bjh guideline

Guidelines for the diagnosis and management of aplastic anaemia

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Introduction

The guideline group was selected to be representative of UK-based medical experts, experienced district general hospital haematologists and a patient representative. MEDLINE and EMBASE were searched systematically for publications in English from 2004 to 2008 using key word aplastic anaemia. The writing group produced the draft guideline which was subsequently revised by consensus by members of the General Haematology Task Force of the British Committee for Standards in Haematology. The guideline was then reviewed by 59 practising UK haematologists, the BCSH (British Committee for Standards in Haematology) and the British Society for Haematology Committee and comments incorporated where appropriate. Criteria used to quote levels and grades of evidence are as outlined in appendix 3 of the Procedure for Guidelines Commissioned by the BCSH (http://www.bcshguidelines.com/process1.asp#App3) and given at the end of this Guideline as Appendix I. The objective of this guideline is to provide healthcare professionals with clear guidance on the diagnosis and management of patients with acquired aplastic anaemia. The guidance may not be appropriate to patients with inherited aplastic anaemia and in all cases individual patient circumstances may dictate an alternative approach. Because aplastic anaemia is a rare disease, many of the statements and comments are based on review of the literature and expert or consensus opinion rather than on clinical studies or trials.

Guidelines update

A previous guideline on the diagnosis and management of aplastic anaemia was published in this journal (Marsh *et al*, 2003). This guideline is an update of the 2003 guideline and is to replace the 2003 guideline (Marsh *et al*, 2003).

Summary of key recommendations

- **Aplastic anaemia (AA) is a rare but heterogeneous disorder. The majority (70–80%) of these cases are categorised as idiopathic because their primary aetiology is unknown. In a subset of cases, a drug or infection can be identified that precipitates the bone marrow failure/aplastic anaemia, although it is not clear why only some individuals are susceptible. In approximately 15–20% of patients the disease is constitutional/inherited, where the disease is familial and/or presents with one or more other somatic abnormalities.**
- **Careful history and clinical examination is important to help exclude rarer inherited forms.**
- **A detailed drug and occupational exposure history should always be taken. Any putative drug should be discontinued and should not be given again to the patient. Any possible association of aplastic anaemia with drug exposure should be reported to the Medicines and Healthcare products Regulatory Agency (MHRA) using the Yellow card Scheme.**
- **All patients presenting with aplastic anaemia should be carefully assessed to:**
  - **(i) confirm the diagnosis and exclude other possible causes of pancytopenia with hypocellular bone marrow.**
  - **(ii) classify the disease severity using standard blood and bone marrow criteria.**
  - **(iii) document the presence of associated paroxysmal nocturnal haemoglobinuria (PNH) and cytogenetic clones. Small PNH clones, in the absence of haemolysis, occur in up to 50% of patients with aplastic anaemia and abnormal cytogenetic clones occur in up to 12% of patients with aplastic anaemia in the absence of myelodysplatic syndrome (MDS).**

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(iv) **exclude a possible late onset inherited bone marrow failure disorder.**

- **A multidisciplinary team (MDT) approach to the assessment and management of newly presenting patients is recommended. A specialist centre with expertise in aplastic anaemia should be contacted soon after presentation to discuss a management plan for the patient.**
- **Best supportive care**

(i) **Prophylactic platelet transfusions should be given when the platelet count is $<10 \times 10^9$/l (or $<20 \times 10^9$/l in the presence of fever).**

(ii) **There is no evidence to support the practice of giving irradiated blood components except for patients who are undergoing bone marrow transplantation (BMT). We would recommend empirically that this practice is extended to patients receiving immunosuppressive therapy.**

(iii) **Transfusion of irradiated granulocyte transfusions may be considered in patients with life-threatening neutropenic sepsis.**

(iv) **The routine use of recombinant human erythropoietin (rHuEpo) in aplastic anaemia is not recommended. A short course of granulocyte colony-stimulating factor (G-CSF) may be considered for severe systemic infection that is not responding to intravenous antibiotics and anti-fungal drugs, but should be discontinued after 1 week if there is no increase in the neutrophil count.**

