Aplastic Anemia

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NOTE FROM THE PUBLISHER:
This publication has been developed without involvement of or review by the American Board of Internal Medicine.
INTRODUCTION

Aplastic anemia (AA) is characterized by bone marrow hypocellularity in association with peripheral blood cytopenias. The incidence of AA is 1 to 3 cases per million persons per year in North America and Europe, a rate which is 2 to 3 times higher in Asia. Although patients can be affected at any age, there are 2 peaks in incidence in early childhood and young adulthood. These 2 peaks in incidence are explained by the heterogeneity of AA, which encompasses both acquired forms and the less common inherited bone marrow failure syndromes. Moreover, AA overlaps with other diseases, such as the myelodysplastic syndromes (MDS) or paroxysmal nocturnal hemoglobinuria (PNH). An understanding of these issues is important to correctly diagnose patients with AA and select an appropriate course of treatment. In this review, a case is provided to illustrate the clinically relevant aspects of AA. Specifically, the differential diagnosis and workup of a patient with pancytopenia will be discussed. In addition, the available options for first-line treatment and relapsed or refractory disease will be described.

EVALUATION OF A PATIENT PRESENTING WITH PANCYTOPENIA

CASE PRESENTATION

A 25-year-old man presents to the emergency department and reports several weeks of fatigue, exertional dyspnea, and easy bruising. The patient has no significant past medical history. He is afebrile, with a blood pressure of 120/80 mm Hg, a regular heart rate of 80 bpm, and 97% oxygen saturation on room air. Physical examination is remarkable for pallor of the conjunctivae, a systolic ejection murmur at the left upper sternal border, and several ecchymoses on his upper and lower extremities. A complete blood count shows a white blood cell (WBC) count of 1.4 × 10^3/µL (4.5–11.0 × 10^3/µL), a hemoglobin level of 7.0 g/dL (normal, 13.0–17.0 g/dL), and a platelet count of 18 × 10^3/µL (normal, 150–450 × 10^3/µL). The absolute neutrophil count is 400/µL (normal, 2000–6500/µL), and the differential shows no WBC forms. Other routine laboratory studies (including electrolytes, creatinine, and liver function tests) are unremarkable.

- What is the differential diagnosis for this patient?

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for a patient presenting with pancytopenia (Table 1) can be categorized in terms of bone marrow cellularity. Pancytopenia that occurs with a hypocellular marrow suggests AA, viral infection (notably HIV infection), or direct toxic injury to the marrow (eg, radiotherapy, chemotherapy). Pancytopenia that occurs with a hypercellular marrow suggests malignant bone marrow infiltration, which is frequently hematologic in origin (eg, leukemias, lymphomas, multiple myeloma, MDS). Solid tumors can also metastasize to the bone marrow; however, pancytopenia is an unusual presenting complaint as the primary tumor or other metastatic sites are often clinically apparent by that time. Also, some hematologic malignancies can present with a hypocellular marrow, most notably hypocellular MDS. Nonmalignant causes of pancytopenia with a hypercellular marrow include infections (tuberculosis or atypical mycobacterial infections, ehrlichiosis, legionellosis, overwhelming sepsis), connective tissue diseases (eg, systemic lupus erythematosus), nutritional deficiencies (vitamin B₁₂ or folate deficiency), sarcoidosis, Kikuchi-Fujimoto disease, and hypothyroidism.

The history, physical examination, and/or results of basic laboratory studies may aid in diagnosing a patient with pancytopenia. For example, AA is usually characterized by an absence of associated clinical findings beside those directly related to the pancytopenia. As splenomegaly is exceedingly rare in AA, its presence should strongly suggest an alternative diagnosis. Laboratory studies also may indicate that pancytopenia is caused by nutritional deficiency. If the patient has mild pancytopenia and is otherwise well, it may be possible to await the results of blood work before undertaking additional studies.

- Which additional diagnostic studies should this patient undergo?
Unless a nutritional deficiency is suspected and documented by laboratory studies, the patient will require a bone marrow biopsy to determine the cause of the pancytopenia. Even in cases where the diagnosis of pancytopenia is suspected on clinical grounds, there may be a coexisting disease, such as a patient presenting with fulminating sepsis (where the sepsis can be the consequence rather than the cause of pancytopenia) or an HIV-positive patient who may have an HIV-related infection or malignancy. In the case patient, there are no clinical findings that suggest the diagnosis and the cytopenias are severe; therefore, an urgent bone marrow biopsy is warranted. As the patient is afebrile and not septic appearing, there is no need for cultures or antibiotics at this time.

