Diagnosis and Management of Acquired Aplastic Anemia: Consensus Statement of Indian Academy of Pediatrics

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Justification: In India, there is a lack of uniformity of treatment strategies for aplastic anemia (AA), and many children are managed only with supportive care due to non-availability of hematopoietic stem cell transplantation (HSCT). **Process:** Eminent national faculty members were invited to participate in the process of forming a consensus statement in Hyderabad in July, 2016. Draft guidelines were circulated to all members, and comments received in a online meeting in October, 2020 were incorporated into the final draft. These were approved by all experts. **Objective**: To facilitate appropriate management of children with acquired aplastic anemia. **Recommendations**: Key recommendations are: *i*) A bone marrow biopsy is must to make a diagnosis of AA; *ii*) Rule out inherited bone marrow failure syndromes (IBMFS), connective tissue disorders, viral infections, paroxysmal nocturnal hemoglobinuria (PNH), drug or heavy metal induced marrow suppression in all cases of AA; *iii*) Conservative approach to transfusions should be followed, with a target to keep hemoglobin >6 g/dL in children with no co-morbidities; *iv*) HLA-matched sibling donor HSCT is the preferred choice of treatment for newly diagnosed very severe/ severe AA; *v*) In absence of HLA-matched family donor, a matched unrelated donor (MUD) transplant or immunosuppressive therapy (IST) should be considered as alternate choice based on physician expertise; *vi*) Fludarabine, cyclophosphamide and anti-thymocyte globulin (ATG) based conditioning with cyclosporine and methotrexate as graft versus host disease (GvHD) prophylaxis is the preferred regimen; *vii*) Horse ATG and cyclosporine are the recommended drugs for IST. One should wait for 3-6 months for the response assessment and consideration of next line therapy.

Keywords: *Anti-thymocyte globulin, Cyclosporine, Hematopoietic stem cell transplant, Immunosuppressive therapy.*

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Survival of children with aplastic anemia (AA) has markedly improved in the developed world, because of advances in hematopoietic stem cell transplantation (HSCT), and immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine, where transplant is not possible. In our country, there is a lack of uniformity of treatment strategies and many children are managed only with supportive care.

OBJECTIVES

The purpose of this guideline is to improve the care of children with acquired aplastic anemia by an early and accurate diagnosis, prompt referral, detailed counseling and definitive therapy in the form of HSCT or IST.

PROCESS

Eminent national faculty members were invited to participate in the process of forming a consensus statement in Hyderabad in July, 2016. Selected members were requested to prepare guidelines on specific subtopics. These guidelines were then incorporated into a draft statement, which was circulated to all members. An online meeting was organized in October, 2020; opinions expressed by the participants were incorporated into the final draft, which was again circulated to all experts and finalized.

RECOMMENDATIONS

Aplastic anemia is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin. Diagnostic criteria of AA include: (1) presence of at least two of the following in the peripheral blood counts; *i*) hemoglobin <100 g/L; *ii*) platelet count $<50 \times 10^9$/L; *iii*) neutrophil count $<1.5 \times 10^9$/L, and (2) low bone marrow cellularity (<25%) [1]. Severity of AA is defined as per the criteria depicted in **Box I** [2,3] (1C).

Box I Severity Classification of Aplastic Anemia

Severe aplastic anemia (SAA)

BM cellularity < 25% (or 25-50% if <30% of BM is hematopoietic cells) and

at least two of the following:

- Peripheral blood neutrophil count $<0.5\times10^9$/L
- Peripheral blood platelet count $<20\times10^9$/L
- Peripheral blood reticulocyte count $<20\times10^9$/L

Very severe aplastic anemia (VSAA)

As above, but peripheral blood neutrophil count must be $<0.2\times10^9$/L

Non-severe aplastic anemia

Hypocellular bone marrow with peripheral blood counts not meeting criteria either for SAA or VSAA

Modified from Reference 1 and 2.

This guideline will focus specifically on early diagnosis and treatment of acquired AA, and will not refer to management of inherited bone marrow failure syndromes (IBFMS) and bone marrow aplasia that occurs secondary to chemotherapy and/or radiation exposure.

