

# 36 Aplastic Anemia

**Abstract:** Aplastic anemia is an uncommon disorder characterized by pancytopenia. Its exact incidence in India is not known, but it is more commonly seen in Asia than in the West. Investigations should include a bone marrow aspirate and biopsy. In children and young adults, Fanconi's anemia should be excluded by chromosomal breakage studies using the patients' blood. In most cases no cause can be found. Supportive therapy is needed in most patients to correct anemia, prevent bleeding and treat any infections. Due to prolonged neutropenia, these patients are prone to fungal infections. The specific therapy varies with the age of the patient and availability of an HLA identical sibling. Allogeneic hematopoietic stem cell transplantation is curative in majority of young patients, if an HLA identical related donor is available. In those without a donor, or the elderly, the best treatment is with immunosuppressive therapy. A combination of anti-thymocyte globulin (ATG) and cyclosporine offers the best results with about 60% response. Unfortunately, the response is often incomplete, relapses are common and evolution into clonal disorders like PNH, myelodysplastic syndrome and leukemias can occur over a period of time. Androgens are much less effective, but are often the only form of therapy offered to patients due to cost constraints. There is no primary role of corticosteroids or hematopoietic growth factors, except as adjunctive therapy in special circumstances. New immunosuppressive therapies are being evaluated for refractory patients.

## INTRODUCTION

Aplastic anemia is an uncommon but potentially serious hematological disorder. It is characterized by pancytopenia secondary to a hypocellular bone marrow. Aplastic anemia accounts for 20-30% cases presenting with pancytopenia in referral centers.<sup>1</sup> The frequency of aplastic anemia seen in hospitals of Asian countries is higher than reported from the West, but the precise incidence of this disorder in India is not known.

## Diagnosis

The diagnosis of aplastic anemia is based on the following: presence of pancytopenia or bicytopenia, with a hypocellular bone marrow and no abnormal infiltrate and no increase in reticulin.<sup>2</sup>

## Clinical Features

The clinical findings of aplastic anemia relate to anemia, thrombocytopenia and neutropenia. Anemia results in pallor, easy fatigability, headache, dyspnea, and tachycardia. Thrombocytopenia manifests with petechiae, ecchymoses, epistaxis, gum bleeding and menorrhagia. The fundus examination may show hemorrhages, at times suggesting an impending intracranial bleed. Neutropenia leads to recurrent infections, oral and gingival ulcerations.

There is no lymphadenopathy or hepatosplenomegaly in aplastic anemia, unless this is due to some infection. Any such finding should indicate an alternative diagnosis.

In children and young adults, an active search should be made of clinical clues which may suggest a hereditary bone marrow failure syndrome like Fanconi's anemia. Features suggestive

of Fanconi's anemia are: hyper-pigmentation and hypopigmentation, Fanconi's facies, café au lait spots, thenar hypoplasia, growth failure, renal anomalies, ear anomaly/impaired hearing, cardiac anomaly, short stature, and microcephaly.

A preceding history of jaundice may suggest post-hepatic aplastic anemia.

A careful drug history should be taken. Although many drugs and chemicals have been implicated in the causation of aplastic anemia, there is no definite evidence of cause and effect.<sup>3</sup> Many drugs have been implicated on the basis of case reports, which may reflect observation and reporting biases. Nevertheless, if patient is taking any of the implicated drugs or is exposed to any chemicals, further exposure should be stopped. In about 85% cases, no cause is identified.

## Investigations

Investigations are done to:

- a. Confirm the diagnosis and determine its severity
- b. Exclude other causes of pancytopenia with a hypocellular marrow
- c. Exclude congenital causes of aplastic anemia
- d. Look for associated paroxysmal nocturnal hemoglobinuria (PNH)
- e. Look for etiology of aplastic anemia.

*Essential investigations:* Complete blood counts, reticulocyte count, peripheral smear examination. Bone marrow aspiration and trephine biopsy (to determine cellularity). Severity of aplastic anemia is based on these parameters (Table 1).

PNH may be associated with aplastic anemia. The most sensitive investigation is flow cytometry, which demonstrates absence of CD55 and CD59 in granulocytes and erythrocytes of any PNH clone. HAM's and sucrose lysis tests are less sensitive and are affected by recent blood transfusions.

Fanconi's anemia should be excluded in all patients < 35 years of age and up to 45 years in those undergoing bone marrow transplant (BMT). This is done on peripheral blood lymphocytes for spontaneous breakage or after stimulation with mitomycin-C or diepoxybutane (DEB). Immunosuppression has no role in Fanconi's anemia.