CASE CONTINUED

A hematology consultation is called. The patient undergoes bone marrow biopsy, which demonstrates a hypocellular marrow (10% cellularity) without evidence of abnormal cells or fibrosis. There is a mild degree of erythroid dysplasia. Flow cytometry and cyogenetic analysis are normal. Further laboratory testing reveals normal vitamin B₁₂ and folate levels, normal thyroid function, and negative test results for HIV, Epstein-Barr virus, and antinuclear antibodies.

- How do these findings help narrow the differential diagnosis?

Pancytopenia in the presence of a hypocellular marrow significantly narrows the differential diagnosis. Having excluded HIV infection and, in the absence of a history of recent exposure to chemotherapy or radiotherapy, the major remaining causes for this patient’s pancytopenia are AA and hypocellular MDS. Features more consistent with a diagnosis of MDS include: prominent dysplasia on marrow or peripheral blood examination (particularly in the myeloid and megakaryocyte lineages); presence of micromegakaryocytes in the blood (a rare but useful finding); circulating blasts or an increased number of marrow blasts (or CD34+ cells on immunohistochemical staining); abnormal localization of immature precursors in the marrow or erythroblast islands (although AA can have islands of hematopoiesis); presence of an increased proportion of ringed sideroblasts in the marrow; marrow fibrosis; cytogenetic abnormalities (especially abnormalities of chromosome 5 or 7, trisomy 8, 20q−, trisomy 21, or complex karyotypes); and a history of exposure to leukemogenic agents (including alkylating agents, topoisomerase II inhibitors, and radiation). However, AA can be associated with macrocytosis, prominent erythroid dysplasia, and cytogenetic abnormalities. Therefore, distinguishing between AA and hypocellular MDS may be challenging in some cases. In this patient, there are no features suggestive of MDS.

As mentioned previously, the diagnosis of AA requires evidence of a hypocellular bone marrow without

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Table 1. Important Causes of Pancytopenia

<table>
<thead>
<tr>
<th>Normocellular or hypocellular marrow</th>
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<tbody>
<tr>
<td>Aplastic anemia</td>
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<tr>
<td>Acquired</td>
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<td>Idiopathic</td>
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<td>Medication-induced</td>
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<td>Posthepatitis</td>
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<td>Pregnancy-related</td>
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<td>Paroxysmal nocturnal hemoglobinuria</td>
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<td>Eosinophilic fasciitis</td>
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<td>Congenital</td>
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<td>Fanconi’s anemia</td>
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<td>Dyskeratosis congenita</td>
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<td>Shwachman-Diamond syndrome</td>
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<td>Direct toxic injury</td>
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<td>Radiation</td>
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<td>Chemotherapy</td>
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<td>Infection</td>
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<td>HIV</td>
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<td>Virally mediated hemophagocytic syndrome</td>
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<td>Malignancy (rare)</td>
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<tr>
<td>Hypocellular myelodysplastic syndrome</td>
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<td>Aleukemic acute leukemia</td>
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<td>Hypercellular marrow</td>
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<td>Malignancy</td>
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<td>Acute leukemia</td>
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<td>Myelodysplastic syndrome</td>
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<td>Lymphoma</td>
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<td>Myeloma</td>
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<tr>
<td>Myelofibrosis</td>
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<td>hairy cell leukemia</td>
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<tr>
<td>Large granular lymphocyte leukemia (rare)</td>
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<tr>
<td>Solid organ tumor with marrow metastasis</td>
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<tr>
<td>Infection</td>
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<td>Overwhelming sepsis</td>
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<tr>
<td>Mycobacteria (tuberculosis or atypical mycobacterial infection)</td>
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<td>Ehrlichiosis</td>
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<tr>
<td>Connective tissue disease</td>
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<td>Systemic lupus erythematous</td>
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<td>Sjögren’s syndrome</td>
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<td>Felty’s syndrome</td>
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<tr>
<td>Nutritional</td>
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<td>Vitamin B₁₂ deficiency</td>
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<td>Folate deficiency</td>
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<tr>
<td>Miscellaneous</td>
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<tr>
<td>Sarcoidosis</td>
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<td>Kikuchi-Fujimoto disease</td>
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<tr>
<td>Hypothyroidism</td>
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<td>Hypersplenism</td>
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Table 2. Diagnostic and Staging Criteria for Aplastic Anemia