Diagnosis

The expert panel felt that there are no specific markers and the diagnosis is reached by exclusion of other reasonable entities. There are three diagnostic steps to define AA: *i*) confirming the suspicion of AA; *ii*) defining the severity of AA and; *iii*) excluding IBMFS and identifiable causes of marrow aplasia.

History and Physical Examination

Most children with AA present with a history pertaining to decrease in the blood cell lines (anemia, leucopenia, neutropenia and thrombocytopenia). There may be a history of lethargy, tiredness, poor appetite and increasing pallor secondary to anemia. The child may present with fever with or without a focus because of neutropenia. They may also give a history of bleeding, especially mucocutaneous bleeding, consequent to thrombocytopenia. There may be a history of blood transfusion prior to presentation. Any history of recent illnesses like viral infections or jaundice (approximately 5-10% of patients with SAA have a preceding seronegative hepatitis) should be elicited [4]. A history of joint pains/swelling, skin rash, photosensitivity, hair loss, mouth ulcers etc may point towards a connective tissue disorder. A detailed drug and occupational exposure history should always be taken (1C). A detailed dietary history may enable to investigate for nutritional megaloblastic anemia as the likely etiology of pancytopenia. A similar history in the family can point towards a IBMFS, or a common environmental exposure. It is essential to perform a detailed examination for every patient that presents with bicytopenia or pancytopenia. AA is clinically characterized by anemia and evidence of mucocutaneous bleeds, in the absence of enlarged lymph nodes, splenomegaly and hepatomegaly. One also needs to identify any stigmata of IBMFS. **Web Table I** gives the various signs which point towards the diagnosis.

Investigations

1. Complete blood count with peripheral smear and reticulocyte count

Following findings may be seen:

i) *Pancytopenia/bicytopenia*: There is moderate to severe leucopenia with relative lymphocytosis and variable degree of neutropenia; In early stages, isolated cytopenia, commonly thrombocytopenia may be seen; and anemia, usually macrocytic. Look for dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells to exclude other causes of pancytopenia.

ii) *Reticulocytopenia*

2. Bone marrow aspiration and/or biopsy

Despite severe thrombocytopenia, adequate pressure is sufficient following a bone marrow aspirate and biopsy to prevent bleeding and there is no need of platelet transfusion before procedure [5]. The marrow is usually hypocellular with prominent fat spaces; few residual haemopoietic cells may be present. A bone marrow aspirate may occasionally be a dry tap. Trephine bone marrow biopsy (at least 2 cm length) is necessary in every AA case to assess the overall cellularity (which may be patchy), to look at the morphology of residual hematopoietic cells and to exclude an abnormal infiltrate. Avoid tangential biopsies as subcortical marrow is normally hypocellular. **Box II** shows the differential diagnosis of pancytopenia according to bone marrow cellularity. Bone marrow cellularity can be estimated using a simple formula (100-age = Bone marrow cellularity in %). The appearance of the marrow in inherited and acquired AA syndromes is identical, and the histological distinction between them is blurred.

Erythropoiesis: Erythroid series in marrow is decreased. AA should be differentiated from myelodysplastic syndrome (MDS). Dyserythropoiesis is not a common finding in immune mediated AA [6].

Megakaryopoiesis: In AA, megakaryocytes are either reduced or absent. Megakaryocytes are the most useful element in differentiating MDS from severe aplastic anemia (SAA) [7]. Small mononuclear or aberrant megakaryocytes are typical of MDS, whereas megakaryocytes are markedly reduced or absent in SAA.

Box II Differential Diagnosis of Pancytopenia

Pancytopenia with hypocellular marrow

- Acquired aplastic anemia
- Inherited bone marrow failure like Fanconi anemia (FA), amegakaryocytic thrombocytopenia, dyskeratosis congenita, Shwachman-diamond syndrome
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Hypoplastic myelodysplastic syndrome

Pancytopenia with cellular marrow

- Primary bone marrow disease like- PNH, Myelodysplasia
- Secondary to systemic disease like- SLE, Hypersplenism, Vitamin B_{12} and folate acid deficiency, Infection

Pancytopenia due to infiltrative bone disorder

- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Myelofibrosis
- Storage disorders

White blood cell (WBC) series: Lymphocytes, macrophages, plasma cells and mast cells appear prominent in bone marrow, while the mature WBC forms, viz granulocytes, are reduced in bone marrow in children with SAA. Dysplastic granulocytes are not seen in AA.