## Differential Diagnosis

This should include other rare causes of pancytopenia with a hypocellular marrow, given below:<sup>2</sup>

- a. Hypocellular myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML)/acute lymphoblastic leukemia (ALL)
- b. Hairy cell leukemia
- c. Lymphomas and myelofibrosis
- d. Mycobacterial infections.

A careful examination of the morphology of the residual cells in the hypoplastic marrow should be seen by an expert in the field.

## Supportive Care

### *Transfusional support*

Packed red cell transfusions must be given to maintain a safe hemoglobin, compatible with the age and associated co-morbid conditions.

Platelets must be given to prevent bleeding and are indicated if the platelet count is <  $10 \times 10^9/L$ .

Platelet sparing efforts like use of tranexamic acid in minor mucosal bleeds and norethisterone in adolescent girls who have achieved menarche are beneficial. Tranexamic acid is used in the dose of 2-4 g/day in 3-4 divided doses.<sup>4</sup>

The adverse effect of repeated blood and platelet transfusions is the development of allo-antibodies. This leads to refractoriness to platelets and febrile reactions after blood transfusions. For those patients who subsequently undergo a BMT, there is a greater risk of graft rejection. Ideally, leukodepleted blood and platelets should be transfused, to minimize alloimmunization.

Blood from siblings and blood relatives should be avoided as there is a risk of fatal transfusion-associated graft versus host disease (GVHD). For those who may undergo BMT, transfusion from family members should be strictly avoided as alloimmunization to family antigens will increase the risk of graft rejection.

### **Treatment of Infection**

Presence of fever with severe neutropenia should be treated as febrile neutropenia, with intravenous antibiotics. As these patients often have prolonged neutropenia, they are prone to fungal infections. Antifungal agents should be introduced early, if fever is not responsive to antibacterial agents. The outcome is poor for those with very severe neutropenia (neutrophils  $< 0.2 \times 10^9/L$ ).

### **Prevention of Infections**

Patients should avoid foods, which may be contaminated with bacteria and fungus. Oral antiseptic mouthwash and oral care is recommended. In patients with severe neutropenia and recurrent infections, prophylactic antibiotics and antifungal agents may be tried.

### **General Measures**

- a. Avoid intramuscular injections as these may give rise to hematomas or abscesses, due to underlying thrombocytopenia and neutropenia, respectively.
- b. Strict food hygiene in the form of cooked clean food. Avoid any open food sold on road side. Ensure a balanced diet with full skin fruits, vegetables and meat. All fruits must be adequately washed before eating. It is preferable to avoid fruits and salads during periods of severe neutropenia.
- c. Avoid crowded places like markets, cinema halls, etc.
- d. Avoid contact games and injuries.
- e. Use soft tooth brush especially if patient is thrombocytopenic.
- f. Psychological support to the patient and family members is of utmost importance, stressing the chronic nature of disease and slow response to treatment.
- g. Avoid hazards of exposure to dust (construction work in buildings), animals and pets during neutropenia.

### **Specific Therapy for Severe Aplastic Anemia**

An algorithm for the management of severe aplastic anemia is given in Figure 1.

### **Hematopoietic Stem Cell Transplant**

The best form of treatment for aplastic anemia is an allogeneic hematopoietic stem cell transplant (HSCT), also called BMT. This is applicable for those who have an HLA identical family donor. With current protocols, 80-90% patients are cured.<sup>3</sup> The advantages are:

- a. Recovery of pancytopenia within 2-3 weeks of stem cell infusion.
- b. Full recovery of blood counts in majority.

- c. Cessation of all medication in a year or two, except in those patients with complications like GVHD.
- d. Little risk of relapse.
- e. Low complication rates in young.

The limitations are:

- a. Need for an HLA identical donor.
- b. Initial high cost of transplant.
- c. Possible complications of GVHD, which are more common in those over 40 years age.
- d. Risk of graft rejection in those who are multiply transfused and alloimmunized.
- e. An initial period of immunocompromised state, requiring special monitoring and care.

The patient and all the siblings must be HLA typed. Blood group compatibility is not required.

The best donor is an HLA identical sibling. The results of HLA identical sibling HSCT are excellent with more than 80% cure. Rarely, another family member (like a parent) may be HLA identical, in communities where there is intermarriage and sharing of HLA genes.