Aplastic anemia

Bone marrow hypocellularity without abnormal cells or fibrosis; and

Presence of 2 of the 3 following features:

- Hemoglobin < 10 g/dL
- Platelet count < 50 × 10^9/L
- Absolute neutrophil count < 1500/µL

Severe aplastic anemia

Bone marrow cellularity < 25% or < 50% with < 30% residual hematopoietic cells

Presence of 2 of the 3 following features:

- Absolute reticulocyte count < 40 × 10^9/µL or corrected reticulocyte count < 1%
- Platelet count < 20 × 10^9/µL
- Absolute neutrophil count < 500/µL

Very severe aplastic anemia

Bone marrow cellularity < 25% or < 50% with < 30% residual hematopoietic cells

Absolute neutrophil count < 200/µL

Presence of 1 of the 2 following features:

- Absolute reticulocyte count < 40 × 10^9/µL or corrected reticulocyte count < 1%
- Platelet count < 20 × 10^9/µL

Data from references 8–10.

*Some investigators use an absolute reticulocyte count < 20 × 10^9/µL.

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In this patient, the bone marrow findings and blood counts are consistent with the diagnosis of SAA.

- What additional information is needed from this patient, and what further tests should be performed?

AA is more of a clinicopathologic diagnosis than a pathophysiologic one. Several mechanisms can lead to the pathologic endpoint that characterizes this disease. Broadly speaking, acquired AA can occur because of direct exogenous damage to the hematopoietic stem cells or from an autoimmune attack against these cells. Con genital AA, in contrast, arises from inherited molecular defects that lead to hematopoietic stem cell loss. Congenital AA encompasses several marrow failure syndromes, including Fanconi’s anemia (FA), dyskeratosis congenita (DKC), and Shwachman-Diamond syndrome (SDS). Our understanding of the molecular basis for these disorders has increased greatly in recent years, and the underlying defects have been mapped to DNA damage repair mechanisms, telomerase regulation, and ribosomal function. As there is no autoimmune phenomenon, patients with congenital AA will not respond to immunosuppressive therapy (IST) and will require allogeneic hematopoietic stem cell transplantation (HSCT). Therefore, additional clinical information obtained from the patient and further testing should be focused on distinguishing between congenital and acquired AA.

Congenital Aplastic Anemia

Three types of clues from the history and physical examination may be used to determine whether newly diagnosed AA is congenital. First, the age of the patient is relevant. The older the age at presentation, the less likely it is that the AA is part of a congenital syndrome. In most cases, the diagnosis of an inherited marrow failure syndrome is made in childhood, based on the typical constellations of features. However, some patients do not develop aplasia until adulthood; moreover, up to one third of patients with FA (the most common cause of congenital AA) do not display obvious congenital anomalies. Therefore, some clinicians would advocate routine workup for FA in patients recently diagnosed with AA who are under age 40 years.

Second, there may be subtle phenotypic clues to an underlying congenital marrow failure syndrome. For example, short stature is not uncommon in inherited marrow failure syndromes. In the case of FA, other diagnostic clues include pigmentation abnormalities (most classically café-au-lait spots), hearing defects, macrocytosis, urogenital abnormalities, or solid tumors occurring at an unusually young age. In the case of DKC, most patients have nail malformations, a reticular rash, or oral leukoplakia. Other manifestations of DKC include epiphora, pulmonary fibrosis, osteoporosis, premature hair loss or
graying, hyperhidrosis, esophageal strictures, or extensive dental caries or loss. Finally, exocrine pancreatic insufficiency, liver abnormalities (which also occur in DKC), and skeletal dysplasia may point to SDS.

Third, a detailed family history is essential, as the suggestive phenotypic abnormalities may not be present in the patient but in a family member. For patients with clinical features that suggest a genetic cause of their AA, further testing is indicated. In patients with suspected FA, chromosomal breakage testing can be performed using mitomycin C or diepoxybutane. A minority of FA patients display somatic mosaicism, in which case diagnosis requires testing of cultured skin fibroblasts rather than hematopoietic cells. If there is a clinical suspicion that one of other inherited marrow failure syndromes is responsible for AA, sequencing of the relevant genes must be undertaken.

