CHAPTER 34
APLASTIC ANEMIA: ACQUIRED AND INHERITED

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SUMMARY

Acquired aplastic anemia is a clinical syndrome in which there is 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. Reticuloctopenia, 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 are the result of the expression of autoimmune cytotoxic T lymphocytes that suppress or destroy primitive CD34+ multipotent hematopoietic cells. The disorder also can occur after (1) 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, chloramphenicol), (4) as a feature of a connective tissue or autoimmune disorder (e.g., lupus erythematosus), or, (5) rarely, in association with pregnancy. The final common pathway may be through cytotoxic T-cell autoimmunity, whether idiopathic or associated with an inciting agent since they all respond in a similar fashion to immunosuppressive therapy. The differential diagnosis of acquired aplastic anemia includes the hypoplastic marrow that can accompany paroxysmal nocturnal hemoglobinuria or hypoplastic oligoblastic (myelodysplastic syndrome) or polyblastic myelogenous leukemia. Allogeneic hematopoietic stem cell transplantation is curative in approximately 80 percent of younger patients with high-resolution human leukocyte antigen (HLA)-matched sibling donors although the posttransplant period may be severely complicated by graft-versus-host disease. The disease may be significantly ameliorated or occasionally cured by immunotherapy, especially a regimen coupling antithymocyte globulin with cyclosporine. However, after successful treatment with immunosuppressive agents, the disease may relapse or evolve into a clonal myeloid disorder, such as paroxysmal nocturnal hemoglobinuria, a clonal cytopenia, or oligoblastic or polyblastic myelogenous leukemia. Several uncommon inherited disorders, including Fanconi anemia, Shwachman-Diamond syndrome, dysertransfusional congenita and others have as a primary manifestation aplastic hematopoiesis.

ACQUIRED APLASTIC ANEMIA

DEFINITION AND HISTORY

Aplastic anemia is a clinical syndrome that results from a marked diminution of marrow blood cell production. The latter results in reticulocytopenia, anemia, granulocytopenia, monocytopenia, and thrombocytopenia. The diagnosis usually requires the presence of pancytopenia with a neutrophil count fewer than 1500/μL (1.5 × 10⁹/L), a platelet count fewer than 50,000/μL (50 × 10⁹/L), a hemoglobin concentration less than 10 g/dL (100 g/L), and an absolute reticulocyte count fewer than 40,000/μL (40 × 10⁹/L), accompanied by a hypocellular marrow without abnormal or malignant cells or fibrosis. For the purpose of therapeutic decision making, comparative clinical trials, and international sharing of data, the disease has been stratified into moderately severe, severe, and very severe acquired aplastic anemia based on the blood counts (especially the neutrophil count) and the degree of marrow hypocellularity (Table 34–1). Most cases of aplastic anemia are acquired; fewer cases are the result of an inherited disorder, such as Fanconi anemia, Shwachman-Diamond syndrome, and others (see “Hereditary Aplastic Anemia” below).

Aplastic anemia was first recognized by Ehrlich 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 was subsequently applied to this disease by Chauffard, a French hematologist, in 1904, and although an anachronistic term because the morbidity is the result of pancytopenia, especially neutropenia and thrombocytopenia, the designation is entrenched in medical usage. For the next 40 years, many conditions that caused pancytopenia were confused with aplastic anemia based on incomplete or inadequate histologic study of the patient’s marrow. The development of improved instruments for percutaneous marrow biopsy in the last half of the 20th century improved diagnostic precision. In 1972, Thomas and his colleagues established that marrow transplantation from a histocompatible sibling could cure the disease. The disease initially was thought to result from an atrophy or chemical injury of primitive marrow hematopoietic cells. The unexpected recovery of marrow recipients who were given immunosuppressive conditioning therapy but who did not engraft with donor stem cells raised the possibility that the disease may not be intrinsic to primitive hematopoietic cells but the result of a suppression of hematopoietic cells by immune cells, notably T lymphocytes. The requirement to treat a recipient of a marrow transplant from an identical twin with immunosuppressive conditioning therapy for optimal results of transplant buttressed this suspicion. This supposition was confirmed by a clinical trial that established antithymocyte globulin (ALG) capable ofameliorating the disease in the majority of patients. Since that time, compelling evidence for a cellular autoimmune mechanism has accumulated (see the main section “Etiology and Pathogenesis” below).

EPIDEMIOLOGY

The International Aplastic Anemia and Agranulocytosis Study and a French study found the incidence rate of acquired aplastic anemia to be about 2 per 1,000,000 persons per year. This approximate annual incidence rate has been confirmed in studies in Spain (Barcelona), Brazil (State of Parana), and Canada (British Columbia). The highest frequency of aplastic anemia occurs in persons between the ages of 15 and 25 years; a second peak occurs between the ages of 65 and 69. Aplastic anemia is more prevalent in the Far East where the incidence is approximately 7 per 1,000,000 in parts of China, approximately 4 per 1,000,000 in sections of Thailand, approximately 5 per 1,000,000 in areas of Malaysia, and approximately 7 per 1,000,000 among children of Asian descent living in a province of Canada.

Acronyms and abbreviations that appear in this chapter include: A, adenine; ALG, antithymocyte globulin; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; ATG, antithymocyte globulin; ATR, ataxia-telangiectasia mutated and rad3-related kinase; BFU-E, erythroid burst-forming units; CD, cluster of differentiation; CFU-GM, colony-forming unit–granulocyte, macrophage; CMV, cytomegalovirus; EBV, Epstein-Barr virus; G, guanine; G-CSF, granulocyte colony-stimulating factor; HHV, human herpes virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IL, interleukin; LDH, lactate dehydrogenase; NMRI, nuclear magnetic resonance imaging; PCP, pneumocystis; PNH, paroxysmal nocturnal hemoglobinuria; SCF, stem cell factor; T, thymine; TNF, tumor necrosis factor; TNT, trinitrotoluene; TPO, thrombopoietin.
Hemoglobin

Marked decrease or absence

<30.0

<50

<90 g/L

Marked decrease or absence

<0.2

<1.5

<100 g/L

<20.0

<80 g/L

<40

<30.0

approximately one.

male-to-female incidence ratio of aplastic anemia in most studies is

results from cytotoxic T-cell-mediated immune suppression of

forming unit–erythroid (BFU–E) are reduced markedly in patients

colony-forming unit–granulocyte-macrophage (CFU-GM) and burst-
cells (multipotential hematopoietic progenitors) and their derivative

Table 34–2 lists the potential causes for aplastic anemia.

diseases, a known association with the onset of acquired aplastic anemia,

an infectious etiology, although no agent, including seronegative hepa-
titis, has been identified. Seronegative viral hepatitis is a forerunner of

has been identified. Seronegative viral hepatitis is a forerunner of

approximately 7 percent of cases of acquired aplastic anemia.

Deficiencies in telomere repair could predis-
pose to aplastic anemia by affecting the size of the multipotential hema-
topoietic cell compartment and by decreasing the multipotential cell’s
response to a marrow injury, and could play a role in the evolution of
aplastic anemia to a clonal myeloid disease by contributing to genome
instability. Thus, reduced hematopoiesis in most cases of aplastic ane-
mia results from cytotoxic T-cell-mediated immune suppression of

\[ \text{TABLE 34–1. Degree of Severity of Acquired Aplastic Anemia} \]

<table>
<thead>
<tr>
<th>Diagnostic Categories</th>
<th>Hemoglobin</th>
<th>Reticulocyte Concentration</th>
<th>Neutrophil Count</th>
<th>Platelet Count</th>
<th>Marrow Biopsy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately severe</td>
<td>&lt;100 g/L</td>
<td>&lt;40 × 10^9/L</td>
<td>&lt;1.5 × 10^9/L</td>
<td>&lt;50 × 10^9/L</td>
<td>Marked decrease of hematopoietic cells.</td>
<td>At the time of diagnosis at least 2 of 3 blood counts should meet these criteria.</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;90 g/L</td>
<td>&lt;30.0 × 10^9/L</td>
<td>&lt;0.5 × 10^9/L</td>
<td>&lt;30.0 × 10^9/L</td>
<td>Marked decrease or absence of hematopoietic cells.</td>
<td>Search for a histocompatible sibling should be made if age permits.</td>
</tr>
<tr>
<td>Very Severe</td>
<td>&lt;80 g/L</td>
<td>&lt;20.0 × 10^9/L</td>
<td>&lt;0.2 × 10^9/L</td>
<td>&lt;20.0 × 10^9/L</td>
<td>Marked decrease or absence of hematopoietic cells.</td>
<td>Search for a histocompatible sibling should be made if age permits.</td>
</tr>
</tbody>
</table>

NOTE: These values are approximations and must be considered in the context of an individual patient’s situation. (In some clinical trials, the blood count thresholds for moderately severe aplastic anemia are higher, e.g., platelet count <100 × 10^9/L and absolute reticulocyte count <50,000 × 10^9/L.) The marrow biopsy may contain the usual number of lymphocytes and plasma cells; “hot spots,” focal areas of erythroid cells, may be seen. No fibrosis, abnormal cells, or malignant cells should be evident in the marrow. Dysmorphic features of blood or marrow cells are not features of acquired aplastic anemia. Ethnic differences in the lower limit of the absolute neutrophil count should be considered. (See Chap. 65.)

twofold or greater incidence in the Orient compared to the Occident may be multifactorial, but a predisposition gene or genes is a likely component. Studies have not established the use of chloramphenicol in Asia as a cause. Poorly regulated exposure of workers to benzene is a factor, but the attributable risk from benzene and other toxic exposures does not explain the magnitude of the difference in the incidence in Asia compared to that in Europe and South America. A relationship to impure water use in Thailand has led to speculation of an infectious etiology, although no agent, including seronegative hepatitis, has been identified. Seronegative viral hepatitis is a forerunner of approximately 7 percent of cases of acquired aplastic anemia. The male-to-female incidence ratio of aplastic anemia in most studies is approximately one.