3. Bone marrow cytogenetics

Obtaining a good bone marrow aspirate may be difficult in a child with hypocellular bone marrow as the number of metaphases observed may be insufficient. In such a situation, we should perform a fluorescent in situ hybridization (FISH) analysis for chromosomes 5 and 7 to exclude MDS. Abnormal cytogenetic clones may be present up to 12% of patients with AA, and are not important for diagnosis or prognosis [8]. These may also arise during the course of the disease. Most frequent anomalies are - trisomy 8, trisomy 6, 5q, anomalies of chromosome 7 and 13 [9].

4. Vitamin B12 and folate levels

If a deficiency of vitamin B12 or folate is documented or suspected based on the peripheral smear or bone marrow findings, then correction must be done before diagnosing AA. Bone marrow aplasia due to vitamin deficiency is exceedingly rare. The levels are extremely sensitive to administration of supplements and hence should be done before administration of the same.

5. Tests of liver function and viral studies

The onset of AA often occurs 2-3 months after an acute episode of hepatitis and is more common in young males [10]. Hepatitis associated AA shows poor prognosis [10]. Viral markers for Hepatitis A, B, C, EBV, HIV, and CMV are indicated but the disease is usually seronegative. Parvovirus usually causes only red cell aplasia and not AA. Viral infections require adaptive immunity for viral clearance that is mediated by antibodies and T-cells. Oligoclonal CD8+ T-cells may target the hematopoietic tissue, which can lead to stem cell death secondary to effects of IFNγ and TNFα. Pro-inflammatory cytokines may also damage the hematopoietic stem cell compartment and cause pancytopenia.

6. Chromosomal breakage analysis (1B)

The diagnosis of Fanconi anemia (FA) is very important, because these patients do not respond to IST, require dose-reductions in transplant conditioning and need careful follow-up for development of non-hematologic malignancies.

Spontaneous breakage (chromatid-type aberrations) can occur in standard cytogenetic preparations, although this phenomenon is highly variable and considered unreliable for diagnostic purposes. Extreme sensitivity of FA cells to the chromosome breaking effect of the cross-linking agents mitomycin C (MMC) and diepoxybutane (DEB) is routinely utilized to diagnose FA by a chromosomal breakage test [11,12]. Chromosomal breakage should be done in all patients below 40 years of age before IST/ HSCT, even if no physical features of FA are identified. Chromosomal breakage should also be tested in sibling donors of affected patients in case of FA. 5-7 mL of venous blood in collected in green topped vacutainers (heparinized sample). If the child / patient has been transfused, wait for at least 2 weeks before obtaining the specimen. Skin fibroblasts have been used for diagnosis of IBFMS in case of recent transfusion of difficulty to culture metaphases but it is not available universally.

Next generation sequencing (NGS) for IBMFS: It is very useful to detect IBMFS, especially if the chromosomal breakage has been negative [13]. The panel recommends carrying out this test, but it is not mandatory. Customized panels are now available in India to rule out IBMFS.

7. Tests to detect a PNH clone (1C)

PNH is an uncommon cause of AA in children. The disease may lead to features of thrombosis, hemolysis, hemoglobinuria and AA. Ham test and sucrose lysis test have been abandoned (as they do not detect clones in neutrophils, monocytes and in transfused patients).

Analysis of glycosylphosphatidylinositol (GPI)-anchored proteins (like CD55 and CD59) by flowcytometry (Fluorescein-labeled proaerolysin, FLAER) is the preferred

method. PNH clone lacks these markers and may be seen in small amounts. They occur in up to 50% of AA patients, which may remain stable, diminish, disappear or increase [14].If a PNH clone is present, evaluate for evidence of intravascular hemolysis (urine hemosiderin and LDH). The presence of less than 50% deficient circulating cells without evidence for thrombosis or significant hemolysis generally does not require PNH-specific therapy and responds well to IST[15].

8. Anti-nuclear antibody and anti-dsDNA

Anti-nuclear antibody (ANA) should be done in all patients presenting with pancytopenia as SLE can present with normocellular, hypocellular or myelofibrosis in marrow with variable cytopenia [16]. Children who develop MAS (macropahge activation syndrome), may have presentation like AA. Anti-dsDNA antibody should be done in cases where ANA is positive or if the index of suspicion is very high.