**Acquired Aplastic Anemia**

Acquired AA is usually idiopathic and is thought to have an autoimmune basis in many cases. However, there are some etiologic associations with acquired AA, such as drug exposure, pregnancy, hepatitis, eosinophilic fasciitis, and PNH. A large epidemiologic study from Thailand also implicated exposure to pesticide and animal fertilizer. Although many drugs have been implicated in AA, the strength of these associations varies. Some drugs and toxins (eg, chloramphenicol, gold, penicillamine, benzene, dipyrone) have a reasonably characterized relationship to AA, whereas others may represent coincidences. Patients must be specifically asked about their use of herbal remedies or over-the-counter medications, including imported medications that are sold in ethnic grocery stores. In patients with drug-related acquired AA, the lag time between exposure and clinical presentation is usually weeks to months.

Although many clinicians may consider assessing hepatitis A, B, and C serologies, the cause of posthepatitis AA has not been attributed to one of the characterized (A to E) hepatitis viruses and, in fact, may not be viral. In these patients, AA usually occurs in the recovery phase of the hepatitis; hence, clinical clues that may indicate the patient has posthepatitis AA include a recent history of jaundice or abnormal but improving liver enzymes. Regardless of whether AA is associated with pregnancy, drug exposure, or hepatitis, the treatment and prognosis are similar to idiopathic AA. In contrast, the detection of an unsuspected PNH clone may be therapeutically relevant; in recent studies, the finding of small PNH clones by flow cytometry seemed to denote a better chance of response of the aplasia to IST.

**Discussion**

In the case patient, further evaluation should include a detailed history that reviews the patient’s use of drugs and supplements as well as his family history for clues that may suggest congenital AA. The evaluation should also include a physical examination targeted to features suggestive of FA, DKC, or SDS. Flow cytometry studies can be undertaken for PNH, and testing for hepatitis serologies could also be considered. Given this patient’s age and the importance of recognizing an inherited marrow failure syndrome, chromosomal breakage testing for FA is also a reasonable choice.

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**TREATMENT**

**CASE CONTINUED**

Physical examination is negative for signs suggestive of congenital AA as well as hepatitis. The patient denies both medication and supplement use. Flow cytometry studies show the presence of a small PNH clone. Chromosomal breakage studies are normal. The patient has no siblings. Based on this information, a diagnosis of idiopathic acquired AA is made.

- What first-line treatment options are available for patients recently diagnosed with AA?

**FIRST-LINE TREATMENT**

Treatment options for patients with recently diagnosed AA depend on whether they have acquired or congenital AA. As noted earlier, patients with congenital AA should not be treated with IST, as there is no underlying autoimmune cause; these patients should undergo upfront allogeneic HSCT if a donor is available. In patients with acquired AA, there are 3 first-line treatment options: IST, allogeneic HSCT, and, potentially, high-dose cyclophosphamide (CYC).

**Immunosuppressive Therapy**

Clinical trials. The rationale behind using IST is that acquired AA appears to have an immunologic basis in many cases. Therefore, immunosuppression can alleviate the antihematopoietic attack and allow the return of normal marrow function. Agents that have proven efficacy in treating acquired AA target T-cell function, implying that the autoimmune attack in acquired AA is T-cell driven. The current standard of care for IST is the combination of antithymocyte globulin (ATG) and a calcineurin inhibitor (cyclosporine A [CsA] or tacrolimus).
The benefit of using ATG for the treatment of AA was first demonstrated in 1970 when ATG was used as a pretransplantation conditioning agent. Randomized controlled trials later confirmed that ATG was superior to supportive care or androgens. CsA is another agent that showed promising activity when used for treating patients with acquired AA. The recommendation for using a combination of ATG and CsA evolved following a randomized clinical trial conducted by the German Aplastic Anemia Study Group (GAASG) that showed improved failure-free survival when CsA was added to antilymphocyte globulin (ALG) and methylprednisolone. Although the overall survival benefit was not statistically significant, this trial still forms the basis for the current IST paradigm in AA. Patients in the combination CsA and ALG treatment arm in the GAASG trial had an outcome very similar to those treated with a similar regimen in a nonrandomized study conducted at the National Institutes of Health (NIH). In patients with SAA or vSAA, the response rate at 4 months was 65%; with median follow-up of over 11 years, this regimen was associated with a 58% actuarial survival. The benefit of combination CsA and ATG over CsA alone was also shown in another randomized trial conducted by the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anemia Working Party. This trial targeted patients without SAA or vSAA and documented an improved response rate and transfusion-free survival for patients treated with the combination regimen. Again, there was no significant difference in overall survival. As of yet, no other agent has proven to be superior, alone or in combination, to the combination of CsA (or tacrolimus) and ATG. Mycophenolate mofetil (MMF), another T-cell targeting agent, has been tried but did not show any benefit.