**Etiology and Pathogenesis**

Table 34–2 lists the potential causes for aplastic anemia.

The final common pathway to the clinical disease is a decrease in blood cell formation in the marrow. The number of marrow CD34+ cells (multipotential hematopoietic progenitors) and their derivative colony-forming unit–granulocyte-macrophage (CFU-GM) and burst-forming unit–erythroid (BFU–E) are reduced markedly in patients with aplastic anemia. Long-term culture-initiating cells, an in vitro surrogate assay for hematopoietic stem cells, also are reduced to approximately 1 percent of normal values. Potential mechanisms responsible for acquired marrow cell failure include (1) direct toxicity to hematopoietic multipotential cells, (2) a defect in the stromal microenvironment of the marrow required for hematopoietic cell development, (3) impaired production or release of essential multilineage hematopoietic growth factors, (4) cellular or humoral immune suppression of the marrow multipotential cells, and (5) progressive erosion of chromosome telomeres. There is little experimental evidence for a stromal microenvironmental defect or a deficit of critical hematopoietic growth factors, and the role of telomerase mutations with consequent telomere shortening is unclear, although present in as much as 40 percent of patients. Deficiencies in telomere repair could predispose to aplastic anemia by affecting the size of the multipotential hematopoietic cell compartment and by decreasing the multipotential cell’s response to a marrow injury, and could play a role in the evolution of aplastic anemia to a clonal myeloid disease by contributing to genome instability. Thus, reduced hematopoiesis in most cases of aplastic anemia results from cytotoxic T-cell-mediated immune suppression of very early CD34+ hematopoietic multipotential progenitor or stem cells. A small fraction of cases is initiated by a toxic exposure, drug exposure, or viral infection, but in these cases the pathogenesis also

**TABLE 34–2. Etiologic Classification of Aplastic Anemia**

<table>
<thead>
<tr>
<th>Acquired</th>
<th>Autoimmune</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>See Table 34–3</td>
</tr>
<tr>
<td>Toxins</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>Chlorinated hydrocarbons</td>
</tr>
<tr>
<td>Organophosphates</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Non-A, -B, -C, -D, -E, or -G hepatitis virus</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>(HIV)</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>Autoimmune/connective tissue disorders</td>
<td></td>
</tr>
<tr>
<td>Eosinophilic fasciitis</td>
<td>Immune thyroid disease (Graves disease, Hashimoto thyroiditis)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Thymoma</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>Radiation</td>
</tr>
<tr>
<td>Radiation</td>
<td>Cytotoxic drug therapy</td>
</tr>
<tr>
<td>Hereditary</td>
<td></td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Dyskeratosis congenita</td>
</tr>
<tr>
<td>Shwachman-Diamond syndrome</td>
<td></td>
</tr>
<tr>
<td>Other rare syndromes (see Table 34–8)</td>
<td></td>
</tr>
</tbody>
</table>
may relate to autoimmunity as there is evidence of immune dysfunction in seronegative hepatitis, after benzene exposure, and many such patients respond to anti–T-cell therapy.\(^24\)

**Autoreactive Cytotoxic T Lymphocytes**

*In vitro* and clinical observations have resulted in the identification of a cytotoxic T-cell-mediated attack on multipotential hematopoietic cells in the CD34+ cellular compartment as the basis for acquired aplastic anemia.\(^25\) Cellular immune injury to the marrow after drug-, viral-, or toxin-initiated marrow aplasia could result from the induction of neoantigens that provoke a secondary T-cell-mediated attack on hematopoietic cells. This mechanism could explain the response to immunosuppressive treatment in cases that follow exposure to an exogenous agent. Spontaneous or mitogen-induced increases in mononuclear cell production of interferon-γ (IFN-γ)\(^26,27\) interleukin (IL)-2,\(^27\) and tumor necrosis factor-α (TNF-α)\(^28,29\) occur. Elevated serum levels of interferon-γ are present in 30 percent of patients with aplastic anemia, and interferon-γ expression has been detected in the marrow of most patients with acquired aplastic anemia.\(^30\) Addition of antibodies to interferon-γ enhances *in vitro* colony growth of marrow cells from affected patients.\(^31\) Long-term marrow cultures manipulated to elaborate exaggerated amounts of interferon-γ markedly reduced the frequency of long-term culture-initiating cells.\(^24\) These observations indicate that acquired aplastic anemia is the result of cellular immune-induced apoptosis of primitive CD34+ multipotential hematopoietic progenitors, mediated by cytotoxic T lymphocytes, in part, through the expression of T-helper type 1 (Th1) inhibitory cytokines, interferon-γ, and TNF-α (Fig. 34–1).\(^32\) The secretion of interferon-γ is a result of the upregulation of transcription regulatory factor T-bet,\(^33\) and apoptosis of CD34+ cells is, in part, mediated through a FAS-dependent pathway.\(^34\) Because HLA-DR2 is more prevalent in patients with aplastic anemia, antigen recognition may be a factor in those patients. A variety of other potential factors have been found in some patients, including nucleotide polymorphisms in cytokine genes, overexpression of perforin in marrow cells, and decreased expression of SAP, a modulator protein that inhibits interferon-γ secretion.\(^24\)

A decrease in regulatory T cells contributes to the expansion of an autoreactive CD8+CD28− T-cell population, which induces apoptosis of autologous hematopoietic multipotential hematopoietic cells.\(^34\) One mouse model of immune-related marrow failure, induced by infusion of parental lymph node cells into F1 hybrid recipients, caused a fatal aplastic anemia. The aplasia could be prevented by immunotherapy or

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**FIGURE 34–1.** Immune pathogenesis of apoptosis of CD34 multipotential hematopoietic cells in acquired aplastic anemia. Antigens are presented to T lymphocytes by antigen-presenting cells (APCs). This triggers T cells to activate and proliferate. T-bet, a transcription factor, binds to the interferon-γ (IFN-γ) promoter region and induces gene expression. SLAM-associated protein (SAP) binds to Fyn and modulates the signaling lymphocyte activation molecule (SLAM) activity on IFN-γ expression, diminishing gene transcription. Patients with aplastic anemia show constitutive T-bet expression and low SAP levels. IFN-γ and tumor necrosis factor-α (TNF-α) upregulate both the T cell’s cellular receptors and the Fas receptor. Increased production of interleukin-2 leads to polyclonal expansion of T cells. Activation of the Fas receptor by the Fas ligand leads to apoptosis of target cells. Some effects of IFN-γ are mediated through interferon regulatory factor 1 (IRF-1), which inhibits the transcription of cellular genes and entry into the cell cycle. IFN-γ is a potent inducer of many cellular genes, including inducible nitric oxide synthase (NOS), and production of nitric oxide (NO) may diffuse its cytotoxic effects. These events ultimately lead to reduced cell cycling and cell death by apoptosis. (Reproduced from Young NS, Calado RT, Scheinberg P: Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 108:2509, 2006, with permission of The American Society of Hematology.)
with monoclonal antibodies to interferon-γ and TNF-α. Another mouse model of aplastic anemia induced by the infusion of lymph node cells histoincompatible for the minor H antigen, H60, resulted from the expansion of H60-specific CD8 T cells in recipient mice. The result was severe marrow aplasia. The effect of the CD8 T cells could be abrogated by either immunosuppressive agents or administration of CD4+CD25+ regulatory T cells, providing additional experimental evidence for the role of regulatory T cells in the prevention of aplastic anemia.

Several putative target antigens on affected hematopoietic cells have been identified. Autoantibodies to one putative antigen, kinectin, have been found in patients with aplastic anemia. T cells, responsive to kinectin-derived peptides, suppress granulocyte-monocye colony growth in vitro. However, in these studies cytotoxic T lymphocytes with that specificity were not isolated from patients.

**Drugs**

Chloramphenicol is the most notorious drug documented to cause aplastic anemia. Although this drug is directly myelosuppressive at very high dose because of its effect on mitochondrial DNA, the occurrence of aplastic anemia appears to be idiiosyncratic, perhaps related to an inherited sensitivity to the nitroso-containing toxic intermediates. This sensitivity may produce immunologic marrow suppression, as a substantial proportion of affected patients respond to treatment with immunosuppressive therapy. The risk of developing aplastic anemia in patients treated with chloramphenicol is approximately 1 in 20,000, or 25 times that of the general population. Although its use as an antibiotic has been largely abandoned in industrialized countries, global reports of fatal aplastic anemia continue to appear with topical or systemic use of the drug.

Epidemiologic evidence established that quinacrine (Atabrine) increased the risk of aplastic anemia. This drug was administered to all U.S. troops in the South Pacific and Asiatic theaters of operations as prophylaxis for malaria during 1943 and 1944. The incidence of aplastic anemia was 7 to 28 cases per 1,000,000 personnel per year in the prophylaxis zones, whereas untreated soldiers had 1 to 2 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 have been reported to increase the risk of aplastic anemia, but owing to incomplete reporting of information and the infrequency of the association, the spectrum of drug-induced aplastic anemia may not be fully appreciated. Table 34–3 is a partial list of drugs that have been implicated.

