bjh review

# An update on the management of severe idiopathic aplastic anaemia in children

**Jeffrey K. Davies[1,3] and Eva C. Guinan[2,4]**

*[1]Department of Medical Oncology, Dana-Farber Cancer Institute, [2]Department of Pediatric Oncology, Dana-Farber Cancer Institute, [3]Department of Medicine, Brigham and Women's Hospital, and [4]Division of Hematology/Oncology, Children's Hospital, Boston, MA, USA*

## Summary

The current outlook for a child with severe idiopathic aplastic anaemia (AA) is very much better than in previous decades. In part, this may reflect better differentiation of idiopathic and inherited marrow failure. For children with idiopathic AA and a human leucocyte antigen (HLA)-matched sibling donor (MSD), allogeneic haematopoietic stem-cell transplantation (AHSCT) is the primary therapy of choice, offering long-term disease-free survival of 90%, although graft-*versus*-host disease remains a cause of long-term morbidity. A greater treatment challenge remains for those children without a MSD. Combination immunosuppressive therapy (IST) is associated with response rates of 70% or more. However, relapse and clonal evolution with transformation to myelodysplasia or acute myeloid leukaemia remain significant problems after IST and long-term event-free survival rates are less impressive. For children who do not have a sustained response to IST, alternate donor AHSCT should be considered. New HLA typing technologies, novel stem cell sources, reduced-intensity conditioning and graft engineering have reduced toxicity and improved the outcome after alternate donor AHSCT. Emerging therapies that capitalise on recent advances in our understanding of the pathophysiology of idiopathic AA and the immunobiology of AHSCT and IST may further improve the long-term outcome of this disease.

**Keywords:** aplastic anaemia, childhood, haematopoietic stem-cell transplantation, immunotherapy, late effects of therapy.

Childhood aplastic anaemia (AA) is rare, with an annual incidence of 2–6 per million in the USA and Europe. The incidence is higher in India and Japan, resulting from differences in population immunogenetics and environmental factors (Kojima, 2002; Locasciulli, 2002).

Correspondence: Eva C Guinan, Department of Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA. E-mail: eva_guinan@dfci.harvard.edu

Important differences exist between adult and childhood AA. A significant proportion of children presenting with AA have an inherited bone marrow failure syndrome (IBMFS), whereas this association is rare in adults (Kurre *et al*, 2005.) Secondly, although childhood and adult idiopathic (non-IBMFS-associated) AA share pathophysiological mechanisms, biological and genetic differences are increasingly recognised. Thirdly, the outcome of a major treatment modality, allogeneic haematopoietic stem-cell transplantation (AHSCT), is better for children than for adults. Finally, the choice of treatment for a child is differentially influenced by long-term sequelae of both the disease and its therapy. This review will focus on the diagnosis and treatment of severe idiopathic AA in childhood. Current concepts in pathophysiology will be presented in relation to therapeutic options. Finally, treatment options will be considered within the context of the limitations of our current knowledge, with special emphasis on the most challenging management issues.

## Diagnosis

Aplastic anaemia is defined as pancytopenia with a hypocellular bone marrow (BM) without infiltration or fibrosis. To diagnose AA *at least two* of the following must be present: (i) haemoglobin <100 g/l, (ii) platelet count $<50 \times 10^9$/l, (iii) neutrophil count $<1{\cdot}5 \times 10^9$/l (International Agranulocytosis and Aplastic Anemia Study Group, 1987). It is critical to evaluate a BM aspirate and trephine biopsy to accurately assess cellularity and to exclude infiltration or fibrosis. Idiopathic AA may be classified based on severity (Camitta *et al*, 1976; Bacigalupo *et al*, 1988a; Table I). This review will subsequently confine discussion to severe (s) and very severe (vs) AA. Unless otherwise stated, the term sAA is used to include both categories.

## What are the diagnoses that need to be excluded?

Many conditions present in childhood with pancytopenia, some of which may also feature a hypocellular marrow.

 
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**Table I.** Classification of aplastic anaemia according to severity.

| Severity | Criteria |
|---|---|
| Severe | Bone marrow cellularity <25% (or 25–50% if <30% residual haemopoeitic cells) *and* at least two of the following:<br>Peripheral blood neutrophil count $<0{\cdot}5 \times 10^9$/l<br>Peripheral blood platelet count $<20 \times 10^9$/l<br>Peripheral blood reticulocyte count $<20 \times 10^9$/l |
| Very severe | As severe, but peripheral blood neutrophil count $<0{\cdot}2 \times 10^9$/l |
| Non-severe | Hypocellular bone marrow with peripheral blood cytopenias not fulfilling criteria for severe or very severe aplastic anaemia |

Conditions that may cause pancytopenia in children and investigations necessary to exclude them are detailed in Table II. Some children will have reversible infectious, metabolic or nutritional causes. Infections may account for 10–20% of children presenting with pancytopenia (Bhatnagar *et al*, 2005). The prevalence and identity of infections causing cytopenias is likely to vary with geographical location. Others will have acute lymphoblastic or myeloid leukaemia (ALL, AML) or myelodysplastic syndrome (MDS). ALL is the most common haematological malignancy to present with pancytopenia and BM hypoplasia. About 1–2% of cases of childhood ALL are preceded by a period of pancytopenia, often with a hypocellular marrow. Subsequent recovery of blood counts may occur, followed by development of overt leukaemia, usually within a few months (Breatnach *et al*, 1981). Careful morphological, immunophenotypic and cytogenetic analysis of the peripheral blood and BM is mandatory to exclude hypoplastic ALL, and consideration should be given to repeating these investigations 6 weeks after presentation. Treatment with immunosuppressive therapy (IST) may delay diagnosis. Hypoplastic MDS must also be excluded. Morphological dysplasia is often not marked, and an adequate determination of BM cytogenetics is essential. Conventional cytogenetic analysis of hypocellular BM often fails; additional fluorescent *in situ* hybridisation (FISH) for chromosomes 5 and 7 and other available tests should be performed. Monosomy 7 and 5q- have been retrospectively detected in presentation material of children diagnosed with AA who went on to develop AML (Fuhrer *et al*, 1998). Childhood pancytopenia with marrow hypoplasia with monosomy 7 carries a high rate of transformation to poor prognosis AML, and recent guidelines suggest that this condition should be treated as MDS (Marsh *et al*, 2003).

