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Idiopathic Aplastic Anemia

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Abstract Aplastic anemia, an uncommon hematological disease, is the paradigm of the human bone marrow failure syndromes. The pathophysiology is immune mediated in most cases, with activated type 1 cytotoxic T cells implicated. The molecular basis of the aberrant immune response and deficiencies in hematopoietic cells is now being defined genetically; examples are telomere repair gene mutations in the target cells and dysregulated T-cell activation pathways. Almost universally fatal just a few decades ago, aplastic anemia can now be cured or ameliorated by stem-cell transplantation or immunosuppressive drug therapy. Immunosuppression with antithymocyte globulins (ATGs) and cyclosporine is effective at restoring blood-cell production in the majority of patients, but relapse and especially evolution of clonal hematologic diseases remain problematic. Allogeneic stem-cell transplant from histocompatible sibling donors is curative in the great majority of young patients with severe aplastic anemia; the major challenges are extending the benefits of transplantation to patients who are older or who lack family donors.

Keywords Aplastic anemia · pancytopenia · $CD34^+$

Definition

The term "aplastic anemia" was introduced by Vaquez and Aubertin in the year 1904. The word "aplastic" is derived from the Greek "a" and "plasso" meaning "without form." "Anemia" is a potentially misleading term, as patients with aplastic anemia fail to form blood cells from all three lineages. The combination of peripheral cytopenias with a decreased or absent bone marrow precursor cells characterizes aplastic anemia. Although there are many known etiologies (Table 95.1), the cause of aplastic anemia is generally difficult to determine in an individual patient, and in the vast majority of cases, no causal etiology can not be found. At times, multiple risk factors can be uncovered in a given patient (Table 95.1).

Epidemiology

A large, prospective study conducted in Europe and Israel between 1980 and 1984 that required stringent case definition and pathologic confirmation reported an annual incidence of aplastic anemia of 2 new cases per 1 million population per year (1). Aplastic anemia occurs two- to three-fold higher in the Far East than in the West. This geographic variation likely stems from environmental rather than genetic risk factors, because the Japanese population in Hawaii manifests similar rates of aplastic anemia as other Americans (2). Studies have not been able to attribute the increased risk of aplastic anemia in the Far East to specific agents, such as chloramphenicol, widely used in Asia (3).

The incidence of acquired aplastic anemia varies bimodally with age, with one peak between ages 15 and 25 years and another peak at older than 60 years of age (4). Aplastic anemia occurs with equal frequency in both genders (1, 2).

Pathophysiology

An immune mechanism was implied decades ago from the recovery of hematopoiesis in patients who failed to engraft after stem-cell transplantation, when renewal of autologous blood-cell production was credited to the conditioning regimen. Also suggestive was that the majority of syngeneic transplantations in which bone marrow was

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TABLE 95.1. A classification of aplastic anemia.

Acquired aplastic anemia
Secondary aplastic anemia
Irradiation
Drugs and chemicals
Regular effects
Cytotoxic agents
Benzene
Idiosyncratic reactions
Chloramphenicol
Nonsteroidal anti-inflammatory drugs
Antiepileptics
Gold
Other drugs and chemicals
Viruses
Epstein–Barr virus (infectious mononucleosis)
Hepatitis virus (non-A, non-B, non-C, non-G hepatitis)
Parvovirus (transient aplastic crisis, some pure red cell aplasia)
Human immunodeficiency virus (acquired immunodeficiency syndrome)
Immune diseases
Eosinophilic fasciitis
Hypoimmunoglobulinemia
Thymoma and thymic carcinoma
Graft-versus-host disease in immunodeficiency
Paroxysmal nocturnal hemoglobinuria
Pregnancy
Idiopathic aplastic anemia
Inherited aplastic anemia
Fanconi's anemia
Dyskeratosis congenita
Shwachman–Diamond syndrome
Reticular dysgenesis
Amegakaryocytic thrombocytopenia
Familial aplastic anemias
Preleukemia (e.g., monosomy 7)
Nonhematologic syndromes (e.g., Down, Dubowitz, Seckel)

infused without conditioning failed (5). The responsiveness of aplastic anemia to immunosuppressive therapies remains the best evidence of an underlying immune pathophysiology: the majority of patients show hematologic improvement after only transient T-cell depletion by antithymocyte globulins (ATGs).