Treatment for Acquired Aplastic Anemia

Supportive Care

The main focus should be on prevention of infections and bleeding and expectant management of treatment-related toxicities and psychological support

i) When to transfuse

Based on concerns with alloimmunization and iron overload, there should be a uniform approach to utilize as few transfusions as possible to keep the patient relatively asymptomatic (1A). Unnecessary transfusion practice should be avoided in AA (1A).

A common problem in multi-transfused patients with AA is that they may develop alloimmunization to leucocytes present in red cell and platelet transfusions by generating HLA or non-HLA (minor histocompatibility) antibodies. This can result in platelet refractoriness, as well as an increased risk of graft rejection and graft versus host disease after allogeneic BMT [17].

It is recommended to give prophylactic platelet transfusions when the platelet count is $<10\times10^9$/L; however, the physician may choose to transfuse only when there is mucosal bleeding (1B). In febrile patients it is desirable to maintain platelet count above 20×10^9/L, however, it may not be possible due to alloimmunization and platelet refractoriness [18] (2C).

During ATG infusion, patients should be transfused optimally to achieve a platelet count of $>30 \times10^9$/L before ATG treatment (2C).

RBC transfusion depends on clinical symptoms, Hb value and quality of life (1A). If Hb levels are less than 6.0 g/L, packed red blood cell (PRBC) transfusion should be given.

Granulocyte apheresis may have an adjunctive role in severe infections in SAA patients as a possible way to bridge the gap between specific treatment and neutrophil recovery [19].

Whenever possible, use leucoreduced blood products to prevent HLA alloimmunization. Irradiated blood product should be used in all AA patients especially who are potential BMT candidates (1C).

Blood and platelet donations from family members should be avoided because the recipient may become sensitized to minor histocompatibility antigens from the potential bone marrow donor resulting in a high risk of graft rejection.

Other practical measures to prevent bleeding include good dental hygiene, the use of oral tranexamic acid and control of menorrhagia with hormone therapy.

ii) Infection control

Patients with AA are at risk of bacterial and fungal infections. The risk of infection depends upon neutrophil count and may vary from patient to patient.

We recommend considering basic principles of asepsis such as: *i*) washing hands before preparing food; *ii*) avoid contamination of food; *iii*) consumption of pasteurized juices and dairy products, and *iv*) consumption of clean, fresh and hygienic home cooked food [20].

Exposure to construction areas should be avoided as this increases the risk of fungal infections. Additionally, it seems reasonable to avoid contact with garbage, compost or potted plants. Hand washing and rubbing with alcohol-based disinfection solution must be used before and after handling the patient by the staff and by visitors. Optimal hand hygiene has been shown to be highly effective in reducing neutropenic infections. Routine prophylactic antimicrobials are not required. Patients with AA are at high risk of fungal infection, including Aspergillus. It is suggested to use prophylactic antifungals for patients with very severe AA (absolute neutrophil count <200/ mm^3). However, definite evidence for this recommendation is lacking [21] (2B). As fluconazole provides no cover against Aspergillus species, the drugs of choice are voriconazole and posaconazole. Azoles are potential inhibitors of cytochrome P450 3A4 enzyme which can lead to increase in the plasma levels of immunosuppressant drugs like cyclosporine thereby resulting in toxicity [22].

Routine prophylaxis against *Pneumocystis jirovecii* or anti-viral prophylaxis is not required [17,20] (2C).

P. jirovecii prophylaxis is essential post BMT for all patients regardless of diagnosis, but not usually required post ATG treatment.

Acyclovir prophylaxis is essential for all transplanted patients and may be used for the first 3-4 weeks after IST with ATG [17,20].

As for all neutropenic patients, fever is an emergency and requires immediate hospitalization and treatment before the results of bacterial investigations are available.

Single anti-pseudomonas antibiotic may be enough in most cases. However, the choice of antibiotic will also depend on whether the infection is hospital acquired or is community acquired. The exact choice depends upon local hospital microbiological sensitivity/resistance pattern. It is recommended that investigations for systemic fungal infection and empirical anti-fungal therapy is introduced into the febrile neutropenia regimen if fever persists for more than 96 hours.