Inherited bone marrow failure syndrome should be excluded in all children presenting with cytopenias and marrow hypoplasia (Table III). Recognition of such disorders is crucial for the management of the child and their family. Diagnosis of IBMFS may be difficult, as clinical and genetic heterogeneity is common. Many children have no family history and some will have no suggestive clinical features at the time of presentation with BM failure. Some IBMFS share a relatively common underlying genetic abnormality facilitating diagnosis (e.g. mutated *C-MPL* in amegakaryocytic thrombocytopenia). In others many separate genetic mutations occur (Fanconi anaemia, FA) or the genetic defect has not been identified (autosomal recessive dyskeratosis congenita), making genetic diagnosis problematic. FA may be diagnosed by the demonstration of diepoxybutane (DEB)-induced chromosomal breakage in peripheral blood lymphocytes. However, a small group of FA patients have DEB-resistant lymphocytes because of spontaneous functional correction resultant from revertant somatic mosaicism (Gregory *et al*, 2001). Increased DEB-induced chromosomal breakage can usually be demonstrated in cultured fibroblasts from such patients. Accurate and timely diagnosis of IBMFS permits the institution of appropriate medical management and education, and recognition of IBMFS has implications for AHSCT and donor selection. Many patients (and affected relatives) are at risk of developing haematological malignancies and solid tumours and life-long monitoring and risk-reduction strategies are required. Management of IBMFS is complex and multi-faceted, requiring the expertise and resources of specialist centres.

## How does the pathophysiology of idiopathic AA relate to therapeutic options?

Significant advances have been made in the understanding of the immune-mediated pathogenesis of sAA in recent years. Haematopoietic colony formation *in vitro* is suppressed by autologous lymphocytes in most idiopathic AA patients. This suppression is mediated by T-cell-helper type 1 (Th1) cytokines [e.g. interferon-γ (IFN-γ)] secreted by cytotoxic T cells (Hoffman *et al*, 1977; Zoumbos *et al*, 1985). Populations of oligoclonally-expanded $CD8^+$ cells have recently been found in idiopathic AA patients, implying a focused immune response directed toward specific autoantigens. Purified oligoclonal T cells selectively kill autologous haematopoietic progenitors. The size of these T-cell populations is quantitatively related to disease activity, and may be useful in predicting relapse after therapy (Risitano *et al*, 2004). The initiation of cytotoxic T-cell activation in idiopathic AA is poorly understood. Certain genetic polymorphisms in Th1 cytokine genes are over-represented in patients with idiopathic AA and genomic-wide transcriptional analysis of $CD4^+$ and $CD8^+$ T cells suggests innate immune system components may be perturbed (Zeng *et al*, 2004; Sloand, 2005). Immune suppression of haematopoiesis is also mediated by $CD4^+$ T cells. $CD4^+$ clones capable of human leucocyte antigen (HLA) class II-restricted lysis of autologous haematopoietic cells have been isolated from AA patients (Nakao *et al*, 1995). While the antigenic target of these autoreactive T cells is unknown, antibody responses specific to kinectin, expressed in all haematopoietic cell lineages including $CD34^+$ cells, have been detected in AA patients (Hirano *et al*, 2003).

 



**Table II.** Differential diagnosis of bi-or pancytopenia in children.

| Category | Condition | Bone marrow appearance | Diagnostic investigation |
|---|---|---|---|
| Aplastic anaemia | Idiopathic | Hypocellular | Exclusion |
| | Associated with IBMFS | Hypocellular | Multiple (Table III) |
| | Pregnancy-associated | Hypocellular | β–hCG |
| | Drug or toxin-associated | Hypocellular | Careful history |
| Megaloblastic anaemia | Acquired deficiency | Hypercellular | B12/folate levels (pretransfusion) |
| | Congenital abnormalities | Hypercellular | Complex metabolic pathway analysis |
| Malignant infiltration | AML | Hypercellular (rarely hypocellular) | BM morphology |
| | ALL | Hyper or hypocellular | Immunocyto/histochemistry |
| | MDS | Hyper or hypocelluar | Immunophenotyping |
| | Hodgkin | Infiltrated, may be hypocellular | Cytogenetics including FISH |
| | Solid tumours | Infiltrated | Molecular analysis |
| | Myelofibrosis | Reticulin fibrosis | |
| | Histiocytic disorders | Hypocelluar, haemophagocytosis | |
| Non-malignant infiltration | Osteopetrosis | Increased bony trabeculae | Trephine biopsy |
| | Storage disorders | Hypercellular, infiltrated | Trephine biopsy |
| Infection | CMV | Hypocellular | Paired serology, IgM and IgG |
| | Influenza A | Hypocellular | PCR for viral DNA |
| | EBV | Hypocellular | |
| | HHV-6 | Hypocellular, haemophagocytosis | |
| | Hepatitis (non A, B or C) | Hypocellular | |
| | HIV | Hyper or hypocellular | |
| | Parvovirus | Hyper/hypocellular giant proerythroblasts | |
| | Tropical infections | Variable | Travel history |
| | | | Immunological assays |
| Metabolic disorders | Anorexia nervosa | Hypocellular ± fat necrosis | Careful history |
| | | | Physical exam |
| | | | Psychiatric evaluation |
| | Hypothermia | Variable | Careful history |
| | | | Physical exam |
| Immune disorders | Evan's syndrome | Hypercellular, increased erythropoiesis | Raised peripheral blood reticulocyte count |
| | Autoimmune lympho-proliferative syndrome | Hypercellular | Immunophenotyping for αβ $TCR^+CD4^-CD8^-$ T cells |
| | | | *In vitro* FAS-mediated apoptosis assay |
| | Thymoma | Hypocellular | Mediastinal imaging |
| | Chronic granulomatous disease | Sea blue histiocytosis | Bone marrow morphology |
| Acquired clonal bone marrow disorder | Paroxysmal nocturnal haemoglobinuria | Variable | Ham's/sucrose lysis test |
| | | | Peripheral blood immunophenotyping for PIG-linked molecules |

IBMFS, inherited bone marrow failure syndrome; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; MDS, myelodysplasia; BM, bone marrow; FISH, fluorescent *in situ* hybridisation; TCR, T-cell receptor; PCR, polymerase chain reaction; HCG, human chorionic gonadotrophin; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV-6, Human herpes virus-6; PIG, phosphatidylinositol glycan.