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

Because aplastic anemia is a rare event with drug use, it may occur because of an underlying metabolic or immunologic predisposition (gene polymorphism) in susceptible individuals. In the case of phenylbutazone-associated marrow aplasia, there is delayed oxidation and clearance of a related compound, acetonilide, as compared to either normal controls or those with aplastic anemia from other causes. This finding suggests excess accumulation of the drug as a potential mechanism for the aplasia. In some cases, drug interactions or synergy may be required to induce marrow aplasia. Cimetidine, a histamine H2-receptor antagonist, is occasionally implicated in the onset of cytopenias and aplastic anemia, perhaps owing to a direct effect on early hematopoietic progenitor cells. This drug accentuates the marrow-suppressive effects of the chemotherapy drug Carmustine. In several instances, it has been reported as a possible cause of marrow aplasia when given with chloramphenicol.

There appears to be little difference in the age distribution, gender, response to immunotherapy, marrow transplantation, or survival whether or not a drug exposure preceded the onset of the marrow aplasia.

**Toxic Chemicals**

Benzenzene was the first chemical linked to aplastic anemia, based on studies in factory workers before the 20th century. Benzene is used as a solvent and is employed 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 acute myelogenous leukemia) in workers exposed to a poorly regulated environment. In studies in China, aplastic anemia among workers was sixfold higher than in the general population.

The U.S. Occupational Safety and Health Administration has lowered the permissible atmospheric exposure limit of benzene to 1 part per million (ppm). Previous to that regulatory change, the frequency of aplastic anemia in workers exposed to greater than 100 ppm benzene was approximately 1 in 100 workers, which decreased to 1 in 1000 workers at 10 to 20 ppm exposure.

Organochlorine and organophosphate pesticide compounds have been suspected in the onset of aplastic anemia and several studies have indicated an increased relative risk, especially for agricultural exposures. The relationships are suspect because dose–disease relationships and other important factors have not been delineated, and several studies have not found an association with environmental exposures. DDt (dichlorodiphenyltrichloroethane), lindane, and chlordane are insecticides that have also been associated with cases of aplastic anemia. Occasional cases still occur following heavy exposure at industrial plants or after its use as a pesticide. 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. Prolonged exposures to petroleum distillates in the form of Stoddard solvent and acute exposure to toluene through the practice of glue sniffing also have been reported to 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. In most cases, these conclusions have not been derived from specific studies but from accumulation of case reports or from patient histories, making conclusions provisional, although the argument for minimizing exposures to potential toxins is logical in any case.

**Viruses**

**Non-A, -B, -C, -D, -E, -G Hepatitis Virus** A relationship between hepatitis and the subsequent development of aplastic anemia has been the subject of a number of case reports, and this association was emphasized by two major reviews in the 1970s. In the aggregate, these 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 (ages 18 to 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, non-C hepatitis. Severe aplastic anemia developed in 9 of 31 patients who underwent liver transplantation for non-A, non-B, non-C hepatitis,
Several lines of evidence indicate there is no causal association with hepatitis C virus, suggesting that an unknown viral agent is involved. \(^{16,75,76}\) Hepatitis virus B or C can be a secondary infection, if carefully screened blood products are not used for transfusion. In 15 patients with posthepatic aplastic anemia, no evidence was found for hepatitis A, B, C, D, E, or G, transfusion-transmitted virus, or parvovirus B19. \(^{76}\) Several reports suggest a relationship of parvovirus B19 to aplastic anemia, \(^{77,78}\) whereas others have not. \(^{79}\) This relationship has not been established (see Chap. 35). The effect of seronegative hepatitis may be mediated through an autoimmune T-cell effect because of evidence of T-cell activation and cytokine elaboration. \(^{24}\) These patients also have a similar response to combined immuno-therapy as does idiopathic aplastic anemia (see “TREATMENT, Combination Immunotherapy”).

**Epstein-Barr Virus** Epstein-Barr virus (EBV) has been implicated in the pathogenesis of aplastic anemia. \(^{80,81}\) The onset usually occurs within 4 to 6 weeks of infection. In some cases, infectious mononucleosis is subclinical, with a finding of reactive lymphocytes in the blood film and serologic results consistent with a recent infection (see Chap. 84). EBV has been detected in marrow cells, \(^{81}\) but it is uncertain whether marrow aplasia results from a direct effect or an immunologic response by the host. Patients have recovered following therapy with antithymocyte globulin. \(^{81}\)

**Other Viruses** Human immunodeficiency virus (HIV) infection frequently is associated with varying degrees of cytopenia. The marrow is

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**TABLE 34-3. Drugs Associated with Aplastic Anemia**

<table>
<thead>
<tr>
<th>Category</th>
<th>High Risk</th>
<th>Intermediate Risk</th>
<th>Low Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesic</td>
<td>Phenacetin, aspirin, salicylamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiarrhythmic</td>
<td>Quinidine, tocainide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiarthritic</td>
<td>Gold salts</td>
<td></td>
<td>Colchicine</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Carbamazepine, hydantoins, felbamate</td>
<td></td>
<td>Ethosuximide, phenacemide, primidone, trimethadione, sodium valproate</td>
</tr>
<tr>
<td>Antihistamine</td>
<td></td>
<td></td>
<td>Chlorpheniramine, pyrilamine, triplexenamine</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td></td>
<td></td>
<td>Captopril, methydopride</td>
</tr>
<tr>
<td>Antiinflammatory</td>
<td>Penicillamine, phenylbutazone, oxyphenbutazone</td>
<td></td>
<td>Diclofenac, ibuprofen, indomethacin, naproxen, sulindac</td>
</tr>
<tr>
<td>Antimicrobial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibacterial</td>
<td>Chloramphenicol</td>
<td></td>
<td>Dapsone, methicillin, penicillin, streptomycin, β-lactam antibiotics</td>
</tr>
<tr>
<td>Antifungal</td>
<td></td>
<td></td>
<td>Amphotericin, flucytosine</td>
</tr>
<tr>
<td>Antiprotozoal</td>
<td></td>
<td></td>
<td>Quinacrine, mepacrine, pyrimethamine</td>
</tr>
<tr>
<td>Antineoplastic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylating agent</td>
<td>Busulfan, cyclophosphamide, melphalan, nitrogen mustard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antimetabolite</td>
<td>Fluorouracil, mercaptopurine, methotrexate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytotoxic antibiotic</td>
<td>Daunorubicin, doxorubicin, mitoxantrone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiprotein</td>
<td></td>
<td></td>
<td>Ticlopidine</td>
</tr>
<tr>
<td>Antithyroid</td>
<td></td>
<td></td>
<td>Carbinazole, methimazole, methylthiouracil, potassium perchlorate, propylthiouracil, sodium thiocyanate</td>
</tr>
<tr>
<td>Sedative and tranquilizer</td>
<td></td>
<td></td>
<td>Chloroquine, chlorpromazine (and other phenothiazines), lithium, meprobamate, methyprylon</td>
</tr>
<tr>
<td>Sulfa derivative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibacterial</td>
<td>Sulfonamides</td>
<td></td>
<td>Numerous sulfonamides</td>
</tr>
<tr>
<td>Diuretic</td>
<td>Acetazolamide</td>
<td></td>
<td>Chlorothiazide, furosemide</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>Allopurinol, interferon, pentoxifylline, penicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Drugs that invariably cause marrow aplasia with high doses are termed high risk; drugs with 30 or more reported cases are listed as moderate risk; others are less often associated with aplastic anemia (low risk).

**SOURCE:** This list was compiled from the AMA Registry, \(^{41}\) publications of the International Agranulocytosis and Aplastic Anemia Study, \(^{42–46}\) other reviews and studies, \(^{24,47–50}\) previous compilations of offending agents, \(^{51}\) and selected reports. An additional comprehensive source for potentially offending drugs can be found in *The Drug Etiology of Agranulocytosis and Aplastic Anemia*, Oxford, UK: Oxford University Press, 1991.
often cellular, but occasional cases of aplastic anemia have been noted. In these patients, marrow hypoplasia may result from viral suppression and from the drugs used to control viral replication in this disorder. Human herpes virus (HHV)-6 has caused severe marrow aplasia subsequent to marrow transplantation for other disorders.

Autoimmune Diseases

The incidence of severe aplastic anemia was sevenfold greater than expected in patients with rheumatoid arthritis. It is uncertain whether the aplastic anemia is related directly to rheumatoid arthritis or to the various drugs used to treat the condition (gold salts, d-penicillamine, and nonsteroidal antiinflammatory agents). Occasional cases of aplastic anemia are seen in conjunction with systemic lupus erythematosus. In vitro studies found either the presence of an antibody or suppressor cell directed against hematopoietic progenitor cells. Patients have recovered after plasmapheresis, glucocorticoids, or cyclophosphamide therapy, which is compatible with an immune etiology.

Eosinophilic fasciitis, an uncommon connective tissue disorder with painful swelling and induration of the skin and subcutaneous tissue, has been associated with aplastic anemia. Although it may be antibody-mediated in some cases, it has been largely unresponsive to therapy. Nevertheless, (1) stem cell transplantation, (2) immunosuppressive therapy using cyclosporine, (3) immunosuppressive therapy using antithymocyte globulin (ATG), or (4) immunosuppressive therapy with ATG and cyclosporine cures or significantly ameliorates the disease in a few patients.

