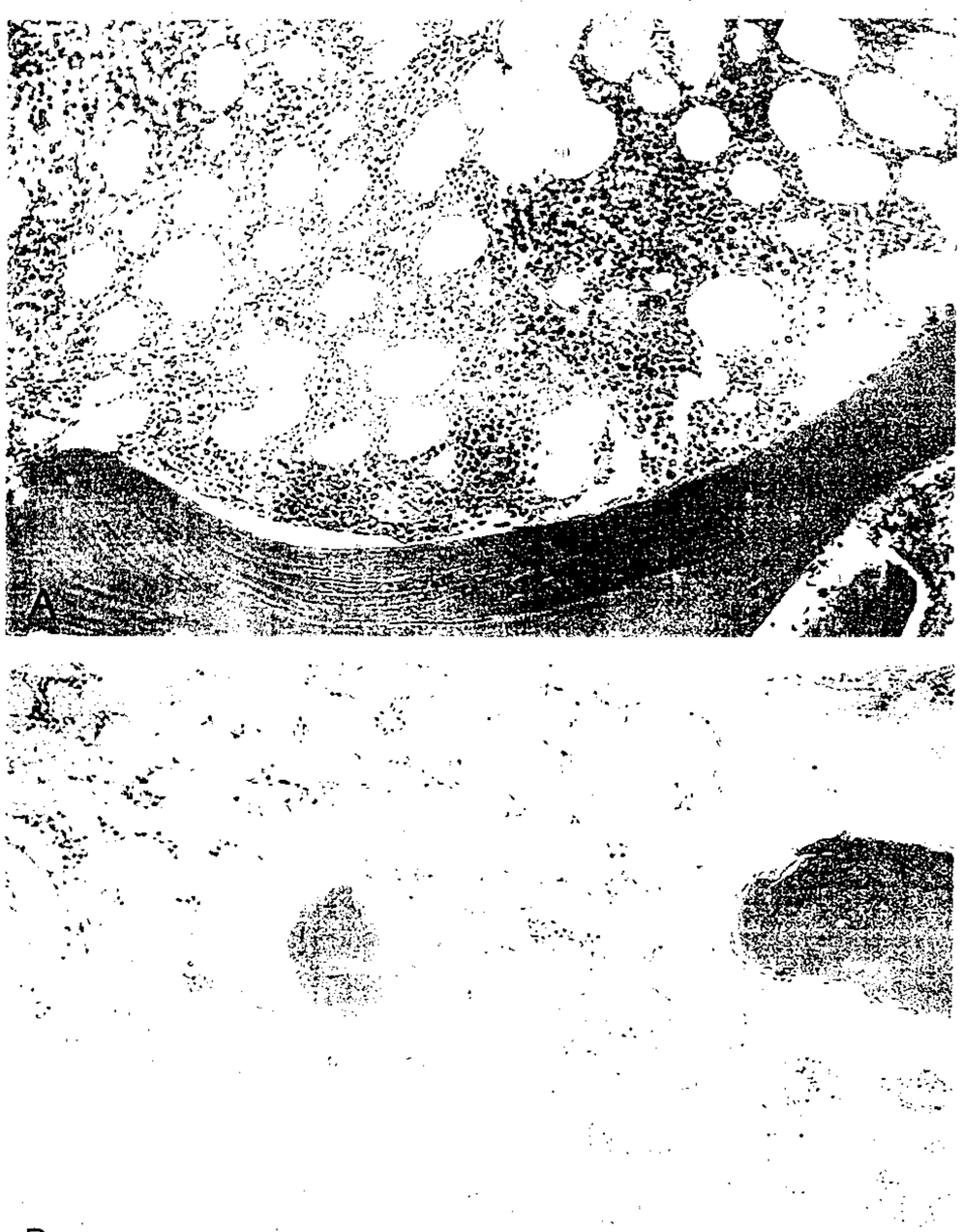

Figure 21-1 A, Normal bone marrow tissue section stained with hematoxylin and eosin. B, Hypoplastic bone marrow tissue section stained with hematoxylin and eosin from a patient with aplastic anemia. (Courtesy of Ann Bell, University of Tennessee, Memphis.)

An abnormal karyotype is infrequent at presentation, but approximately one fourth of aplastic anemia patients develop chromosome abnormalities over the course of the disease, particularly monosomy 7 and trisomy 8.[34] Conventional cytogenetics may underestimate the incidence of karyotype abnormalities. Detection of chromosome abnormalities with conventional culture techniques is difficult and insensitive in aplastic anemia owing to the hypocellularity of the bone marrow and scarcity of cells in metaphase. Newer methods that employ interphase fluorescent in situ hybridization using DNA probes for specific chromosomes have a greater sensitivity and have the ability to use nondividing cells.[5,34]

Treatment and Prognosis

Severe aplastic anemia requires immediate treatment to prevent the dire consequences of serious pancytopenia. If a potential causative agent is suspected, it should be discontinued. To keep blood counts at safe levels, platelets generally are administered when the platelet count decreases to less than 10×10^9/L, whereas RBCs are given when the patient becomes symptomatic.[5] One of the most important early decisions that must be made is whether a patient is a candidate for a hematopoietic stem cell transplant. Hematopoietic stem cell transplant is the treatment of choice for patients with severe aplastic anemia who are younger than age 40 and have an HLA-identical sibling.[3,5] For patients older than age 40 or of any age without an HLA-identical sibling, immunosuppressive therapy, consisting of antithymocyte globulin with cyclosporine is the preferred therapy.[3,5] The antithymocyte globulin decreases the number of activated T cells, and the cyclosporine inhibits their function, suppressing the autoimmune reaction against the stem cells. For patients with severe disease who are not responsive to immunosuppression, hematopoietic stem cell transplant from an HLA-matched unrelated donor may be an option, but survival is not as favorable compared with an HLA-identical sibling.[3,5] Recombinant humanized antibody to the interleukin-2 receptor is under evaluation as a possible therapy.[35]

Other supportive therapy includes antibiotic and antifungal prophylaxis in cases of prolonged neutropenia. Growth factors such as recombinant human erythropoietin are not recommended for primary treatment in that they are generally ineffective and can have serious side effects.[5] Patients with nonsevere aplastic anemia may not require treatment but must be monitored periodically for cytopenia and abnormal cells.

Long-term survival is achieved in 80% to 90% of younger patients who receive a hematopoietic stem cell transplant from an identical sibling.[3] In patients treated with immunosuppression, 75% show an improvement in cytopenia, but 12% to 30% eventually relapse.[3] The presence of monosomy 7 denotes a poor prognosis, whereas trisomy 8 has a good prognosis.[34] Over the course of the disease, 10% to 25% of patients treated with immunosuppression develop paroxysmal nocturnal hemoglobinuria, and 10% to 20% progress to myelodysplastic syndrome or leukemia.[32,34]

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advances in drug therapy have increased survival, with over one-third of patients now surviving for 5 years, compared with only one-quarter 10 years ago. The outlook may improve further with new drugs and combinations of treatments.

APLASTIC ANAEMIA

Primary idiopathic acquired aplastic anaemia

This is a rare disorder in Europe and North America, with 2–4 new cases per million population per annum. The disease is much more common in certain other parts of the world: for example, east Asia. The basic problem is failure of the pluripotent stem cells, producing hypoplasia of the bone marrow with a pancytopenia in the blood. The diagnosis rests on exclusion of other causes of secondary aplastic anaemia (see below) and rare congenital causes, such as Fanconi's anaemia.

Clinical features and investigations

Patients present with symptoms of bone marrow failure, usually anaemia or bleeding, and less commonly, infections. An FBC demonstrates pancytopenia, low reticulocytes and often macrocytosis. Bone marrow aspiration and trephine reveal hypocellularity.

Management

All patients will require blood product support and aggressive management of infection. The prognosis of severe aplastic anaemia managed with supportive therapy only is poor and more than 50% of patients die, usually in the first year. The curative treatment for patients under 30 years of age with severe idiopathic aplastic anaemia is allogeneic HSCT if there is an available donor (p. 1017). Those with a compatible sibling donor should proceed to transplantation as soon as possible; they have a 75–90% chance of long-term cure. In older patients, immunosuppressive therapy with ciclosporin and antithymocyte globulin gives 5-year survival rates of 75%. Such patients may relapse or other clonal disorders of haematopoiesis may evolve, such as paroxysmal nocturnal haemoglobinuria (p. 1031), myelodysplastic syndrome (p. 1041) and acute myeloid leukaemia (p. 1036). They must be followed up long-term.

Secondary aplastic anaemia

Causes of this condition are listed in Box 24.65. It is not practical to list all the drugs which have been suspected of causing aplasia. It is important to check the reported side-effects of all drugs taken over the preceding months. In some instances, the cytopenia is more selective and affects only one cell line, most often the neutrophils. Frequently, this is an incidental finding, with no ill health. It probably has an immune basis but this is difficult to prove.

The clinical features and methods of diagnosis are the same as for primary idiopathic aplastic anaemia. An underlying cause should be treated or removed but otherwise management is as for the idiopathic form.

24.65 Causes of secondary aplastic anaemia

- Drugs
  - Cytotoxic drugs
  - Antibiotics – chloramphenicol, sulphonamides
  - Antirheumatic agents – penicillamine, gold, phenylbutazone, indometacin
  - Antithyroid drugs
  - Anticonvulsants
  - Immunosuppressants – azathioprine
- Chemicals
  - Benzene toluene solvent misuse – glue-sniffing
  - Insecticides – chlorinated hydrocarbons (DDT), organophosphates and carbamates (pp. 220 and 222)
- Radiation
- Viral hepatitis
- Pregnancy
- Paroxysmal nocturnal haemoglobinuria

MYELOPROLIFERATIVE NEOPLASMS

These make up a group of chronic conditions characterised by clonal proliferation of marrow precursor cells, and include polycythaemia rubra vera (PRV), essential thrombocythaemia, myelofibrosis, and chronic myeloid leukaemia (p. 1039). Although the majority of patients are classifiable as having one of these disorders, some have overlapping features and there is often progression from one to another, e.g. PRV to myelofibrosis. The recent discovery of the molecular basis of these disorders will lead to changes in classification and treatment; a mutation in the gene on chromosome 9 encoding the signal transduction molecule *JAK-2* has been found in more than 90% of PRV cases and 50% of those with essential thrombocythaemia and myelofibrosis.

Myelofibrosis

In myelofibrosis, the marrow is initially hypercellular, with an excess of abnormal megakaryocytes which release growth factors, e.g. platelet-derived growth factor, to the marrow microenvironment, resulting in a reactive proliferation of fibroblasts. As the disease progresses, the marrow becomes fibrosed.

Most patients present over the age of 50 years, with lassitude, weight loss and night sweats. The spleen can be massively enlarged due to extramedullary haematopoiesis (blood cell formation outside the bone marrow), and painful splenic infarcts may occur.

The characteristic blood picture is leucoerythroblastic anaemia, with circulating immature red blood cells (increased reticulocytes and nucleated red blood cells) and granulocyte precursors (myelocytes). The red cells are shaped like teardrops (teardrop poikilocytes), and giant platelets may be seen in the blood. The white count varies from low to moderately high, and the platelet count may be high, normal or low. Urate levels may be high due to increased cell breakdown, and folate deficiency is common. The marrow is often difficult to

WILLIAMS HEMATOLOGY

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CHAPTER 33

APLASTIC ANEMIA

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MARSHALL A. LICHTMAN

***Aplastic anemia* is a clinical syndrome manifested as a deficiency of red cells, neutrophils, monocytes, and platelets in the blood, and fatty replacement of the marrow with a near absence of hematopoietic precursor cells. Reticulocytopenia, neutropenia, monocytopenia, and thrombocytopenia, when severe, are life-threatening because of the risk of infection and bleeding, complicated by severe anemia. Most cases occur without an evident precipitating cause and result from expression of autoreactive T lymphocytes that suppress or destroy primitive hematopoietic cells. The disorder also can occur (1) after prolonged high-dose exposure to certain toxic chemicals (e.g., benzene), (2) after specific viral infections (e.g., Epstein-Barr virus), (3) as an idiosyncratic response to certain pharmaceuticals (e.g., ticlopidine), (4) as a feature of a connective tissue or autoimmune disorder (e.g., lupus erythematosus), or (5) rarely in association with pregnancy. Aplastic hematopoiesis is the primary manifestation of several uncommon inherited disorders (e.g., Fanconi anemia). Differential diagnosis includes other causes of a hypoplastic marrow, which can occur in paroxysmal nocturnal hemoglobinuria or hypoplastic myelogenous leukemia. Allogeneic stem cell transplantation is curative in approximately 80 percent of younger patients with a suitable donor, although the posttransplant period may be marred by graft-versus-host disease. The disease can be significantly ameliorated or rarely cured by anti–T cell therapy, especially with antithymocyte globulin and cyclosporine. After successful treatment with immunosuppressive agents, the disease has a propensity to evolve into a clonal hematopoietic disorder, such as paroxysmal nocturnal hemoglobinuria, a clonal cytopenia, or oligoblastic or polyblastic myelogenous leukemia.**

DEFINITION AND HISTORY

Aplastic anemia is a clinical syndrome that results from marked diminution of marrow blood cell production. The decreased production results in reticulocytopenia, anemia, granulocytopenia, monocytopenia, and thrombocytopenia. Severe aplastic anemia is defined as pancytopenia accompanied by a markedly hypocellular marrow and two of the following three features: (1) a corrected reticulocyte count less than 1 percent, (2) fewer than 500/μl granulocytes, or (3) fewer than 20,000/μl platelets. Most cases of aplastic anemia are acquired. Fewer cases are the result of an inherited disorder, such as Fanconi anemia.

Acronyms and abbreviations that appear in this chapter include: ALG, antilymphocyte globulin; ATG, antithymocyte globulin; BFU-E, burst forming unit–erythroid; CFU-GM, colony forming unit–granulocyte-macrophage; CMV, cytomegalovirus; DDT, dichlorodiphenyltrichloroethane; EBV, Epstein-Barr virus; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; HLA, human leukocyte antigen; IFN-γ, interferon gamma; IL, interleukin; MRI, magnetic resonance imaging; PCP, pentachlorophenol; PNH, paroxysmal nocturnal hemoglobinuria; SCF, stem cell factor; TNT, trinitrotoluene.