(v) **Prophylactic antibiotic and antifungal drugs should be given to patients with neutrophil count $<0{\cdot}5 \times 10^9$/l. Intravenous amphotericin should be introduced into the febrile neutropenia regimen early if fevers persist despite broad spectrum antibiotics.**

(vi) **Iron chelation therapy should be considered when the serum ferritin is >1000 μg/l.**

- **Definitive treatment**

(1) **Infection or uncontrolled bleeding should be treated first before giving immunosuppressive therapy. This also applies to patients scheduled for BMT, although it may sometimes be necessary to proceed straight to BMT in the presence of severe infection as a BMT may offer the best chance of early neutrophil recovery.**

(2) **Haemopoietic growth factors such as rHuEpo or G-CSF should not be used on their own in newly diagnosed patients in an attempt to 'treat' the aplastic anaemia.**

(3) **Prednisolone should not be used to treat patients with aplastic anaemia because it is ineffective and encourages bacterial and fungal infection.**

(4) **Allogeneic BMT from a human leucocyte antigen (HLA)-identical sibling donor is the initial treatment of choice for newly diagnosed patients if they have severe or very severe aplastic anaemia, are <40 years old and have an HLA-compatible sibling donor. There is no indication for using irradiation-based conditioning regimens for patients undergoing HLA-identical sibling BMT for aplastic anaemia. The recommended source of stem cells for transplantation in aplastic anaemia is bone marrow.**

(5) **Immunosuppressive therapy is recommended for (i) patients with non-severe aplastic anaemia who are transfusion dependent (ii) patients with severe or very severe disease who are >40 years old and (iii) younger patients with severe or very severe disease who do not have an HLA-identical sibling donor. The standard immunosuppressive regimen is a combination of antithymocyte globulin (ATG) and ciclosporin. ATG must only be given as an in-patient. Ciclosporin should be continued for at least 12 months after achieving maximal haematological response, followed by a very slow tapering, to reduce the risk of relapse. The routine use of long term G-CSF, or other haemopoietic growth factors, after ATG and ciclosporin, is not recommended outside the setting of prospective clinical trials.**

(6) **Matched unrelated donor (MUD) BMT may be considered when a patient has severe aplastic anaemia, has no matched sibling donor but a matched unrelated donor, is <50 years old (or 50–60 years old with good performance status), and has failed at least one course of ATG and ciclosporin. The optimal conditioning regimen for MUD BMT is uncertain, but currently a fludarabine, non-irradiation-based regimen is favoured for younger patients.**

- **There is a high risk (around 33%) of relapse of aplastic anaemia in pregnancy. Supportive care is the mainstay of treatment in pregnancy and the platelet count should be maintained $>20 \times 10^9$/l, if possible. It is safe to use ciclosporin in pregnancy.**

1. Definition and clinical presentation

Aplastic anaemia is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin. For a comprehensive update on the pathophysiology, the reader is directed to a recent review (Young *et al*, 2006). These guidelines will focus specifically on idiosyncratic acquired aplastic anaemia, and will not refer to the inevitable and predictable aplasia that occurs after chemotherapy and/or radiotherapy. The incidence of acquired aplastic anaemia in Europe and North America is around 2 per million population per year (Issaragrisil *et al*, 2006; Montané *et al*, 2008). The incidence is 2–3 times higher in East Asia. There is a biphasic age distribution with peaks from 10 to 25 years and >60 years. There is no significant difference in incidence between males and females (Heimpel, 2000). Congenital aplastic anaemia is very rare, the commonest type being Fanconi anaemia, which is inherited as an autosomal recessive disorder in most cases.