A chest radiograph should be included as part of the investigation of new or persistent fever with respiratory symptoms, and computed tomography (CT) chest may be also done if there is a high index of clinical suspicion for aspergillus pneumonia.

iii) Hemopoietic growth factors

The routine use of recombinant human erythropoietin (EPO) or G-CSF is not recommended. A short course of subcutaneous G-CSF at a dose of 5 microgram/kg per day may be considered for severe systemic infections that are not responding to intravenous antibiotics and antifungals. If there is no response by 1 week, then discontinue G-CSF. GM-CSF is not recommended for the treatment [23]. The addition of eltrombopag to IST may be considered [24]. However, the routine use of eltrombopag cannot be recommended as definite evidence is lacking in children.

iv) Iron chelation

Frequent transfusions in SAA may result in significant iron overload and its complications. Subcutaneous desferrioxamine (DFO) or oral deferasirox should be considered when the serum ferritin is >1000 ng/ml [18].

Definitive Treatment (Fig. 1)

i) When and whom to treat [25]

Severe and very severe AA almost always requires treatment, both immediate and definitive. For patients with non-severe AA, as defined by lack of blood count criteria for SAA, observation is often appropriate, especially when they are not transfusion-dependent. Moderate AA patients who progress to severe pancytopenia and meet the criteria for SAA or become transfusion-dependent can then be treated accordingly.

It is necessary that before specific treatment is given, the patient is stabilized (bleeding controlled and free from infection).

ii) Choice of definitive treatment

Hematopoiesis can be restored in severe AA with HSCT or IST. Transplantation is the preferred treatment when matched sibling donor available.

a) Matched-related HSCT

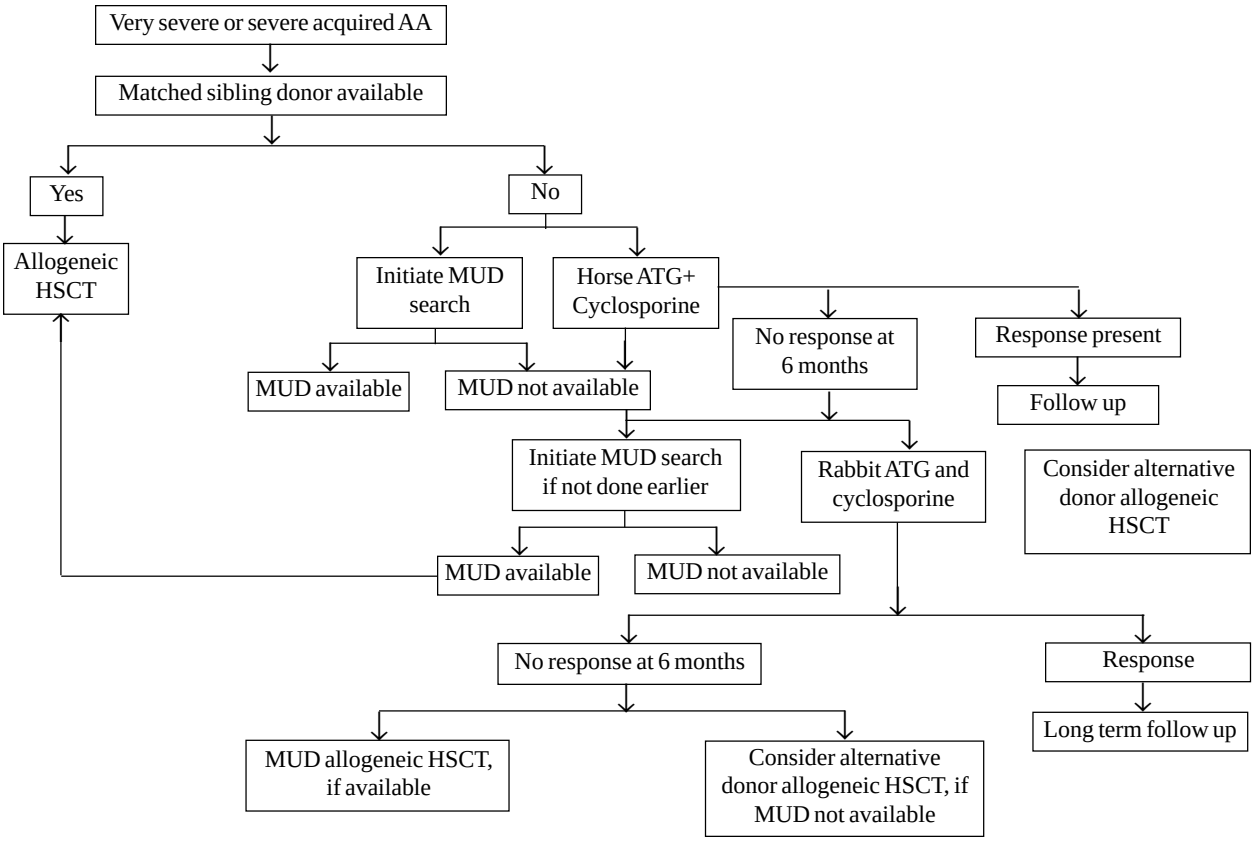
Allogeneic BMT from an HLA-identical sibling donor (matched sibling donor, MSD) is the initial treatment of choice for newly diagnosed patients with AA if they have *i*) Severe or VSAA *ii*) Non-severe AA and in whom treatment is indicated [25] (1A).