Aplastic anemia was first recognized by Ehrlich[1] in 1888. He described a young pregnant woman who died of severe anemia and neutropenia. Autopsy examination revealed a fatty marrow with essentially no hematopoiesis. The name *aplastic anemia* subsequently was applied to this disease by the French hematologist Chauffard[2] in 1904. Although the term is anachronistic because morbidity is the result of pancytopenia, the designation is entrenched in medical usage. For the following 30 years, many conditions that caused pancytopenia were confused with aplastic anemia based on incomplete or inadequate histologic study of the patient's marrow.[3] The development of improved instruments for percutaneous marrow biopsy in the last half of the 20th century improved diagnostic precision. The disease initially was thought to result from atrophy of primitive marrow hematopoietic cells. The unexpected recovery of marrow recipients who were given immunosuppressive conditioning but who did not engraft with donor stem cells raised the possibility that the disease was not intrinsic to primitive hematopoietic cells but resulted from suppression of hematopoietic cells by immune cells.[4] This supposition was confirmed by a clinical trial that established antilymphocyte globulin (ALG) alone as effective therapy for aplastic anemia.[5]

EPIDEMIOLOGY

Retrospective analysis in the United States estimated the incidence of aplastic anemia at two to five cases per 1,000,000 population per year.[6] The International Aplastic Anemia and Agranulocytosis Study and a French study brought the number closer to two per 1,000,000 persons per year.[7,8] The highest frequency of aplastic anemia occurs in persons aged 15 to 25 years; a second peak occurs at age 65 to 69 years.[7] Aplastic anemia is more prevalent in the Far East, where the incidence is approximately seven per 1,000,000 in China,[10] approximately four per 1,000,000 in Thailand,[11] and approximately five per 1,000,000 in Malaysia.[12] The explanation for the twofold or greater incidence in the Orient compared to the Occident is unclear. A study showing that the incidence of aplastic anemia in Hawaiians of Japanese ancestry is similar to that observed in the West does not support a genetic basis.[13] Use of chloramphenicol in Asia probably is not an explanation given that the occurrence of aplastic anemia remained high even after decreased use of the agent.[14,15] Poorly regulated exposure of workers to benzene may be a factor.[16]

ETIOLOGY AND PATHOGENESIS

Table 33-1 lists potential causes of aplastic anemia. The final common pathway to the clinical disease is decreased blood cell formation. The numbers of marrow colony forming unit–granulocyte-macrophage (CFU-GM) and burst forming unit–erythroid (BFU-E) are reduced markedly in patients with aplastic anemia.[17–22] The number of long-term culture-initiating cells is reduced to approximately 1 percent of normal values.[23] CD34+ hematopoietic cells, the fraction in which hematopoietic stem cells may reside, are correspondingly low.[24,25] Potential mechanisms responsible for acquired marrow cell failure include (1) direct toxicity to hematopoietic stem cells, (2) a defect in the stromal microenvironment of the marrow required for hematopoietic cell development, (3) impaired production or release of essential hematopoietic growth factors, and (4) cellular or humoral immune suppression of marrow progenitor cells. Little experimental evidence for a stromal microenvironmental defect or a deficit of critical hematopoietic growth factors exists. Thus, reduced hematopoiesis appears to represent an acquired toxic injury to primitive hematopoietic cells or, alternatively, immune suppression of hematopoietic progenitor cells. The accumulated evidence points primarily to suppression of hematopoiesis by autoreactive T lymphocytes.[26,27] The inheritance of mutations in genes such as *TERC* or *TERT* results in impaired mainte-

Acquired
- Idiopathic (autoimmune)
  - *TERC*, *TERT*, *TERF* 1 & 2, *TIN2* susceptibility mutations
- Drugs (see Table 33-2)
- Toxins
  - Benzene
  - Chlorinated hydrocarbons
  - Organophosphates
- Viruses
  - Epstein-Barr virus
  - Non-A, non-B, non-C, non-D, non-E, and non-G hepatitis virus
  - Human immunodeficiency virus
- Paroxysmal nocturnal hemoglobinuria
- Autoimmune/connective tissue disorders
  - Eosinophilic fasciitis
  - Immune thyroid disease (Graves disease, Hashimoto thyroiditis)
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
  - Thymoma
- Pregnancy
- Iatrogenic
  - Radiation
  - Cytotoxic drug therapy

Hereditary
- Fanconi anemia
- Dyskeratosis congenita
- Shwachman-Diamond syndrome
- Other rare syndromes (see Table 33-4)

nance of telomere length. Shortened telomere length in the hematopoietic cells may heighten susceptibility to immune or other injury. These mutations may, therefore, predispose to the development of aplastic anemia.[278,279]

Early studies showed that marrow lymphocytes or blood or marrow mononuclear cells from patients with aplastic anemia inhibited colony growth when the cells were cocultured with normal marrow.[17,20,28–30] Inhibition could have resulted from transfusion sensitization rather than autoimmunity.[31,32] However, culture studies in patients with aplastic anemia prior to transfusion[33] or before and after successful treatment[34,35] were highly suggestive of T cell–mediated suppression of marrow cell development. Furthermore, some marrow transplant recipients recovered from marrow aplasia without engraftment after the initial immunosuppressive preparative treatment, a finding compatible with successful treatment of a suppressor cell population.[36] Also, transplantation of a patient with aplastic anemia from an identical twin often resulted in engraftment failure unless a conditioning regimen (immunosuppression) was administered prior to transplantation. Because the latter treatment is not required to prevent graft rejection between identical twins, the requirement also supported the need to eradicate in the recipient a cell population that interfered with the growth of normal hematopoiesis. Taken together, these *in vitro* and *in vivo* observations support a T cell–mediated mechanism for genesis of aplastic anemia.[39] Immune injury to the marrow after exposure to toxin-induced marrow aplasia could result from the release of antigens that provoke a secondary T cell–mediated injury to hematopoietic cells. This mechanism could explain the need for immunosuppressive treatment after exposure to an exogenous agent. Levels of cytokines with inhibitory effects on hematopoiesis increase in the marrow of patients with severe aplastic anemia. Spontaneous or mitogen-induced increases in mononuclear cell production of interferon gamma (IFN-γ),[40,41] interleukin (IL) 2,[42] and tumor necrosis factor alpha[43,44] occur. Elevated serum levels of IFN-γ have been found in 30 percent of patients with aplastic anemia, and IFN-γ expression has been detected in the marrow of most patients with acquired aplastic anemia.[45] Addition of antibodies to IFN enhances *in vitro* colony growth of marrow cells from affected patients.[40] This indicates a role for IFN-γ in either the initiation or propagation of the aplastic anemia defect. Aplastic anemia now is considered to result from immune inhibition of primitive hematopoietic progenitors, mediated in part by inhibitory cytokines released by cytotoxic T lymphocytes. Several putative target antigens on affected hematopoietic cells have been identified. Autoantibodies to kinectin, one putative antigen, have been found in patients with aplastic anemia. T cells, which are responsive to kinectin-derived peptides, suppress granulocyte-monocyte colony growth *in vitro*. However, in these studies cytotoxic T lymphocytes with that specificity were not isolated from patients.[273]

Chloramphenicol is the most notorious drug documented to cause aplastic anemia. Although this drug is directly myelosuppressive at very high dose because of mitochondrial toxicity, the occurrence of aplastic anemia appears to be idiosyncratic, perhaps related to an inherited sensitivity to a nitroso-containing toxic intermediate.[46] This sensitivity may produce immunologic marrow suppression, given the substantial numbers of affected patients who respond to immunosuppressive therapy.[47] The risk of developing aplastic anemia in patients treated with chloramphenicol is approximately one in 20,000, or 10 to 50 times that of the general population.[6,48,49] Unfortunately, fatal aplastic anemia with topical or systemic drug use still is reported.[50,51]

Epidemiologic evidence established that quinacrine (Atabrine) increased the risk of aplastic anemia.[52] This drug was administered to all US troops in the South Pacific and Asiatic theaters of operations as prophylaxis for malaria during 1943 and 1944. The incidence of aplastic anemia was seven to 28 cases per 1,000,000 personnel per year in the prophylaxis zones, whereas untreated soldiers had one to two cases per 1,000,000 personnel per year. The aplasia occurred during administration of the offending agent and was preceded by a characteristic rash in nearly half the cases. Many other drugs reportedly increase the risk of aplastic anemia, but the spectrum of drug-induced aplastic anemia may not be fully appreciated because of incomplete reporting of information and the infrequency of the association. Table 33-2 lists the drugs that have been associated with aplastic anemia.

Many of the drugs induce selective cytopenias, such as agranulocytosis, which usually are reversible after the offending agent is discontinued. These reversible reactions are not correlated with the risk of aplastic anemia, which casts doubt on the effectiveness of routine monitoring of blood counts as a strategy to avoid aplastic anemia.

Aplastic anemia remains a rare event that can occur because of an underlying genetic, metabolic, or immunologic predisposition in susceptible individuals. Delayed oxidation and clearance of acetanilide, a related compound, occur in patients with phenylbutazone-associated marrow aplasia compared to either normal controls or patients with aplastic anemia resulting from other causes.[53] This finding suggests excess accumulation of the drug as a potential mechanism for the aplasia. Drug interactions or synergy may be required to induce marrow aplasia in some cases. Cimetidine, a histamine H_2-receptor antagonist, occasionally is implicated in the onset of cytopenias and aplastic anemia, perhaps because of a direct effect on hematopoietic stem cells.[54,55]

DRUGS ASSOCIATED WITH APLASTIC ANEMIA

Category	High Risk	Intermediate Risk	Low Risk
Analgesic			Phenacetin, aspirin, salicylamide
Antiarrhythmic			Quinidine, tocainide
Antiarthritics		Gold salts	Colchicine
Anticonvulsant		Carbamazepine, hydantoins, felbamate	Ethosuximide, phenacemide, primidone, trimethadione, sodium valproate
Antihistamine			Chlorpheniramine, pyrilamine, tripelennamine
Antihypertensive			Captopril, methyldopa
Antiinflammatory		Penicillamine, phenylbutazone, oxyphenbutazone	Diclofenac, ibuprofen, indomethacin, naproxen, sulindac
Antimicrobial			
Antibacterial		Chloramphenicol	Dapsone, methicillin, penicillin, streptomycin, β-lactam antibiotics
Antifungal			Amphotericin, flucytosine
Antiprotozoal		Quinacrine	Chloroquine, mepacrine, pyrimethamine
Antineoplastic drugs			
Alkylating agents	Busulfan, cyclophosphamide, melphalan, nitrogen mustard		
Antimetabolites	Fluorouracil, mercaptopurine, methotrexate		
Cytotoxic antibiotics	Daunorubicin, doxorubicin, mitoxantrone		
Antiplatelet			Ticlopidine
Antithyroid			Carbimazole, methimazole, methylthiouracil, potassium perchlorate, propylthiouracil, sodium thiocyanate
Sedative and tranquilizer			Chlordiazepoxide, chlorpromazine (and other phenothiazines), lithium, meprobamate, methyprylon
Sulfonamides and derivatives			Numerous sulfonamides
Antibacterial			
Diuretic		Acetazolamide	Chlorothiazide, furosemide
Hypoglycemic			Chlorpropamide, tolbutamide
Miscellaneous			Allopurinol, interferon, pentoxifylline

NOTE: Drugs that invariably cause marrow aplasia with high doses are termed *high risk*; drugs with at least 30 reported cases are listed as *moderate risk*; others are less often associated with aplastic anemia (*low risk*).
SOURCES: This list was compiled from the AMA Registry,[125] publications of the International Agranulocytosis and Aplastic Anemia Study,[126–130] other reviews and studies,[87,131–133,183] previous compilations of offending agents,[134,135] and selected reports. An additional comprehensive source for potentially offending drugs can be found in reference 272.

This drug accentuates the marrow-suppressive effects of the chemotherapy drug carmustine.[56] In several instances, cimetidine reportedly was a possible cause of marrow aplasia when cimetidine was given with chloramphenicol.[51]

TOXIC CHEMICALS

Benzene was the first chemical linked to aplastic anemia, based on studies of factory workers before the 20th century.[57] Benzene is used as a solvent in the manufacture of chemicals, drugs, dyes, and explosives. It has been a vital chemical in the manufacture of rubber and leather goods and has been used widely in the shoe industry, leading to an increased risk for aplastic anemia and leukemia in workers in these industries.[58,59] In China, toxic effects of benzene were found in 0.5 percent of exposed workers; occurrence of aplastic anemia among workers was sixfold higher than in the general population.[16]

The US Occupational Safety and Health Administration has lowered the permissible exposure limit to benzene to 1 ppm,[60] after exposure to 100 ppm was shown to be associated with leukopenia in about one third of workers.[61] Other hematologic abnormalities, such as hemolytic anemia, marrow hyperplasia, myeloid metaplasia, and acute myelogenous leukemia, have been observed in patients exposed to benzene.[16,58,59,62]

Chlorinated hydrocarbons and organophosphate compounds have been implicated in the onset of aplastic anemia.[63] Chlorophenothane (dichlorodiphenyltrichloroethane [DDT]), lindane, and chlordane are the most common insecticides involved. Aplastic anemia was reported following use of lindane in home vaporizers for disinfection. This practice continued until the 1970s, when more than 30 case reports of aplastic anemia led to curtailment of chlorophenothane use.[64] Cases still occur occasionally after heavy exposure at industrial plants or after its use as a pesticide.[65] Lindane is metabolized in part to pentachlorophenol (PCP), another potentially toxic chlorinated hydrocarbon that is manufactured for use as a wood preservative. Many cases of aplastic anemia and related blood disorders have been attributed to PCP over the past 25 years.[64–67] Prolonged exposures to petroleum distillates in the form of Stoddard solvent[68] and acute exposure to toluene through glue sniffing[69] reportedly cause marrow aplasia. Trinitrotoluene (TNT), an explosive used extensively during World Wars I and II, is absorbed readily by inhalation and through the skin. Fatal cases of aplastic anemia were observed in munitions workers exposed to TNT in Great Britain from 1940 to 1946.[70]

VIRUSES

NON-A, NON-B, NON-C, NON-D, NON-E, AND NON-G HEPATITIS VIRUSES

A number of case reports have studied the relationship between hepatitis and subsequent development of aplastic anemia. The association was emphasized by two major reviews in the 1970s.[71,72] In aggregate, the reports summarized findings in more than 200 cases. In many instances, the hepatitis was improving or had resolved when the aplastic

anemia was noted 4 to 12 weeks later. Approximately 10 percent of cases occurred more than 1 year after the initial diagnosis of hepatitis. Most patients were young (aged 18–20 years), two thirds were male, and their survival was short (10 weeks). Although hepatitis A and B have been implicated in aplastic anemia in a small number of cases, most cases are related to non-A, non-B, and non-C hepatitis.[73,74] Severe aplastic anemia developed in nine of 31 patients who underwent liver transplantation for non-A, non-B, or non-C hepatitis but in none of 1463 patients transplanted for other indications.[75] Several lines of evidence indicate no association with hepatitis C virus, which suggests an unknown viral agent is involved.[76–78] No evidence of hepatitis A, B, C, D, E, or G, transfusion-transmitted virus or parvovirus B19 was found in 15 patients with posthepatitic aplastic anemia.[79]

EPSTEIN-BARR VIRUS

EBV has been implicated in the pathogenesis of aplastic anemia.[80] Onset usually occurs within 4 to 6 weeks of infection. Infectious mononucleosis is subclinical in some cases. Reactive lymphocytes are noted on the blood film, and serologic results are consistent with a recent infection. EBV has been detected in marrow cells,[81] but whether marrow aplasia results from a direct effect or is an immunologic response by the host is uncertain. Some patients have recovered after antithymocyte globulin therapy.[76,81]

OTHER VIRUSES

Human immunodeficiency virus infection frequently is associated with varying degrees of cytopenia. The marrow often is cellular, but occasional cases of aplastic anemia have been noted.[82–84] In these patients, marrow hypoplasia may result from viral suppression and from the drugs used to control viral replication in this disorder.

A number of other viruses have been implicated in the pathogenesis of marrow failure. B19 parvovirus, the cause of fifth disease, leads to transient erythroid aplasia but is not known to induce aplastic anemia.[76,85] Human herpes virus 6 has caused severe marrow aplasia subsequent to bone marrow transplantation for other disorders.[86]

AUTOIMMUNE/CONNECTIVE TISSUE DISEASES

The incidence of severe aplastic anemia was sevenfold greater than expected in patients with rheumatoid arthritis.[87] Whether the aplastic anemia is related directly to rheumatoid arthritis or to the various drugs used to treat the condition (gold salts, D-penicillamine, nonsteroidal agents) is uncertain. Occasional cases of aplastic anemia are seen in conjunction with systemic lupus erythematosus.[88] *In vitro* studies found either an antibody[89] or a suppressor cell[90,91] directed against hematopoietic progenitor cells. Patients have recovered after plasmapheresis,[89] glucocorticoids,[91] or cyclophosphamide therapy,[90,92] suggesting an immune etiology.

Eosinophilic fasciitis is an uncommon connective tissue disorder with painful swelling and induration of the skin and subcutaneous tissue associated with aplastic anemia.[93,94] The disorder is antibody mediated in some cases but is largely unresponsive to therapy.[93] Stem cell transplantation, immunosuppressive therapy using cyclosporine,[93] antithymocyte globulin (ATG), or ATG and cyclosporine has cured or significantly ameliorated the disease in a few patients.[94]

Severe aplastic anemia has been reported in association with immune thyroid disease[95,96] and thymoma.[97,98]

Cases of pregnancy-associated aplastic anemia have been reported, but the relationship between the two conditions is not clear.[99–101] In some patients, pregnancy exacerbates preexisting aplastic anemia, which improves after the pregnancy is terminated.[99,100] In other cases, the aplasia develops during pregnancy, with recurrences during subsequent pregnancies.[100,101] Termination of pregnancy or delivery may improve marrow function, but the disease may progress to a fatal outcome even after delivery.[99–101] Therapy can include elective termination of early pregnancy, supportive care, immunosuppressive therapy, or marrow transplantation after delivery. Pregnancy in women previously treated with immunosuppression for aplastic anemia can result in the birth of a normal newborn.[102] In a series of 36 pregnancies, 22 were uncomplicated, 7 were complicated by a relapse of the marrow aplasia, and 5 without aplasia required red cell transfusion during delivery.[102] One death as a result of cerebral thrombosis occurred in a patient with paroxysmal nocturnal hemoglobinuria (PNH) and aplasia.