Patients with aplastic anaemia most commonly present with symptoms of anaemia and skin or mucosal haemorrhage or visual disturbance due to retinal haemorrhage. Infection is a

less common presentation. There is no lymphadenopathy or hepatosplenomegaly (in the absence of infection) and these findings strongly suggest another diagnosis (Gordon-Smith, 1991). In children and young adults, the findings of short stature, café au lait spots and skeletal anomalies should alert the clinician to the possibility of a congenital form of aplastic anaemia, Fanconi anaemia, although Fanconi anaemia can sometimes present in the absence of overt clinical signs. Patients with Fanconi anaemia most commonly present between the ages of 3 and 14 years but can occasionally present later in their 30s [up to 32 years in males and 48 years in females reported by Young & Alter, (1994)]. The findings of leucoplakia, nail dystrophy and pigmentation of the skin are characteristic of another inherited form of aplastic anaemia, dyskeratosis congenita, with a median age at presentation of 7 years (range 6 months to 26 years) (Dokal, 2000; Walne & Dokal, 2009). Some affected patients may have none of these clinical features and the diagnosis is made later after failure to respond to immunosuppressive therapy (Vulliamy & Dokal, 2006). A preceding history of jaundice, usually 2–3 months before, may indicate a post-hepatitic aplastic anaemia (Gordon-Smith, 1991; Young & Alter, 1994).

Many drugs and chemicals have been implicated in the aetiology of aplastic anaemia, but for only very few is there reasonable evidence for an association from case control studies, and even then it is usually impossible to prove causality (Baumelou *et al*, 1993; Young & Alter, 1994; Heimpel, 1996; Kauffmann *et al*, 1996; Issaragrissil *et al*, 1997), (see Table I). A careful drug history should be obtained, detailing all drug exposures for a period beginning 6 months and ending 1 month prior to presentation (Heimpel, 1996; Kauffmann *et al*, 1996). If at presentation the patient is taking several drugs which may have been implicated in aplastic anaemia, even if the evidence is based on case report(s) alone, then all the putative drugs should be discontinued and the patient should not be re-challenged with the drugs at a later stage after recovery of the blood counts. The MHRA should be informed using the Yellow Card Scheme on every occasion that a patient presents with aplastic anaemia where there is a possible drug association (website: http://www.yellowcard.gov.uk).

Similarly, a careful occupational history of the patient may reveal exposure to chemicals or pesticides that have been associated with aplastic anaemia, as summarised in Table II.

Table I. Currently licenced drugs which have been reported as a rare association with aplastic anaemia. Evidence based on case reports or uncontrolled series (Young & Alter, 1994) or case control studies (Baumelou *et al*, 1993; Issaragrisil *et al*, 2006; Issaragrissil *et al*, 1997; Kauffmann *et al*, 1996).

Antibiotics	Chloramphenicol*, Sulphonamides, Cotrimoxazole, Linezolid
Anti-inflammatory	Gold, Penicillamine, Phenylbutazone, Indomethacin, Diclofenac, Naproxen, Piroxicam, Sulphasalazine
Anti-convulsants	Phenytoin, Carbamazepine
Anti-thyroids	Carbimazole†, Thiouracil
Anti-depressants	Dothiepin, Phenothiazines
Anti-diabetics	Chlorpropamide, Tolbutamide
Anti-malarials	Chloroquine
Others‡	Mebendazole, Thiazides, Allopurinol

*No association with chloramphenicol tablets was observed in recent study from Thailand (Issaragrisil *et al*, 2006). There is no evidence for an association between chloramphenicol eye drops and aplastic anaemia (Gordon-Smith *et al*, 1995; Lancaster *et al*, 1998; Wilholm *et al*, 1998).
†More likely to cause neutropenia.
‡From epidemiological study in Thailand (Issaragrisil *et al*, 2006).

Recommendations

(i) Aplastic anaemia is a rare disorder. Most cases are idiopathic, but careful history and clinical examination is important to identify rarer inherited forms.
(ii) Although most cases of aplastic anaemia are idiopathic, a careful drug and occupational exposure history should be taken.
(iii) Any putative drug should be discontinued and should not be given again to the patient. Any possible association of aplastic anaemia with drug exposure should be reported to the MHRA using the Yellow card Scheme.

2. Investigations required for diagnosis

The following investigations are required to (i) confirm the diagnosis (ii) exclude other possible causes of pancytopenia with a hypocellular bone marrow (iii) exclude inherited aplastic anaemia (iv) screen for an underlying cause of aplastic anaemia and (v) document or exclude a co-existing abnormal cytogenetic clone or a PNH clone. See Table III for a summary of investigations required for the diagnosis of aplastic anaemia.