Matched-sibling transplantation is always preferred. Ideally patient should be free from infections before transplant. The expected overall response rate with matched related donor HSCT is ~90% [25].

The transplant physician may proceed with BMT in the presence of active infection, particularly fungal infection, as the transplant offers the best chance of early neutrophil recovery. Delaying the transplant may actually risk progression of the fungal infection. The conditioning regimens and GVHD prophylaxis described below refer specifically to patients with acquired AA. Patients with FA and other types of inherited AA need special consideration and should not follow these pathways, as the conditioning regimen and GVHD prophylaxis are completely different. Current guidelines from European Society for Blood and Marrow Transplantation (EBMT) [26] and the British Society for Standards in Hematolgy [27] call for a combination of fludarabine-cyclophospamide with ATG or alemtuzumab.

The recommended post-transplant immunosuppression usually comprises of two drugs, cyclosporine and short course methotrexate. The starting dose of cyclosporine is 3-5 mg/kg/day in 2 divided doses. The cyclosporine levels should be maintained between 150-200 microgram /L, to avoid toxicity. Therapeutic cyclosporine should be continued for at least nine months before gradually reducing the dose to zero over the following three months.

Source and dose of stem cells: It is recommended that bone marrow stem cells should be used [28] but the transplant physician may decide to use peripheral blood stem cells (PBSC) based on the experience or logistics. It is recommended that at least 3×10^6/kg of CD34 stem cells



HSCT: hematopoietic stem cell transplantation; MUD: matched unrelated donor; ATG: anti-thymocyte globulin.

Fig. 1 Management algorithm for pediatric aplastic anemia.

should be given [29]. In the absence of a MSD, a center may choose to proceed with IST or MUD transplant as per the physician expertise. MUD has encouraging outcomes and may be used in case there is a 10/10 match with the recipient and the logistics like finances and timing can be appropriately coordinated [28]. The Indian Stem Cell Registries have made this increasingly possible [30]. The current cost of arranging a MUD donation through an Indian registry is approximately 10 lakhs. If there is no MSD or MUD available, and the IST course has failed, alternative donor transplant options like haploidentical transplant may be considered [31-34].

Complications from HSCT: Post-HSCT, children need regular monitoring. Short-term complications include chemotherapy related side effects (e.g. hemorrhagic cystitis, infections), acute GVHD, and graft failure. Long-term complications include chronic GVHD, delayed immune reconstitution and relapse.

b) Immunosuppressive therapy. Indications are:

i) Patients with non-severe AA who are dependent on red cell and/or platelet transfusions and no matched sibling donor available

ii) Patients with severe or very severe disease who lack an HLA-compatible sibling donor or where HSCT is not feasible

For those patients with non-severe AA who are not dependent on either red cell or platelet transfusions, and maintain safe blood counts, it is reasonable to observe the blood count and monitor the patient regularly without initially starting IST.

Horse (or equine) ATG infusions combined with oral cyclosporine remains the standard first line IST [35] (1A). The expected overall response rate with IST is 60-75% with a long-term survival of 80-90% [25,36].