IATROGENIC CAUSES

Although marrow toxicity from cytotoxic chemotherapy or radiation directly damages stem cells and more mature cells and results in marrow aplasia, most patients with acquired aplastic anemia cannot relate an exposure that would be responsible for marrow damage.

Chronic exposure to low doses of radiation or use of spinal radiation for ankylosing spondylitis is associated with an increased, but delayed, risk of developing aplastic anemia and acute leukemia.[103,104] Patients who were given thorium dioxide (Thorotrast) as an intravenous contrast medium suffered numerous late complications, including malignant liver tumors, acute leukemia, and aplastic anemia.[105] Chronic radium poisoning with osteitis of the jaw, osteogenic sarcoma, and aplastic anemia was seen in workers who painted watch dials with luminous paint when the workers moistened the brushes orally.[106]

Acute exposures to large doses of radiation are associated with development of marrow aplasia and a gastrointestinal syndrome.[107,108] Total body exposure between 1 and 2.5 Gy leads to gastrointestinal symptoms and depression of leukocyte counts, but most patients recover. A 4.5-Gy dose leads to death in half of individuals (LD_{50}) as a result of marrow failure. Higher doses of approximately 10 Gy are universally fatal unless the patient receives extensive supportive care followed by marrow transplantation. Aplastic anemia associated with nuclear accidents was seen after the Chernobyl nuclear power station disaster in the Ukraine in 1986.[109]

Antineoplastic drugs such as alkylating agents, antimetabolites, and certain cytotoxic antibiotics have the potential to produce marrow aplasia. In general, the effect is transient, is an extension of the drugs' pharmacologic action, and resolves within several weeks of completing chemotherapy. Severe hypoplasia, although unusual, can follow use of the alkylating agent busulfan and persist for extended intervals. Patients may develop marrow aplasia 2 to 5 years after discontinuing alkylating agent therapy. These cases often evolve into hypoplastic myelodysplastic syndromes.

STROMAL MICROENVIRONMENT AND GROWTH FACTORS

Short-term clonal assays for marrow stromal cells show variable defects in stromal cell function. Serum levels of stem cell factor (SCF) were either moderately low or normal in several studies of aplastic anemia.[110–112] Although SCF augments the growth of hematopoietic colonies from aplastic marrows,[113] its use in patients has not led to clinical remissions. Flt-3 ligand, another early-acting growth factor, is 30- to 100-fold elevated in the serum of patients with aplastic anemia.[114] Fibroblasts grown from patients with severe aplastic anemia have subnormal cytokine production. However, serum levels of granulocyte colony stimulating factor (G-CSF),[115] erythropoietin,[116] and thrombopoietin[117] usually are high. Synthesis of IL-1, an early stimulator of hematopoiesis, is decreased in mononuclear cells from patients with aplastic anemia.[118] Studies of the microenvironment have shown relatively normal stromal cell proliferation and growth factor

production.[119–121] These findings, coupled with the limited response of patients with aplastic anemia to growth factors, suggest cytokine deficiency is not the etiologic problem in most cases. The most compelling argument is that most patients transplanted for aplastic anemia are cured with allogeneic donor stem cells and autologous stroma.[37,122–124]

HEREDITARY APLASTIC ANEMIA

FANCONI ANEMIA

DEFINITION AND HISTORY

Fanconi anemia, the most common form of constitutional aplastic anemia, was described in three brothers by Fanconi[136] in 1927. It is inherited as an autosomal recessive condition that results from defects in genes that modulate DNA stability.

EPIDEMIOLOGY

Since its initial description, more than 1300 cases of Fanconi anemia have been recorded through reports in the literature or from an International Fanconi Anemia Registry.[137] Fanconi anemia is estimated to be present in one in 1,000,000 individuals; although it occurs more frequently in Afrikaners of European descent and in southern Italy.

ETIOLOGY AND PATHOGENESIS

Eleven complementation groups, defined by somatic cell hybridization, are associated with development of Fanconi anemia.[138] The complementation groups are designated *FANCA, B, C, D1, D2, E, F, G, I, J,* and *L*. Table 33-3 lists the gene mutations corresponding to eight complementation groups. The great majority of patients have mutations of *FANCA, C,* or *G*.[139] The A and C gene products, which are cytoplasmic proteins, have been proposed to form a complex with the products of genes B, E, F, and G, which are adaptors or phosphorylators. The complex translocates to the nucleus, where it protects the cell from DNA cross-linking and likely participates in DNA repair by interacting with *BRCA2*, Rad51, and possibly *BRCA1*.[139] Normal function is disturbed in the presence of a mutant gene product, leading to damaging effects in sensitive tissues, including hematopoietic cells. Mutation of the D product appears to affect tissue cells through a different mechanism, perhaps downstream from the complex.[140,141] An eighth gene, *FANCL*, has been identified. The gene product is necessary for *FANCD2* monoubiquitination, and its mutation can lead to Fanconi anemia.[274] Two additional genes, *FANCI* and *FANCJ*, have been described in a group of patients with Fanconi anemia.[275] FANCI anemia also involves a defect in monoubiquitination of *FANCD2*.

In addition to the genetic defects leading to DNA instability and an inability to repair DNA, tumor necrosis factor (TNF) alpha and TNF gamma are overexpressed in the marrow of Fanconi anemia patients.[276] The excess TNF-α may play a role in suppression of erythropoiesis in these patients.

CLINICAL FEATURES

The onset of marrow failure, usually during the last half of the first decade of life, is gradual. Anemia, weakness, fatigue, dyspnea on exertion, thrombocytopenia, epistaxis, purpura, or other unexpected bleeding are the principal findings. Hepatosplenomegaly is not a feature of the disease. *Café au lait* spots, an abnormal skin pigmentation consisting of flat, light brown lesions ranging from 1 to 12 cm in diameter, may be evident. Growth retardation results in short stature. Skeletal anomalies, especially dysplastic radii and thumbs, occur in half the patients. Heart, kidney, and eye defects may be present. Microcephaly and mental retardation may be a feature. Hypogonadism may be evident. Hematologic and visceral manifestations are combined in more than one third of patients, but some may have cytopenias and inconspicuous somatic changes, whereas others may have somatic anomalies with no or a nominal disorder of blood cell formation. Some who carry the gene may be virtually unaffected.[140,142] In a review of the more than 1300 patients reported in the literature, 100 patients (14%) without anomalies were identified by chromosome breakage studies (see "Laboratory Features," below) as a result of affected siblings.[143] In the past, children in Fanconi families with onset of aplastic anemia without congenital abnormalities were thought to have a different disorder termed *Estren-Dameshek syndrome*.[144] However, children whose lymphocytes show sensitivity to diepoxybutane are considered to have Fanconi anemia without skeletal abnormalities.

TABLE 33-3 FANCONI ANEMIA GENES

Gene	Approximate Incidence among Fanconi Anemia Patients (%)	Chromosomal Location	Exon Number	Amino Acid Residues
FANCA	70*	16q24.3	43	1455
FANCC	10	9q22.3	14	558
BRCA2†	1	13q12.3	27	3418
FANCD2	1	3p25.3	44	1451
FANCE	5	6p21.3	10	536
FANCF	2	11p15	1	374
FANCG	10	9p13	14	622
FANCL	1	2p16.1	?	373

* There are more than 100 mutant FANCA alleles, approximately 40 percent of which are large intragenic deletions.
Whereas FA alleles of *BRCA2* are found in FA-D1 cells, a sufficient number of FANCB cells have not been tested at this time to conclude that *BRCA2* mutations account for both FA-B and FA-D1.
† Although a *BRCA2* null genotype is an embryonic lethal phenotype, certain homozygous *BRCA2* mutations that lead to C-terminal truncations lead to FA of the D1 complementation group.
Reproduced with permission from reference 139.

LABORATORY FEATURES

Blood counts and marrow cellularity often are normal until the patient is 5 to 10 years old, when pancytopenia develops over an extended interval. Macrocytosis with anisocytosis and poikilocytosis may be present before cytopenia occurs. Thrombocytopenia may precede the development of granulocytopenia and anemia. The marrow becomes hypocellular, and *in vitro* colony assays reveal decreased CFU-GM and BFU-E.[142]

Random chromatid breaks are present in myeloid cells, lymphocytes, and chorionic villus biopsy samples. This chromosome damage is intensified after exposure to DNA cross-linking agents such as mitomycin C or diepoxybutane. The hypersensitivity of the chromosomes of marrow cells or lymphocytes to the latter agent is used as a diagnostic test for the condition. Cell cycle progression is prolonged at the G_2-to M transition, and the cells are more susceptible to oxygen toxicity when cultured *in vitro*.[145] It is important to test the lymphocytes from pediatric patients with aplastic anemia for sensitivity to diepoxybutane, since therapy for Fanconi anemia differs from that used for aplastic anemia.

In the near future, clinical laboratories will be able to genotype suspected patients. Determining the specific gene mutation responsible in a patient (see Table 33-3) is important because it confirms the diagnosis, identifies the genotype linked to *BRCA2* which may predispose to a cancer (breast, ovary), and permits carrier detection.[146]

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of Fanconi anemia includes other causes of aplastic anemia, particularly familial syndromes associated with skeletal anomalies and other dysmorphic features. Other familial types of aplastic anemia have been reported with or without associated anomalies. In instances in which no sensitivity to DNA damaging agents is observed, the syndrome does not represent Fanconi anemia. Several uncommon syndromes of this type are described below and in Table 33-4.

THERAPY AND PROGNOSIS

Most patients with Fanconi anemia do not respond to ATG or cyclosporine. Patients improve with androgen preparations, often for as long as several years. Cytokines can improve blood counts, but studies in a mouse model suggest the effects will not be sustained.[162] Relapses occur gradually. Death from progressive marrow failure or conversion to myelodysplastic syndrome, acute myelogenous leukemia (approximately 10 percent of patients), or development of a variety of other cancers occurs eventually by age 10 to 20 years.[137] The presence of a clonal cytogenetic abnormality or marrow morphology consistent with myelodysplasia markedly reduces the 5-year survival rate.[163] Allogeneic stem cell transplantation is curative for Fanconi anemia.[164,165] A marked reduction in dosage of the marrow-conditioning regimen of cyclophosphamide and radiation is necessary because of the undue sensitivity of the tissues to alkylating agents.[164] Early studies of gene therapy that introduce normal cDNA into cells from patients may restore resistance to DNA-damaging agents.[166,167] Difficulties in this approach include the paucity of stem cells in these patients and the toxicity of the gene transfer methodology.

DYSKERATOSIS CONGENITA, SHWACHMAN-DIAMOND SYNDROME, AND OTHER INHERITED SYNDROMES

Dyskeratosis congenita and Shwachman-Diamond syndrome (pancreatic insufficiency with neutropenia) are two rare disorders that may evolve into aplastic anemia. Dyskeratosis usually is inherited as a recessive X chromosome–linked disorder, although rare cases are autosomal dominant or recessive. Mutations of the *DKC1* gene are responsible for the X-linked form and mutations of the *TERC* gene for the autosomal dominant form.[151,153,154] *DKC1* encodes dyskerin, which is a protein component of the telomerase complex, and hTR is the RNA component of telomerase. Dyskeratosis congenita likely results from defective telomerase activity resulting from mutations in these two genes.[151,153,154] The disease is reflected in reticulate skin pigmentation, leukoplakia, and dystrophic nails. A variety of noncutaneous anomalies are observed. Skin and mucosal lesions appear in adolescence, and aplastic anemia usually develops in early adulthood.[158]

Shwachman-Diamond syndrome results from a mutation in the *SBDS* gene and is manifest by pancreatic insufficiency and steatorrhea.[158] Neutropenia is present in virtually all patients and neutropenia and thrombocytopenia in about one third to one half of patients. Thus, a substantial plurality of patients has bicytopenia or tricytopenia with hypoplastic marrows. A significant risk of progression to myelogenous leukemia exists.[169,170] Severe hematopoietic dysfunction can be treated successfully with allogeneic stem cell transplantation.

Table 33-4 lists other rare syndromes associated with aplastic anemia. Reticular dysgenesis appears to result from a stem cell defect, given that lymphoid and myeloid precursors are affected. Seckel syndrome involves the *ATR* gene, and marrow cells exhibit heightened sister chromatid exchange.[156,171] The genetic bases of marrow failure in the other syndromes are unknown.

CLINICAL FEATURES

The onset of symptoms of aplastic anemia may be gradual. Pallor, weakness, dyspnea, and fatigue result from the decrease in red cells. Dependent petechiae, bruising, epistaxis, vaginal bleeding, and unexpected bleeding at other sites secondary to thrombocytopenia are frequent presenting signs of the underlying marrow disorder. Rarely, the symptoms are more dramatic. Fever, chills, and pharyngitis or other sites of infection result from neutropenia. Physical examination generally is unrevealing, except for evidence of anemia (e.g., conjunctival

TABLE 33-4 RARE SYNDROMES ASSOCIATED WITH APLASTIC ANEMIA

DISORDER	FINDINGS	INHERITANCE	GENE	REFERENCES
Ataxia-pancytopenia	Cerebellar atrophy Pancytopenia	Autosomal dominant	Unknown	146–148
Dubowitz syndrome	Growth failure Microcephaly Abnormal facies Pancytopenia, AML, ALL	Autosomal recessive	Unknown	149,150
Dyskeratosis congenita	Skin pigmentation Leukoplakia Dystrophic nails Pancytopenia	X-linked recessive Rarely: Autosomal dominant Autosomal recessive	*DKC1* *TERC* Unknown	151–154
Reticular dysgenesis	Lymphopenia Granulocytopenia (mostly seen in males) Often anemia	X-linked recessive	Unknown	155
Seckel syndrome	Growth failure Microcephaly Abnormal facies Occasional pancytopenia AML (1 case)	Autosomal recessive	*ATR* (and *RAD3* related gene)	156,157
Shwachman-Diamond	Pancreatic insufficiency and neutropenia Pancytopenia in 1/3 to 1/2 AML	Autosomal recessive	*SBDS*	158–160
[illegible]	Radial/ulnar abnormalities Pancytopenia, AML	Autosomal dominant	Unknown	161

A number of other isolated cases of familial aplastic anemia with or without associated anomalies that are not consistent with Fanconi anemia have been reported (see reference 143).
AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia.

TABLE 33-5 APPROACH TO DIAGNOSIS

History and physical examination
Initial laboratory studies
- Complete blood counts, reticulocyte count, and examination of blood film
- Marrow aspiration and biopsy
- Marrow cytogenetics to evaluate clonal myeloid disease
- Red cell hemoglobin F content and DNA stability test as markers of Fanconi anemia
- Immunophenotyping of red and white cells, especially for CD59 to exclude paroxysmal nocturnal hemoglobinuria
- Direct and indirect Coombs test to rule out immune cytopenia
- Serum lactate dehydrogenase and uric acid that may reflect neoplastic cell turnover
- Liver function tests to assess evidence of recent hepatitis virus exposure
- Screening tests for hepatitis viruses A, B, and C
- Screening tests for cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus
- Serum B_{12} and red cell folic acid levels to rule out megaloblastic pancytopenia
- Serum iron, iron-binding capacity, and ferritin as a baseline prior to chronic transfusion therapy

and cutaneous pallor, resting tachycardia), cutaneous bleeding (e.g., ecchymoses and petechiae), or gingival bleeding and intraoral purpura. Lymphadenopathy and splenomegaly are not features of aplastic anemia; such findings suggest an alternative diagnosis such as leukemia or lymphoma.