Severe aplastic anemia also has been reported coincident with immune thyroid disease (Graves disease) and the aplasia has been reversed with treatment of the hyperthyroidism. Aplastic anemia has occurred in association with thymoma. Autoimmune renal disease and aplastic anemia have occurred concurrently. The underlying relationship may be the role of cytotoxic T lymphocytes in the pathogenesis of several autoimmune diseases and in aplastic anemia.

Pregnancy

There are a number of reports of pregnancy-associated aplastic anemia, but the relationship between the two conditions is not always clear. In some patients, preexisting aplastic anemia is exacerbated with pregnancy, only to improve following termination of the pregnancy. In other cases, the aplasia develops during pregnancy with recurrences during subsequent pregnancies. Termination of pregnancy or delivery may improve the marrow function, but the disease may progress to a fatal outcome even after delivery. Therapy may 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. In this latter study of 36 pregnancies, 22 were uncomplicated, 7 were complicated by a relapse of the marrow aplasia, and 5 without marrow aplasia required red cell transfusion during delivery. One death occurred from cerebral thrombosis in a patient with paroxysmal nocturnal hemoglobinuria (PNH) and marrow aplasia.

Iatrogenic Causes

Although marrow toxicity from cytotoxic chemotherapy or radiation produces direct damage to stem cells and more mature cells, resulting 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. 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. Chronic radiation poisoning with osteitis of the jaw, osteogenic sarcoma, and aplastic anemia was seen in workers who painted watch dials with luminous paint when they moistened the brushes orally.

Acute exposures to large doses of radiation are associated with the development of marrow aplasia and a gastrointestinal syndrome. Total body exposure to between 1 and 2.5 Gy leads to gastrointestinal symptoms and depression of leukocyte counts, but most patients recover. A dose of 4.5 Gy leads to death in half the individuals (LD50) owing to marrow failure. Higher doses in the range of 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 disaster that occurred at the Chernobyl nuclear power station in the Ukraine in 1986.

Antineoplastic drugs such as alkylating agents, antimetabolites, and certain cytotoxic antibiotics have the potential for producing marrow aplasia. In general, this is transient, is an extension of their pharmacologic action, and resolves within several weeks of completing chemotherapy. Although unusual, severe marrow aplasia can follow use of the alkylating agent, busulfan, and may persist indefinitely. Patients may develop marrow aplasia 2 to 5 years after discontinuation of alkylating agent therapy. These cases often evolve into hypoplastic myelodysplastic syndromes.

Stromal Microenvironment and Growth Factors

Short-term clonal assays for marrow stromal cells have shown variable defects in stromal cell function. Serum levels of stem cell factor (SCF) have been either moderately low or normal in several studies of aplastic anemia. Although SCF augments the growth of hematopoietic colonies from aplastic anemia patient's marrow, its use in patients has not led to clinical remissions. Another early acting growth factor, FLT-3 ligand, is 30- to 100-fold elevated in the serum of patients with aplastic anemia. Fibroblasts grown from patients with severe aplastic anemia have subnormal cytokine production. However, serum levels of granulocyte colony-stimulating factor, erythropoietin, and thrombopoietin (TPO) are usually high. Synthesis of IL-1, an early stimulator of hematopoiesis, is decreased in mononuclear cells from patients with aplastic anemia. Studies of the microenvironment have shown relatively normal stromal cell proliferation and growth factor production. These findings, coupled with the limited response of patients with aplastic anemia to growth factors, suggest that 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.

A rare exception is the homozygous or mixed heterozygous mutation of the TPO receptor gene, MPL, which can cause amegakaryocytic thrombocytopenia that evolves, later, into aplastic anemia (see Chap. 119).

### CLINICAL FEATURES

The onset of symptoms of aplastic anemia may be gradual with pallor, weakness, dyspnea, and fatigue as a result of the anemia. 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, it may be more dramatic with fever, chills, and pharyngitis or other sites of infection resulting from neutropenia and monocytopenia. Physical examination generally is unrevealing except for evidence of anemia (e.g., conjunctival and cutaneous pallor, resting tachycardia) or cutaneous bleeding (e.g., ecchymoses and petechiae), gingival bleeding and intraoral purpura. Lymphadenopathy and
splenomegaly are not features of aplastic anemia; such findings suggest an alternative diagnosis such as a clonal myeloid or lymphoid disease.

**LABORATORY FEATURES**

**Blood Findings**

Patients with aplastic anemia have varying degrees of pancytopenia. Anemia is associated with a low reticulocyte index. The relative reticulocyte count is usually less than 1 percent and may be zero despite the high levels of erythropoietin. Absolute reticulocyte counts are usually fewer than 40,000/μL (40 × 10⁹/L). Macrocyes may be present. The absolute neutrophil and monocyte count are low. An absolute neutrophil count fewer than 500/μL (0.5 × 10⁹/L) along with a platelet count fewer than 30,000/μL (30 × 10⁹/L) is indicative of severe disease and a neutrophil count below 200/μL (0.2 × 10⁹/L) denotes very severe disease (see Table 34–1). Lymphocyte production is thought to be normal, but patients may have mild lymphopenia. Platelets function normally. Significant qualitative changes of red cell, leukocyte, or platelet morphology are not features of classical 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 will fail shortly thereafter (days to weeks) and permit a definitive diagnosis. Table 34–4 is a plan for initial laboratory investigation.

**Plasma Findings**

The plasma contains high levels of hematopoietic growth factors, including erythropoietin, thrombopoietin, and myeloid colony-stimulating factors. Plasma iron values are usually high, and ⁵⁹Fe clearance is prolonged, with decreased incorporation into red cells.

**Marrow Findings**

**Morphology** The 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 present. 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 it is not unusual to see mild macronormoblastic erythropoiesis, presumably as a result of the high levels of erythropoietin. Marrow biopsy is essential to confirm the overall hypocellularity (Fig. 34–2), as a poor yield of spicules and cells occurs in marrow aspirates in other disorders, especially if fibrosis is present.

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

**Progenitor Cell Growth** In vitro CFU-GM and BFU–E colony assays reveal a marked reduction in progenitor cells.

**Cytogenetic Studies** Cytogenetic analysis may be difficult to perform owing to low cellularity; thus, multiple aspirates may be required to
provide sufficient cells for study. The results are normal in aplastic anemia. Clonal cytogenetic abnormalities in otherwise apparent aplastic anemia is indicative of an underlying hypocellular clonal myeloid disease.127

**Imaging Studies** Magnetic resonance imaging can be used to distinguish between marrow fat and hematopoietic cells.128 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.128

### Differential Diagnosis

Any disease that can present with pancytopenia may mimic aplastic anemia if only the blood counts are considered. Measurement of the reticulocyte count and an examination of the blood film and marrow biopsy are essential early steps to arrive at a diagnosis. A reticulocyte percentage of 0.5 percent to zero is strongly indicative of aplastic erythrophagocytosis, and when 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 approximately 5 to 10 percent of patients with myelodysplastic syndromes who present with a hypoplastic rather than a hypercellular marrow. Myelodysplasia should be considered if there is abnormal blood film morphology consistent with myelodysplasia (e.g., poikilocytosis, basophilic stippling, neutrophils with the pseudo-Pelger-Huet anomaly). Marrow erythroid precursors in myelodysplasia may have dysmorphic features. Pathologic sideroblasts are inconsistent with aplastic anemia and a frequent feature of myelodysplasia. Granulocyte precursors may have reduced or abnormally granulated. Megakaryocytes may have abnormal nuclear lobulation (e.g., unilobular micromegakaryocytes; see Chap. 88). If clonal cytogenetic abnormalities are found, a clonal myeloid disorder, especially myelodysplastic syndrome or hypocellular myelogenous leukemia is likely. Magnetic resonance imaging (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.

A hypocellular marrow frequently is associated with PNH. PNH is characterized by an acquired mutation in the **PIG-A** gene that encodes an enzyme that is required to synthesize mannolipids. The latter deficiency prevents the synthesis of the glycosyl-phosphatidylinositol anchor precursor. This moieties anchors several proteins, including inhibitors of the complement pathway to blood cell membranes, and its absence accounts for the complement-mediated hemolysis in PNH. As many as 50 percent of patients with otherwise typical aplastic anemia may have a PNH cell population as large as 5 to 10 percent of patients with myelodysplastic syndromes who present with a hypoplastic rather than a hypercellular marrow. Myelodysplasia should be considered if there is abnormal blood film morphology consistent with myelodysplasia (e.g., poikilocytosis, basophilic stippling, neutrophils with the pseudo-Pelger-Huet anomaly). Marrow erythroid precursors in myelodysplasia may have dysmorphic features. Pathologic sideroblasts are inconsistent with aplastic anemia and a frequent feature of myelodysplasia. Granulocyte precursors may have reduced or abnormally granulated. Megakaryocytes may have abnormal nuclear lobulation (e.g., unilobular micromegakaryocytes; see Chap. 88). If clonal cytogenetic abnormalities are found, a clonal myeloid disorder, especially myelodysplastic syndrome or hypocellular myelogenous leukemia is likely. Magnetic resonance imaging (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.

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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, and any residual hematopoiesis is presumably 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, especially if immunotherapy is used, whereas hematopoietic stem cell transplantation may either ablate threatening minor clones or establish more robust hematopoiesis, an environment less conducive to clonal evolution.