2.1. Full blood count, reticulocyte count, blood film and % HbF

The full blood count (FBC) typically shows pancytopenia although usually the lymphocyte count is preserved. In most cases the haemoglobin level, neutrophil and platelet counts are all uniformly depressed, but in the early stages isolated cytopenia, particularly thrombocytopenia, may occur. Anaemia is accompanied by reticulocytopenia, and macrocytosis is commonly noted. Careful examination of the blood film is essential to exclude the presence of dysplastic neutrophils and abnormal platelets, blasts and other abnormal cells, such as hairy cells (as seen in hairy cell leukaemia). The monocyte count may be depressed but the absence of monocytes should alert the clinician to a possible diagnosis of hairy cell leukaemia. In aplastic anaemia, anisopoikilocytosis is common and neutrophils may show toxic granulation. Platelets are reduced in number and mostly of small size. Fetal haemoglobin (HbF) should be measured pre-transfusion in children as this is an important prognostic factor in

Table II. Occupational and environmental exposures as potential aetiological agents in aplastic anaemia.

Benzene and other solvents (evidence based on large industrial studies (Yin *et al*, 1987; Smith, 1996; Yin *et al*, 1996; Issaragrisil *et al*, 2006)
Agricultural pesticides: Organochlorines e.g. Lindane, Organophosphates, Pentachlorophenol [Muir *et al*, 2003 (case control study), Fleming & Timmeny, 1993; Roberts, 1997 (literature reviews of case reports)], DDT and Carbamates (Issaragrisil *et al*, 2006)
Cutting oils and lubricating agents (Muir *et al*, 2003)
Non-bottled water, non-medical needle injury, farmers exposed to ducks and geese, animal fertiliser (Issaragrisil *et al*, 2006)
Recreational drugs: methylenedioxy-methamphetamine, MDMA, Ecstasy, [evidence based on case reports, (Marsh *et al*, 1994b; Clark & Butt, 1997)]

Table III. Summary of investigations required for the diagnosis of aplastic anaemia.

1. FBC and reticulocyte count
2. Blood film examination
3. HbF% in children
4. Bone marrow aspirate and trephine biopsy, including cytogenetics
5. Peripheral blood chromosomal breakage analysis to exclude Fanconi anaemia if <50 years
6. Flow cytometry for GPI-anchored proteins (see note below concerning Ham test)*
7. Urine haemosiderin if Ham test positive or GPI-anchored protein deficiency
8. Vitamin B12 and folate
9. Liver function tests
10. Viral studies: Hepatits A, B and C, EBV, HIV (CMV, see page 5)
11. Anti-nuclear antibody and anti-dsDNA
12. Chest X-ray
13. Abdominal ultrasound scan and echocardiogram
14. Peripheral blood gene mutation analysis for dyskeratosis congenita *DKC1*, *TERC*, ?*TERT*) if clinical features or lack of response to immunosuppressive therapy

FBC, full blood count; HbF, fetal haemoglobin; GPI, glycerophosphatidylinositol; EBV, Epstin–Barr virus; HIV, human immunodeficiency virus; CMV, cytomegalovirus.

*The Ham test and sucrose lysis test have been abandoned in most centres as diagnostic tests for PNH as they are both less sensitive and less quantitative than flow cytometry (Parker *et al*, 2005).

paediatric myelodysplastic syndrome (MDS) which may feature in the differential diagnosis of pancytopenia in children.