LABORATORY FEATURES

BLOOD FINDINGS

Patients with aplastic anemia have varying degrees of pancytopenia. Anemia is associated with a low reticulocyte index. The reticulocyte count usually is less than 1.0 percent and may be zero despite the high levels of erythropoietin.[172] Macrocytes may be present. The total leukocyte count and platelet counts are low. The differential white cell count reveals a decrease in neutrophils and monocytes. An absolute neutrophil count less than 500×10^6/liter and a platelet count less than $20{,}000 \times 10^6$/liter are indicative of severe disease. A neutrophil count less than 200×10^6/liter indicates very severe disease. Lymphocyte production is thought to be normal, but patients may have mild lymphopenia. Platelets are reduced but function normally. Significant qualitative changes of red cell, leukocyte, or platelet morphology are not features of classic acquired aplastic anemia. On occasion, only one cell line is depressed initially, which may lead to an early diagnosis of red cell aplasia or amegakaryocytic thrombocytopenia. In such patients, other cell lines fail shortly thereafter (days to weeks) and permit a definitive diagnosis (Table 33-5).

PLASMA FINDINGS

Plasma contains high levels of hematopoietic growth factors, including erythropoietin, thrombopoietin, and myeloid colony stimulating factors.[115–117] Serum iron values usually are high, and ^{59}Fe clearance is prolonged, with decreased incorporation into circulating red cells.[173]

MARROW FINDINGS

MORPHOLOGY

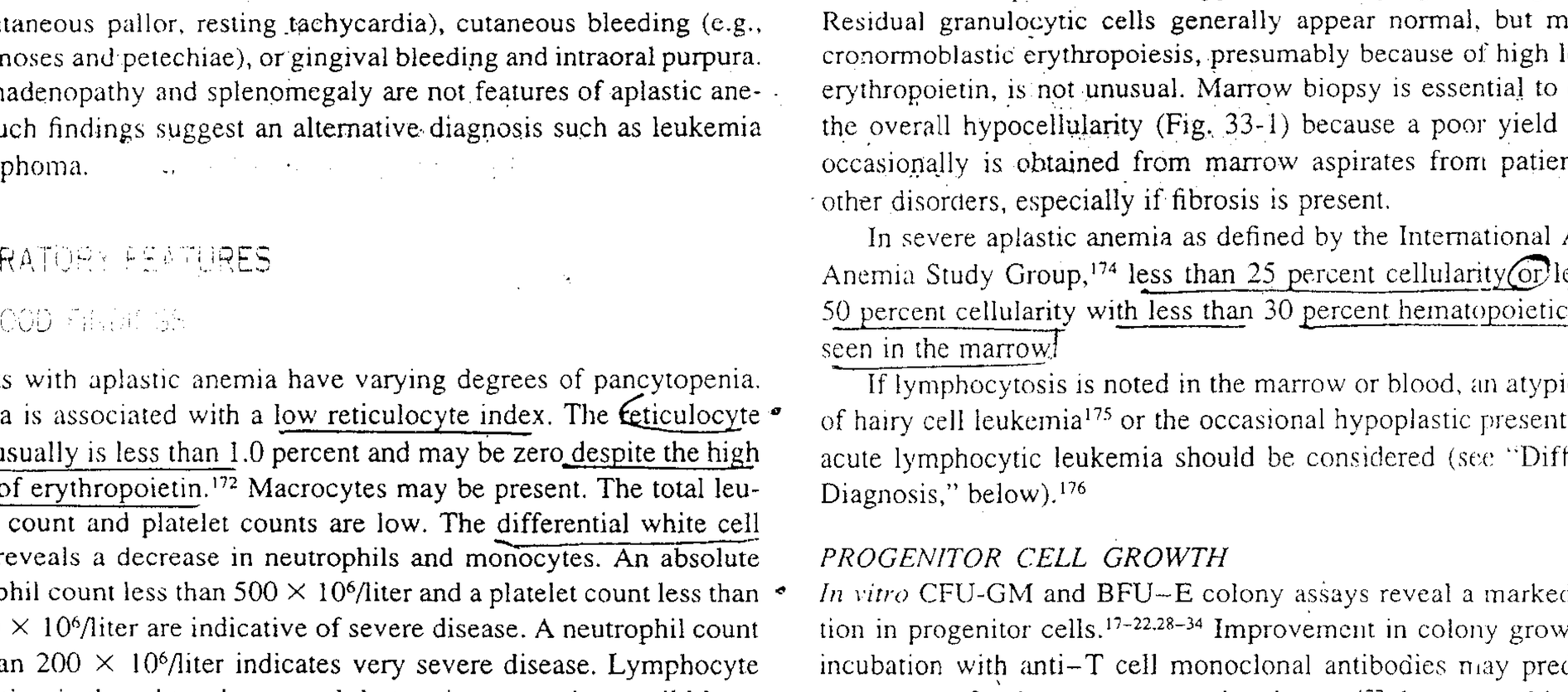
Marrow aspirate typically contains numerous spicules with empty, fat-filled spaces and relatively few hematopoietic cells. Lymphocytes, plasma cells, macrophages, and mast cells may be prominent, reflecting a lack of other cells rather than an increase in these elements. On occasion, some spicules are cellular or even hypercellular ("hot spots"), but megakaryocytes usually are reduced. These focal areas of residual hematopoiesis do not appear to be of prognostic significance. Residual granulocytic cells generally appear normal, but mild macronormoblastic erythropoiesis, presumably because of high levels of erythropoietin, is not unusual. Marrow biopsy is essential to confirm the overall hypocellularity (Fig. 33-1) because a poor yield of cells occasionally is obtained from marrow aspirates from patients with other disorders, especially if fibrosis is present.

In severe aplastic anemia as defined by the International Aplastic Anemia Study Group,[174] less than 25 percent cellularity or less than 50 percent cellularity with less than 30 percent hematopoietic cells is seen in the marrow.

If lymphocytosis is noted in the marrow or blood, an atypical case of hairy cell leukemia[175] or the occasional hypoplastic presentation of acute lymphocytic leukemia should be considered (see "Differential Diagnosis," below).[176]

PROGENITOR CELL GROWTH

In vitro CFU-GM and BFU–E colony assays reveal a marked reduction in progenitor cells.[17–22,28–34] Improvement in colony growth after incubation with anti–T cell monoclonal antibodies may predict improvement after immunosuppressive therapy[177]; however, this has not been a universal finding.



Marrow biopsy in aplastic anemia. *(A)* Normal marrow biopsy. *(B)* Marrow in aplastic anemia is devoid of hematopoietic cells and contains only scattered lymphocytes and stromal cells.

CYTOGENETIC STUDIES

Cytogenetic analysis may be difficult because of low cellularity; thus, multiple aspirates may be required to provide sufficient cells for study. The results of analysis are normal in aplastic anemia. Clonal cytogenetic abnormalities in otherwise apparent aplastic anemia are indicative of an underlying hypoproliferative clonal myeloid disease.[178]

Magnetic resonance imaging (MRI) can be used to distinguish between marrow fat and hematopoietic cells.[179] This may be a more useful overall estimate of marrow hematopoietic cell density than morphologic techniques and may help differentiate hypoplastic myelogenous leukemia from aplastic anemia.[180,181]

DIFFERENTIAL DIAGNOSIS

Any disease that can present with pancytopenia may mimic aplastic anemia if only the blood counts are considered. Measurement of reticulocyte count and examination of the blood film and marrow biopsy are essential early steps to arrive at a diagnosis. A reticulocyte percentage of 0.5 to zero is strongly indicative of aplastic erythropoiesis and, coupled with leukopenia and thrombocytopenia, points to aplastic anemia. Absence of qualitative abnormalities of cells on the blood film and a markedly hypocellular marrow are characteristic of acquired aplastic anemia. The disorders most commonly confused with severe aplastic anemia include the myelodysplastic syndromes in the approximately 5 to 10 percent of patients who present with a hypoplastic rather than a hypercellular marrow. Myelodysplasia should be considered if abnormal blood film morphology consistent with myelodysplasia (e.g., poikilocytosis, basophilic stippling, granulocytes with the pseudo-Pelger-Hüet anomaly) is observed. Marrow erythroid precursors in myelodysplasia may have dysmorphic features. Pathologic sideroblasts are inconsistent with aplastic anemia and are a frequent feature of myelodysplasia. Granulocyte precursors may have reduced or abnormal granulation. Megakaryocytes may have abnormal nuclear lobulation (e.g., unilobular micromegakaryocytes) (see Chap. 86). A clonal myeloid disorder, especially myelodysplastic syndrome or hypocellular myelogenous leukemia, is likely if clonal cytogenetic abnormalities are found. MRI studies of bone may be useful in differentiating severe aplastic anemia from clonal myeloid syndromes. The former gives a fatty signal and the latter a diffuse cellular pattern.[179–181]

A hypocellular marrow frequently is associated with PNH. PNH is characterized by an acquired mutation in the PIG-A gene that encodes a glycosyl-phosphatidylinositol anchor protein (CD59). This protein anchors protein inhibitors of the complement pathway to blood cell membranes, and its absence accounts for complement-mediated hemolysis in PNH. As many as 50 percent of patients with otherwise typical aplastic anemia have evidence of glycosyl-phosphatidylinositol molecule defects and diminished anchor protein on leukocytes and red cells as judged by flow cytometry, analogous to that seen in PNH.[182] The absence of this anchor protein may make the PNH clone of cells resistant to the acquired immune attack on normal marrow components, or the anchor protein on normal cells provides an epitope that initiates an aberrant T cell attack, leaving the PNH clone relatively resistant[183] (see Chap. 38).

Occasionally, apparent aplastic anemia is the prodrome to childhood acute lymphoblastic leukemia. Careful examination of marrow cells by light microscopy or flow cytometry can uncover a population of leukemic lymphoblasts.[176] Hairy cell leukemia rarely is preceded by a period of marrow hypoplastia.[175] Use of tartrate-resistant acid phosphatase or immunophenotyping by flow cytometry for CD25 may uncover the presence of hairy cells. Other clinical features may be distinctive (see Chap. 93). Organomegalies such as lymphadenopathy, hepatomegaly, or splenomegaly are inconsistent with the atrophic (hypoproliferative) features of aplastic anemia.

RELATIONSHIP AMONG APLASTIC ANEMIA, PAROXYSMAL NOCTURNAL HEMOGLOBINURIA, AND CLONAL MYELOID DISEASES

In addition to the diagnostic difficulties occasionally presented by patients with hypoplastic myelodysplastic syndromes, hypoplastic acute myelogenous leukemia (AML), or PNH and hypocellular marrows, a more fundamental relationship may exist among these three diseases and aplastic anemia. The development of clonal cytogenetic abnormalities such as monosomy 7 or trisomy 8 in a patient with aplastic anemia portends the evolution of a myelodysplastic syndrome or acute leukemia. Occasionally, these cytogenetic markers are transient. Hematologic improvement has occurred in cases with disappearance of monosomy 7.[184] Persistent monosomy 7 carries a poor prognosis compared to trisomy 8.[185,186]

As many as 15 to 20 percent of patients with aplastic anemia have a 5-year probability of developing myelodysplasia.[184] If any transformation to a clonal myeloid disorder that occurs up to 6 months after treatment is excluded, to avoid misdiagnosis among the hypoplastic myeloid diseases, the frequency of a clonal disorder was nearly 15 times greater in patients treated with immunosuppression compared to patients treated with marrow transplantation after 39 months of observation.[187] This finding suggests that immune suppression by anti-T cell therapy enhances the evolution of a neoplastic clone or that the therapy does not suppress the intrinsic tendency of aplastic anemia to evolve to a clonal disease but provides the increased longevity of the patient required to express that potential. The latter interpretation is more likely given that patients successfully treated solely with androgens develop clonal disease as frequently as patients treated with immunosuppression.[188] Transplantation may reduce the potential to clonal evolution in patients with aplastic anemia by reestablishing robust hematolymphopoiesis.

Telomere shortening may play a pathogenetic role in the evolution of aplastic anemia into myelodysplasia. Patients with aplastic anemia had shorter telomere lengths than matched controls, and patients with aplastic anemia with persistent cytopenias had greater telomere shortening over time than matched controls. Three of five patients with telomere lengths less than 5 kb developed clonal cytogenetic changes, whereas patients with longer telomeres did not develop such diseases.[189]

The relationship of PNH to aplastic anemia remains enigmatic. Because hematopoietic stem cells lacking the phosphatidylinositol anchor proteins are present in very small numbers in many or all normal persons,[190] it is not surprising that more than 50 percent of patients with aplastic anemia may have a PNH cell population as detected by immunophenotyping.[182] The probability of patients with aplastic anemia developing a clinical syndrome consistent with PNH is 10 to 20 percent, and this is not a consequence of immunosuppressive treatment.[184] Patients may present with the hemolytic anemia of PNH and later develop progressive marrow failure, so any pathogenetic explanation must consider both types of development of aplastic marrows in PNH. The PIG-A mutation may confer either a proliferative or a survival advantage to PNH cells.[191] A survival advantage could result if the anchor protein or one of its ligands served as an epitope for the T lymphocyte cytotoxicity inducing the marrow aplasia. In this case, the presenting event could reflect either cytopenias or the sensitivity of red cells to complement lysis and hemolysis, depending on the intrinsic proliferative potential of the PNH clone.

Within our current state of knowledge, aplastic anemia is an autoimmune process. Any residual hematopoiesis presumably is polyclonal. This is a critical distinction from hypoplastic leukemia and PNH, which are clonal (neoplastic) diseases. The environment of the aplastic marrow, however, may favor the eventual evolution of a mutant (malignant) clone.

TREATMENT

Severe anemia, bleeding from thrombocytopenia, and, rarely at the time of diagnosis, infection secondary to granulocytopenia and monocytopenia require prompt attention to remove potential life-threatening conditions and improve patient comfort (Table 33-6). More specific treatment of marrow aplasia involves three principal options: (1) allogeneic stem cell transplantation, (2) combination immunosuppressive therapy, or (3) high-dose cyclophosphamide. Selection of the specific mode of treatment depends on several factors, including the patient's age and condition and the availability of a suitable stem cell donor. In general, transplantation is the preferred treatment for children and most otherwise healthy adults. Histocompatibility testing is of particular importance because it establishes whether a sibling donor is available to the patient for transplantation. The preferred stem cell source is a histocompatible sibling matched at the human leukocyte antigen (HLA)-A, B, and DR loci. The outcome is compromised if there is a mismatch at one or more loci, and immunosuppression with combined therapy is the preferred treatment.

USE OF BLOOD PRODUCTS

Sparing use of red cell and platelet transfusions in potential transplant recipients has been recommended to minimize sensitization to histocompatibility antigens. However, this recommendation has become less important since ATG and cyclophosphamide have been used as the preparative regimen for transplantation in aplastic anemia because the combination markedly reduces the problem of graft rejection.[192]

Cytomegalovirus (CMV)-negative blood products should be given to potential transplant recipients to minimize problems with CMV infections after transplantation. This restriction is no longer necessary once a patient is CMV positive. Leukocyte-depletion filters decrease the risk of CMV transmittal.

RED CELL TRANSFUSION

Packed red cells given to alleviate symptoms of anemia are indicated when hemoglobin values are less than 7 to 8 g/dl unless comorbid medical conditions require a higher hemoglobin concentration. These products should be leukocyte depleted to lessen leukocyte and platelet sensitization and to reduce subsequent transfusion reactions. Blood products should be irradiated in those patients requiring immunosuppression. It is important not to transfuse patients with red cells (or platelets) from family members if transplantation is remotely possible, because this approach may sensitize patients to minor histocompatibility antigens, increasing the risk of graft rejection after marrow transplantation. Following a marrow transplant or in individuals in whom transplantation is not a consideration, family members may be ideal donors for platelet products. Because each unit of red cells adds approximately 200 to 250 mg of iron to the total body iron, transfusion-induced iron overload may occur over the long term. This is not a major problem in patients who respond to transplantation or immunosuppressive therapy but is an issue in nonresponders who require continued transfusion support. In the latter case, consideration should be given to iron chelation therapy with deferoxamine (see Chap. 46).

PLATELET TRANSFUSION

Assessment of the risk of bleeding in each patient is important. Most patients tolerate platelet counts of 8 to 10 $\times$ 10^9/liter without undue bruising or bleeding, unless a systemic infection is present.[193,194] Administration of ε-aminocaproic acid, 100 mg/kg per dose every 4 hours (maximum dose 5 g) orally or intravenously, may reduce the bleeding tendency.[193] Pooled random-donor platelets can be used until sensitization ensues, although use of single-donor platelets from the onset to minimize sensitization to HLA or platelet antigens is preferable. Single-donor apheresis products or HLA-matched platelets may be required later.