Treatment regimens: dosing and toxicities. ATG can be derived either from rabbits or horses. At present, there is no clear reason to favor one over the other. However, a Chinese randomized trial suggested the superiority of the horse. Other trials are ongoing to determine whether rabbit or horse ATG is superior.

ATG is administered in the inpatient setting, with high-dose corticosteroids (eg, methylprednisolone 1 mg/kg/day during the period of ATG infusion, with subsequent taper) to decrease the risk of allergic reaction. Almost all patients develop fever in response to ATG treatment, with a subset of patients also developing renal failure, rash, cardiovascular instability, or third-spacing. The regimen used at the NIH was equine ATG at 40 mg/kg/day for 4 days, with CsA administered orally at 12 mg/kg/day in divided doses (adjusted to maintain trough levels of 200–400 ng/mL). An alternative regimen used in Europe consists of ATG 15 mg/kg/day for 5 days, with CsA at 5 mg/kg/day (adjusted to maintain trough levels of 75–200 ng/mL). Importantly, early tapering of CsA is often associated with relapse of the aplasia. Therefore, CsA should not be tapered earlier than 6 months. It is also important to remember that many patients (15%–30%) will need indefinite therapy.

Outcomes. IST carries several significant adverse outcomes. First, the relapse rate is high: 45% and 35% of patients relapsed in the GAASG and NIH trials, respectively. More importantly, patients with AA treated with IST are at increased risk for developing clonal abnormalities, including PNH, MDS, or acute myeloid leukemia (AML). The latter 2 complications are the leading causes of death in patients who survive beyond 3 years after undergoing IST. In large series of patients treated with IST, the rate for developing PNH was between 15% and 20%, while the rate for developing MDS or AML was between 10% and 20%. It is unclear why acquired AA is associated with such a high rate of malignant evolution and what role IST plays in promoting this evolution. Again, this phenomenon emphasizes the difficulties in distinguishing AA from hypoplastic MDS at presentation. It is possible that some cases of AA evolving into MDS actually represent hypoplastic MDS evolving to overt disease. However, our current understanding of acquired AA and MDS is that they are overlapping but distinct diseases; the distinction between them has some therapeutic relevance, which will be discussed later. Patients treated with IST are also at increased risk for the development of avascular necrosis, although this outcome may be related to the dose of steroids administered concomitantly. Finally, there is an increased risk of secondary solid tumors, with a reported incidence of 2%.
response rate and lower relapse rate when growth colony-stimulating factor was added to the combination CsA and ATG regimen. At this time, it is difficult to make a firm recommendation for or against the use of growth factors; ongoing randomized trials should clarify the issue.

**Allogeneic Hematopoietic Stem Cell Transplantation**

The efficacy of HSCT in treating patients with AA was first shown in the 1960s. In 1976, a randomized trial demonstrated the survival benefit of HSCT over supportive care, which at the time consisted of supportive transfusions or androgen treatment. Since then, the prognosis of patients treated with HSCT has improved considerably.

Graft failure is an important concern in patients undergoing HSCT for AA. These patients experience graft failure more frequently than patients who undergo transplantation for any other indication. Graft failure occurs more frequently in AA because the conditioning regimens for AA are nonmyeloablative, and, more importantly, because of the antihematopoietic immune activity that is already present in the host at the time of transplantation. Antihematopoietic immune activity can cause graft rejection, just as it can cause hematopoiesis in the host. In the early experience with transplantation, the incidence of graft failure was as high as 30% in patients with AA, even when using matched sibling donors. Several factors have contributed to reducing this rate, which is currently between 5% and 10%.

The first factor is related to the choice of conditioning regimen. As graft failure is likely to be mediated by the residual host immune system, it stands to reason that intensifying the conditioning regimen would be beneficial. Initially, intensifying the conditioning regimens was accomplished by adding radiation, which reduced the incidence of graft failure in many series. In several other studies, there was no survival advantage to using radiation as a conditioning regimen, as its benefits in diminishing graft failure were offset by greater toxicity, such as pulmonary disease, acute graft-versus-host disease (GVHD), and secondary malignancies. The introduction of ATG resolved this issue. First employed in the reconditioning of patients who had rejected their grafts, ATG moved into the initial conditioning regimen. The combination of CYC and ATG led to excellent outcomes. In a recent study of 81 patients receiving a CYC/ATG-conditioned graft from matched-related donors (MRD), the rate of graft failure was 4%, moderate-to-severe acute GVHD was 24%, and chronic GVHD was 26%. With median follow-up of almost 10 years, the 15-year overall survival was 81% for children and 71% for adults. The superiority of CYC/ATG conditioning over CYC with radiation was confirmed by a French retrospective study of patients transplanted from matched siblings. In this study, use of radiation in combination with CYC was associated with higher GVHD rates and lower overall survival as compared with using CYC/ATG (55% versus 95%). In both series, patients conditioned with CYC/ATG had excellent long-term survival.