- Immunosuppression administration

Anti-thymocyte globulin (ATG): It must be given via central line in a hospitalized patient with monitoring for infusion reactions and other side effects. Perform an ATG skin test for hypersensitivity with intradermal testing with

0.02 mL of a 1:1000 v/v (volume/volume) saline dilution of ATG with a separate saline control injection of similar volume. Read the result after 10 minutes: a wheal at the ATG site 3 or more mm larger in diameter than that at the saline control site suggests clinical sensitivity and an increased possibility of a systemic allergic reaction. Increasingly intravenous (IV) test dose has replaced skin testing. We can add 0.1 mL of ATG in 100 mL saline and start the first few drops at a very slow rate, which can be gradually increased to complete the test dose in 30 minutes [18].

Platelets should be maintained at more than $>30\times10^9$/L before ATG treatment and during the ATG administration period, but should not be given concurrently with ATG administration because of the anti-platelet activity of ATG [18].

Horse ATG is administered at a dose of 40 mg/kg as intravenous infusion in a glass ornon-PVC bottle over 12-18 hours, daily for 4 days. ATG can be diluted in normal saline or 5% Dextrose + 0.45% sodium chloride solution (DNS). The concentration of the diluted solution should not exceed 4 mg of ATG/mL. ATG administration should not start late in the day or on weekends when hospitals may be short-staffed as infusion reactions are more common on day 1 of ATG. Premedication before each ATG dose with acetaminophen and diphenhydramine is conventional, and common infusion reactions are managed symptomatically.

Prednisone (1 mg/kg) or methylprednisolone (2mg/kg) is started on day 1 and continued for 2 weeks, as prophylaxis for serum sickness. In the presence of life-threatening reactions, the ATG infusion is withheld temporarily until alarming signs and symptoms subside. Depending on the severity of reactions, reinitiate ATG at the normal or a slower infusion rate (sometimes over 24 hours) in a monitored setting. Increased liver enzymes tend to normalize over several days, and ATG may be infused despite mild to moderate elevation in transaminases.

Serum sickness typically occurs between day 7 and 14 from the start of ATG treatment. If serum sickness occurs, intravenous hydrocortisone 1-2 mg/kg/dose six hourly (max 100 mg) should be commenced. The common symptoms of serum sickness include arthralgia, myalgia, rash, fever, edema, mild proteinuria and platelet consumption often necessitating increased platelet transfusion support.

Cyclosporine (CsA): Initiate CsA on day 1 of IST or after completion of steroids to a target trough level between 150 and 200 ng/mL, starting at a dose of 5 mg/kg per day. Many patients develop hypertension during CsA treatment and amlodipine is the preferred anti-hypertensive drug because of minimal overlap with CsA toxicities. Bothersome gingival hyperplasia can improve on a short course of azithromycin or metronidazole ointment or dose reduction of CsA. Calcium channel blockers have been associated with worse gingival hyperplasia when combined with CsA [37]. Continue careful monitoring of renal function and adjustment of dosing to achieve target CsA levels. Continue CsA if modest increases in creatinine. More serious compromise of kidney function from baseline (1.5 times the baseline) may require temporary cessation of CsA with later reintroduction at lower doses. Consider withholding CsA in case of microangiopathic anemia and posterior reversible encephalopathy syndrome (PRES). Avoid concomitant use of other nephrotoxic agents.

There is a significant risk of relapse with rapid tapering of CsA and we recommend that CsA should be continued for at least 12 months followed by very slow tapering over 6 months to 12 months [29]. Blood pressure, renal and liver function tests should also be monitored regularly while on CsA.

v) What more can we do?

Eltrombopag has been used in adult AA patients, either alone or with IST. Along with IST, the response rates upto 80% have been reported [24,38]. Eltrombopag can be administered at a dose of 150 mg daily in patients who are 12 years of age or older, at a dose of 75 mg daily in patients who are 6 to 11 years of age, and at a dose of 2.5 mg per kilogram of body weight per day in patients who are 2 to 5 years of age along with ATG and cyclosporine [24]. However, the data in pediatric age group is still not entirely convincing [38]. Therefore, it cannot be recommended as a routine. Romiplostim is being tried for stimulation of hematopoiesis in AA. However, there is no enough data to routinely recommend its usage for this indication. More studies are needed to explore the role of thrombopoietin receptor agonists for patients with AA.

vi) When do we consider for second course of ATG?