### TREATMENT

#### Approach to Therapy

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 34–5). More specific treatment of the marrow aplasia involves two principal options: (1) syngeneic or allogeneic hematopoietic stem cell transplantation or (2) combination immunosuppressive therapy with ATG and cyclosporine. The selection of the specific mode of treatment depends on several factors, including the patient's age and condition and the availability of a suitable allele-level HLA-matched hematopoietic stem cell donor. In general, transplantation is the preferred treatment for children and most otherwise healthy younger adults. Early histocompatibility testing of siblings is of particular importance because it establishes whether there is an optimal donor available to the patient for transplantation. The preferred stem cell source is a histocompatible sibling matched at the HLA-A, B, C, and DR loci.

#### Supportive Care

##### The Use of Blood Products

Although it was recommended that red cell and platelet transfusions be used sparingly in potential transplant recipients to minimize sensitization to histocompatibility antigens, this has become less important since ATG and cyclophosphamide have been used as the preparative regimen for transplantation in aplastic anemia, as their use has markedly reduced the problem of graft rejection.140

Cytomegalovirus (CMV)-reduced risk red cells and platelets should be given to a potential transplant recipient to minimize problems with CMV infections after transplantation. Once a patient is shown to be CMV-positive, this restriction is no longer necessary. Leukocyte-depletion filters or CMV serotesting are equivalent methods of decreasing the risk of transmitting CMV.

**Red Cell Transfusion** Packed red cells to alleviate symptoms of anemia usually are indicated at hemoglobin values below 8 g/dL (80 g/L), 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 and radiated to reduce the potential for a graft-versus-host reaction. It is important not to transfuse patients with red cells (or platelets) from family members if transplantation within the family is remotely possible, as this approach may sensitize patients to minor histocompatibility antigens, increasing the risk of graft rejection after marrow transplantation. Following a marrow transplant, or in those 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, over the long-term transfusion-induced iron overload may occur. This is not a major problem in patients who respond to transplantation or immunosuppressive therapy, but it is an issue in nonresponders who require continued transfusion support. In the latter case, consideration should be given to iron chelation therapy. Newer oral agents will make this procedure easier to effect (see Chap. 47).141

**Platelet Transfusion** It is important to assess the risk of bleeding in each patient. Most patients tolerate platelet counts of 10,000/μL (10 × 10^9/L) without undue bruising or bleeding, unless a systemic infection is present.142,143 A traumatic injury or surgery requires transfusion to >50,000/μL or >100,000/μL, respectively. 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.144 Pooled random-donor platelets may be used until sensitization ensues, although it is preferable to use single-donor platelets from the onset to minimize sensitization to HLA or platelet antigens. Subsequently, single-donor apheresis products or HLA-matched platelets may be required.

Platelet refractoriness is a major problem with long-term transfusion support.145 This may occur transiently, with fever or infection, or as a chronic problem secondary to HLA sensitization. In the past, this 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 percent of patients receiving chronic transfusions.145,146 Patients should also get ABO-identical platelets because this enhances platelet survival and further decreases refractoriness to platelet transfusion. Single-donor HLA-matched apheresis-harvested platelets may be necessary in previously pregnant or transfused patients who are already allosensitized or who become so after treatment with leukoreduced platelets. The frequency of either of these events is less than 10 percent. Approaches to chronic platelet transfusion are discussed in Chap. 141. The utility of TPO receptor agonists in the thrombocytopenia of aplastic anemia is yet to be determined.

**Management of Neutropenia** Neutropenic precautions should be applied to hospitalized patients with a severe depression of the neutrophil count. The level of neutrophils requiring precautions is fewer than 500/μL (0.5 × 10^9/L). One approach is to use private rooms, with requirements for face masks and handwashing with antiseptic soap. Unwashed fresh
fruits and vegetables should be avoided as they are sources of bacterial contamination. It is uncommon for patients with aplastic anemia to present with a significant infection. When patients with aplastic anemia become febrile, cultures should be obtained from the throat, sputum (if any), blood, urine, stool, and any suspicious lesions. Broad-spectrum bacteriocidal antibiotics should be initiated promptly, without awaiting culture results. 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 (notably methicillin- and oxacillin-resistant strains), Staphylococcus 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. 22).

In the past, leukocyte transfusions were used on a daily basis to reduce the short-term mortality from infections. It was unusual to detect more than 100 to 200 neutrophils per microliter for more than a few hours after transfusion. The yield of neutrophils can be increased by administering granulocyte colony-stimulating factor (G-CSF) to the donor, but most physicians avoid using white cell products because present-day antibiotics are usually sufficient to treat a patient for 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 if blood cultures remain positive in spite of antibiotic treatment. Leukocyte transfusion is more effective in children and adults with smaller body size, as transfused leukocytes have a smaller distribution space, which results in higher blood and tissue concentrations.

Hematopoietic Stem Cell Transplantation
Prompt therapy usually is indicated for patients with severe aplastic anemia. The major curative approach is hematopoietic stem cell transplantation from a histocompatible sibling. This treatment modality is described in Chap. 21. Only 20 to 30 percent of patients in the United States have compatible sibling donors (related to average family size). In the unusual case of an identical twin donor, conditioning is required to obliterate the immune disease in the recipient, but it can be limited to cyclophosphamide. In this setting, an 80 to 90 percent survival is expected. Marrow stem cells seem to perform better than blood stem cells when used as a source for patients with aplastic anemia, although this is under continued study. The results of transplantation are best in patients younger than age 20 years (80 to 90 percent long-term survival) but decrease every decade of age thereafter. Posttransplant mortality is increased and survival decreased with increasing age (Fig. 34–3). In patients older than age 40 years, survival in matched sibling transplant is reduced to approximately 50 percent. There are still uncertainties about the optimal conditioning program in younger and older patients. ATG, cyclophosphamide, total-body radiation, and fludarabine are among the agents being studied. The longer the delay between diagnosis and transplant, the less salutary the outcome, probably as a result of a greater number of transusions and a higher likelihood of pretransplant infection. Acute and chronic graft-versus-host disease are serious complications, and therapy to prevent or ameliorate them is a standard part of posttransplant treatment. Transplants have been performed using stem cells from partially matched siblings or unrelated, histocompatible donors recruited through the National Marrow Donor Program or similar organizations in other countries. Umbilical cord blood is an alternative source of stem cells from unrelated donors (or, rarely, siblings) for transplantation in children. The use of high-resolution, HLA typing of a matched, unrelated donor markedly improves the prognosis for transplantation. High-resolution DNA matching at HLA-A, -B, -C, and -DRB1 (8 of 8 allele) is considered the lowest level of matching consistent with the highest level of survival. If there is an HLA mismatch at one or more loci, especially HLA-A or -DRB1, the outcome is compromised and immunosuppression with combined therapy may be preferred initially, depending on patient age, cytomegalovirus status, and disease severity. The use of hematopoietic stem cell transplantation can be considered for patients who do not respond or who no longer respond to immunotherapy. If the patient in question is a candidate for stem cell transplantation based on all relevant factors, transplantation could be considered at any age for a patient with a syngeneic donor; transplantation could be considered as a first-choice therapy up to age 50 years for a patient with an HLA allele-level matched unrelated donor; and transplantation could be considered a first-choice therapy if an allele-level HLA-matched unrelated donor is available for patients younger than age 20 years.

Components of Anti–T-Lymphocyte (Immunosuppressive) Therapy
Antilymphocyte Serum and Antithymocyte Globulin
ATG and ALG act principally by reducing cytotoxic T cells. This involves ATG-induced apoptosis through both FAS and TNF pathways. Cathespin B also plays a role in T-cell cytotoxicity at clinical concentrations of ATG, but may involve an independent apoptosis pathway. ATG and ALG also release hematopoietic growth factors from T cells. Horse and rabbit ATG are licensed in the United States. Skin tests against horse serum should be performed prior to administration. If positive, the patient may be desensitized. ATG therapy is given daily for 4 to 10 days with doses of 15 to 40 mg/kg. Fever and chills are common during the first day of treatment. Concomitant treatment with glucocorticoids, such as
methylprednisolone or dexamethasone lessens the reaction to ATG. Studies are under way to compare equine to rabbit ATG in the immunotherapy of aplastic anemia.

ATG treatment may accelerate platelet destruction, reduce the absolute neutrophil count, and cause a positive direct antiglobulin test. This effect may lead to an increase in transfusion requirements during the 4- to 10-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 17 after treatment. Approximately one-third of patients no longer require transfusion support after treatment with ATG alone.159–161

Of 358 patients responding to immunosuppressive therapy, principally ATG alone, 74 (21%) relapsed after a mean of 2.1 years. The actuarial incidence of relapse was 35 percent at 10 years.162 Similar results were observed when 227 patients were treated with immunosuppression, primarily ATG alone.163 The actuarial survival at 15 years was 38 percent following immunosuppression.162 However, a combination of immunosuppressive agents provides more effective therapy than ATG alone (see “Combination Immunotherapy” below).

Twenty-eight (22%) of 129 patients treated with ALG developed myelodysplasia, leukemia, paroxysmal nocturnal hemoglobinuria, or combined disorders.164 This tendency to relapse and to develop clonal hematologic disorders was reviewed by the European Cooperative Group for Bone Marrow Transplantation in 468 patients, most of whom received ATG.165 The risk of a hematologic complication increased continuously and reached 57 percent at 8 years after immunosuppressive therapy. A further survey found 42 (5%) malignancies in 860 patients treated with immunosuppression, whereas only 9 (1%) malignancies were seen in 748 patients who received marrow transplants.166

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 1984,167 several groups have used cyclosporine as either (1) primary treatment,168–171 (2) in patients refractory to ATG or glucocorticoids,169–174 (3) in combination with granulocyte colony-stimulating factors,175,176 or (4) in varying combinations with other modes of therapy.177 Cyclosporine is administered orally at 10 to 12 mg/kg per 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 below 2 mg/dL. Cyclosporine also may cause moderate hypertension, a variety of neurological 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 may range from achieving transfusion independence to complete remission. Approximately 25 percent of patients respond to this agent when used alone, but the response rate has ranged from 0 to 80 percent in various reports.177 Although immunosuppression with ALG or ATG has been used the longest and has a seemingly better response rate, there are certain advantages to cyclosporine. This 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.178 In this crossover study of newly diagnosed patients, survival of approximately 65 percent was observed 12 months after diagnosis.