2.2. Bone marrow examination

Both a bone marrow aspirate and trephine biopsy are required. Bone marrow aspiration and biopsy may be performed in patients with severe thrombocytopenia without platelet support, providing that adequate surface pressure is applied (Kelsey *et al*, 2003). Fragments are usually readily obtained from the aspirate. Difficulty obtaining fragments should raise the suspicion of a diagnosis other than aplastic anaemia. The fragments and trails are hypocellular with prominent fat spaces and variable amounts of residual haemopoietic cells. Erythropoiesis is reduced or absent, dyserythropoiesis is very common and often marked, so this alone should not be used to make a diagnosis of MDS. Megakaryocytes and granulocytic cells are reduced or absent; dysplastic megakaryocytes and granulocytic cells are not seen in aplastic anaemia. Lymphocytes, macrophages, plasma cells and mast cells appear prominent. In the early stages of the disease, one may also see prominent haemophagocytosis by macrophages, as well as background eosinophilic staining representing interstitial oedema. A trephine is crucial to assess overall cellularity, to assess the morphology of residual haemopoietic cells and to exclude an abnormal infiltrate. In most cases the trephine is hypocellular throughout but sometimes it is patchy, with hypocellular and cellular areas. Thus, a good quality trephine of at least 2 cm is essential. A 'hot spot' in a patchy area may explain why sometimes the aspirate is normocellular. Care should be taken to avoid tangential biopsies as subcortical marrow is normally 'hypocellular'. Focal hyperplasia of erythroid or granulocytic cells at a similar stage of maturation may be observed. Sometimes lymphoid aggregates occur, particularly in the acute phase of the disease or when the aplastic anaemia is associated with systemic autoimmune disease, such as rheumatoid arthritis or systemic lupus erythematosus. The reticulin is not increased and no abnormal cells are seen. Increased blasts are not seen in aplastic anaemia, and their presence either indicates a hypocellular MDS or evolution to leukaemia (Marin, 2000; Tichelli *et al*, 1992; Bennett & Orazi, 2009).

2.3. Definition of disease severity based on the FBC and bone marrow findings

To define aplastic anaemia there must be at least two of the following (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 Anaemia Study Group, 1987). The severity of the disease is graded according to the blood count parameters and bone marrow findings as summarised in

Table IV. Definition of severity of aplastic anaemia.

Severe AA (Camitta *et al*, 1975)	BM cellularity <25%, or 25–50% with <30% residual haemopoietic cells* 2/3 of the following: Neutrophil count $<0{\cdot}5 \times 10^9$/l Platelet count $<20 \times 10^9$/l Reticulocyte count $<20 \times 10^9$/l
Very severe AA (Bacigalupo *et al*, 1988)	As for severe AA but neutrophils $<0{\cdot}2 \times 10^9$/l
Non-severe AA	Patients not fulfilling the criteria for severe or very severe aplastic anaemia

*Cellularity should be determined by comparison with normal controls (Tuzuner & Bennett, 1994).

Table IV (Camitta *et al*, 1975; Bacigalupo *et al*, 1988). However, because of routine and more accurate automated reticulocyte counting, this will over-estimate the level of reticulocyte count used in the historical Camitta criteria (Camitta *et al*, 1975) for defining disease severity. The assessment of disease severity is important in treatment decisions but has less prognostic significance today in terms of correlation with response to ATG treatment (Scheinberg *et al*, 2009). Patients with bi-or tri-lineage cytopenias that are less severe than this are not classified as aplastic anaemia. However, they should have their blood counts monitored to determine whether they will develop aplastic anaemia with time.

2.4. Liver function tests and viral studies

Liver function tests should be performed to detect antecedent hepatitis, but in post-hepatitic aplastic anaemia the serology is most often negative for all the known hepatitis viruses. The onset of aplastic anaemia occurs 2–3 months after an acute episode of hepatitis and is more common in young males (Brown *et al*, 1997). Blood should be tested for hepatitis A antibody, hepatitis B surface antigen, hepatitis C antibody and Epstein–Barr virus (EBV). Cytomegalovirus (CMV) and other viral serology should be assessed if BMT is being considered. Parvovirus causes red cell aplasia but not aplastic anaemia. Human immunodeficiency virus (HIV) is not a recognised cause of aplastic anaemia, but it can cause isolated cytopenias. We would recommend that prior to a diagnosis of aplastic anaemia, appropriate investigations to exclude alternative aetiologies of cytopenias (B12, red cell folate and HIV) should be performed.