Platelet refractoriness is a major problem with long-term transfusion support. The refractoriness may occur transiently, with fever or infection, or as a chronic problem secondary to HLA sensitization. In the past, the problem occurred in approximately 50 percent of patients after 8 to 10 weeks of transfusion support. Filtration of blood and platelet concentrates to remove leukocytes reduces this problem to approximately 15 to 20 percent of patients receiving chronic transfusions.[196] In some patients refractory to platelets, this problem can be overcome by administering high-dose intravenous γ-globulin[197,198] or by immunoabsorbent pheresis, using a column to remove circulating immunoglobulin G complexes.[199]

MANAGEMENT OF NEUTROPENIA

Neutropenic precautions should be applied to hospitalized patients with a severely depressed neutrophil count. The level of neutrophils requiring precautions is debated but is approximately less than 0.75 $\times$ 10^9/liter. One approach is use of private rooms, with requirements for face masks and hand washing with antiseptic soap. Unwashed fresh fruits and vegetables should be avoided because they are sources of bacterial contamination. Patients with aplastic anemia uncommonly present with significant infection. When patients with aplastic anemia become febrile, cultures should be obtained from the throat, sputum (if any), blood, urine, and any suspicious lesions. Broad-spectrum antibiotics should be initiated promptly, without awaiting culture results (see Chap. 20). The choice of antibiotics depends on the prevalence of organisms and their antibiotic sensitivity in the local setting. Organisms of concern usually include *Staphylococcus aureus*, *S. epidermidis* (in patients with venous access devices), and gram-negative organisms. Patients with persistent culture-negative fevers should be considered for antifungal treatment (see Chap. 20).

In the past, leukocyte transfusions were used on a daily basis to reduce the short-term mortality from infections. Detection of more than 100 to 200 neutrophils per microliter for more than a few hours after transfusion was unusual. Neutrophil yield can be increased by administering granulocyte-macrophage colony stimulating factor

TABLE [illegible] MANAGEMENT OF APLASTIC ANEMIA

- Discontinue any potential offending drug and use an alternative class of agents if essential
- Anemia: transfusion of leukocyte-depleted red cells as required for severe anemia
- Severe thrombocytopenia or thrombocytopenic bleeding: use ε-aminocaproic acid; transfusion of platelets as required
- Infectious precautions if severe neutropenia
- Infection: cultures, broad-spectrum antibiotics if specific organism not identified, G-CSF in dire cases; if low body weight and profound infection (e.g., gram-negative bacteria, fungus) are present, consider granulocyte transfusion from a G-CSF–pretreated donor
- Assessment for allogeneic stem cell transplantation: histocompatibility testing of patient, parents, and siblings

G-CSF, granulocyte colony stimulating factor.

(GM-CSF) or G-CSF to the donor,[200] but most physicians avoid using white cell products because present-day antibiotics usually are sufficient to treat an episode of sepsis. Notable exceptions include documented invasive aspergillosis unresponsive to amphotericin (particularly in the posttransplant setting), infections with organisms resistant to all known antibiotics, and disorders in which blood cultures remain positive despite antibiotic treatment.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Prompt therapy usually is indicated for patients with severe disease. The major curative approach is hematopoietic stem cell transplantation from a histocompatible sibling.[37,122] This treatment modality is described in Chap. 22. Only approximately 30 percent of patients in the United States have compatible sibling donors. The outcome is compromised if there is a mismatch at one or more loci, and immunosuppression with combined therapy is preferred. Transplants have been performed using stem cells from partially matched siblings or from unrelated histocompatible donors recruited through the National Marrow Donor Program or similar organizations in other countries.[201] Umbilical cord blood is an alternative source of stem cells from unrelated donors (or rarely siblings) for transplantation in children.[202] Use of high-resolution DNA-based HLA typing of a matched unrelated donor markedly improves the prognosis for transplantation from an unrelated donor.[203] This may be the preferred treatment for patients who no longer respond to immunotherapy rather than repeated courses of immunosuppression.[203]

COMPONENTS OF ANTI-T LYMPHOCYTE (IMMUNOSUPPRESSIVE) THERAPY

ANTILYMPHOCYTE GLOBULIN AND ANTITHYMOCYTE GLOBULIN

ATG and ALG act principally by reducing cytotoxic T cells. The process involves ATG-induced apoptosis through both Fas and TNF pathways.[204] Cathepsin B also plays a role in T cell cytotoxicity at clinical concentrations of ATG but may involve an independent apoptosis pathway.[271] ATG and ALG also release hematopoietic growth factors from T cells.[205,206] Horse and rabbit ATGs are licensed in the United States. Skin tests against horse serum should be performed prior to administration.[207] If the result is positive, the patient may be desensitized. ATG therapy with doses of 15 to 40 mg/kg is given daily for 4 to 10 days. Fever and chills are common during the first day of treatment. Concomitant treatment with glucocorticoids, such as methylprednisolone (1 mg/kg/day), lessens the reaction to ATG.

ATG treatment may accelerate platelet destruction, reduce the absolute neutrophil count, and cause a positive direct antiglobulin test. This effect may increase transfusion requirements during the 4- to10-day treatment interval. Serum sickness, characterized by spiking fevers, skin rashes, and arthralgias, occurs commonly 7 to 10 days from the first dose. The clinical manifestations of serum sickness can be diminished by increasing the glucocorticoid dose from day 10 to day 14 after treatment. Approximately one third of patients no longer require transfusion support after treatment with ATG alone.[208–210]

Of 188 patients responding to immunosuppressive therapy, principally ATG alone, 74 (21 percent) relapsed after a mean of 2.1 years. The actuarial incidence of relapse was 35 percent at 10 years.[211] Similar results were observed when 227 patients were treated with immunosuppression primarily ATG alone.[212] The actuarial survival at 5 years was 58 percent following immunosuppression.[211] However, a combination of immunosuppressive agents provides more effective therapy than ATG alone (see "Combination Immunotherapy," below).

Twenty-two percent of 129 patients treated with ALG developed myelodysplasia, leukemia, paroxysmal nocturnal hemoglobinuria, or combined disorders.[213] The tendency to relapse and develop clonal hematologic disorders was reviewed by the European Cooperative Group for Bone Marrow Transplantation in 468 patients, most of whom received ATG.[214] The risk of a hematologic complication increased continuously and reached 57 percent at 8 years after immunosuppressive therapy. A further survey found 42 (5 percent) malignancies in 860 patients treated with immunosuppression, whereas only 9 (1 percent) malignancies were seen in 748 patients who received marrow transplants.[215]

CYCLOSPORINE

Administration of cyclosporine, a cyclic polypeptide that inhibits IL-2 production by T lymphocytes and prevents expansion of cytotoxic T cells in response to IL-2, is another approach to immunotherapy. After the initial report of its ability to induce remission in 1984,[216] several groups have utilized cyclosporine (1) as primary treatment,[217–220] (2) in patients refractory to ATG or glucocorticoids,[218–223] (3) in combination with G-CSFs,[224,225] or (4) in varying combinations with other modes of therapy.[226] Cyclosporine is administered orally at 10 to 12 mg/kg/day for at least 4 to 6 months. Dosage adjustments may be required to maintain trough blood levels of 200 to 400 ng/ml. Renal impairment is common and may require increased hydration or dose adjustments to keep creatinine values less than 2 mg/dl. Cyclosporine may cause moderate hypertension, a variety of neurologic manifestations, and other side effects. Several drug classes interact with cyclosporine to either increase (e.g., some antibiotics and antifungals) or decrease (e.g., some anticonvulsants) blood levels. Responses usually are seen by 3 months and range from achieving transfusion independence to complete remission. Approximately 25 percent of patients respond to this agent used alone, but the reported response rate ranges from 0 to 80 percent.[226]

Although immunosuppression with ALG or ATG has been used the longest and has a seemingly better response rate, cyclosporine has certain advantages. The drug does not require hospitalization or use of central venous catheters. Fewer platelet transfusions are required during the first few weeks of therapy compared to treatment with ALG or ATG. A French cooperative trial showed equal effectiveness of ATG plus prednisone compared to cyclosporine.[227] In this crossover study of newly diagnosed patients, survival of approximately 65 percent was observed 12 months after diagnosis. Improved *in vitro* tests for predicting responsiveness may help tailor specific therapy for each patient in the future.[222]

HIGH-DOSE GLUCOCORTICOIDS

Marrow recovery can occur after very high doses of glucocorticoids.[228,229] Methylprednisolone in the range from 500 to 1000 mg daily for 3 to 14 days has been successful, but the side effects can be severe. Side effects include marked hyperglycemia and glycosuria, electrolyte disturbances, gastric irritation, psychosis, increased infections, and aseptic necrosis of the hips. Glucocorticoids at lower doses commonly are used only as a component of combination therapy for aplastic anemia. In this role, they are useful in modulating the adverse reactions to ATG and in providing additional lymphocyte suppression.

COMBINATION IMMUNOTHERAPY

Combination treatment of severe aplastic anemia usually includes ATG 40 mg/kg/day for 4 days, cyclosporine 10 to 12 mg/kg/day for 6 months, and methylprednisolone 1 mg/kg/day for 2 weeks.[230] The cyclosporine dose is adjusted to maintain a trough level of 200 to 400 ng/ml. Prophylaxis for *Pneumocystis carinii* with daily trimethoprim-

sulfamethoxazole (160/800 mg qd orally) or with monthly pentamidine inhalations should be considered for these patients as they receive immunosuppressive therapy.

Addition of cyclosporine to the combination of ALG and glucocorticoids improves response rates to approximately 70 percent.[231,232] Although G-CSF used alone in patients with aplastic anemia may be detrimental,[233] G-CSF added to ALG, glucocorticoids, and cyclosporine appeared to permit 5-year actuarial survival of greater than 80 percent in 100 patients with very severe aplastic anemia.[234] Response usually is defined as a significant improvement in red cells, white cells, and platelets to eliminate risk of infection and bleeding and the requirement for red cell transfusions.

Five-year survival after completion of combination immunosuppressive therapy may approximate that after stem cell transplantation.[235] Forty-eight children treated between 1983 and 1992 had a 10-year survival of approximately 75 percent after marrow transplantation and approximately 75 percent after combined immunosuppressive therapy, although there were only half the number of severely affected patients in the immunosuppressive therapy group.[236] Thus, immunosuppression may be preferable for patients who are older than 30 years and for patients who experience a delay in finding a suitable donor. Marrow transplants are curative for aplastic anemia, whereas more frequent sequelae occur after immunosuppressive therapy,[237–239] notably a substantial rate of evolution to a myelodysplastic syndrome or acute myelogenous leukemia.

A recent National Institutes of Health protocol was designed to increase immune tolerance by specific deletion of activated T lymphocytes that target primitive hematopoietic progenitor cells.[240] Concurrent administration of cyclosporine with ATG may diminish the ATG tolerizing effect so that, in this program, cyclosporine is introduced at a later time. Addition of new immunosuppressive agents, such as mycophenolate mofetil, rapamycin, or monoclonal antibodies to the IL-2 receptor, may provide more effective induction of tolerance so that the activated lymphocytes spare the targeted hematopoietic stem cells.[240]

HIGH-DOSE CYCLOPHOSPHAMIDE

High-dose cyclophosphamide has been used as a form of immunosuppression.[241] Although it seems inappropriate to administer high doses of chemotherapy to patients with severe marrow aplasia, this approach was based on observations of autologous recovery after preparative therapy for allogeneic transplants.[241] Ten patients received cyclophosphamide at 45 mg/kg/day intravenously for 4 days with or without cyclosporine for an additional 100 days. Gradual neutrophil and platelet recovery ensued over 3 months. Seven patients responded completely and remain in remission 11 years after treatment. Interest in high-dose cyclophosphamide treatment has been renewed because hematopoietic stem cells have high levels of aldehyde dehydrogenase and are relatively resistant to cyclophosphamide.[242,243] Thus, cyclophosphamide in this situation is more immunosuppressive than it is myelotoxic. The most extensive trial of high-dose cyclophosphamide resulted in complete response by 65 percent of patients at 50 months.[244] However, the role of this regimen as initial therapy is not clear because early toxicity may exceed that of the ATG and cyclosporine combination.[245] The probability of a durable remission may be superior, but insufficient data exist to allow conclusion on whether ATG and cyclosporine or high-dose cyclophosphamide provides better long-term results. Cyclophosphamide, also, is effective in inducing marrow recovery in some patients who have not responded to immunosuppressive therapy.[280]

ANDROGENS

Randomized trials have not shown efficacy when androgens were used as primary therapy for severe or moderately severe aplastic anemia.[174,246] These agents have been replaced by immunosuppression or stem cell transplantation.

Androgens stimulate the production of erythropoietin, and their metabolites stimulate erythropoiesis when they are added to marrow cultures *in vitro*.[247] High doses of androgens were beneficial in some patients with moderately severe aplasia.[248] Large series of patients in whom survival seemed improved compared with historical controls have been reported, but this finding could have resulted from improved supportive care.[188]

Androgens, if used, should be continued for at least 3 to 6 months because responses may require prolonged treatment. Nandrolone decanoate administered at 400 mg intramuscularly per week is one approach. Local hematomas can be minimized by firm local pressure for 30 minutes after the injection. Long-term survivors after androgen therapy have essentially the same progression to clonal hematologic disorders as patients treated with immunosuppressive agents.[188]

CYTOKINES

Despite their effectiveness in accelerating recovery from chemotherapy, cytokines have been far less effective in achieving long-term benefits in patients with severe aplastic anemia. Daily treatment with GM-CSF[249–251] or G-CSF[252] has improved marrow cellularity and increased neutrophil counts approximately 1.5- to 10-fold. Unfortunately, the blood counts return to baseline within several days of cessation of therapy in nearly all patients. Although occasional patients show evidence of trilineage marrow recovery with long-term therapy,[252–254] the vast majority of patients do not respond. In fact, physicians have been cautioned not to use hematopoietic growth factors as primary therapy.[233] Therapy with myeloid growth factors probably is best reserved for episodes of severe infection or as a preventive measure prior to dental work or other procedures that can compromise mucosal barriers. Prophylactic use of growth factors is not warranted. G-CSF at a dose of 5 μg/kg by subcutaneous injection is easiest to administer and seems to be associated with the fewest side effects. The drug can be given daily or fewer times per week, depending on the response. Newer pegylated preparations have greater longevity and usually are administered at less frequent, every-other-week intervals.

IL-1, a potent stimulator of marrow stromal cell production of other cytokines, and IL-3 have been ineffective in small numbers of patients with severe aplastic anemia.[255,256] These disappointing results with cytokines are not unexpected, given that previous work has found high serum levels of growth factors in patients with aplastic anemia. Moreover, the majority of patients have suppression of very primitive progenitors, which may be unresponsive to factors that act on more mature progenitor cells.

SPLENECTOMY

Removal of the spleen does not increase hematopoiesis but may increase neutrophil and platelet counts twofold to threefold and improve survival of transfused red cells or platelets in highly sensitized individuals.[257] Surgical morbidity and mortality in patients with few platelets and white cells makes splenectomy a questionable therapeutic procedure. Because more successful methods of therapy that attack the fundamental problem are available, splenectomy is rarely used today.