Combination Immunotherapy Combination treatment of severe aplastic anemia usually includes, for example, ATG, 40 mg/kg per day, for 4 days; cyclosporine, 10 to 12 mg/kg per day, for 6 months and methylprednisolone, 1 mg/kg per day, for 2 weeks.179 The dose of cyclosporine is adjusted to maintain a trough level of 200–400 ng/mL. Prophylaxis for Pneumocystis carinii with daily trimethoprim-sulfamethoxazole or with monthly pentamidine inhalations should be considered for these patients as they receive immunosuppressive therapy. The addition of cyclosporine to the combination of ALG and glucocorticoids improves response rates to approximately 70 percent of patients (Table 34–6).180,181 G-CSF added to the combined immunosuppressive therapy does not increase response rate or survival.182 Response is usually 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.

<table>
<thead>
<tr>
<th>Year of Publication</th>
<th>Principal Drugs Used</th>
<th>No. Pts (Age range, yrs)</th>
<th>Significant Response No. (%)</th>
<th>Survival at 5/10 Years (%)</th>
<th>Relapse at 5 Years (Cum%)</th>
<th>Comments</th>
<th>Reference</th>
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<td>2008</td>
<td>ATG + CYA</td>
<td>77 (&lt;18)</td>
<td>57 (74)</td>
<td>85/80</td>
<td>25</td>
<td>8.5% evolved to clonal myeloid disease</td>
<td>180</td>
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<tr>
<td>2007</td>
<td>ATG + CYA</td>
<td>44 (NR)</td>
<td>31 (70)</td>
<td>NR/88</td>
<td>NR</td>
<td>All cases were associated with hepatitis</td>
<td>181</td>
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<tr>
<td>2007</td>
<td>ATG + CYA</td>
<td>47 (19–75)</td>
<td>31 (66)</td>
<td>80/NR</td>
<td>NR</td>
<td>No late clonal diseases at 5 years</td>
<td>182</td>
</tr>
<tr>
<td>2007</td>
<td>ATG + CYA + G-CSF</td>
<td>48 (19–74)</td>
<td>37 (77)</td>
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<td>NR</td>
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<td>182</td>
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<tr>
<td>2006</td>
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<td>No late clonal diseases at 10 years</td>
<td>183</td>
</tr>
<tr>
<td>2006</td>
<td>ATG + CYA + G-CSF + rhuEPO</td>
<td>30 (5–68)</td>
<td>22 (73)</td>
<td>80/75</td>
<td>NR</td>
<td>One patient developed clonal myeloid disease</td>
<td>183</td>
</tr>
</tbody>
</table>

ATG, antithymocyte globulin; Cum%, cumulative percent; CYA, cyclosporine A; G-CSF, granulocyte colony-stimulating factor; No. Pts, number of patients; NR, not reported; rhuEPO, recombinant human erythropoietin.

NOTE: In some cases, response, survival, and relapse percentages are very close approximations, read off the published graphs. Significant response combines complete and partial remissions, which usually means the platelet and red cell count are high enough to avoid transfusions and neutrophil count over a critical level. Some protocols used short periods of glucocorticoid treatment to ameliorate reactions to ATG. There is a significant frequency of relapses or progression to clonal myeloid disease after 5 to 10 years postimmunotherapy.
The 5-year survival after completion of combination immunosuppressive therapy may approximate that after stem cell transplantation.184 Forty-eight children treated between 1983 and 1992 had a 10-year survival of approximately 75 percent for marrow transplantation and approximately 75 percent for combined immunosuppressive therapy, although there were only half the number of severely affected patients in the immunosuppressive therapy group.185 Thus, immunosuppression may be preferable for patients who are older than 30 years of age and in those who may experience a delay in finding a suitable donor. Marrow transplants are, however, curative for aplastic anemia, whereas more frequent sequelae have been found after immunosuppressive therapy.186–188 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.24 Concurrent administration of cyclosporine with ATG may diminish the ATG effect so that in this program cyclosporine is introduced at a later time. The addition of new immunosuppressive agents, such as mycophenolate mofetil, rapamycin, or monoclonal antibodies, to the IL-2 receptor may be more effective in decreasing cytotoxic T cells, sparing the targeted hematopoietic stem cells.24

For the 30 to 40 percent of patients who relapse after immunotherapy, retreatment with ATG and cyclosporine is effective in 50 to 60 percent of them.189,190

**High-Dose Glucocorticoid Treatment** Marrow recovery can occur after very high doses of glucocorticoids.191,192 Methylprednisolone in the range of 500 to 1000 mg daily for 3 to 14 days has been successful, but the side effects, which include marked hyperglycemia and glycosuria, electrolyte disturbances, gastric irritation, psychosis, increased infections, and aseptic necrosis of the hips, can be severe. Glucocorticoids at lower doses commonly are used only as a component of combination therapy for aplastic anemia to ameliorate the toxic effects of ATG and in providing additional lymphocyte suppression.

**High-Dose Cyclophosphamide Therapy** High-dose cyclophosphamide has been used as a form of immunosuppression.193 Although it would seem 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.6 Ten patients received cyclophosphamide at 45 mg/kg per 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 remained in remission 11 years after treatment. High-dose cyclophosphamide treatment may spare hematopoietic stem cells, which have high levels of aldehyde dehydrogenase and are relatively resistant to cyclophosphamide.194,195 Thus, cyclophosphamide in this situation may be more immunosuppressive than myelotoxic. The most extensive trial of high-dose cyclophosphamide resulted in 65 percent of patients responding completely at 50 months.196 However, the role of this regimen as initial therapy is not clear because of early toxicity that may exceed that of the ATG-cyclosporine combination.197 The probability of a durable remission may be superior, but there are insufficient data (comparative clinical trials) to conclude whether high-dose cyclophosphamide provides better long-term results than ATG and cyclosporine. The latter approach is favored at this time.

**Rituximab** A case report of the successful use of the anti-CD20 humanized mouse antibody rituximab has provided preliminary evidence for its potential effectiveness in treating aplastic anemia.198 Clinical trials should examine its efficacy compared to standard immunotherapy (ATG and cyclosporine), in patients refractory to standard therapy, or as a third drug in an immunotherapy regimen. The role of B lymphocytes in the pathogenesis of aplastic anemia has not been defined.

**Androgens** Randomized trials have not shown efficacy when androgens were used as primary therapy for severe or moderately severe aplastic anemia.199,200 Androgens stimulate the production of erythropoietin, and their metabolites stimulate erythropoiesis when added to marrow cultures in vitro. High doses of androgens were beneficial in some patients with moderately severe aplasia.199 Series of patients were reported in which survival seemed improved as compared with historical controls, but this could have resulted from improved supportive care.196 Masculinization and other androgen side effects can be severe. Long-term survivors after androgen therapy have essentially the same progression to clonal hematologic disorders as patients treated with immunosuppressive agents.196 These agents have been replaced by immunosuppression or autologous hematopoietic stem cell transplantation.

**Cytokines** Despite their effectiveness in accelerating recovery from chemotherapy, these agents have been far less effective in achieving long-term benefits in patients with severe aplastic anemia. Daily treatment with G-CSF201,202 has improved marrow cellularity and increased neutrophil counts approximately 1.5- to 10-fold. Unfortunately, in nearly all patients, the blood counts return to baseline within several days of cessation of therapy. Although occasional patients show evidence of trilineage marrow recovery with long-term therapy, the vast majority do not respond. Therapy with myeloid growth factors is probably best reserved for episodes of severe infection or as a preventive measure prior to dental work or other procedures that would compromise mucosal barriers in patients who have not responded to stem cell transplant or immunotherapy. Prophylactic use of growth factors is not warranted. G-CSF in a dose of 5 μg/kg 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.202,203 These disappointing results with cytokines are not unexpected, as 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 individual factors that act on more mature progenitor cells.

**Splenectomy** Removal of the spleen does not increase hematopoiesis but may increase neutrophil and platelet counts two- to threefold and improve survival of transfused red cells or platelets in highly sensitized individuals.206 The surgical morbidity and mortality in patients with few platelets and white cells makes this a questionable therapeutic procedure. Because there are more successful methods of therapy that attack the fundamental problem, this approach would not be used today.

**Other Therapy** High doses of intravenous gamma globulin have been given to small numbers of patients with severe aplastic anemia.207,208 Because of its success in treating certain cases of antibody-mediated pure red cell aplasia. Some improvement was noted in 4 of 6 patients treated. Another treatment that is occasionally successful is lymphocytapheresis to deplete T cells.209,210

**Course and Prognosis**

At diagnosis, the prognosis is largely related to the absolute neutrophil and platelet count. The absolute neutrophil count is the most important prognostic feature, with a count of fewer than 500/μL (0.5 × 10^9/L) considered severe aplastic anemia and a count of fewer than 200/μL (0.2 ×
10^9/L) very severe aplastic anemia, the latter associated with a poor response to immunotherapy and usually a dire prognosis if early successful allogeneic transplant is not available. In the past, the prognosis appeared worse when the disease followed hepatitis.59,70 But more comprehensive results with immunosuppression196 or hematopoietic stem cell transplantation211 show an equivalent response to that seen with idiopathic or drug-induced cases.