**Table III.** Inherited bone marrow failure syndromes associated with pancytopenia.

| Syndrome | Age/gender | Haematological presentation | Associated clinical features | Gene mutation | Inheritance pattern | Specific diagnostic investigation |
|---|---|---|---|---|---|---|
| Fanconi anaemia | Usually first decade equal gender distribution | Typically thrombocytopenia followed by progressive pancytopenia with marrow hypoplasia | Skin pigmentation, abnormal thumbs/radii, renal/urinary tract malformations | >12 *FANC* genes identified | Autosomal recessive/ X-linked | Increased chromosomal breakage by DNA cross-linkers in haematopoeitic cells (90%) or fibroblasts (100%) |
| Dyskeratosis congenita | Second decade Male> female | Macrocytosis Thrombocytopenia Pancytopenia with marrow hypoplasia | Dyskeratotic nails, reticular rash, oral lesions | *DKC1* *TERC* ? | X-linked Autsomal dominant Autosomal recessive | None (shortened telomeres seen in some cases) |
| Shwachman–Diamond syndrome | 0–5 years Equal gender distribution | Neutropenia, pancytopenia with marrow hypoplasia | Short stature Pancreatic exocrine insufficiency | *SBDS* | Autosomal Recessive | Decreased serum trypsinogen/isoamylase levels |
| Amegakaryocytic thrombocytopenia | 0–5 years Equal gender distribution | Thrombocytopenia with absent megakaryocytes, Pancytopenia with marrow hypoplasia | Bleeding | *C-MPL* | Autosomal Recessive | None |

AML, acute myeloid leukaemia; MDS, myelodysplasia.

Immunosuppressive agents used in the treatment of AA exert their action at least in part by reducing suppression of haematopoiesis by autoreactive immune cells. Anti-thymocyte globulin (ATG), a polyclonal anti-T-cell antibody-rich serum fraction produced by immunising mammals against human thymic tissue or thymocytes, is both lympholytic, leading to a severe and dose-dependant lymphopenia, and immunomodulatory. It may produce a state of immunological tolerance by preferential destruction of activated T cells (Young *et al*, 2006). ATG also stimulates haematopoietic growth factor and cytokine release from various cell types (Taniguchi *et al*, 1990). Other immunosuppressive agents used in conjunction with ATG have different mechanisms. Ciclosporin A (CSA) selectively inhibits T-cell activation and proliferation by inhibiting calcineurin and downstream nuclear factor $\kappa$B-mediated transcriptional regulation. Mycophenolate mofetil (MMF) inhibits inosine monophosphate dehydrogenase, selectively targeting lymphocyte purine metabolism. In addition to direct haematopoietic activity, granulocyte colony-stimulating factor (G-CSF) may also have an immunomodulatory action. Normal human T cells express G-CSF receptors. When administered to mobilise peripheral blood stem cells (PBSC) in healthy donors, G-CSF causes a shift from Th1 to Th2 cytokine responses (Franzke *et al*, 2003). Thus G-CSF could synergise with IST by reducing Th1 autoimmune effectors directed at haematopoietic cells in patients with AA.

Up to 30% of patients with sAA do not have detectable evidence for an immune aetiology and will not respond to IST, suggesting that alternate or additional pathophysiological mechanisms exist. One such mechanism may be telomeric shortening. Telomeres, structures that protect chromosomes in human somatic cells from recognition as damaged DNA, are gradually lost after repeated cell division, resulting in arrested proliferation, apoptosis and genomic instability. Haematopoietic stem cells, which must retain proliferative capacity, maintain telomere integrity via the telomerase ribonucleoprotein complex consisting of telomerase reverse transcriptase (*TERT*) and its integral RNA template (*TERC*). Abnormal telomere shortening in haematopoietic cells occurs in some patients with idiopathic AA (Ball *et al*, 1998). Telomere shortening is consistent with the concept of haematopoietic progenitor cell exhaustion, and suggests that defective telomerase maintenance might have a role in the aetiology of sAA. Mutations in both the *TERT* and *TERC* genes are infrequently found in adults with idiopathic AA or their unaffected family members (Yamaguchi *et al*, 2003, 2005). A retrospective analysis of 300 children with idiopathic AA found *TERC* mutations in <1% of cases (Field *et al*, 2006). Other, as yet undetermined, genes might be mutated in patients with AA and shortened telomeres, or telomeric shortening may occur as a result of increased division in a reduced pool of haematopoietic precursors. Although correcting reduced telomerase activity represents a potential therapeutic target, the importance of telomeric shortening in the pathogenesis of childhood AA remains unclear. Allogeneic transplantation has curative


 

potential by replacing both myeloid and lymphoid compartments and thus can correct both immune and constitutional defects (including shortened telomeres) potentially important in the pathogenesis of AA.

Increases in our understanding of the pathophysiology of idiopathic AA may lead to the development of novel treatment modalities. Profiling of the transcriptome of $CD4^+$ and $CD8^+$ T cells and of haematopoietic progenitors in patients with idiopathic AA has revealed hundreds of differentially expressed genes that may serve as new therapeutic targets (Zeng *et al*, 2004, 2006).

## What is the significance of the presence of a PNH clone?

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired condition caused by somatic mutation of the X-linked phosphatidylinositol glycan A gene (*PIGA*), resulting in deficient expression of glycosylphosphatidylinositol-anchored proteins (GAPs). PNH, characterised by intravascular haemolysis and thrombosis, is rare in its classical form in children; only small case series have been published (Ware *et al*, 1991; van den Heuvel-Eibrink *et al*, 2005). However, 70% of these children had co-existing AA (AA/PNH). Thrombosis should be considered as a cause of abdominal pain in such children. The treatment of BM failure with IST and ATG has been reported to precipitate severe acute haemolysis in adults with AA/PNH and chronic intravascular haemolysis; some investigators recommend avoidance of ATG in such patients (Ebenbichler *et al*, 1996). Haemolysis has been successfully treated with the novel anti-complement antibody eculizumab in adults with classical PNH and this treatment warrants investigation in children with AA/PNH as it might permit safer subsequent administration of ATG (Hall *et al*, 2003).