2.5. Vitamin B12 and folate levels

Vitamin B12 and folate levels should be measured to exclude megaloblastic anaemia which, when severe, can present with pancytopenia. If a deficiency of B12 or folate is documented, this should be corrected before a final diagnosis of aplastic anaemia is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare.

2.6. Autoantibody screen

The occurrence of pancytopenia in systemic lupus erythematosus may (i) be autoimmune in nature occurring with a cellular bone marrow or (ii) be associated with myelofibrosis or rarely (iii) occur with a hypocellular bone marrow. Blood should be tested for anti-nuclear antibody and anti-DNA antibody in all patients presenting with aplastic anaemia.

2.7. Tests to detect a PNH clone

Paroxysmal nocturnal haemoglobinuria should be excluded by performing flow cytometry (Dacie & Lewis, 2001; Parker *et al*, 2005). The Ham test and sucrose lysis test have been abandoned by most centres as diagnostic tests for PNH. Analysis of glycosylphosphatidylinositol (GPI)-anchored proteins, such as CD55 and CD59 by flow cytometry, is a sensitive and quantitative test for PNH enabling the detection of small PNH clones which occur in up to 50% of patients with aplastic anaemia, the proportion depending on the sensitivity of the flow cytometric analysis used (Dunn *et al*, 1999; Socie *et al*, 2000; Sugimori *et al*, 2005). Such small clones are most easily identified in the neutrophil and monocyte lineages in aplastic anaemia and will be detected by flow cytometry and not by the Ham test. If the patient has had a recent blood transfusion, the Ham test may be negative whereas a population of GPI-deficient red cells may still be detected by flow cytometry. However, the clinical significance of a small PNH clone in aplastic anaemia as detected by flow cytometry remains uncertain. Such clones can remain stable, diminish in size, disappear or increase. What is clinically important is the presence of a significant PNH clone with clinical or laboratory evidence of haemolysis. Urine should be examined for haemosiderin to exclude intravascular haemolysis which is a constant feature of haemolytic PNH. Evidence of haemolysis associated with PNH should be quantified with the reticulocyte count, serum bilirubin, serum transaminases and lactate dehydrogenase (LDH).

2.8. Cytogenetic investigations

Cytogenetic analysis of the bone marrow should be attempted although this may be difficult in a very hypocellular bone marrow and often insufficient metaphases are obtained. In this situation, one should consider fluorescence *in situ* hybridization (FISH) analysis for chromosomes 5 and 7 in particular. It was previously assumed that the presence of an abnormal cytogenetic clone indicated a diagnosis of MDS and not aplastic anaemia, but it is now evident that abnormal cytogenetic clones may be present in up to 12% of patients with otherwise typical aplastic anaemia at diagnosis (Appelbaum *et al*, 1989; Tichelli *et al*, 1996; Gupta *et al*, 2006). The presence of abnormal cytogenetics at presentation in children, especially monosomy 7, should alert to the likelihood of MDS. Abnormal cytogenetic clones may also arise during the course of the disease (Socie *et al*, 2000). The management of a patient with aplastic anaemia who has an abnormal cytogenetic clone is discussed in *Section 9*.

2.9. Screen for inherited disorders

Peripheral blood lymphocytes should be tested for spontaneous and diepoxybutane (DEB) or mitomycin C (MMC)-induced chromosomal breakage to identify or exclude Fanconi anaemia. This should be performed in all patients who are BMT candidates. Siblings of Fanconi anaemia patients should also be screened. For all other patients, it is difficult to set an upper age limit for Fanconi anaemia screening because the age at diagnosis may sometimes occur in the fourth decade, and rarely in the fifth decade, of life (Alter, 2007). Dyskeratosis congenita may be excluded by identifying a known mutation but there are probably many mutations yet to be identified (Vulliamy *et al*,

2005; Walne & Dokal, 2009). Along with measuring telomere lengths, this is not currently available as a routine clinical service.

2.10. Radiological investigations

- A chest X-ray is useful at presentation to exclude infection and for comparison with subsequent films.
- Routine X-rays of the radii are no longer indicated as all young patients should have peripheral blood chromosomes analysed to exclude a diagnosis of Fanconi anaemia.
- Abdominal ultrasound: the findings of an enlarged spleen and/or enlarged lymph nodes raise the possibility of a malignant haematological disorder as the cause of the pancytopenia. In younger patients, abnormal or anatomically displaced kidneys are features of Fanconi anaemia.