A second course of ATG is recommended if there is no response or relapse after the first course. This should not be given earlier than 6 months after the first course because it usually takes around 3 to 6 months before a response occurs [26,27]. Second course of immunosuppression is given with rabbit ATG and cyclosporine [26,27]. Rabbit ATG is given for 5 days as a daily intravenous infusion over 12-18 hours. The daily dose of rabbit ATG is 3.75 mg/kg body weight.

A test dose of 2.5 mg for rabbit ATG, diluted in 100 mL normal saline and infused over 1 hour, is often given beforehand and if a severe systemic reaction or

Box III Response Criteria For Aplastic Anemia

Response criteria for severe aplastic anemia

None: Still severe

Partial response: Transfusion independence and no longer meeting criteria for severe disease

Complete response: Hemoglobin normal for age, neutrophil count >1.5×10^9/L, platelet count >150×10^9/L

Response criteria for non-severe aplastic anemia

None: Worse or not meeting criteria below

Partial response: Transfusion independence (if previously dependent) or doubling or normalization of at least one cell line or increase of baseline hemoglobin of >30 g/L (if initially <60) or increase of baseline neutrophils of >0.5 x10^9/L (if initially <0.5) or increase of baseline platelets of >20x10^9/L (if initially <20)

Complete response: Hemoglobin normal for age, neutrophil count >1.5 x10^9/L, platelet count >150 x10^9/L

Modified from reference 35.

anaphylaxis occurs, further doses of that preparation of ATG must not be given.

vii) How to assess response to IST

Response should be confirmed by two or more blood counts at least 4 weeks apart. [35]. **Box III** shows the response criteria for severe and non-severe AA [35].

viii) Refractory SAA

When child is refractory to IST and no matched sibling donor is available; MUD BMT is indicated in those who have no matched siblings and failed at least one course of ATG and CsA [39] (1B). If MUD is not available/feasible, a second course of IST with rabbit ATG (1B) or haplo-identical HSCT can be considered based on center preference (2B).

What Not to Do in AA

Supportive measures alone, growth factors, androgens or cyclosporine (CsA) alone are not definitive therapies. Patients where ATG or transplant are not possible early in disease course, cyclosporine with or without androgens can be tried, with a response rate of 20-30%.

Corticosteroids are of unproven benefit and inferior in efficacy to conventional immunosuppression regimens, but they are more toxic and should not be used as therapy in SAA. Infectious complications especially fungal infections and other life-threatening complications can occur after unnecessary steroid use.

Follow-Up

Patients with AA should be followed up indefinitely to monitor for relapse and later clonal disorders, such as MDS, leukemia, PNH and solid tumors.

CONCLUSIONS

Acquired aplastic anemia is a disease characterized by immune mediated bone marrow failure leading to pancytopenia and hypocellular marrow. A standard of care should follow in diagnosis and treatment of acquired aplastic anemia. MSD BMT is ideal when available. IST should be combined (ATG + CsA) and not monotherapy, when no matched sibling donor available. MUD transplant can be considered when at least one course of IST fails.

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Web Table I Signs Pointing Towards Diagnosis of Pancytopenia

Head	Microcephaly – FA
Eye	Pallor, jaundice Microphthalmia, epicanthal fold, strabismus - FA
Ears	Small and dysplastic ears Hearing defects- FA
Face	Elfin face -FA
Skin	Skin bleeds Malar rash – SLE Hyperpigmentation - FA/ DKC café au lait spots - FA Hypopigmented area - FA
Tongue	Oral leukoplakia – DKC
Neck	Sprengel shoulder, Klippel-Fiel anomaly - FA
Upper limb	Absence or hypoplastic radius - FA
Lower Limbs:	Toe syndactyly, abnormal toes Congenital hip dislocation - FA
Hand anomalies	Thenar hypoplasia, clinodactyly of 5th digit, syndactyly, hyperextensible thumbs - FA Knuckle pigmentation - vitamin B 12 and Folic acid deficiency Nail changes (dystrophy) - DKC
Short stature	FA
Cardiac	VSD - FA
Renal	Horseshoe shaped kidney and ureter abnormalities- FA
Gonads	In males: undescended testes, hypospadias, micropenis- FA In females: Hypogenitalia, bicornuate uterus, abnormal menses

FA: Fanconi Anemia, SLE: Systemic lupus erythematosus, DKC: Dyskeratosis congenital, VSD: Ventricular septal defect