In addition to individuals with AA/PNH, 20–40% of adults with sAA without laboratory or clinical evidence of classic PNH have clonal populations of cells with reduced expression of GAPs ('PNH clones') detectable by flow cytometry (Maciejewski *et al*, 2001). So far, no large cohorts of children with sAA systematically tested for the presence of PNH clones by flow cytometry are available. However, 40% of children with AA had GAP-deficient cells detectable in BM by immunohistochemistry (Rizk *et al*, 2002). Such clones may possess growth advantages in a BM microenvironment subjected to immune suppression and their significance is unknown. In reports combining adults and children with AA, neither the presence at diagnosis nor the subsequent emergence of PNH clones were associated with altered response rate or overall survival after IST (De Lord *et al*, 1998; Piaggio *et al*, 1999).

## What is the importance of cytogenetic abnormalities present at diagnosis?

Bone marrow hypoplasia with monosomy 7 or 5q- at diagnosis is usually treated as MDS, even in the absence of morphological dysplasia. Some children with AA have other cytogenetic abnormalities at diagnosis without morphological dysplasia; such abnormalities do not necessarily confer a poor prognosis. Many (but not all) centres treat such children as conventional AA patients, including close cytogenetic and morphological monitoring of the BM. Cytogenetic abnormalities at diagnosis were found in (4%) (seven of 159 successful karyotypings) of 200 Japanese children with AA. In this study, FISH was not used to detect abnormalities undetectable by G-banding, and abnormalities may have been present in some of those in whom cytogenetic analysis failed: thus the true incidence of cytogenetic abnormalities may be higher. Only children with del(13) and del(20) did not respond to primary IST (Ohga *et al*, 2002).

The relationship of trisomies and response to IST reported in adults (Gupta *et al*, 2006) has yet to be examined in children. Cytogenetic abnormalities present at diagnosis may persist after IST despite haematological responses; such persistence has been associated with an increased risk of transformation to AML in some but not all studies (Mikhailova *et al*, 1996; Geary *et al*, 1999).

## What is the natural history of AA?

The one-year mortality for patients with sAA treated with transfusion only was >80% in an older, retrospective series (Williams *et al*, 1978). Early spontaneous remissions of sAA in childhood are reported but rare, often associated with the identification and treatment of infection such as Hepatitis A (Smith *et al*, 1978). Some data suggests that the natural history of AA may be heterogeneous with a more benign outcome in some patients, and that this may not be restricted to those with non-severe AA. In a large cohort of (mostly) sAA patients diagnosed in the 1980s and treated only with androgens, mortality was 58% at 2 years, 60% at 5 years and 65% at 12 years. The actuarial risk of death related to AA in those that survived to 5 years was 14% at 15 years after diagnosis; some had late spontaneous recovery of cytopenias: most reported good quality of life (Najean & Haguenauer, 1990).

AA may relapse or occur *de novo* during pregnancy, and may improve spontaneously after delivery or termination (Goldstein & Coller, 1975; Meletis *et al*, 1998).

The natural history of non-severe AA in children is more benign (Howard *et al*, 2004).

## What supportive care should be given?

Blood products should be administered as needed to ensure patient safety. Children should receive leucocyte-depleted products to reduce the chance of alloimmunisation and cytomegalovirus (CMV)-negative products should be given (unless known to be CMV-seropositive) unless AHSCT is not a potential option. The placement of an indwelling vascular access device should be considered in all children with sAA to facilitate transfusion and administration of intravenous med-

 

ications and to ease repeated phlebotomy. Transfusion management should include an ongoing plan for detection of transfusion-related iron overload and its complications. Ideally iron chelation should commence when the serum ferritin is >2000–2500 μg/l, although each patient should be assessed individually. Families should be counselled to avoid paediatric multivitamins containing iron.

Menarche should be discussed with all pubertal or immediately prepubertal girls, as onset of menstruation may be associated with sudden and severe haemorrhage. A plan for medical suppression of menstruation should be made before this occurs. Equally, menstrual suppression should be initiated in all actively menstruating girls.

Haematopoietic growth factors, such as G-CSF, stimulate haematopoietic precursors and can occasionally result in a temporary rise in neutrophil counts. Such growth factors may be useful in patients with severe neutropenia with symptomatic infections although no survival benefit has been demonstrated (Kojima *et al*, 1991). The long-term use of G-CSF has been associated in some patient cohorts with acquisition of cytogenetic abnormalities and the evolution of AA to MDS and AML (Kojima *et al*, 2002a). Prevention of infection remains a difficult issue for children with sAA. Morbidity and mortality from bacterial and fungal infections is related to the degree of neutropenia and its duration. However, non-absorbable antibiotics and antifungals are unpalatable and compliance is poor and no evidence-based algorithms have been developed in this population. The importance of antimicrobial prophylaxis should thus be determined on an individual basis in the context of degree of neutropenia, and the history of infection. No consensus exists regarding the dietary and social limitations that should be advised other than avoidance of siblings and classmates with active infections and contact with pets that might increase the risk of exposure to zoonotic infections. Issues of sexual practice as they relate to potential for infectious and haemorrhagic complications should be discussed in a culturally and age appropriate manner.

## A simple treatment algorithm

Barring specific contraindications, patients with sAA should proceed straight to AHSCT if a fully matched sibling donor (MSD) is available. If not, a registry search for an unrelated donor should be initiated immediately and combination IST should be administered. Further treatment options depend on the response to IST and the availability of alternate stem cell donors (Fig 1).

## What is the current outcome of matched sibling donor AHSCT?

New conditioning regimens, graft-*versus*-host disease (GvHD) prophylaxis and better supportive care have significantly improved failure-free survival after MSD AHSCT for AA. A recent retrospective study of 37 children with idiopathic sAA receiving MSD AHSCT reported a 10-year failure-free survival rate of 97% (Kojima *et al*, 2000a). AHSCT should be performed as soon as possible after diagnosis. Prior to universal leucodepletion of blood products, significant preAHSCT transfusion and alloimmunisation was strongly associated with graft rejection (Storb *et al*, 1977). Delayed MSD AHSCT with preceding IST and transfusion support increases the incidence of graft rejection in children with AA (Kobayashi *et al*, 2006).