In early laboratory experiments, removal of lymphocytes from aplastic bone marrows improved colony numbers in tissue culture and their addition to normal marrow inhibited hematopoiesis in vitro (6). The effector cells were identified by immunophenotyping as activated cytotoxic T cells expressing Th1 cytokines, especially interferon-γ. In general, patients at presentation demonstrate oligoclonal expansions of a few subfamilies of those T cells, which diminish or disappear with successful therapy. Original clones re-emerge with relapse, sometimes accompanied by new clones, consistent with spreading of the immune response. Occasionally, a large clone persists in remission, perhaps evidence of T-cell tolerance (7). The impact of T-cell attack on marrow can be modeled in vitro. It has been shown that interferon-γ in increasing doses reduces numbers of human hematopoietic progenitors assayed by inducing apoptosis in CD34 target cells (8).

A number of hypothesis have been made for the unclear activation of T cells in aplastic anemia patients, most of whom are associated with alterations in nucleotide sequence (e.g., polymorphisms in cytokine genes like interferon-γ) or in gene regulation: SAP gene encodes a small modulator protein that inhibits interferon-γ production whose levels are markedly diminished in a majority of acquired aplastic anemia cases (9). Perforin gene which is an important cytolytic, T cell mediated, APCs is diminished and assumptually responsible in the uncontrolled expansion of cytotoxic T cells and CD34 destruction in few idiopathic aplastic patients.

The aforementioned process in which hematopoietic cells are immunely T-cell mediated and destroyed leads to marrow failure. The pallor of the marrow biopsy core or empty spicules of an aspirate, few or no CD34 cells on flow cytometry, and minimal numbers of colonies derived from committed progenitors in semisolid media all reflect the severe reduction in hematopoietic cells that defines the disease. The few hematopoietic cells that are seen in the marrow of aplastic patients experience cell destruction through apoptotic mechanisms. The same process was reproduced in normal CD34 cells exposed to interferon-γ (10).

One peculiar feature of white blood cells in aplastic anemia is telomeres length. Telomeres are short in one-third to one-half of patients (11). The first hypothesis that blamed telomere shortening on stem-cell exhaustion was dismissed by the discovery of mutations in genes that repair and protect telomeres. The working premise in these days is that those mutations are genetic risk factors in acquired aplastic anemia, probably because they confer a quantitatively reduced hematopoietic stem-cell compartment that may also be qualitatively inadequate to sustain immune-mediated damage.

Finally, to date, there is no satisfying mechanism to explain clonal escape evolving to other hematologic diseases that are characterized by proliferation of distinctive cell clones, as in paroxysmal nocturnal hemoglobinuria (PNH) or myelodysplasia (MDS).

Clinical Manifestations

The patient with aplastic anemia occasionally comes to medical attention because of the fatigue and even cardiopulmonary compromise associated with progressive anemia. However, more common presentations are recurrent infections due to profound neutropenia or mucosal hemorrhage due to thrombocytopenia. Increased menstrual flow is a common complaint in premenopausal women. Major hemorrhage from any organ can occur in aplastic anemia but is usually not seen until late in the course of the disease

and is generally associated with infections, or traumatic therapeutic procedures (e.g., intravenous line placements).

The infections in aplastic anemia patients are typically bacterial, including sepsis, pneumonia, and urinary tract infection. However, invasive fungal infection is a common cause of death, especially in subjects with prolonged and severe neutropenia.

The physical examination is generally unremarkable except for bruising and petechiae, as noted above. Hepatosplenomegaly and lymphadenopathy are usually absent.

Diagnosis

Signs and symptoms at presentation are illustrated in Table 95.2.