Therefore, CYC (eg, 200 mg/m² divided in 4 daily doses) in combination with ATG (eg, 90 mg/kg divided in 3 daily doses) is the current standard of care for MRD transplant conditioning.

The benefit of using ATG in the setting of HSCT concurs with the immune hypothesis of the pathogenesis of AA. The antihematopoietic activity in AA appears to be largely T-cell dependent, as evidenced by laboratory and clinical data. As mentioned earlier, the standard of care for IST is the combination of a calcineurin inhibitor (CsA or tacrolimus) and ATG, which are both T-cell targeting drugs. Thus, specifically targeting the T-cell population during transplant conditioning should maximize efficacy and minimize toxicity, as compared with the broadly myelotoxic effects of radiation. Unsurprisingly, the newest agents being studied in transplantation conditioning, fludarabine and alemtuzumab, are also T-cell targeting agents. Results with fludarabine-containing conditioning regimens are encouraging and consistent. The experience with alemtuzumab is more preliminary but also deserving of further study. Finally, it should be noted that patients with certain forms of congenital AA, particularly FA, are highly sensitive to alkylating agents and radiation and require dose adjustments.

The second important factor contributing to improved transplantation outcome is the choice of GVHD prophylaxis regimens. Initially, these regimens consisted of single-agent methotrexate (MTX). The benefit of adding CsA to MTX was first suggested by a 1982 retrospective study that compared patients undergoing MRD HSCT who had either received CsA for GVHD prophylaxis or MTX. Engraftment rates and overall survival were superior in the CsA-treated patients, although GVHD was actually more common in the CsA group. This study suggests that the benefit of CsA in AA transplantation may involve suppressing the host immune system and thereby preventing early graft rejection instead of improving GVHD control. Again, this emphasizes the importance of T-cell suppression in the treatment of AA. The benefit of CsA for survival was confirmed in other retrospective studies and in 2 randomized prospective trials that compared single-agent MTX with combination MTX and CsA. Currently, no
other regimen has shown clear superiority over the combination of calcineurin inhibitor (CsA or tacrolimus) and MTX. Other GVHD prophylaxis regimens that have been considered include T-cell depletion, which may be associated with an increased risk of graft failure and therefore should be avoided outside of a clinical trial. Whether GVHD prophylaxis regimens using combinations of other T-cell targeting agents can improve on the results with combination CsA and MTX remains to be determined. There are isolated reports that CsA and MMF prophylaxis is an acceptable regimen, but the experience is as of yet too limited to reliably compare this regimen to CsA and MTX.

Of note, most studies of HSCT for AA have used unmanipulated bone marrow as the source of the graft. In recent years, there has been an explosive increase in the use of peripheral blood stem cells (PBSC) in HSCT for other indications. PBSC transplantations have been performed in AA patients, and some successes have been reported. However, a retrospective EBMT study concluded that use of PBSC, despite providing faster engraftment, was associated with an increased incidence of chronic GVHD and a significantly lower 2-year survival compared with marrow grafts. At present, it appears to be safer to use bone marrow grafts.

**Potential First-Line Treatment**

An interesting phenomenon occurred in the early stem cell transplantation experience. Some patients who rejected their graft after high-dose CYC conditioning had a remission of their AA together with autologous bone marrow recovery, which concurs with the hypothesis that acquired AA is immune mediated. High-dose CYC may eliminate the antihematopoietic clone and allow marrow recovery. Based on this observation, high-dose CYC without stem cell rescue has been championed as an alternative to ATG-based IST. Single-institution results at Johns Hopkins were encouraging.

A small follow-up randomized trial conducted by the NIH demonstrated that patients receiving high-dose CYC experienced increased toxicity (in particular invasive fungal infections), prompting early closure of the trial. With admittedly incomplete accrual, there was no evidence of improved remission rates or of a decreased risk of clonal progression with longer follow-up. Therefore, the role of high-dose CYC as first-line therapy for acquired AA is still undefined. At present, most centers do not use high-dose CYC treatment.