OTHER THERAPY

High doses of intravenous γ-globulin have been given to small numbers of patients with severe aplastic anemia[258,259] because of its success in treating certain cases of antibody-mediated pure red cell aplasia. Some improvement was noted in four of six patients treated. Another treatment that occasionally is successful is lymphocytapheresis administered to deplete T cells.[260,261]

COURSE AND PROGNOSIS

At diagnosis, the prognosis is largely related to the absolute neutrophil count and the platelet count. The absolute neutrophil count is the most important prognostic feature. A count of less than 500/μl (0.5×10^9/liter) is considered severe aplastic anemia, and a count less than 200/μl (0.2×10^9/liter) is associated with a poor response to immunotherapy and a dire prognosis if early successful allogeneic transplant is not available. In the past, the prognosis appeared worse when the disease occurred after hepatitis.[71,72] Results with immunosuppression[262] or marrow transplant[263] are equivalent to results seen in idiopathic or drug-induced cases. Children appear to respond better than adults.[264] Constitutional aplastic anemia responds temporarily to androgens and glucocorticoids but usually is fatal unless it is treated by transplantation.[142,164]

Before marrow transplantation and immunosuppressive therapy, greater than 25 percent of patients with severe aplastic anemia died within 4 months of diagnosis; half died within 1 year.[265,266] Marrow transplantation is curative for approximately 80 percent of patients younger than 20 years, approximately 70 percent of patients aged 20 to 40 years, and approximately 50 percent of patients older than 40 years.[267] Unfortunately, as many as 40 percent of transplant survivors suffer the deleterious consequences of chronic graft-versus-host disease,[212] and the risk of subsequent cancer can be as high as 11 percent in older patients or after cyclosporine therapy prior to stem cell transplantation.[277] The best outcomes occur in patients who have not been exposed to immunosuppressive therapy prior to transplantation, not exposed and sensitized to blood cell products, and not subjected to irradiation in the conditioning regimen for transplantation.[277]

Combined immunosuppressive therapy with ATG and cyclosporine leads to marked improvement in at least 70 percent of patients.[268] Although some patients have normal blood counts, many continue having moderate anemia or thrombocytopenia. The disease may progress over 10 years to paroxysmal nocturnal hemoglobinuria, a myelodysplastic syndrome, or acute myelogenous leukemia in as many as 40 percent of patients who initially responded to immunosuppressive therapy.[211,213–215,237–239] In 168 transplanted patients, actuarial survival at 15 years was 69 percent, and in 227 patients receiving immunosuppressive therapy survival was only 38 percent.[269]

Treatment with high-dose cyclophosphamide produces early results similar to the results seen with the combination of ATG and cyclosporine.[245,270] However, cyclophosphamide has greater early toxicity and slower hematologic recovery but may generate more durable remissions.[245]

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19th Edition

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139e Hematopoietic Cell Transplantation

Frederick R. Appelbaum

This is a digital-only chapter. It is available on the DVD that accompanies this book, as well as on Access Medicine/Harrison's Online, and the eBook and "app" editions of HPIM 19e.

Bone marrow transplantation was the original term used to describe the collection and transplantation of hematopoietic stem cells, but with the demonstration that 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 Center for International Blood and Marrow Transplant Research (*http://www.cibmtr.org*) estimates that about 65,000 transplants are performed each year.

SECTION 3 DISORDERS OF HEMOSTASIS

140 Disorders of Platelets and Vessel Wall

Barbara A. Konkle

Hemostasis is a dynamic process in which the platelet and the blood vessel wall play key roles. Platelets become activated upon adhesion to von Willebrand factor (VWF) and collagen in the exposed subendothelium after injury. Platelet activation is also mediated through shear forces imposed by blood flow itself, particularly in areas where the vessel wall is diseased, and is also affected by the inflammatory state of the endothelium. The activated platelet surface provides the major physiologic site for coagulation factor activation, which results in further platelet activation and fibrin formation. Genetic and acquired influences on the platelet and vessel wall, as well as on the coagulation and fibrinolytic systems, determine whether normal hemostasis or bleeding or clotting symptoms will result.

THE PLATELET

Platelets are released from the megakaryocyte, likely under the influence of flow in the capillary sinuses. The normal blood platelet count is 150,000–450,000/μL. The major regulator of platelet production is the hormone thrombopoietin (TPO), which is synthesized in the liver. Synthesis is increased with inflammation and specifically by interleukin 6. TPO binds to its receptor on platelets and megakaryocytes, by which it is removed from the circulation. Thus a reduction in platelet and megakaryocyte mass increases the level of TPO, which then stimulates platelet production. Platelets circulate with an average life span of 7–10 days. Approximately one-third of the platelets reside in the spleen, and this number increases in proportion to splenic size, although the platelet count rarely decreases to <40,000/μL as the spleen enlarges. Platelets are physiologically very active, but are anucleate, and thus have limited capacity to synthesize new proteins.

Normal vascular endothelium contributes to preventing thrombosis by inhibiting platelet function (Chap. 78). When vascular endothelium is injured, these inhibitory effects are overcome, and platelets adhere to the exposed intimal surface primarily through VWF, a large multimeric protein present in both plasma and in the extracellular matrix of the subendothelial vessel wall. Platelet adhesion results in the generation of intracellular signals that lead to activation of the platelet glycoprotein (Gp) IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor and resultant platelet aggregation.

Activated platelets undergo release of their granule contents, which include nucleotides, adhesive proteins, growth factors, and procoagulants that serve to promote platelet aggregation and blood clot formation and influence the environment of the forming clot. During platelet aggregation, additional platelets are recruited to the site of injury, leading to the formation of an occlusive platelet thrombus. The platelet plug is stabilized by the fibrin mesh that develops simultaneously as the product of the coagulation cascade.

THE VESSEL WALL

Endothelial cells line the surface of the entire circulatory tree, totaling $1–6 \times 10^{13}$ cells, enough to cover a surface area equivalent to about six tennis courts. The endothelium is physiologically active, controlling vascular permeability, flow of biologically active molecules and nutrients, blood cell interactions with the vessel wall, the inflammatory response, and angiogenesis.

The endothelium normally presents an antithrombotic surface (Chap. 78) but rapidly becomes prothrombotic when stimulated, which promotes coagulation, inhibits fibrinolysis, and activates platelets. In many cases, endothelium-derived vasodilators are also platelet inhibitors (e.g., nitric oxide) and, conversely, endothelium-derived vasoconstrictors (e.g., endothelin) can also be platelet activators. The net effect of vasodilation and inhibition of platelet function is to promote blood fluidity, whereas the net effect of vasoconstriction and platelet activation is to promote thrombosis. Thus, blood fluidity and hemostasis are regulated by the balance of antithrombotic/prothrombotic and vasodilatory/vasoconstrictor properties of endothelial cells.

DISORDERS OF PLATELETS

THROMBOCYTOPENIA

Thrombocytopenia results from one or more of three processes: (1) decreased bone marrow production; (2) sequestration, usually in an enlarged spleen; and/or (3) increased platelet destruction. Disorders of production may be either inherited or acquired. In evaluating a patient with thrombocytopenia, a key step is to review the peripheral blood smear and to first rule out "pseudothrombocytopenia," particularly in a patient without an apparent cause for the thrombocytopenia. Pseudothrombocytopenia (Fig. 140-1*B*) is an in vitro artifact resulting from platelet agglutination via antibodies (usually IgG, but also IgM and IgA) when the calcium content is decreased by blood collection in ethylenediamine tetraacetic (EDTA) (the anticoagulant present in tubes [purple top] used to collect blood for complete blood counts [CBCs]). If a low platelet count is obtained in EDTA-anticoagulated blood, a blood smear should be evaluated and a platelet count determined in blood collected into sodium citrate (blue top tube) or heparin (green top tube), or a smear of freshly obtained unanticoagulated blood, such as from a finger stick, can be examined.

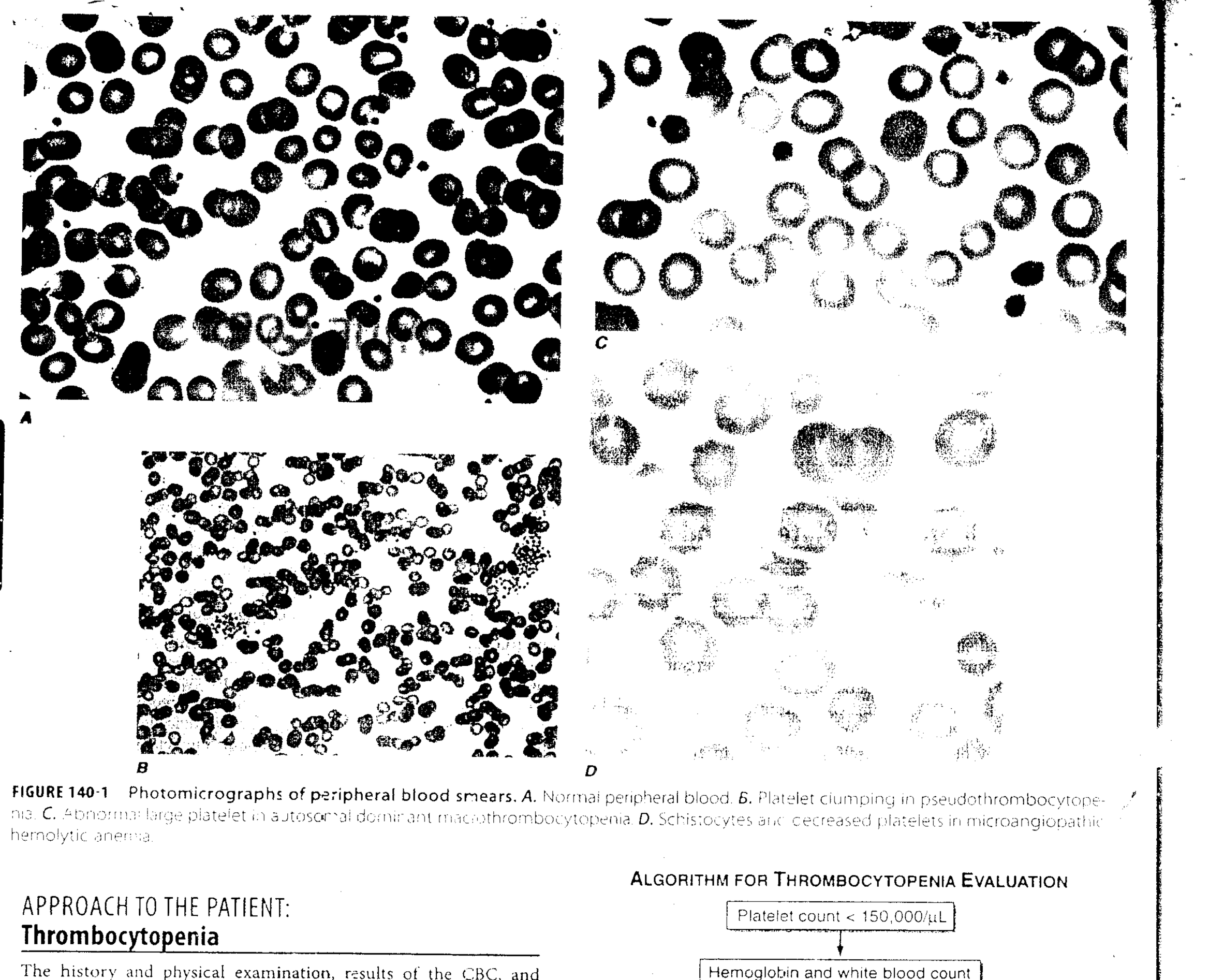

FIGURE 140-1 Photomicrographs of peripheral blood smears. *A.* Normal peripheral blood. *B.* Platelet clumping in pseudothrombocytopenia. *C.* Abnormal large platelet in autosomal dominant macrothrombocytopenia. *D.* Schistocytes and decreased platelets in microangiopathic hemolytic anemia.

APPROACH TO THE PATIENT: Thrombocytopenia

The history and physical examination, results of the CBC, and review of the peripheral blood smear are all critical components in the initial evaluation of thrombocytopenic patients (Fig. 140-2). The overall health of the patient and whether he or she is receiving drug treatment will influence the differential diagnosis. A healthy young adult with thrombocytopenia will have a much more limited differential diagnosis than an ill hospitalized patient who is receiving multiple medications. Except in unusual inherited disorders, decreased platelet production usually results from bone marrow disorders that also affect red blood cell (RBC) and/or white blood cell (WBC) production. Because myelodysplasia can present with isolated thrombocytopenia, the bone marrow should be examined in patients presenting with isolated thrombocytopenia who are older than 60 years of age. While inherited thrombocytopenia is rare, any prior platelet counts should be retrieved and a family history regarding thrombocytopenia obtained. A careful history of drug ingestion should be obtained, including nonprescription and herbal remedies, because drugs are the most common cause of thrombocytopenia.

The physical examination can document an enlarged spleen, evidence of chronic liver disease, and other underlying disorders. Mild to moderate splenomegaly may be difficult to appreciate in many individuals due to body habitus and/or obesity but can be easily assessed by abdominal ultrasound. A platelet count of approximately 5000–10,000 is required to maintain vascular integrity in the microcirculation. When the count is markedly decreased, petechiae

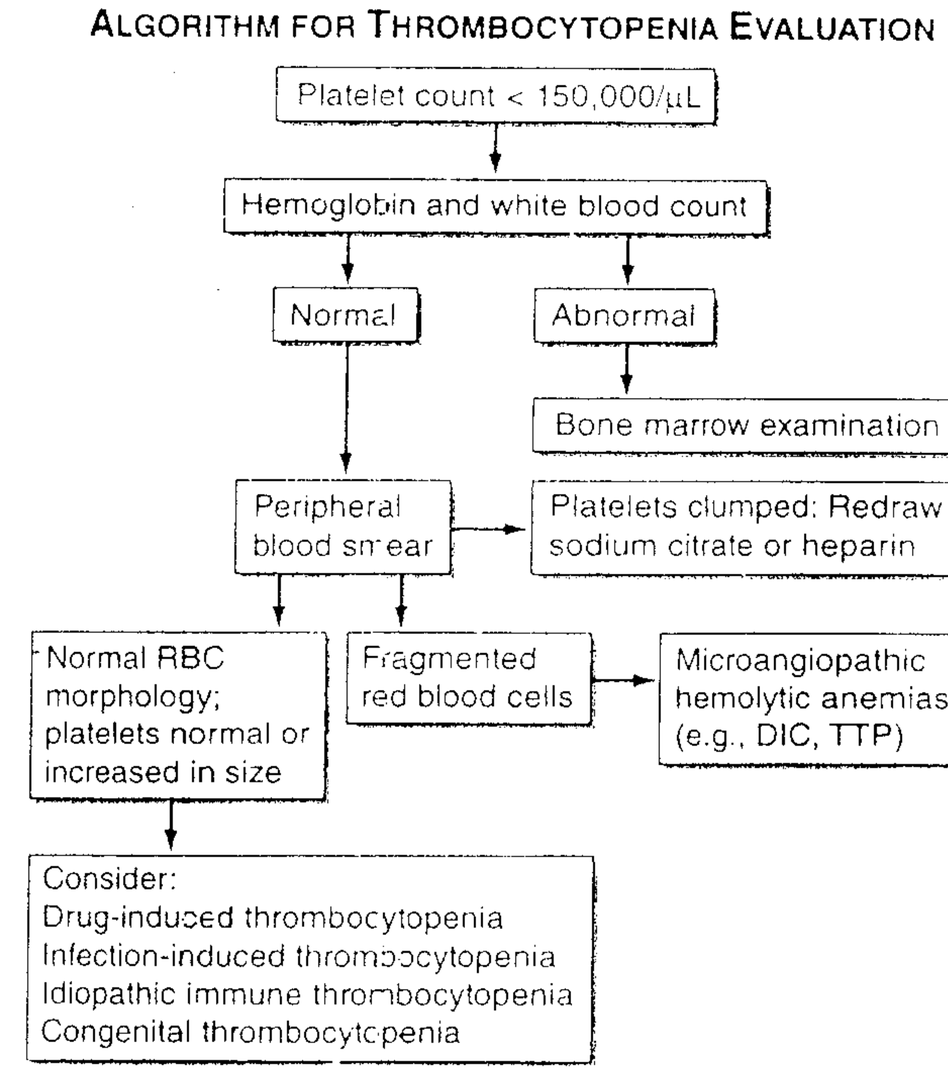

FIGURE 140-2 **Algorithm for evaluating the thrombocytopenic patient.** DIC, disseminated intravascular coagulation; RBC, red blood cell; TTP, thrombotic thrombocytopenic purpura.

first appear in areas of increased venous pressure, the ankles and feet in an ambulatory patient. Petechiae are pinpoint, nonblanching hemorrhages and are usually a sign of a decreased platelet number and not platelet dysfunction. Wet purpura, blood blisters that form on the oral mucosa, are thought to denote an increased risk of life-threatening hemorrhage in the thrombocytopenic patient. Excessive bruising is seen in disorders of both platelet number and function.