## What is the best conditioning regimen?

Early regimens containing high-dose cyclophosphamide (CY) led to high rates of graft rejection (Champlin *et al*, 1989). The addition of total body irradiation (TBI) and use of higher

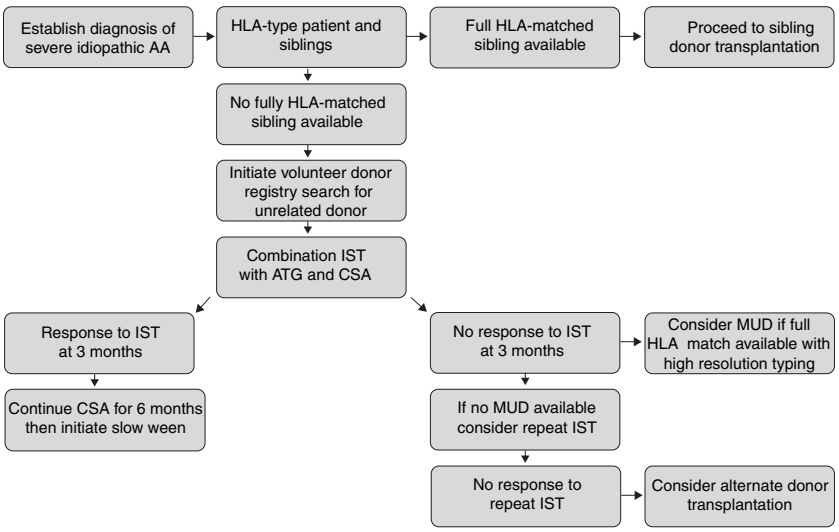


**Fig 1.** A simple algorithm for the treatment of severe aplastic anaemia in childhood. AA, aplastic anaemia; HLA, human leucocyte antigen; IST, immunosuppressive therapy; ATG, anti-thymocyte globulin; CSA, ciclosporin A; MUD, matched unrelated donor.

stem-cell doses improved engraftment rates, but increased toxicity. Highly immunosuppressive but radiation-free conditioning regimens containing CY and ATG have permitted excellent engraftment rates with lower toxicity (McCann *et al*, 1994). In a recent report of 81 children and adults with sAA undergoing MSD AHSCT conditioned with CY and ATG, 96% had sustained engraftment with 3% developing severe acute GvHD and 26% chronic GvHD. Overall survival was 88% with a median follow-up of 9 years (Kahl *et al*, 2005). The European Bone Marrow Transplant (EBMT) consortium recommend CY 200 mg/kg total dose and ATG daily for 4 d (Bacigalupo *et al*, 2005). Interestingly, a prospective, randomised study of AA patients of all ages undergoing MSD AHSCT using CY with or without ATG produced equivalent rates of engraftment, late rejection and GvHD with overall survival of 80% in both arms (Champlin *et al*, 2003).

There is no consensus yet regarding the optimum conditioning regimen for MSD umbilical cord blood (UCB) cell transplantation for childhood sAA.

## What is the best stem cell source?

Bone marrow, which may contain stromal cells with potential pro-engraftment and immunomodulatory effects, has traditionally been the stem cell source of choice. BM may indeed be the preferred source, but not for reasons related to engraftment. In a recent combined retrospective International Bone Marrow Transplant Registry (IBMTR)/EBMT study there was no difference in graft failure rates amongst AA patients receiving MSD BM or PBSC. However, BM recipients had significantly less chronic GvHD and better overall survival (Schrezenmeier *et al*, 2003). There are also practical reasons to favour BM in the paediatric population. Most MSDs of children are also children, from whom BM may be easier to obtain. The use of allogeneic BM does not require the administration of growth factor to the donor, which may, even in short courses, be associated with a small but finite risk of acute and long-term complications (Bennett *et al*, 2006). Donation of both BM and PBSC by a child requires consent (and in some countries independent medical assessment), which may raise specific ethical issues.

## Umbilical cord blood transplantation from matched sibling donors

The first reported use of UCB was from a sibling donor to transplant a patient with FA (Gluckman *et al*, 1989). Although, most subsequent reported experience is with unrelated donors, there is limited experience with MSD UCB transplants in children with both malignant and non-malignant disorders (Wagner *et al*, 1995). Better rates of engraftment (>80%) have been achieved with higher doses of nucleated cells (Locatelli *et al*, 1999). Unfortunately there is no potential for additional cells doses to be obtained if graft failure occurs.

A joint study by Eurocord and the IBMTR compared the outcome of 113 children who received UCB transplants from MSDs to children receiving MSD BM transplants. In this study (which contained eight children with idiopathic AA who received UCB transplants) MSD UCB transplantation was associated with slower neutrophil engraftment but less acute and chronic GvHD, although overall survival was the same in both groups (Rocha *et al*, 2000).

Arrangements should be made to store UCB from siblings of children with severe AA wherever possible for potential future use, particularly in those who lack existing MSDs and/or matched unrelated donors (MUDs). Antenatal HLA typing can be performed but this strategy is most commonly employed in families of children with IBMFS.

## What is the best GvHD prophylaxis?

Twenty-year follow-up confirms that the addition of CSA to short course methotrexate (MTX) reduced acute GvHD and improved overall survival in a randomised study of patients of all ages with sAA undergoing MSD AHSCT (Sorror *et al*, 2005). CSA and short course MTX remain the standard for GvHD prophylaxis (Schrezenmeier *et al*, 2000). Tacrolimus, an alternate calcineurin inhibitor resulted in reduced rates of acute GvHD (when used in conjunction with short course MTX) when compared with CSA in a randomised study in adults with haematological malignancies receiving MSD AHSCT (Ratanatharathorn *et al*, 1998). Tacrolimus has been used in paediatric patients but has not been directly compared with CSA in randomised studies in patients with AA. Tacrolimus has a similar toxicity profile to CSA. Age-dependent pharmacokinetics of calcineurin inhibitors, such as CSA and tacrolimus, suggest that careful therapeutic level monitoring within the paediatric population is required (Yee *et al*, 1988; Przepiorka *et al*, 2000). MMF has been used successfully in conjunction with tacrolimus as GvHD prophylaxis in paediatric patients, sparing the use of MTX (which can delay engraftment). There is not enough evidence to specifically support the use of these newer agents as GvHD prophylaxis for MSD AHSCT for childhood AA outside the setting of clinical trials (Osunkwo *et al*, 2004).