2.11. Differential diagnosis of pancytopenia and a hypocellular bone marrow

The above investigations should exclude causes of a hypocellular bone marrow with pancytopenia other than aplastic anaemia. These include:

- Hypocellular MDS/acute myeloid leukaemia (AML) can sometimes be difficult to distinguish from aplastic anaemia. The following features of MDS are not found in aplastic anaemia: dysplastic cells of the granulocytic and megakaryocytic lineages, blasts in the blood or marrow (Tuzuner *et al*, 1995; Jaffe *et al*, 2001; Bennett & Orazi, 2009). In trephine specimens, increases in reticulin associated with residual areas of haemopoiesis suggest hypocellular MDS rather than aplastic anaemia. The presence of abnormal localisation of immature precursors (ALIPs) is difficult to interpret in this context because small collections of immature granulocytic cells may be seen in the bone marrow in aplastic anaemia when regeneration occurs. As discussed previously, dyserythropoiesis is very common in aplastic anaemia.
- Hypocellular acute lymphoblastic leukaemia (ALL) occurs in 1–2% of cases of childhood ALL. Overt ALL usually develops within 3–9 months of the apparent bone marrow failure. In contrast to aplastic anaemia, the neutropenia is usually more pronounced than the thrombocytopenia and sometimes there is an increase in reticulin within the hypocellular bone marrow (Chessells, 2001). Immunophenotyping may help confirm the diagnosis. Treatment should not be deferred in severe aplastic anaemia in children just in case they turn out to have ALL. For all new paediatric cases of aplastic anaemia, a national central morphology review is planned under the aegis of the Medical Research Council Childhood Leukaemia Working Party Subgroup for rare haematological diseases.
- Hairy cell leukaemia classically presents with pancytopenia but the accompanying monocytopenia is a constant feature of this disorder. It is usually difficult or impossible to aspirate on bone marrow fragments. In addition to the typical interstitial infiltrate of hairy cells with their characteristic 'fried egg' appearance in the bone marrow trephine, there is always increased reticulin. Immunophenotyping reveals $CD20^+$, $CD11c^+$, $CD25^+$, $FMC7^+$, $CD103^+$ tumour cells that are typically $CD5^-$, $CD10^-$ and $CD23^-$. Although splenomegaly is a common finding in hairy cell leukaemia, it may be absent in 30–40% of cases.
- Lymphomas, either Hodgkin lymphoma or non-Hodgkin lymphoma and myelofibrosis may sometimes present with pancytopenia and a hypocellular bone marrow. The bone marrow biopsy should be examined very carefully for foci of lymphoma cells or fibrosis which may be seen in only a small part of the trephine. Since lymphocytes are often prominent in aplastic anaemia, immunophenotyping should be performed. Myelofibrosis is usually accompanied by splenomegaly and the absence of an enlarged spleen in the presence of marrow fibrosis should alert one to secondary malignancy. Marker studies and gene rearrangement studies will help to confirm the diagnosis of lymphoma.
- Mycobacterial infections can sometimes present with pancytopenia and a hypocellular bone marrow, this is seen more commonly with atypical mycobacteria. Other bone marrow abnormalities include granulomas, fibrosis, marrow necrosis and haemophagocytosis. Demonstrable acid alcohol fast bacilli (AAFB) and granulomas are often absent in Mycobacterium tuberculosis infection. AAFB are more frequently demonstrated in atypical mycobacterial infections where they are often phagocytosed by foamy macrophages. The bone marrow aspirate should be sent for AAFB culture if tuberculosis is suspected (Bain *et al*, 2001).
- Anorexia nervosa or prolonged starvation may be associated with pancytopenia. The bone marrow may show hypocellularity and gelatinous transformation (serous degeneration/atrophy) with loss of fat cells as well as haemopoietic cells, and increased ground substance which stains a pale pink on haematoxylin/eosin stain (Bain *et al*, 2001). The pink ground substance may also be seen as on an May–Grünwald–Giemsa stained aspirate. Some degree of fat change may also be seen in aplastic anaemia, especially early in its evolution.
- Occasionally aplastic anaemia can present with an isolated thrombocytopenia, and pancytopenia develops later. Such patients can initially be misdiagnosed as autoimmune immune thrombocytopenia (ITP) but bone marrow examination in aplastic anaemia shows hypocellularity with reduced or absent megakaryocytes, which is not seen in ITP.
- A recent comprehensive review on aplastic anaemia in children discusses in more detail conditions that may present with pancytopenia and a hypocellular bone marrow in children (Davies & Guinan, 2007).