**Transfusions**

It has been well established that transfusions of blood product prior to transplantation worsen the outcome, which most likely results from alloimmunization in patients with active antihematopoietic immunity. The detrimental effect of transfusion is partially responsible for the high rates of graft failure observed in the early days of transplantation. Since then, advances in transfusion practice (eg, use of leukopoor, irradiated red blood cells, use of single donor apheresis platelets) have attenuated this risk, which has decreased the antigenic exposure that accompanies transfusion and has likely decreased the negative impact of transfusion on outcomes. Despite these advances, even a single transfusion may have a negative effect on the eventual outcome of HSCT. Therefore, it is important to minimize exposure to blood products until the time of transplantation as well as avoid directed donation from family members to prevent the patient from becoming sensitized to antigens from a future marrow donor. For young patients, lower hemoglobin levels may be tolerable, especially as the anemia tends to develop slowly and hence be less symptomatic. For the case patient whose counts were low but not life threatening and who had tolerable symptoms, it was preferable not to transfuse at presentation. Broadly speaking, transfusions should be avoided whenever possible in patients presenting with pancytopenia of an unknown cause. Of course, this guideline should not be interpreted as a strict contraindication to transfusion, and clinical judgment must always be exercised to balance the risk of bleeding and cardiopulmonary compromise against the risks of transfusion.

- Which of these first-line treatment options should the case patient receive?

**Choice of First-Line Therapy**

When approaching a patient with newly diagnosed acquired SAA, the clinician must decide whether to pursue IST or HSCT. Several issues factor into this decision. The first is donor availability. Historically, the results of HSCT using MRD have been considerably better than those using alternative donors (including matched unrelated donors [MUD] and mismatched donors). Patient age is also an important prognostic factor. Other adverse prognostic factors that have consistently emerged from multivariate analyses are omission of CsA from the GVHD prophylaxis regimen, earlier year of transplantation, longer time from diagnosis to transplantation, and gender mismatching. For example, a young patient with a matched sibling may have a 10-year survival rate in excess of 80% after HSCT, whereas an older patient with a mismatched donor may have a survival rate closer to 30%.

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Moreover, HSCT is associated with toxicities that may, even when not fatal, significantly impair quality of life. Chief among these are acute and chronic GVHD, the latter of which affects 20% to 50% of long-term survivors. Transplant survivors are also at increased risk for growth abnormalities (in children), infertility, cataracts, hypothyroidism, and secondary solid tumors (with a 20-year risk around 2%, or higher in patients who have received radiation). The relative risks and benefits of IST and HSCT have been weighed in detailed analysis, most notably by the EBMT. Their recommendation is to reserve upfront transplantation for patients younger than age 40 years with an available matched sibling donor. Other patients, including older patients or those without a MRD, should receive IST as first-line therapy. Although more recent data on transplantation results may lead clinicians to consider slightly broader indications for transplantation, the EBMT recommendations continue to form the backbone of current clinical practice. The case patient is young but does not have a sibling donor; therefore, the first choice of treatment would be IST, using a combination of ATG and a calcineurin inhibitor. Moreover, this patient’s small PNH clone may indicate a better prognosis after IST.

**CASE CONTINUED**

The hematologist informs the patient of his treatment options, and he agrees to undergo IST with combination ATG and CsA. Four weeks after completing therapy, there is no appreciable improvement in absolute neutrophil count, platelet count, or hemoglobin levels.

- **How should this patient be managed at this point?**

  In the various studies that examined IST, responses tended to be slow, even when using the optimal medication regimen. In the GAASG trial, the median time to response was 61 days in the combination arm, and the response curves plateaued at around 4 months. Therefore, clinicians should wait 3 to 4 months before declaring IST a failure. However, it would be reasonable to begin an early search for a MUD for this patient. As discussed earlier, there is currently not enough evidence to support the use of recombinant growth factors in the treatment of AA.

**CASE CONTINUED**

The patient’s blood counts begin to improve 6 weeks following the first IST course. Over the course of 2 years, he continues to take CsA and does well. At his 2-year follow-up visit, routine laboratory work demonstrates that the counts have decreased: absolute neutrophil count, 400/µL; hemoglobin level, 9 g/dL; and platelet count, 80 × 10³/µL.

- **Is a repeat marrow biopsy warranted in this patient?**

  In this context, the worsening WBC counts most likely represent relapsed AA. However, the association of AA with clonal diseases, in particular MDS and AML, must always be considered. Therefore, a repeat marrow biopsy with cytogenetic analysis should be performed to exclude these complications prior to deciding on a course of action.