## What are the prognostic factors for outcome for MSD AHSCT?

Retrospective series of sAA patients of all ages have shown better overall survival in multivariate analysis of younger patients (<15 years), those conditioned with CY-ATG and those transplanted without any form of prior treatment (Ades *et al*, 2004).

IBMTR data support the importance of age on outcome and suggest that duration of aplasia prior to MSD AHSCT, clinical status and transfusion history are also relevant. These registry data did not suggest that outcome was related to severity of AA treated with MSD AHSCT (Horowitz, 2000).

 

Series of MSD patients conditioned with CY/ATG have either lacked multivariate analysis (Storb *et al*, 2001) or found age to be the only factor significantly impacting on outcome (Kahl *et al*, 2005).

## Long-term effects of AHSCT

Unlike intensive AHSCT regimens that frequently perturb growth and development, non-TBI containing regimens for AA often result in normal growth, attainment of predicted adult heights and well-preserved fertility (Eapen *et al*, 2000; Sanders, 2004). Nonetheless, routine assessments of growth and development, dermatological status, endocrine and pulmonary function and bone mineral density should be made and appropriate counselling, including that related to fertility, should be provided to patients and families. Attention to immunisation status is important. Delayed infectious complications, dermatological issues (including scleroderma), cataracts, pulmonary insufficiency and bone and joint problems may present after MSD AHSCT for AA (Deeg *et al*, 1998). Some of these toxicities are related to the IST needed for chronic GvHD. Despite the benefits of reduced-intensity conditioning and the routine use of CSA for GvHD prophylaxis, the cumulative incidence of chronic GvHD after sibling AHSCT for AA is over 40% (Ades *et al*, 2004). AA patients with chronic GvHD have an impaired quality of life and reduced overall survival (Deeg *et al*, 1996a).

The development of secondary malignancies is another concern after AHSCT for AA (Pierga *et al*, 1994). In a series that combined 621 patients with idiopathic AA from Paris and Seattle, the actuarial risk of developing a secondary malignancy was 14% at 20 years, with an early excess of lymphoproliferative disorders that declined after 2 years and a sustained excess of solid tumours, particularly after irradiation-based conditioning or azathioprine for chronic GvHD (Deeg *et al*, 1996b).

## Immunosuppressive therapy

Combining ATG and CSA has considerably improved upon ATG alone, resulting in overall response rates in children of 80% (Bacigalupo *et al*, 1988a).

However, major areas of uncertainty remain regarding combination of IST. They include how and when response should be assessed, the role of additional G-CSF, the impact of severity on response rates and duration and long-term sequelae of IST.

## What factors predict response after IST?

Little paediatric-specific information is reported regarding predictors of response to IST. Unsurprisingly, the presence of a population of cytotoxic T cells with increased levels of intracellular IFN-$\gamma$ in patients with sAA has recently been shown to be associated with response to combined IST (Sloand *et al*, 2002). Interestingly, patients with idiopathic AA and evidence of non-immune pathogenesis of aplasia (in the form of *TERC* mutations and/or telomere shortening) have exhibited poor responses to IST (Yamaguchi *et al*, 2005).

Response to IST has recently been associated with the presence of low frequency PNH clones in patients with AA, although the PNH clone may be a surrogate marker. *In vitro* hyper-responsiveness of PNH clones to IST has not been convincingly demonstrated (Sugimori *et al*, 2006). Unlike MDS, responses to IST in AA patients are not associated with the *DRB1**1502 allele (Sauntharajah *et al*, 2002).

Early retrospective analyses of combined IST demonstrated worse survival in patients with vsAA than those with sAA (Locasciulli *et al*, 1990). A large prospective multicentre trial of CSA and ATG in 151 children with idiopathic sAA (97 of whom had vsAA) reported better overall 5-year survival in children with vsAA (93%) *versus* sAA (81%). Children with vsAA were all given additional G-CSF, which has immunomodulatory effects and may augment IST (*vide supra*) (Fuhrer *et al*, 2005).

## How and when should response after IST be measured?

Response to IST is categorised as complete, partial or no response. These categories are based on the combination of peripheral blood counts and transfusion requirement (Table - IV; Schrezenmeier, 1999). Improvement in peripheral blood counts after IST occurs slowly, with response rates increasing over time: 86 children treated with ATG and CSA had overall response rates of 61%, 74% and 80% at 3, 6 and 12 months respectively (Fuhrer *et al*, 1998). It is therefore important to allow sufficient time (3–6 months) for responses to occur after IST. Administering two closely spaced courses of IST did not improve frequency or kinetics of responses in children (Matloub *et al*, 1997).

## What combination of IST should be given?

Randomised studies, combining adults and children, reported improved response rates (but not overall survival) with the addition of CSA to ATG (65% at 6 months *versus* 31%;

**Table IV.** Criteria for response after immunosuppressive therapy for severe aplastic anaemia in children.

| Response category | Criteria |
|---|---|
| Complete response | No transfusion support |
| | Haemoglobin normal for age and gender |
| | Absolute neutrophil count $>1{\cdot}5 \times 10^9$/l |
| | Platelet count $>150 \times 10^9$/l |
| Partial response | No transfusion support |
| | Does not meet criteria for severe aplastic anaemia |
| No response | Meets criteria for severe aplastic anaemia |

Frickhofen *et al*, 2003). Combined IST with ATG and CSA is now considered the treatment of choice for children with idiopathic sAA without a MSD (Bacigalupo *et al*, 1988a). A minority of patients who respond to IST with ATG and CSA, relapse upon CSA withdrawal. Some such patients may recapture and maintain responses if a very slow wean of CSA is performed (Fuhrer *et al*, 1998).

The effect of G-CSF when administered with combined IST is the focus of an ongoing multicentre EBMT trial, although the excellent outcomes in the recent German study support the routine use of G-CSF as an adjunct to IST in children with vsAA (Fuhrer *et al*, 2005).