The possible presence of aplastic anemia is suggested by the complete blood count, which reveals pancytopenia along with absolute reticulocytopenia, suggestive of bone marrow failure. The red blood cells are usually normocytic but occasionally may be macrocytic. Examination of the peripheral blood smear shows that the remaining elements, while reduced, are morphologically normal.

Aspiration and biopsy of the bone marrow, along with cytogenetic analysis, are pathognomonic and usually provide sufficient information to establish the diagnosis: in most cases the marrow shows hypocellularity with a decrease in all elements, although significant residual cellularity is present in some patients because of lymphocytes.

In those few patients in whom there is a discordant relationship between cellularity and peripheral blood findings, cellularity often diminishes rapidly and a second evaluation will reveal the classic marrow picture. The marrow space is composed mostly of fat cells and marrow stroma. The residual hematopoietic cells are morphologically normal and there is no malignant infiltrates or fibrosis. Bone marrow cytogenetics is typically normal for patients initially presenting with aplastic anemia. In contrast, cytogenetic abnormalities are frequently found in myelodysplastic bone marrows and may be helpful in distinguishing aplastic anemia from hypoplastic MDS.

TABLE 95.2. Presenting symptoms of aplastic anemia.

Symptoms	Number of patients
Bleeding	41
Anemia	27
Bleeding and anemia	14
Bleeding and infection	6
Infection	5
Routine examination	8
Total	101

Adapted from Williams DM, Lynch RE, Cartwright GE. Drug induced aplastic anemia. *Semin Hematol* 1973; 10: 195.

The severity of aplastic anemia was classified (12) in an effort to make possible the comparison of diverse groups of patients and different therapeutic approaches. Diagnosis of severe aplastic anemia requires that the patient have a marrow biopsy showing <25% of normal cellularity or marrow showing <50% normal cellularity, in which fewer than 30% of the cells are hematopoietic and at least two of the following are satisfied: a granulocyte count <500/μl, a platelet count <20,000/μl, and an absolute reticulocyte count <40,000/μl. Very severe aplastic anemia is further defined by a granulocyte count <200/μl.

Treatment: Curative Treatment

Immunosuppression

For aplastic anemia that is severe, as defined above, definitive therapies are immunosuppression or stem-cell transplantation.

Immunosuppressive therapies are most widely used because of lack of histocompatible sibling donors, patient age, and the immediate cost of transplantation. The most acceptable immunosuppression regimen today is an ATG used in combination with cyclosporine (13). ATGs, which are produced by immunizing animals against human thymocytes, probably are immunomodulatory as well as lymphocytotoxic, perhaps by producing a state of tolerance by preferential depletion of activated T cells. Cyclosporine's selective effect on T-cell function is due to direct inhibition on the expression of nuclear regulatory proteins, resulting in reduced T-cell proliferation and activation. Although severe aplastic anemia can respond to cyclosporine alone, it is less effective than ATG alone or ATG plus cyclosporine.

Reported hematologic response rates vary, at least in part due to lack of consensus on parameters (transfusion independence, absolute or relative improvement in blood counts) and defined landmarks. Improvement in blood counts, so that the criteria for severity are no longer met, highly correlates with termination of transfusions, freedom from neutropenic infection, and better survival (14). By this standard, about 60% of patients are responders at 3 or 6 months after initiation of ATG treatment (15). Responders have much better survival prospects than do non-responders and the outcomes are related to patient age: 5-year survival of >90% of children has been reported in recent trials, compared with about 50% survival for adults older than 60 years in the collective European experience (16).

Relapse, defined as a requirement for additional immunosuppression, is not uncommon, occurring in 30–40% of responding patients. Reinstitution of cyclosporine usually reverses declining blood counts, and when required, a second round of ATG is usually effective. As much as it known today, relapse does not confer a poor prognosis.

Molecular analysis of T cell suggests that the major reason for relapse is incomplete eradication of pathogenic clones by ATG.

The addition of other immunosuppressive agents (mycophenolate mofetil, sirulimus) to the ATG–cyclosporine regimen has not shown any superiority in hematologic response, relapse, or evolution rates.