If a matched sibling is available, young patients must be referred to an appropriate BMT center for counseling and treatment. In India, related HSCTs are being performed in many centers.<sup>5</sup>

The results of unrelated HSCT are inferior as the risks of graft failure and graft versus host disease are very high and 5 years survival estimated at 39%.<sup>6</sup> In India, unrelated transplants are not being routinely performed at present, because there are no large unrelated donor registries, complications are more, experience is limited, and cost is prohibitive. Hence, the issue of unrelated transplants is not being addressed in this article.

For patients older than 40 years, immunosuppression is preferred. HSCT is considered a second option in case immunosuppressive therapy fails.

## **Immunosuppressive Therapy**

Immunosuppressive therapy consists of a combination of antithymocyte globulin (ATG) and cyclosporine A (CsA). Response rates are about 60% at 3 or 6 months after horse ATG.<sup>3,7</sup> It is effective in both young and old patients.<sup>8,9</sup>

Active infection is a contraindication. Patients should be referred early to an appropriate center as there are different preparations of ATG with varying dosage schedules. The following precautions are needed:

- a. Admission is required
- b. Corticosteroids are added to prevent serum sickness
- c. Reactions during ATG transfusion are common and rarely anaphylaxis can occur
- d. Platelet support is essential, as ATG can cause a fall in platelet counts
- e. Serum sickness occurs in about 40% patients in the second and third week

Cyclosporine is usually started after steroids are tapered off, usually after 3 weeks of ATG. The response with ATG/cyclosporine is seen after 3-4 months. Cyclosporine should be tapered off very gradually after a minimum of 6 months. If there is no response to one course of ATG, or there is a relapse, a second or third course may be given. Patients who show no response to two courses of ATG are unlikely to respond to a third course.<sup>10</sup> If cyclosporine is used alone, the response rates are about 30%.

## **Technical Aspects of ATG Administration**

### *Preparation of the Patient*

- a. All patients less than 35 years of age should have a stress cytogenetics test done to rule out Fanconi's anemia as immunosuppressive therapy is ineffective in patients with Fanconi's anemia.
- b. Patient should have no evidence of infection prior to administration of ATG as this medication is intensely immunosuppressive.
- c. Central venous access is required as ATG can cause peripheral venous sclerosis. A peripherally inserted line is usually not useful.
- d. Platelet and packed red cell transfusion should be given to keep platelets above  $20 \times 10^9/L$  and hemoglobin above 7 gm/dl.
- e. It is desirable to use leukodepleted blood products. Irradiated blood products are desirable to prevent transfusion-associated graft versus host (TA-GVHD) as there is profound immune suppression. TA-GVHD is fatal, if it occurs. If related blood products are given, then irradiation is mandatory.
- f. ATG should be instituted under care of a qualified medical team familiar with this treatment.

### **ATG Administration**

- a. Horse ATG is available in India containing 250 mg in a 5 ml vial. The recommended dose used is 40 mg/kg/day diluted in normal saline infused over 8 hours, however, one should follow the manufacturer's instructions. Many centers, including our own at AIIMS, often use lower doses due to cost constraints with reasonable responses. (The international brand used most often is ATGAM from Pfizer. An Indian product is also available from Bharat Serums and Vaccines).
- b. The infusion should be done using glass bottles or commercially available high quality bags (like the Baxter bags) as ATG tends to stick to the sides of an ordinary intravenous fluid plastic bags.
- c. Reconstituted solution should be used as early as possible. If there is any delay in administration, it should be kept refrigerated and brought back to room temperature before infusion.
- d. Premedication with paracetamol, chlorpheniramine and hydrocortisone or prednisolone is given prior to starting ATG infusion daily to prevent allergic reactions.
- e. The first vial of ATG should be administered very slowly. The patient should be observed carefully for allergic reactions, especially anaphylaxis.
- f. ATG infusion should be stopped if severe allergic reactions develop like hypotension, dyspnea or anaphylaxis.

*Serum sickness:* This generally occurs 10-14 days post ATG. The presenting features are fever, skin rash, arthralgia and arthritis. Prophylaxis with oral prednisolone 1mg/kg/day should be given till day 14 and tapered over 7 days to prevent serum sickness. If serum sickness develops, then treatment should be with intravenous hydrocortisone. Safe analgesics should be prescribed for arthralgia.