The addition of MMF to ATG and CSA did not improve response rates or survival in a large recent non-randomised study containing both adults and children (Scheinberg *et al*, 2006).

Large recent paediatric series of combined IST are summarised in Table V.

## Who should get repeat courses of IST?

Twenty per cent of children fail to respond to a first course of combined IST. 30% of primary non-responders achieve a complete response with a second course of IST and nearly 80% achieve transfusion-independence (Di Bona *et al*, 1999). Re-treatment 3–6 months after a first unsuccessful IST course is therefore advisable. In contrast, response to a third course of IST has been restricted to those responding previously; thus the administration of a third course of IST to patients without prior response cannot be recommended (Gupta *et al*, 2005a).

In general, the majority of patients relapsing after a primary response to IST respond to a second course (Rosenfeld *et al*, 1995). A retrospective EBMT study of adults and children with sAA responding to a primary course of IST reported an actuarial incidence of relapse of 35% at 14 years after IST. Relapse was not related in this cohort to the severity of disease, age, or gender. Half of relapsing patients responded to a second course of IST, most of which were sustained long term. Responses after relapse were associated with early response to previous IST. The survival of relapsing patients who responded again to IST was similar to patients who did not relapse and significantly better than the patients not reaching a second response after relapse (Schrezenmeier *et al*, 1993).

## Clonal evolution after IST

Perturbation of the BM microenvironment by IST and resultant immune suppression may favour the development of clonal haematopoietic populations over time. This process, clonal evolution, may result from a relative survival advantage of clones over other haematopoietic cells suppressed by autologous cytotoxic T cells or outgrowth of cells not regulated by IST. There is some degree of overlap between the diagnoses of AA, MDS and PNH and the clinical syndrome may be dictated by the relative size of clonal cell populations. Clonal cytogenetic abnormalities developed with a cumulative incidence of 14% after a median of 37 months (range 9–81) in a cohort of over 100 children with normal cytogenetics at the time of diagnosis of AA treated with IST (with or without G-CSF; Kojima *et al*, 2002a). Monosomy 7 was the most common abnormality, with a variety of structural and numerical abnormalities of other chromosomes accrued by other patients. All patients acquiring monosomy 7 developed morphological evidence of MDS and had a poor outcome. The rate of clonal evolution appears constant over time and is independent of response to IST (Frickhofen *et al*, 2003). Transient chromosomal abnormalities are infrequently observed. In an adult series numerical and/or structural abnormalities of chromosome 7 accounted for 40% of cases, with other abnormalities occurring less frequently. Unlike primary

**Table V.** Recent large trials of immunosuppressive therapy for severe aplastic anaemia in children.

| Source | Patients | Median age (years) | Immunosuppressive Therapy | Response at 6 months | Relapse (F/U) | Survival (F/U) | Reference |
|---|---|---|---|---|---|---|---|
| Japan | 119 | 9 | ATG<br>CSA<br>Danazol ± GCSF | 55–77%<br>(CR + PR) | 22% (3 years) | 88% (3 years) | (Kojima *et al*, 2000b) |
| Germany | 146 | 9 | ATG<br>CSA<br>G-CSF* | 61%<br>(CR) | 14% (5 years) | 89% (5 years) | (Fuhrer *et al*, 2005) |
| Europe | 50 | <16 | ATG<br>CSA<br>Methylprednisolone<br>G-CSF | 77%<br>(CR + PR) | 12%† (3 years) | 88% (4 years) | (Bacigalupo *et al*, 2000) |

ATG, antithymocyte globulin; CSA, cyclosporin A; G-CSF, granulocyte colony-stimulating factor; CR, complete response; PR, partial response; F/U, follow up.

*G-CSF if neutophil count $<0{\cdot}5 \times 10^9$/l.

†Relapse is for children and adults combined.

 

MDS, abnormalities of chromosome 5 and 20 were infrequent. Acquisition of chromosome 7 abnormalities was seen most often in patients who had failed to respond to IST. In contrast, trisomy 8 developed in patients with good haematological responses to IST who often required chronic immunosuppression with CSA. Leukaemic transformation occurred mostly in patients with abnormalities of chromosome 7 or complex cytogenetic alterations (Maciejewski *et al*, 2002). Thus, the prognostic significance of clonal evolution in adults after IST may vary according to the specific acquired cytogenetic abnormality. Similar information is not yet available in paediatric populations.

## Who should be considered for alternate donor transplantation?

Children with idiopathic sAA who lack a MSD and fail to respond to IST should be considered for alternate donor AHSCT. Early attempts to use MUDs resulted in long-term survival rates of only 30–40% (Bacigalupo *et al*, 1988b; Margolis *et al*, 1996; Henslee-Downey *et al*, 1997). Prolonged prior duration of aplasia, high rates of graft rejection and GvHD, and increased toxicity resulting from more aggressive conditioning, contributed to this poor outcome. However, earlier AHSCT, improvements in HLA-typing technology and novel conditioning regimens have led to greatly improved outcomes after alternate donor AHSCT for AA in recent years.

## MUD *versus* mismatched donor transplantation?

Alternate donors include MUDs, mismatched (MM) UDs and mismatched related donors (MMRDs). While a retrospective analysis of 318 AA patients receiving low resolution HLA-typed alternate donor transplants demonstrated no significant differences in multivariate analysis in rates of graft failure, GvHD or overall survival according to donor type (MUD, MMUD or MMRD; Passweg *et al*, 2006), superior survival of AA patients with use of fully matched UDs (by high resolution HLA typing) rather than HLA class I MMUDs (Kojima *et al*, 2002b) has been reported. Thus, the prognostic importance of single-antigen donor mismatches may therefore vary according to the HLA typing technology used as well as the locus of individual HLA antigen mismatch. It seems prudent to select a fully matched UD; however mismatched donors should be considered if a MUD is unavailable.