Hematopoietic Stem Cell Transplantation

Allogeneic transplant from a matched sibling donor cures the great majority of patients with high 5-year survival rates (17). Despite this, graft-versus-host disease (GVHD) remains a serious problem for older patients (40–45% $>$ 20 years of age), even with routine cyclosporine prophylaxis. Chronic GVHD rises the risk of death and often requiring years of immunosuppressive therapy (18). Even with resolution, chronic GVHD remains a risk factor for late complications such as growth and endocrine system effects, pulmonary disease, cataracts, neurological dysfunction, and secondary malignancy. Addition of ATG and more recently its substitution by alemtuzumab (monoclonal antibody against CD52, which is found on mature lymphocyts) (18) may reduce the frequency and severity of acute GVHD, a predictor of chronic GVHD.

As the outcome in aplastic patients who have failed a single round of ATG has been poor and the matched sibling donor available is only 20–30% of cases, alternative sources of hematopoietic stem cells have been sought. The outcomes of 318 alternative donor transplants performed from 1988 to 1998 recently have been summarized for the European registry (19): the rejection rate was 15%, the GVHD 2–4 grades, 48%, and 5-year survival was estimated at 39%. The mortality rate is about twice that observed in matched sibling transplants. On the contrary, retrospective analysis from the Japan Marrow Donor Program suggested that patients with the most favorable characteristics and conditioned with a minimal dose of radiation might anticipate survival comparable with matched sibling transplants (20). In current practice, unrelated transplant is offered for children who have failed a single course of immunosuppression and to adults who are refractory to multiple courses of ATG and alternative therapies such as androgens. Studies with longer follow-up of larger numbers of patients are crucial to establish the optimal conditioning regimen and to define which patients will benefit and especially how early unrelated transplantation should be performed.

Very few clinical trials have specifically addressed moderate disease in which the course and treatment are less clear. As for the course, some patients progress to severe disease, whereas others remain stable and may not require intervention. The two most acceptable modes of treatment options are immunosuppressive and androgen therapies.

Supportive Care

The initial management in the majority of aplastic anemia patients consists of blood transfusions, platelet concentrates, and treatment and prevention of infection. All blood products should be filtrated to reduce the risk of alloimunization and irradiated to prevent grafting of live donor lymphocyte.

Although it is generally accepted that prophylactic platelet transfusions can reduce the risk of hemorrhage, the guidelines for such treatments remain an area of controversy. Multicenter, randomized trials in newly diagnosed acute myelocytic leukemia (AML) patients found no significant difference in risk of major bleeding between patients randomized to receive prophylactic platelet transfusions at threshold platelet counts of 20,000/μl versus 10,000/μl. Use of the lower platelet threshold significantly reduced platelet use. Platelet transfusions at platelet counts below 5000–10,000/μl in stable outpatients with chronic severe aplastic anemia were feasible and safe in recent studies (21). In practice, the decision for platelet transfusion must be individualized and take into account the number of platelets, the personal tendency of the patient to bleed, and whether is the patient at increased risk of bleeding (e.g., fever, infection). Whereas severe granulocytopenia may last for years, the cellular immune functions of aplastic anemia patients remain intact. Neutropenia (and perhaps monocytopenia) increases the risk of bacterial infection in aplastic anemia. Because neutropenia precludes the development of an inflammatory response, signs and symptoms of infection can be deceptively minimal. Despite all of that, the use of prophylactic antibiotics has no demonstrated role in the otherwise well patient with aplastic anemia. In the context of fever and neutropenia, complete evaluation and cultures of all possible sites should generally be followed by the administration of broad-spectrum parenteral antibiotics until the fever abates and all cultures are negative. Deficiency of hemopoietic growth factors (such aserythropoietin) is not the cause of the bone-marrow failure in aplastic anaemia; concentrations of hemopoietic growth factors are very high in patients with the disorder, in a compensatory attempt to increase blood production. Hence, these factors should not be used routinely.

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