**CASE CONTINUED**

The patient undergoes repeat bone marrow biopsy. The results reveal aplasia without any increased dysplasia or blast infiltration.

- **How should the relapsed AA be treated in this patient?**

  **TREATMENT FOR RELAPSED/REFRACTORY DISEASE**

  There is a well-described response rate to retreatment with ATG after relapse. In a retrospective European study of 73 patients who either relapsed after or were refractory to ATG treatment, a second course of ATG (in combination with CsA in some patients) led to a response in 64% of patients. The response rate was similar for relapsing and primary refractory patients. After a median follow-up of over 9 years, the 10-year overall survival rate in retreated patients was 55%. High-dose CYC has also been used in the relapsed/refractory setting by the Johns Hopkins group. In a published series of 17 patients, the response rate (complete and partial) was 53%, and the 5-year overall survival rate was 52%, with a median follow-up of 29 months.

**CASE CONTINUED**

The patient undergoes another course of ATG, but his WBC counts fail to recover, even after several months.

- **How should this patient be treated after IST has not had any effect on relapsed AA?**

  The likelihood of responding to a third course of ATG after having failed the second course is low. At this point, allogeneic HSCT is the most likely cure for this patient. By this point, a search for a MUD should have been completed and possible donors identified. Historically, the outcomes of MUD HSCT have been poorer than those using matched siblings. Presumably, the higher rate of graft failure in MUD transplantation results from a greater antigen disparity at minor
histocompatibility loci. Graft failure affects the choice of conditioning regimen. ATG is useful in this context as well, as demonstrated by a retrospective Japanese study. In this study, survival was superior when ATG was used in the conditioning regimen, with a 5-year overall survival rate ranging from 61% to 75% in patients who were treated with ATG versus 24% to 53% in patient who did not receive ATG.67 In a multicenter prospective study of patients receiving grafts from alternative (nonmatched sibling) donors, Deeg and colleagues76 attempted to define the optimal total body irradiation (TBI) dose (in combination with CYC and ATG) in this setting. Graft failure rates remained acceptably low with a radiation dose as low as 200 cGy, and decreasing the TBI dose did not compromise survival. In fact, for MUD allografts, survival was highest in the 200 cGy cohort, although this did not achieve statistical significance. This conclusion is also supported by a Japanese study, which demonstrated excellent survival (90%) for the small group of patients who received 500 cGy or less of radiation in addition to CYC and ATG.67 There are also preliminary data supporting the use of fludarabine and possibly alemtuzumab for MUD transplantation.77,78 Interestingly, recent patient series using allele-level human leukocyte antigen (HLA) matching have reported improved transplantation outcomes of MUD transplants. Transplantation using molecularly MUD and modern conditioning regimens may yield overall survival rates between 60% and 80% in young patients.76,77,79 The benefit of allele-level HLA matching has recently been confirmed in a retrospective French study, in which young patients with fully HLA-matched unrelated donors had a 5-year survival rate of 78%.80 These improved survival rates could be due in part to allele-level HLA typing, as opposed to the serologic typing that was used in most older series. Because of the historically poor outcomes of HSCT with alternative donor transplants, most patients receiving MUD transplantation in clinical trials have previously failed at least 1 course (usually multiple courses) of IST. The literature on MRD transplants has repeatedly demonstrated that longer time to HSCT and prior immunosuppression are adverse risk factors.44,56,67,81,82 Therefore, the quoted survival rates after MUD HSCT could underestimate the true survival rate of young patients transplanted with allele-level MUD allografts as first-line therapy. Therefore, it would not be unreasonable to consider HSCT as first-line therapy for the case patient, although this approach would run counter to current EBMT recommendations.16 Regardless, the best choice for the patient after a repeat course of IST for relapsed AA has failed is allogeneic HSCT, assuming a suitable donor is available.

**CASE CONCLUSION**

A matched unrelated donor is found in the registry, and the patient undergoes allogeneic transplantation. He engrafts successfully, with full return of WBC counts. Three years posttransplantation, the patient is alive, free of disease, and has a normal performance status.

**CONCLUSION**

This case illustrates some of the difficulties facing the clinician when diagnosing and managing patients with AA. However, this case also emphasizes that, with the tremendous advances in therapy and supportive care that have occurred over the last 40 years, AA has become an eminently curable disease, with most patients being long-term survivors who can resume a normal life.

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**REFERENCES**


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