Before marrow transplantation and immunosuppressive therapy, more than 25 percent of the patients with severe aplastic anemia died within 4 months of diagnosis; half succumbed within 1 year.12,211 Marrow transplantation is curative for approximately 80 to 90 percent of patients younger than 20 years of age, approximately 70 percent if between the ages of 20 and 40 years, and approximately 50 percent if older than age 40 years.151,214 Unfortunately, as many as 40 percent of transplant survivors suffer the deleterious consequences of chronic graft-versus-host disease.151 and the risk of subsequent cancer can be as high as 10 percent in older patients or after immunotherapy prior to hematopoietic stem cell transplantation.215 The best outcomes occur in those patients who have an allele-based HLA-matched sibling, have not been exposed to immunosuppressive therapy prior to transplantation, have not been exposed and sensitized to blood cell products, have had marrow rather than a blood suppressive therapy prior to transplantation, have not been exposed and...
Fanconi families with an onset of aplastic anemia without congenital somatic abnormalities were thought to have a different disorder termed Estren-Dameshek syndrome. However, these children, whose lymphocytes show sensitivity to diepoxybutane, are considered to have Fanconi anemia without skeletal abnormalities.

**Laboratory Features**

Blood counts and marrow cellularity are often normal until 5 to 10 years of age, when pancytopenia develops over an extended interval. Macrocytosis with anisocytosis and poikilocytosis may be present before any cytopenia occurs. Thrombocytopenia may precede the development of granulocytopenia and anemia. The marrow becomes hypocellular, and in vitro colony assays reveal a decrease in CFU-GM and BFU–E. 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 this condition. Cell-cycle progression is prolonged at the G2 to M transition, and the cells are more susceptible to oxygen toxicity when cultured in vitro. It is important to test the lymphocytes from pediatric patients with aplastic anemia for sensitivity to diepoxybutane, because therapy for Fanconi anemia differs from that used for acquired 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 34–7) is important because it confirms the diagnosis, identifies the genotype linked to BRCA2 that may predispose to a cancer (e.g., breast, ovary), and permits carrier detection.

**Differential Diagnosis**

The differential diagnosis of Fanconi anemia includes other causes of aplastic anemia, particularly those familial syndromes.

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**TABLE 34–7. Gene Mutations Found in Fanconi Anemia**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome Location</th>
<th>% of Patients</th>
<th>Inheritance</th>
<th>Protein Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCA</td>
<td>16q24.3</td>
<td>~65%</td>
<td>AR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCB</td>
<td>Xp22.31</td>
<td>rare</td>
<td>XLR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCC</td>
<td>9q22.3</td>
<td>~10%</td>
<td>AR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCD (BRCA2)</td>
<td>13q12.3</td>
<td>rare</td>
<td>AR</td>
<td>RAD51 Recruitment</td>
</tr>
<tr>
<td>FANCD2</td>
<td>3p25.3</td>
<td>rare</td>
<td>AR</td>
<td>Monoubiquitinated protein</td>
</tr>
<tr>
<td>FANCE</td>
<td>6p21.3</td>
<td>~10%</td>
<td>AR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCF</td>
<td>11p15</td>
<td>rare</td>
<td>AR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCG (KCC9)</td>
<td>9p13</td>
<td>~10%</td>
<td>AR</td>
<td>FA Core Complex</td>
</tr>
<tr>
<td>FANCI (KIAA1794)</td>
<td>15q25–26</td>
<td>rare</td>
<td>AR</td>
<td>Monoubiquitination of FANCD2</td>
</tr>
<tr>
<td>FANJ (BACH1/BRIPI)</td>
<td>17q22.3</td>
<td>rare</td>
<td>AR</td>
<td>5’ to 3’ DNA helicase/ATPase</td>
</tr>
<tr>
<td>FANL (PHF9/ POG)</td>
<td>2q16.1</td>
<td>rare</td>
<td>AR</td>
<td>FA Core Complex, E3 Ubiquitin ligase</td>
</tr>
<tr>
<td>FANCM (Hel)</td>
<td>14q21.3</td>
<td>rare</td>
<td>AR</td>
<td>FA Core Complex, ATPase/translocase, DNA helicase motifs</td>
</tr>
<tr>
<td>FANCN (PALB2)</td>
<td>16q12.1</td>
<td>rare</td>
<td>AR</td>
<td>Regulation of BRCA2 localization</td>
</tr>
</tbody>
</table>

*AR, autosomal recessive; ATPase, adenosine triphosphatase; XLR, X-linked recessive.

*There are more than 100 mutant FANCA alleles, approximately 40% of which are large intragenic deletions. This table was made using material from references 221–223.

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**FIGURE 34–4.** Representation of the “FA/BRCA pathway.” Following DNA damage when a replication fork encounters a DNA cross-link, ATR (ataxia telangiectasia and rad3-related protein) is activated. This leads to the activation of the FA pathway as well as cell-cycle checkpoint activation via the ATM (Ataxia Telangiectasia Mutated) protein. Activation of the FA pathway leads to the formation of the “FA core complex” (consisting of the FA proteins A, B, C, E, F, G, L, and M). This activated FA core complex leads to the monoubiquitination of FANCD2 (FANCD2-Ub) and FANCI (I-Ub). The I-Ub/FANCD2-Ub complex is then targeted to the chromatin containing the cross link where it interacts with BRCA2 and possibly other DNA repair proteins (e.g., RAD51, I, N) leading to the repair of the DNA damage. Proteins mutated in the different FA subtypes are shown in yellow. (Reproduced from reference 221 with permission of Elsevier.)
TABLE 34–8. Other Rare Syndromes Associated with Aplastic Anemia

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Findings</th>
<th>Inheritance</th>
<th>Mutated Gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia-pancytopenia (myelocerebellar disorder)</td>
<td>Cerebellar atrophy and ataxia; aplastic pancytopenia; ± monosomy 7; increased risk of AML</td>
<td>AD</td>
<td>Unknown</td>
<td>256–258</td>
</tr>
<tr>
<td>Congenital amegakaryocytic thrombocytopenia</td>
<td>Thrombocytopenia; absent or markedly decreased marrow megakaryocytes; hemorrhagic propensity; elevated thrombopoietin; propensity to progress to aplastic pancytopenia; propensity to evolve to clonal myeloid disease</td>
<td>AR (compound heterozygotes)</td>
<td>MPL</td>
<td>259, 260</td>
</tr>
<tr>
<td>DNA ligase IV deficiency</td>
<td>Pre- and postnatal growth delay; dysmorphic facies; aplastic pancytopenia</td>
<td>AR (compound heterozygotes)</td>
<td>LIG4</td>
<td>261–263</td>
</tr>
<tr>
<td>Dubowitz syndrome</td>
<td>Intrauterine and post-partum growth failure; short stature; microcephaly; mental retardation; distinct dysmorphic facies; aplastic pancytopenia; increased risk of AML and ALL</td>
<td>AR</td>
<td>Unknown</td>
<td>264, 265</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td>Microcephaly; dystrophic facies; short stature; immunodeficiency; radiation sensitivity; aplastic pancytopenia; predisposition to lymphoid malignancy</td>
<td>AR</td>
<td>NBS1</td>
<td>266, 267</td>
</tr>
<tr>
<td>Reticular dysgenesis (type of severe immunodeficiency syndrome)</td>
<td>Lymphopenia; anemia and neutropenia; corrected by hematopoietic stem cell transplantation</td>
<td>XLR</td>
<td>Unknown</td>
<td>268, 269</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td>Intrauterine and post-partum growth failure; microcephaly; characteristic dysmorphic facies (bird-headed profile); aplastic pancytopenia; ? increased risk of AML</td>
<td>AR</td>
<td>ATR (and RAD3-related gene); PCNT</td>
<td>270–273</td>
</tr>
<tr>
<td>WT syndrome</td>
<td>Radial/ulnar abnormalities; aplastic pancytopenia; increased risk of AML</td>
<td>AD</td>
<td>Unknown</td>
<td>274</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; AR, autosomal recessive; XLR, X-linked recessive.

**NOTE:** The listed clinical findings in each syndrome are not comprehensive. The designated clinical findings may not be present in all cases of the syndrome. Isolated cases of familial aplastic anemia with or without associated anomalies that are not consistent with Fanconi anemia or other defined syndromes have been reported.227

associated with skeletal anomalies and other dysmorphic features. Other familial types of aplastic anemia have been reported with or without associated anomalies. In those 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 are tabulated in Table 34–8.