Infection-Induced Thrombocytopenia Many viral and bacterial infections result in thrombocytopenia and are the most common noniatrogenic cause of thrombocytopenia. This may or may not be associated with laboratory evidence of disseminated intravascular coagulation (DIC), which is most commonly seen in patients with systemic infections with gram-negative bacteria. Infections can affect both platelet production and platelet survival. In addition, immune mechanisms can be at work, as in infectious mononucleosis and early HIV infection. Late in HIV infection, pancytopenia and decreased and dysplastic platelet production are more common. Immune-mediated thrombocytopenia in children usually follows a viral infection and almost always resolves spontaneously. This association of infection with immune thrombocytopenic purpura is less clear in adults.

Bone marrow examination is often requested for evaluation of occult infections. A study evaluating the role of bone marrow examination in fever of unknown origin in HIV-infected patients found that for 86% of patients, the same diagnosis was established by less invasive techniques, notably blood culture. In some instances, however, the diagnosis can be made earlier; thus, a bone marrow examination and culture are recommended when the diagnosis is needed urgently or when other, less invasive methods have been unsuccessful.

Drug-Induced Thrombocytopenia Many drugs have been associated with thrombocytopenia. A predictable decrease in platelet count occurs after treatment with many chemotherapeutic drugs due to bone marrow suppression (Chap. 103e). Drugs that cause isolated thrombocytopenia and have been confirmed with positive laboratory testing are listed in Table 140-1, but all drugs should be suspect in a patient with thrombocytopenia without an apparent cause and should be stopped, or substituted, if possible. A helpful website, Platelets on the Internet (*http://www.ouhsc.edu/platelets/ditp.html*), lists drugs and supplements reported to have caused thrombocytopenia and the level of evidence supporting the association. Although not as well studied, herbal and over-the-counter preparations may also result in thrombocytopenia and should be discontinued in patients who are thrombocytopenic.

TABLE 140-1 DRUGS REPORTED AS DEFINITELY OR PROBABLY CAUSING ISOLATED THROMBOCYTOPENIA[a]

Abciximab	Mirtazapine
Acetaminophen	Naproxen
Amiodarone	Oxaliplatin
Amlodipine	Penicillin
Ampicillin	Phenytoin
Carbamazepine	Piperacillin
Ceftriaxone	Quinine
Cephamandole	Quinidine
Ciprofloxacin	Ranitidine
Diazepam	Rosiglitazone
Eptifibatide	Roxifiban
Furosemide	Sulfisoxazole
Gold	Suramin
Haloperidol	Tirofiban
Heparin	Tranilast
Ibuprofen	Trimethoprim/sulfamethoxazole
Lorazepam	Vancomycin

[a]Based on scoring requiring a compatible clinical picture and positive laboratory testing.

Source: Adapted from DM Arnold et al: J Thromb Haemost 11:169, 2013.

Classic drug-dependent antibodies are antibodies that react with specific platelet surface antigens and result in thrombocytopenia only when the drug is present. Many drugs are capable of inducing these antibodies, but for some reason, they are more common with quinine and sulfonamides. Drug-dependent antibody binding can be demonstrated by laboratory assays, showing antibody binding in the presence of, but not without, the drug present in the assay. The thrombocytopenia typically occurs after a period of initial exposure (median length 21 days), or upon reexposure, and usually resolves in 7–10 days after drug withdrawal. The thrombocytopenia caused by the platelet Gp IIb/IIIa inhibitory drugs, such as abciximab, differs in that it may occur within 24 h of initial exposure. This appears to be due to the presence of naturally occurring antibodies that cross-react with the drug bound to the platelet.

Heparin-Induced Thrombocytopenia Drug-induced thrombocytopenia due to heparin differs from that seen with other drugs in two major ways. (1) The thrombocytopenia is not usually severe, with nadir counts rarely <20,000/μL. (2) Heparin-induced thrombocytopenia (HIT) is not associated with bleeding and, in fact, markedly increases the risk of thrombosis. HIT results from antibody formation to a complex of the platelet-specific protein platelet factor 4 (PF4) and heparin. The anti-heparin/PF4 antibody can activate platelets through the FcγRIIa receptor and also activate monocytes and endothelial cells. Many patients exposed to heparin develop antibodies to heparin/PF4, but do not appear to have adverse consequences. A fraction of those who develop antibodies will develop HIT, and a portion of those (up to 50%) will develop thrombosis (HITT).

HIT can occur after exposure to low-molecular-weight heparin (LMWH) as well as unfractionated heparin (UFH), although it is more common with the latter. Most patients develop HIT after exposure to heparin for 5–14 days (Fig. 140-3). It occurs before 5 days in those who were exposed to heparin in the prior few weeks or months (<~100 days) and have circulating anti-heparin/PF4 antibodies. Rarely, thrombocytopenia and thrombosis begin several days after all heparin has been stopped (termed *delayed-onset HIT*). The "4T's" have been recommended to be used in a diagnostic algorithm for HIT: *t*hrombocytopenia, *t*iming of platelet count drop, *thrombosis* and other sequelae such as localized skin reactions, and other causes of thrombocytopenia not evident. Application of the 4T scoring system is very useful in excluding a diagnosis of HIT but will result in overdiagnosis of HIT in situations where thrombocytopenia and thrombosis due to other etiologies are common, such as in the intensive care unit. A scoring model based on broad expert opinion (the HIT Expert Probability [HEP] Score) has improved operating characteristics and may provide better utility as a scoring system.

LABORATORY TESTING FOR HIT HIT (anti-heparin/PF4) antibodies can be detected using two types of assays. The most widely available is an enzyme-linked immunoassay (ELISA) with PF4/polyanion complex as the antigen. Because many patients develop antibodies but do not develop clinical HIT, the test has a low specificity for the diagnosis

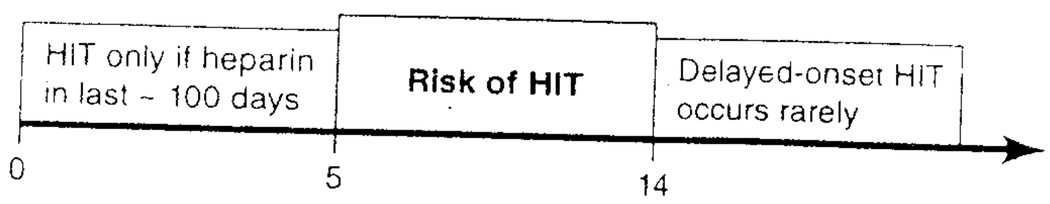

FIGURE 140-3 Time course of heparin-induced thrombocytopenia (HIT) development after heparin exposure. The timing of development after heparin exposure is a critical factor in determining the likelihood of HIT in a patient. HIT occurs early after heparin exposure in the presence of preexisting heparin/platelet factor 4 (PF4) antibodies, which disappear from circulation by ~100 days following a prior exposure. Rarely, HIT may occur later after heparin exposure (termed delayed-onset HIT). In this setting, heparin/PF4 antibody testing is usually markedly positive. HIT can occur after exposure to either unfractionated (UFH) or low-molecular-weight heparin (LMWH).

of HIT. This is especially true in patients who have undergone cardiopulmonary bypass surgery, where approximately 50% of patients develop these antibodies postoperatively. IgG-specific ELISAs increase specificity but may decrease sensitivity. The other assay is a platelet activation assay, most commonly the serotonin release assay, which measures the ability of the patient's serum to activate platelets in the presence of heparin in a concentration-dependent manner. This test has lower sensitivity but higher specificity than the ELISA. However, HIT remains a clinical diagnosis.

TREATMENT HEPARIN-INDUCED THROMBOCYTOPENIA

Early recognition is key in treatment of HIT, with prompt discontinuation of heparin and use of alternative anticoagulants if bleeding risk does not outweigh thrombotic risk. Thrombosis is a common complication of HIT, even after heparin discontinuation, and can occur in both the venous and arterial systems. Patients with higher anti-heparin/PF4 antibody titers may have a higher risk of thrombosis. In patients diagnosed with HIT, imaging studies to evaluate the patient for thrombosis (at least lower extremity duplex Doppler imaging) are recommended. Patients requiring anticoagulation should be switched from heparin to an alternative anticoagulant. The direct thrombin inhibitors (DTIs) argatroban and lepirudin are effective in HITT. The DTI bivalirudin and the antithrombin-binding pentasaccharide fondaparinux are also effective but not yet approved by the U.S. Food and Drug Administration (FDA) for this indication. Danaparoid, a mixture of glycosaminoglycans with anti-Xa activity, has been used extensively for the treatment of HITT; it is no longer available in the United States but is in other countries. HIT antibodies cross-react with LMWH, and these preparations should not be used in the treatment of HIT.

Because of the high rate of thrombosis in patients with HIT, anticoagulation should be considered, even in the absence of thrombosis. In patients with thrombosis, patients can be transitioned to warfarin, with treatment usually for 3–6 months. In patients without thrombosis, the duration of anticoagulation needed is undefined. An increased risk of thrombosis is present for at least 1 month after diagnosis; however, most thromboses occur early, and whether thrombosis occurs later if the patient is initially anticoagulated is unknown. Options include continuing anticoagulation until a few days after platelet recovery or for 1 month. Introduction of warfarin alone in the setting of HIT or HITT may precipitate thrombosis, particularly venous gangrene, presumably due to clotting activation and severely reduced levels of proteins C and S. Warfarin therapy, if started, should be overlapped with a DTI or fondaparinux and started after resolution of the prothrombotic state.

Immune Thrombocytopenic Purpura Immune thrombocytopenic purpura (ITP; also termed *idiopathic thrombocytopenic purpura*) is an acquired disorder in which there is immune-mediated destruction of platelets and possibly inhibition of platelet release from the megakaryocyte. In children, it is usually an acute disease, most commonly following an infection, and with a self-limited course. In adults, it is a more chronic disease, although in some adults, spontaneous remission occurs, usually within months of diagnosis. ITP is termed *secondary* if it is associated with an underlying disorder; autoimmune disorders, particularly systemic lupus erythematosus (SLE), and infections, such as HIV and hepatitis C, are common causes. The association of ITP with *Helicobacter pylori* infection is unclear.

ITP is characterized by mucocutaneous bleeding and a low, often very low, platelet count, with an otherwise normal peripheral blood cells and smear. Patients usually present either with ecchymoses and petechiae, or with thrombocytopenia incidentally found on a routine CBC. Mucocutaneous bleeding, such as oral mucosa, gastrointestinal, or heavy menstrual bleeding, may be present. Rarely, life-threatening, including central nervous system, bleeding can occur. Wet purpura (blood blisters in the mouth) and retinal hemorrhages may herald life-threatening bleeding.

LABORATORY TESTING IN ITP Laboratory testing for antibodies (serologic testing) is usually not helpful due to the low sensitivity and specificity of the current tests. Bone marrow examination can be reserved for those who have other signs or laboratory abnormalities not explained by ITP or in patients who do not respond to initial therapy. The peripheral blood smear may show large platelets, with otherwise normal morphology. Depending on the bleeding history, iron-deficiency anemia may be present.

Laboratory testing is performed to evaluate for secondary causes of ITP and should include testing for HIV infection and hepatitis C (and other infections if indicated). Serologic testing for SLE, serum protein electrophoresis, immunoglobulin levels to potentially detect hypogammaglobulinemia, selective testing for IgA deficiency or monoclonal gammopathies, and testing for *H. pylori* infection should be considered, depending on the clinical circumstance. If anemia is present, direct antiglobulin testing (Coombs' test) should be performed to rule out combined autoimmune hemolytic anemia with ITP (Evans' syndrome).

TREATMENT IMMUNE THROMBOCYTOPENIC PURPURA

The treatment of ITP uses drugs that decrease reticuloendothelial uptake of the antibody-bound platelet, decrease antibody production, and/or increase platelet production. The diagnosis of ITP does not necessarily mean that treatment must be instituted. Patients with platelet counts >30,000/μL appear not to have increased mortality related to the thrombocytopenia.

Initial treatment in patients without significant bleeding symptoms, severe thrombocytopenia (<5000/μL), or signs of impending bleeding (such as retinal hemorrhage or large oral mucosal hemorrhages) can be instituted as an outpatient using single agents. Traditionally, this has been prednisone at 1 mg/kg, although Rh_0(D) immune globulin therapy (WinRho SDF), at 50–75 μg/kg, is also being used in this setting. Rh (D) immune globulin must be used only in Rh-positive patients because the mechanism of action is production of limited hemolysis, with antibody-coated cells "saturating" the Fc receptors, inhibiting Fc receptor function. Monitoring patients for 8 h after infusion is now advised by the FDA because of the rare complication of severe intravascular hemolysis. Intravenous gamma globulin (IVIgG), which is pooled, primarily IgG antibodies, also blocks the Fc receptor system, but appears to work primarily through different mechanism(s). IVIgG has more efficacy than anti-Rh (D) in postsplenectomized patients. IVIgG is dosed at 1–2 g/kg total, given over 1–5 days. Side effects are usually related to the volume of infusion and infrequently include aseptic meningitis and renal failure. All immunoglobulin preparations are derived from human plasma and undergo treatment for viral inactivation.

For patients with severe ITP and/or symptoms of bleeding, hospital admission and combined-modality therapy is given using high-dose glucocorticoids with IVIgG or anti-Rh_0(D) therapy and, as needed, additional immunosuppressive agents. Rituximab, an anti-CD20 (B cell) antibody, has shown efficacy in the treatment of refractory ITP, although long-lasting remission only occurs in approximately 30% of patients.

Splenectomy has been used for treatment of patients who relapse after glucocorticoids are tapered. Splenectomy remains an important treatment option, however, more patients than previously thought will go into remission over time. Observation, if the platelet count is high enough, or intermittent treatment with anti-Rh_0(D) or IVIgG, or initiation of treatment with a TPO receptor agonist (see below) may be a reasonable approach to see if the ITP will resolve. Vaccination against encapsulated organisms (especially pneumococcus, but also meningococcus and *Haemophilus influenzae*, depending on patient age and potential exposure) is recommended before splenectomy. Accessory spleen(s) are a very rare cause of relapse.

TPO receptor agonists are now available for the treatment of ITP. This approach stems from the finding that many patients with ITP do not have increased TPO levels, as was previously hypothesized. TPO

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660 ation of membrane attack complex (MAC). In an interna-
661 placebo-controlled, randomized trial of 87 patients (so far
ly controlled therapeutic trial in PNH) who had been selected
rounds of having severe hemolysis making them transfusion-
dependent, eculizumab proved effective and was licensed in 2007. Eculizumab, by abrogating complement-dependent intravascular hemolysis, significantly improves the quality of life of PNH patients. One would expect that the need for blood transfusion would also be abrogated; indeed, this is the case in about one-half of patients, in many of whom there is also a rise in hemoglobin levels. In the remaining patients, however, the anemia remains sufficiently severe to require blood transfusion. One reason for this is that, once the distal complement pathway is blocked, red cells no longer destroyed by the MAC become opsonized by complement (C3) fragments and undergo extravascular hemolysis (Fig. 129-10). The extent to which this happens depends in part on a genetic polymorphism of the complement receptor CR1. Based on its half-life, eculizumab must be administered intravenously every 14 days. The only form of treatment that currently can provide a definitive cure for PNH is allogeneic BMT. When an HLA-identical sibling is available, BMT should be offered to any young patient with severe PNH; the availability of eculizumab has decreased significantly the proportion of patients receiving BMT.

For patients with the PNH-AA syndrome, immunosuppressive treatment with antithymocyte globulin and cyclosporine A may be indicated, especially in order to relieve severe thrombocytopenia and/or neutropenia in patients in whom these were the main problem(s); of course, this treatment will have little or no effect on hemolysis. Any patient who has had venous thrombosis or who has a genetically determined thrombophilic state in addition to PNH should be on regular anticoagulant prophylaxis. With thrombotic complications that do not resolve otherwise, thrombolytic treatment with tissue plasminogen activator may be indicated.