## What is the best stem cell source?

Most published studies use unmanipulated BM. The use of unrelated donor PBSC has been associated in some studies with an increased incidence of extensive chronic GvHD (Remberger *et al*, 2005). Any graft-*versus*-leukaemia effect that extensive chronic GvHD may confer has not been shown to benefit patients with AA. An IBMTR analysis of children and adolescents undergoing AHSCT with PBSC or BM for haematological malignancy demonstrated an increase in chronic GVHD, treatment-related mortality, treatment failure and mortality with PBSC (Eapen *et al*, 2004) Similar data confined to AA is currently unavailable. A number of groups have reported encouraging results using $CD34^+$ selected PBSC as a source of haematopoietic progenitors from alternate donors for AA patients and even from haploidentical parents (Kremens *et al*, 2001; Benesch *et al*, 2004; de la Rubia *et al*, 2005).

## Newer conditioning regimens

Use of conventional MSD conditioning regimens using alternate donors resulted in poor engraftment (Deeg *et al*, 1994) and alternative approaches have subsequently been explored (Table VI). Low-dose TBI (200 cGy) improved survival when added to ATG and CY (200 mg/kg total dose) in a cohort of children with sAA receiving BM transplants (Deeg *et al*, 2006). Substitution of Campath 1H for ATG in combination with high-dose CY (200 mg/kg total dose) and low-dose TBI (300cGy) in eight children with sAA resulted in 100% engraftment and minimal toxicity (Vassiliou *et al*, 2001). Fludarabine (FLU) may permit irradiation-free engraftment. FLU/low-dose CY/ATG resulted in a 2-year actuarial risk of graft failure and survival of 5% and 84% respectively in 19 children with sAA transplanted from alternate donors. This regimen is currently recommended by the EBMT for alternate donor transplantation for AA (Bacigalupo *et al*, 2005).

## GvHD prophylaxis

CSA and short MTX are most widely used as GvHD prophylaxis with FLU/CY/ATG conditioning. UD AHSCT for childhood AA with FLU/CY and *in vivo* Campath 1H followed by CSA results in little GvHD and permits the omission of MTX (Gupta *et al*, 2005a). The role of alternative pharmacological agents for GvHD prophylaxis in this patient group remains undetermined. While the use of tacrolimus is widespread with reasonable paediatric experience, the use of agents such as sirolimus and MMF in AA AHSCT should be limited to clinical trials or children intolerant of calcineurin inhibitors.

## Unrelated donor umbilical cord blood transplantation

Unrelated donor UCB transplantation has been used extensively in children with both malignant and non-malignant conditions (Rubinstein *et al*, 1998). In a matched pair analysis combining children with haematological malignancies and AA, the use of MUD UCB was associated with a similar outcome to MUD marrow, suggesting that unrelated UCB should be

**Table VI.** Alternate donor allogeneic stem-cell transplantation for severe idiopathic aplastic anaemia in children (selected recent studies).

| Source | Patients | Donor | Conditioning therapy | Graft failure (%) | GvHD acute/chronic | Survival (F/U) | Reference |
|---|---|---|---|---|---|---|---|
| UK | 8 | MUD<br>MMUD | CY<br>Campath<br>Low-dose TBI<br>(300 cGy) | 0 | 25%/0% | 100% (3 years) | (Vassiliou *et al*, 2001) |
| Austria | 9 | MUD<br>MMUD | CY<br>ATG/OKT3<br>TLI/TBI | 0* | 0%/0% | 89% (4 years) | (Benesch *et al*, 2004) |
| UK | 7† | MUD | CY<br>Campath<br>Fludarabine | 0 | 0%/10% | 70% (2 years) | (Gupta *et al*, 2005b) |
| Europe | 19‡ | MUD | CY<br>ATG<br>Fludarabine | 5 | 11%/27%§ | 84% | (Bacigalupo *et al*, 2005) |
| USA<br>Germany<br>UK | 47¶ | MUD<br>MMUD | CY<br>ATG<br>Low-dose TBI<br>(600/400/300 cGy) | 5** | 74%/55%** | 73% (5 years) | (Deeg *et al*, 2006) |

(M)MUD, (Mis) matched unrelated donor; CY, cyclophosphamide; TB/LI, total body/lymphoid irradiation; ATG, antithymocyte globulin; cGy, centiGray; GvHD, graft-*versus*-host disease.
*$CD34^+$ selected PBSC used.
† Three patients had Fanconi anaemia.
‡ ⩽14 years old.
§Data from entire cohort of 38 adults and children.
¶<20 years old.
**Data from entire cohort of 87 adults and children.

considered as a possible alternative to unrelated marrow (Barker *et al*, 2001). Small series of adults with idiopathic AA receiving UD UCB transplants after conditioning with CY/ATG were reported to show 80% engraftment (Mao *et al*, 2005). Experience in paediatric idiopathic AA is much more limited, with detailed information only available in a few case reports (Schwinger *et al*, 1999; Ohga *et al*, 2006). Experience is much wider for IBMFS.

The establishment of UCB banks will extend the availability of UCB to children with AA. However, concerns exist regarding increased infectious complications after UD UCB transplantation, and there is no consensus regarding the optimal cell dose and conditioning regime (Barker *et al*, 2005).

## New approaches to graft engineering in HLA mismatched transplantation

Several groups have developed strategies to selectively remove or disable donor alloreactive T cells to reduce GvHD without impairing pathogen-specific immunity after haploidentical donor AHSCT. Three studies have demonstrated the feasibility of these approaches in children with IBMFS and severe idiopathic AA (Guinan *et al*, 1999; Andre-Schmutz *et al*, 2002; Amrolia *et al*, 2006). Protocols improving the safety of haploidentical transplantation would greatly increase the pool of donors for AHSCT available to children with AA and this remains an exciting area of translational research.

## Conclusions and future challenges

Improved understanding of the immunopathogenesis of idiopathic AA should enable the development of therapies that manipulate molecular pathways involved in the activation of auto-reactive T cells and their suppressive effect on haematopoietic progenitor cells. In the meantime, for those with a MSD, AHSCT offers an excellent chance of long-term disease-free survival, although this treatment is still limited by the long-term complications of GvHD and secondary malignancy. For those patients who lack a MSD, combination IST offers increasingly good long-term responses, but treatment failure, disease recurrence and clonal evolution remain major concerns.

Recent encouraging results with alternate donor AHSCT have improved the outlook for patients without a MSD who do not respond to IST. Further innovations in conditioning regimens and stem-cell manipulation are needed to routinely produce acceptable outcomes.

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