**Therapy and Course**

Most patients with Fanconi anemia do not respond to ATG or cyclosporine but do improve with androgen preparations, often for as long as several years. Cytokines may provide some improvement in blood counts, but their effect may wane. Studies in a mouse model also suggest that cytokine effects may not be sustained.230 The cumulative median survival is about 20 years from progressive marrow failure, conversion to myelodysplastic syndrome, acute myelogenous leukemia (approximately 10 percent of patients), or the development of a variety of other cancers such as genitourinary system, digestive system (especially liver), head and neck.231 Multiple cancers in an individual patient also occur. Cancers may occur as late as the fifth decade of life and precede the diagnosis of Fanconi anemia in 25 percent of patients.232 The presence of a clonal cytogenetic abnormality or marrow morphology consistent with myelodysplasia markedly reduces the 5-year survival.232 Allogeneic hematopoietic stem cell transplantation is curative for the marrow manifestations of Fanconi anemia.232–235 A marked reduction in dosage of the marrow-conditioning regimen of cyclophosphamide and radiation is necessary owing to the undue sensitivity of the tissues to DNA-damaging exposures. The risk of cancer is so high that, where practical, surveillance should be used, for example, frequent pelvic exams in females, hepatic ultrasonography to detect adenomas, and careful oropharyngeal examinations. Therapy of cancer in patients with Fanconi anemia needs to consider the marked sensitivity of their cells to DNA cross-linking agents and radiotherapy. Normal cDNA has been transferred into cells from patients with restoration of resistance to DNA damaging agents.236,237 Difficulties in this approach include the paucity of stem cells in these patients, as well as the potential toxicity of the gene transfer methodology.

**DYSKERATOSIS CONGENITA**

**Definition**

This inherited disorder is characterized by cutaneous and mucous membrane abnormalities, progressive marrow insufficiency, and a predisposition to malignant transformation. It is much more common in males than in females, and occurs in about 1 per 1 million population.231,238

**Pathogenesis**

Dyskeratosis usually is inherited as a recessive X-chromosome–linked disorder although rare cases can have autosomal dominant or autosomal recessive inheritance (Table 34–9). The disease is a reflection of telomere complex dysfunction,239,240 and it results from defective telomerase activity resulting from mutations in the telomerase-related genes (Fig. 34–5).240 The telomerase complex maintains the length of telomeres, which are nucleotide tandem repeat structures residing at the termini of eukaryotic chromosomes (e.g., 5′-TTAGGG-3′). Telomerase restores the G-rich telomere repeats that are lost as a result of end-processing during
normal cell division. Combined with protein, located at the ends of chromosomes, they maintain chromosome integrity by preventing end-to-end chromosome fusion, preventing chromosome degradation, and preventing chromosome instability.

In dyskeratosis congenita, the telomeres are markedly shortened resulting in genomic instability and cell (including marrow cell) apoptosis. Rapidly proliferating cells are at highest risk for dysfunction. Mutations of the \( \text{DKC1} \) gene are responsible for the X-linked recessive form. \( \text{DKC1} \) encodes dyskerin, which is a conserved multifunctional protein component of the telomerase complex. Mutations of the \( \text{TERT}, \text{TERC}, \) and \( \text{TINF2} \) genes are the principal abnormalities in the autosomal dominant form. \( \text{TERC} \) is the RNA component of the telomerase reverse transcriptase that \( \text{TERT} \), the reverse transcriptase, uses to synthesize the 6-bp repeats on the 3' end of telomeric DNA. \( \text{TINF2} \) is a component of the shelterin complex. The latter permits the distinction of telomeres from sites of DNA damage, preventing their otherwise inappropriate processing. Recessive mutations in \( \text{NOP10} \), which encodes small ribonucleoproteins associated with the telomerase complex, have been described in a consanguineous family.\(^{241}\) Homozygous recessive mutations in the telomerase reverse transcriptase (\( \text{TERT} \)) produce a severe variant, referred to as the Høyeråal-Hreidarsson syndrome.\(^{275}\)

### Clinical Findings

The cutaneous findings usually appear after 5 years of age and include reticulated, tan to gray, hypopigmented and hypopigmented cutaneous macules; alopecia of scalp, eyelashes, and eyebrows; adermatoglyphia (loss of dermal ridges on fingers and toes); hyperkeratosis of palms and soles; mucosal leukoplaikia in 75 percent of patients; and dystrophic nails in more than 85 percent of patients.\(^{221,238,239}\) Other mucosal sites, such as conjunctiva, lacrimal duct, esophagus, urethra, vagina, and anus, can be involved, sometimes with stenosis and, for example, dysphagia or dysuria. Pulmonary vascular involvement occurs in a significant minority of affected children. Aplastic anemia usually develops in late childhood or early adulthood and is evident in the classical blood and marrow findings described under acquired aplastic anemia, above. Female carriers of X-linked dyskeratosis congenital may have slight abnormalities such as a dystrophic nail, a single area of hypopigmentation, or slight leukoplaikia.\(^{238}\)

### Diagnosis

The diagnosis results from the combination of phenotypic findings and blood cell deficiencies. Genetic analysis for telomerase complex gene mutations should be used to confirm the clinical conclusion. Shortened telomere length in leukocytes also can be assessed by flow cytometric fluorescence in situ hybridization studies.\(^{242}\)

### Management

Stem cell hematopoietic transplantation has had inconsistent results because of frequent and severe posttransplantation complications.\(^{243}\) Nonmyeloablative transplantation might improve results.\(^{244,245}\) Transplantation might improve the cytopenias but not the abnormalities of other organs or the frequency of secondary nonhematopoietic cancer.
Course and Prognosis

The incidence of squamous cell carcinoma of mucosal sites is increased and they often originate in the leukoplakia in the skin, gastrointestinal, or genitourinary tracts. These usually develop between the ages of 20 and 30 years. Mortality from neutropenic infection or thrombocytopenic hemorrhage occurs in about two-thirds of patients with aplastic anemia. Median survival is about 30 years.

**SHWACHMAN-DIAMOND SYNDROME**

**Definition**

An uncommon inherited disorder that is estimated to occur once in every 100,000 births, manifesting exocrine pancreatic insufficiency with secondary steatorrhea, blood cell deficiencies, and skeletal abnormalities. It was first described in 1964.

**Pathogenesis**

Shwachman-Diamond syndrome results from mutations in the SBDS gene on chromosome 7q11, which induces accelerated cellular apoptosis via the FAS pathway. The resulting hyperproliferation may account for the abnormal telomere shortening that has been documented in the leukocytes in this condition. The pathogenetic mechanism that (1) prevents development of pancreatic acinar cells, (2) results in abnormal bone morphogenesis, and (3) causes marrow impairment of blood cell production is not understood. SBDS knockdown in experimental animals affects expression of genes involved in brain, bone, and marrow development, and may be the result of the gene's role in RNA processing. The mutations also result in abnormalities in neutrophil motility and chemotaxis, but pus formation in vivo seems adequate.

**Clinical Findings**

Pancreatic insufficiency, steatorrhea, and neutropenia are present in most patients at the time of diagnosis. Pallor may reflect anemia and easy bruising; epistaxis or bleeding from other sites reflect thrombocytopenia. Neutropenia occurs in approximately 95 percent, anemia in approximately 50 percent, and thrombocytopenia in approximately 35 percent of patients. Thus, a substantial plurality of patients has bony involvement or tricytopenia with an hypoplastic marrow. Fetal hemoglobin levels are elevated in approximately 75 percent of the patients, perhaps secondary to erythroid hypoplasia. Genetic abnormalities involving chromosomes 7 and 20 have been described in marrow cells. Nutritional inadequacies related to intestinal malabsorption result in a failure to thrive. Short stature is characteristic. Skeletal abnormalities are present in most patients, notably osteopenia, but also syndactyly, supernumerary metatarsals, coxa vera deformity, and dental enamel defects and caries. Delayed puberty is common. The neutropenia and chemotactic abnormality may result in recurrent infections, including sinusitis, otitis, pneumonia, osteomyelitis, and others. Pancreatic cell lipase production improves with age, and as many as half the patients may have improvement in lipid absorption in the small bowel with time.

**Management**

Supportive care, particularly with supplemental pancreatic enzymes, to provide proper nutrition, and appropriate and prompt treatment of bacterial infections with antibiotics is important. Many agents, including G-CSF, glucocorticoids, pancreatic extract, vitamins, have been tried to improve the neutropenia with erratic results. Some agents have potential risks, such as G-CSF fostering clonal evolution and glucocorticoids fostering immunodeficiency. Severe hematopoietic dysfunction and cytopenias can be corrected with allogeneic hematopoietic stem cell transplantation.

**Course and Prognosis**

Death from overwhelming sepsis is common. These patients, especially males, have a significant risk of progression to a myelodyplastic syndrome or acute myelogenous leukemia. Survival is a function of the severity of the cytopenias. If the cytopenias are mild, survival is not uncommon into the fourth or fifth decade of life. If symptomatic pancytopenia, especially neutropenia, is present, median survival is about 20 to 30 years.

**OTHER INHERITED APLASTIC ANEMIAS**

Several other rare syndromes are associated with aplastic pancytopenia, and these are described in Table 34–8. Congenital amegakaryocytic thrombocytopenia results from mutations in the thrombopoietin receptor gene, MPL. Reticular dysgenesis results from a pluripotential stem cell defect as both lymphoid and myeloid progenitors are affected. The Seek syndrome results from mutations in the ATR gene, and marrow cells exhibit heightened sister chromatid exchange. The ataxia-telangiectasia mutated and rad3-related (ATR) kinase orchestrates cellular responses to DNA damage and replication stress. The genetic basis of marrow failure in these four syndromes that involve aplastic pancytopenia is not yet known, but may be related to defects in the ATR-dependent DNA damage-repair pathway. Most of these syndromes can be treated by marrow transplantation, but this step, if successful, does not correct somatic abnormalities, only the hematopoietic defect. The restoration of robust hematopoiesis by transplantation may decrease their propensity to undergo clonal evolution to a clonal myeloid or, in some cases, lymphoid disorder.

**REFERENCES**


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PART VI: The Erythrocyte


183. Br J Haematol