PART 7 Oncology and ...atology

ANEMIA DUE TO ACUTE BLOOD LOSS

Blood loss causes anemia by two main mechanisms: (1) by the direct loss of red cells; and (2) if the loss of blood is protracted, it will gradually deplete iron stores, eventually resulting in iron deficiency. The latter type of anemia is covered in Chap. 126; here we are concerned with the former type, i.e., *posthemorrhagic anemia*, which follows *acute* blood loss. This can be *external* (e.g., after trauma or obstetric hemorrhage) or *internal* (e.g., from bleeding in the gastrointestinal tract, rupture of the spleen, rupture of an ectopic pregnancy, subarachnoid hemorrhage). In any of these cases, after the sudden loss of a large amount of blood, there are three clinical/pathophysiologic stages. (1) At first, the dominant feature is hypovolemia, which poses a threat particularly to organs that normally have a high blood supply, like 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, because 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, for example, 7 g/dL, it means that about half of the entire blood has been lost. (3) Provided bleeding does not continue, the bone marrow response will gradually ameliorate the anemia.

The diagnosis of acute posthemorrhagic anemia (APHA) is usually straightforward, although sometimes internal bleeding episodes (e.g., after a traumatic injury), even when large, may not be immediately obvious. 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.

TREATMENT **ANEMIA DUE TO ACUTE BLOOD LOSS**

With respect to treatment, a two-pronged approach is imperative. (1) In many cases, the blood lost needs to be replaced promptly. Unlike with many chronic anemias, when 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; because the body is not adapted to the anemia, blood transfusion takes priority. (1) While the emergency is being confronted, it is imperative to stop the hemorrhage and to eliminate its source.

A special type of APHA is blood loss during and immediately after surgery, which can be substantial (e.g., up to 2 L in the case of a radical prostatectomy). Of course with elective surgical procedures, the patient's own stored blood may be available (through preoperative autologous blood donation), and in any case, blood loss ought to have been carefully monitored/measured. The fact that this blood loss is iatrogenic dictates that ever more effort should be invested in optimizing its management.

A Holy Grail of emergency medicine for a long time has been the idea of a blood substitute that would be universally available, suitable for all recipients, easy to store and to transport, safe, and as effective as blood itself. Two main paths have been pursued: (1) fluorocarbon synthetic chemicals that bind oxygen reversibly, and (2) artificially modified hemoglobins, known as hemoglobin-based oxygen carriers (HBOCs). Although there are numerous anecdotal reports of the use of both approaches in humans, and although HBOCs have reached the stage of phase 2–3 clinical trials, no "blood substitute" has yet become standard treatment.

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130 Bone Marrow Failure Syndromes Including Aplastic Anemia and Myelodysplasia

Neal S. Young

The hypoproliferative anemias are normochromic, normocytic, or macrocytic and are characterized by a low reticulocyte count. Hypoproliferative anemia is also a prominent feature of hematologic diseases that are described as bone marrow failure states; these include aplastic anemia, myelodysplastic syndrome (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 [ITP] or due to splenomegaly), and granulocytes (as in the immune leukopenias). Marrow damage and dysfunction also may be secondary to infection, inflammation, or cancer.

Hematopoietic failure syndromes are classified by dominant morphologic features of the bone marrow (Table 130-1). Although practical distinction among these syndromes usually is clear, 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.

It is important that the internist and general practitioner recognize the marrow failure syndromes, as their prognosis may be poor if the

TABLE 130-1 DIFFERENTIAL DIAGNOSIS OF PANCYTOPENIA

Pancytopenia with Hypocellular Bone Marrow	
Acquired aplastic anemia	
Constitutional aplastic anemia (Fanconi anemia, dyskeratosis congenita)	
Some myelodysplasia	
Rare aleukemic leukemia	
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
	B_{12}, folate deficiency
Myelofibrosis	Overwhelming infection
Some aleukemic leukemia	Alcohol
Myelophthisis	Brucellosis
Bone marrow lymphoma	Sarcoidosis
Hairy cell leukemia	Tuberculosis
	Leishmaniasis
Hypocellular Bone Marrow ± Cytopenia	
Q fever	
Legionnaires' disease	
Anorexia nervosa, starvation	
Mycobacteriurn	

patient is untreated; effective therapies are often available but sufficiently complicated in their choice and delivery so as to warrant the care of a hematologist or oncologist.

APLASTIC ANEMIA

DEFINITION

Aplastic anemia is pancytopenia with bone marrow hypocellularity. Acquired aplastic anemia is distinguished from iatrogenic aplasia, marrow hypocellularity after intensive cytotoxic chemotherapy for cancer. Aplastic anemia can also be constitutional: the genetic diseases Fanconi anemia and dyskeratosis congenita, although 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. 129) 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 older adults.

ETIOLOGY

The origins of aplastic anemia have been inferred from several recurring clinical associations (Table 130-2); unfortunately, these relationships are not reliable in an individual patient and may not be etiologic. In addition, although 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 130-2 CLASSIFICATION OF APLASTIC ANEMIA AND SINGLE CYTOPENIAS

Acquired	Inherited
Aplastic Anemia	
Secondary	Fanconi 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, Dubowitz, Seckel)
Parvovirus B19 (transient aplastic crisis, PRCA)	
HIV-1 (AIDS)	
Immune diseases	
Eosinophilic fasciitis	
Hyperimmunoglobulinemia	
Large granular lymphocytosis (LGL)	
Thymoma/thymic carcinoma	
Graft-versus-host disease in immunodeficiency	
Paroxysmal nocturnal hemoglobinuria (PNH)	
Pregnancy	
Idiopathic	
Cytopenias	
PRCA (see Table 130-4)	Congenital PRCA (Diamond-Blackfan anemia)
Neutropenia/agranulocytosis	
Idiopathic	Kostmann syndrome
Drugs, toxins	Shwachman-Diamond syndrome
LGL	Reticular dysgenesis
Pure white cell aplasia (+/– thymoma)	
Thrombocytopenia	
Drugs, toxins	Amegakaryocytic thrombocytopenia
Idiopathic amegakaryocytic	Thrombocytopenia with absent radii

Abbreviation: 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 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. Whereas 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: epidemiologic, clinical, and laboratory data link benzene to aplastic anemia, acute leukemia, and blood and marrow abnormalities. For leukemia, incidence is correlated with cumulative exposure, but susceptibility must also be important, because only a minority of even heavily exposed workers develop 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.

.al Examination Petechiae and ecchymoses are typical, and reti- ıemorrhages may be present. Pelvic and rectal examinations can :n be deferred but, when performed, should be undertaken with ₀ eat 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 anemia; peculiar nails and leukoplakia suggest dyskeratosis congenita; early graying (and use of hair dyes to mask it!) suggests a telomerase defect.

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 (RBCs) 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 all 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, whereas "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. Granulomas 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 anemia. Very short telomere length (available commercially) strongly suggests the presence of a telomerase or shelterin mutation, which can be pursued by family studies and nucleotide sequencing. Chromosome studies of bone marrow cells are often revealing in MDS and should be negative in typical aplastic anemia. Flow cytometry offers a sensitive diagnostic test for PNH. Serologic studies may show evidence of viral infection, such as Epstein-Barr virus and HIV. Posthepatitis aplastic anemia is seronegative. The spleen size should be determined by computed tomography (CT) scanning or ultrasound if the physical examination of the abdomen is unsatisfactory. Occasionally MRI may be helpful to assess the fat content of 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 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 SLE, or miliary tuberculosis on chest radiograph (Table 130-1).

Diagnostic problems can occur with atypical presentations and among related hematologic diseases. Although pancytopenia is most common, some patients with bone marrow hypocellularity have depression of only one or two of three blood lines, with later progression to pancytopenia. The bone marrow in constitutional aplastic anemia is morphologically indistinguishable 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. Historically, provision first of RBC and later of platelet transfusions and effective antibiotics were of some benefit, but few patients show spontaneous recovery. The major prognostic determinant is the blood count. Severe disease has been 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). In the era of effective immunosuppressive therapies, absolute numbers of reticulocytes (>25,000/μL) and lymphocytes (>1000/μL) may be better predictors of response to treatment and long-term outcome.

TREATMENT APLASTIC ANEMIA

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. Glucocorticoids are not of value as primary therapy. 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 younger patient with a fully histocompatible sibling donor (**Chap. 139e**). 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, but limited numbers of blood products probably do not greatly affect outcome. For allogeneic transplant from fully matched siblings, long-term survival rates for children are approximately 90%. Transplant morbidity and mortality are increased among adults, due to the higher risk of chronic GVHD and serious infections.

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. High-resolution matching at HLA and more effective conditioning regimens and GVHD prophylaxis have led to improved survival rates in patients who proceed to alternative donor transplant, in some series approximating results with conventional sibling donors. Patients will be at risk for late complications, especially a higher rate of cancer, if radiation is used as a component of conditioning.

IMMUNOSUPPRESSION

The standard regimen of antithymocyte globulin (ATG) in combination with cyclosporine induces hematologic recovery (independence from transfusion and a leukocyte count adequate to prevent infection) in 60–70% of patients. Children do especially well, whereas older adult patients often suffer complications due to the presence of comorbidities. An early robust hematologic response 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 bone marrow cellularity returns toward normal 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 approximately 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 administered as intravenous infusions over 4 days. ATG binds to peripheral blood cells; therefore, platelet and granulocyte numbers may decrease further during active treatment. Serum sickness, a flulike illness with a characteristic cutaneous eruption and arthralgia, often develops approximately 10 days after initiating treatment. Methylprednisolone is administered with ATG to 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 high dose, with subsequent adjustment according to blood levels obtained every 2 weeks; rough levels should be between 150 and 200 ng/mL. The most important side effects are nephrotoxicity, hypertension, seizures, and opportunistic infections, especially *Pneumocystis jiroveci* (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, whereas 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.

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.

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. Sex hormones upregulate telomerase gene activity in vitro, which is possibly also their mechanism of action in improving marrow function. For patients with moderate disease, especially if a telomere defect is present, or those with severe pancytopenia in whom immunosuppression has failed, a 3- to 4-month trial is appropriate.

Hematopoietic growth factors (HGFs) such as erythropoietin and granulocyte colony-stimulating factor (G-CSF) are not definitive therapy for severe aplastic anemia, and even their roles as adjuncts to immunosuppression are not clear. In research protocols, thrombopoietin mimetics have shown surprising activity in patients with refractory aplastic anemia, with patterns of blood count recovery suggesting that they act as stem cell stimulants.

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. A major reason for the improved prognosis in aplastic anemia has been the development of better antifungal drugs and the timely institution of such therapy when infection is suspected. Granulocyte transfusions using G-CSF–mobilized peripheral blood may be effective in the treatment of overwhelming or refractory infections. 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 to maintain the platelet count >10,000/μL (oozing from the gut 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.

RBCs 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 approximately the fiftieth transfusion 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 marrow shows corresponding absence or decreased numbers of specific precursor cells: aregenerative anemia as in PRCA (see below), thrombocytopenia with amegakaryocytosis (Chap. 140), and neutropenia without marrow myeloid cells in agranulocytosis (Chap. 80). 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 older adults 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 of the single-lineage failure syndromes, progression to pancytopenia or leukemia is unusual.

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TABLE 130-4 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 anemia)
- Acquired pure red cell aplasia
  - Cancer
    - 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
  - Viruses
    - Persistent B19 parvovirus, hepatitis, adult T cell leukemia virus, Epstein-Barr virus
  - Pregnancy
  - Drugs
    - Especially phenytoin, azathioprine, chloramphenicol, procainamide, isoniazid
  - Antibodies to erythropoietin
  - Idiopathic

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 130-4. 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; mutations in ribosome protein genes are etiologic. Temporary red cell failure occurs in transient aplastic crisis of hemolytic anemias due to acute parvovirus infection (Chap. 221) and in transient erythroblastopenia of childhood, which occurs in normal children.

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 complicate chronic lymphocytic leukemia. Some patients may be hypogammaglobulinemic. Infrequently (compared to agranulocytosis), PRCA can be due to an idiosyncratic drug reaction. Subcutaneous administration of erythropoietin (EPO) has provoked PRCA mediated by neutralizing antibodies.

Like aplastic anemia, PRCA results from diverse mechanisms. Antibodies to RBC 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 and 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 RBC 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. 130-2), 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.

TREATMENT PURE RED CELL APLASIA

History, physical examination, and routine laboratory studies may disclose an underlying disease or a drug exposure. Thymoma should be sought by radiographic procedures. Tumor excision

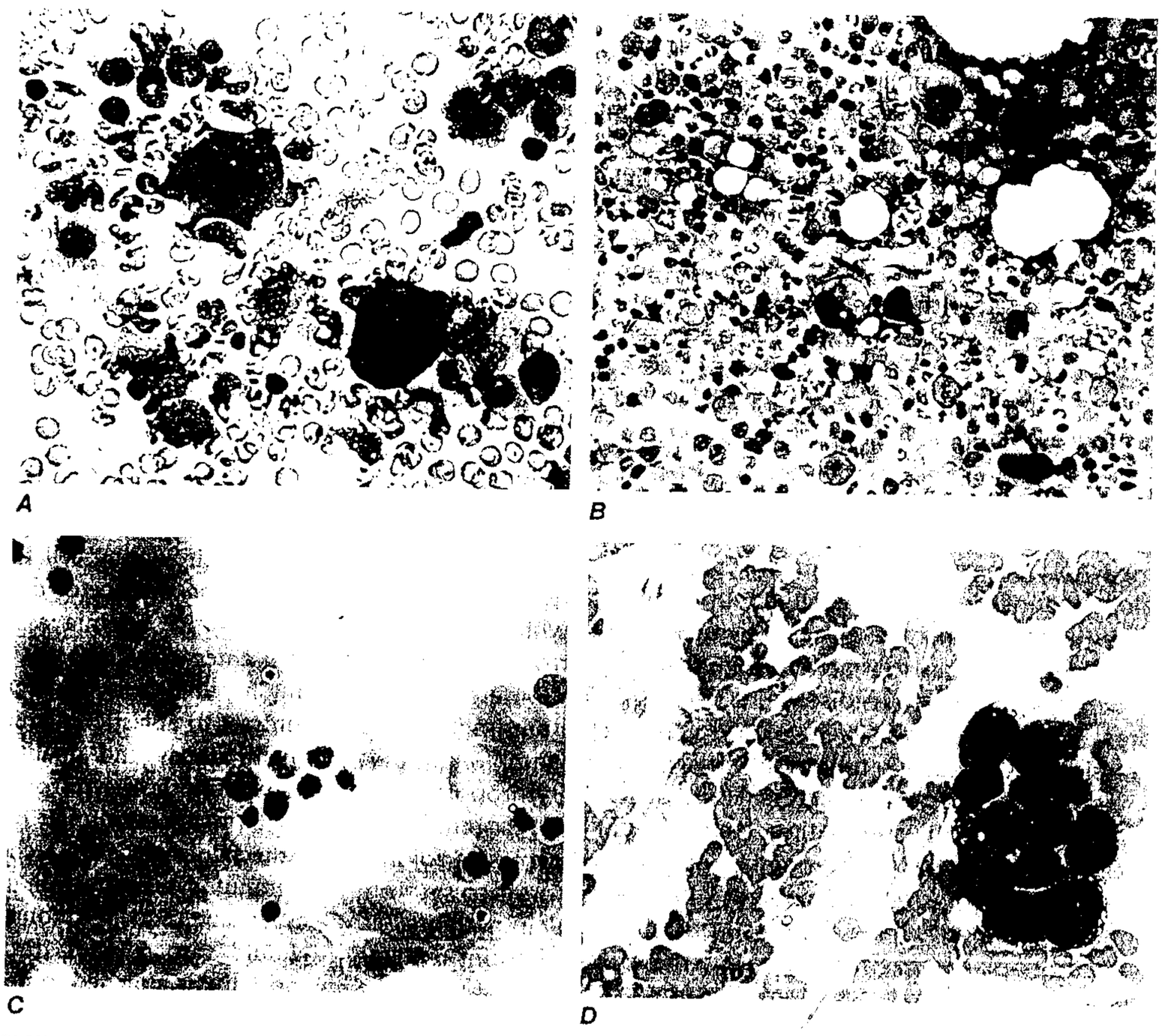

FIGURE 130-2 Pathognomonic cells in marrow failure syndromes. ***A.*** Giant pronormoblast, the cytopathic effect of B19 parvovirus infection of the erythroid progenitor cell. ***B.*** Uninucleate megakaryocyte and microblastic erythroid precursors typical of the 5q–myelodysplasia syndrome. ***C.*** 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.