*Cyclosporine:* Most centers start oral cyclosporine on day 21 of ATG, after stopping prednisolone, as combined administration is more immunosuppressive and both drugs can lead to hypertension. The initial dose is 5 mg/kg/day in two divided doses to maintain a trough level of 150-250 ng/ml in adults and 100-150 ng/ml in children. Monitoring of liver and renal functions, and blood pressure is important. Cyclosporine can alter the levels of other drugs administered concomitantly. It is continued for a minimum of 6 months to assess the response.

### **Response to Immunosuppression**

The response criteria for immunosuppressive therapy are given in Table 2.

The disadvantages of ATG/cyclosporine are:

- a. Response rates are significantly less as compared to HSCT.
- b. Patients need intensive blood component support till response occurs.
- c. In most cases, the responses are incomplete.
- d. Relapses occur in 12-30% of patients.<sup>3</sup>
- e. Transformation to clonal disorders like PNH, myelodysplastic syndrome (MDS) and leukemia can occur with the passage of time.<sup>11</sup>

### **Androgens**

Androgens are not recommended in current literature as other options are better. Due to cost considerations, many patients in India cannot afford HSCT or immunosuppressive therapy. Androgens may be tried and show response in about 15% of aplastic anemia, majority of whom are non-severe. The agents, which are available, are oxymetholone or stanozolol in doses of 1.5 to 2 mg/day for many months.

### **Role of Colony Stimulating Factors**

There is little role of colony stimulating factors like G-CSF, as there is hardly any marrow to stimulate. In severe unresponsive infections, a short course of G-CSF may be tried. If there is a rise in neutrophil counts, G-CSF should be continued till infection resolves. If there is no improvement in counts, G-CSF should be stopped after a maximum of 7 days.<sup>12</sup> Some reports suggest that their use may be harmful as there may be transformation to MDS or leukemia.

There is no role of erythropoietin in aplastic anemia. The normal body response results in very high erythropoietin levels in these patients.

### **Role of Corticosteroids**

Corticosteroids should not be used for primary treatment of aplastic anemia. They have no benefit and predispose patients to infection and gastrointestinal bleeding.

### **New Immunosuppressive Drugs**

Many immunomodulatory drugs are being evaluated in refractory patients. These include high dose cyclophosphamide, mycophenolate mofetil, sirolimus, and alemtuzumab (monoclonal antibody to CD 52). At present these should only be used in the context of a clinical trial.

### **Treatment of Non-Severe Aplastic Anemia**

The treatment principles are similar to those of severe aplastic anemia, but initiation is with less toxic therapy. For a stable patient, it may be enough to remove any potential etiological factor. Androgens are likely to be more effective than in severe aplastic anemia. Immunosuppression may be started with cyclosporine alone. ATG or HSCT is not administered till the counts fall and suggest a rapid evolution to more severe disease.

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## Multiple Choice Questions

- 1. The following is true of chromosomal fragility/breakage studies:**
  - A. Fanconi's anemia shows a positive result
  - B. It is needed in old patients with aplastic anemia
  - C. Positive result suggests good response to immunosuppression
  - D. It has no value in the investigation of aplastic anemia
- 2. The following is true of severe aplastic anemia:**
  - A. Antithymocyte globulin therapy is the treatment of choice for patients older than 40 years
  - B. Cyclosporin is curative in 70 percent cases
  - C. Injection G-CSF should be given to all severe aplastic anemias, as it improves response and survival rates
  - D. High dose steroids should be the initial therapy
- 3. The therapeutic principles of severe aplastic anemia are:**
  - A. Cyclosporine is the treatment of choice for patients younger than 20 years
  - B. Antithymocyte globulin therapy is indicated when a HLA identical sibling is not available
  - C. Injection erythropoietin should be given to all severe aplastic anemias
  - D. Mycophenolate is the latest approved therapy
- 4. In a patient with pancytopenia, clinical features suggestive of aplastic anemia are:**
  - A. Splenomegaly
  - B. Hepatomegaly
  - C. Lymphadenopathy
  - D. No organomegaly
- 5. The following is true regarding transfusion from blood relatives in aplastic anemia:**
  - A. It improves the chance of successful hematopoietic stem cell transplant
  - B. It is essential to ensure safe blood transfusion
  - C. Irradiation of blood is not needed as it is related
  - D. It may lead to transfusion associated graft versus host disease, a fatal complication
- 6. The following is true of allogeneic hematopoietic stem cell transplant in aplastic anemia:**
  - A. The risk of relapse and evolution to clonal disorders is high
  - B. An unrelated transplant is an easy option in India, with its vast population
  - C. The main risks are graft rejection and graft versus host disease
  - D. Age is not a limiting factor