A MDT meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should also be given to review of blood and bone marrow

slides by a specialist centre, especially if there are unusual morphological features or where there is any doubt about the diagnosis.

Recommendations

(i) All new patients presenting with aplastic anaemia should be carefully assessed to:
- **confirm the diagnosis and exclude other possible causes of pancytopenia with hypocellular bone marrow**
- **classify the disease severity using standard blood and bone marrow criteria**
- **document the presence of associated PNH and cytogenetic clones**
- **exclude a possible late onset inherited bone marrow failure disorder**

(ii) A MDT approach to the above assessment is recommended and also to formulate an appropriate management plan for the patient.

(iii) If there is doubt about the diagnosis and/or management plan, referral of the case for specialist advice and/or review of the blood and bone marrow morphology slides at a specialist centre, is encouraged.

3. Supportive care

3.1. Transfusional support

Support with red cell and platelet transfusions is essential for patients with aplastic anaemia to maintain a safe blood count. It is recommended to give prophylactic platelet transfusions when the platelet count is $<10 \times 10^9$/l (or $<20 \times 10^9$/l in the presence of fever) (Grade C recommendation; level IV evidence), rather than giving platelets only in response to bleeding manifestations (Kelsey *et al*, 2003). Prediction of bleeding is difficult in an individual patient. Fatal haemorrhage, usually cerebral, is more common in patients who have $<10 \times 10^9$/l platelets, extensive retinal haemorrhages, buccal haemorrhages or rapidly spreading purpura. However, cerebral haemorrhage may be the first major bleed in patients who have none of these other bleeding manifestations (Gordon-Smith, 1991). For invasive and surgical procedures, platelet transfusion(s) must be given to achieve appropriate levels as recommended by BCSH guidelines, and a pre-procedure platelet count checked to ensure that level has been achieved.

A common problem in multi-transfused patients with aplastic anaemia, compared with leukaemia patients, is that they may develop alloimmunisation 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 after allogeneic BMT (Kaminsky *et al*, 1990). Routine pre-storage leucocyte depletion of all units of red cells and platelets in the UK is likely to reduce the risk of alloimmunisation (Killick *et al*, 1997; Ljungman, 2000). In a retrospective, single centre study, the incidence of HLA alloimmunisation was reported to be 50% in patients with aplastic anaemia who had received blood products prior to the introduction of pre-storage leucocyte depletion in the UK compared with only 12% for patients who received only leucocyte depleted blood products (Killick *et al*, 1997). Patients who become refractory to platelet transfusions should be screened for HLA antibodies. However, other causes of platelet refractoriness, such as infection and drugs, should be excluded. If a patient does become sensitised to random donor platelets resulting in platelet refractoriness, HLA-matched platelets should be used (grade C recommendation; level IV evidence). Red cell and platelet transfusions should be given to maintain a safe haemoglobin level (>80 g/l, although this will depend on co-morbidities) and platelet count and not be withheld for fear of sensitising the patient. Directed blood and platelet donations from family members are not permitted within the National Blood Service, and the recipient may become sensitised to minor histocompatibility antigens from the potential bone marrow donor resulting in a high risk of graft rejection. In exceptional circumstances, a family donor may provide the most compatible platelets if a patient has developed multi-specific HLA antibodies and requires platelets urgently.

Apart from platelet transfusional support, other important practical measures to help prevent bleeding include good dental hygiene, the use of oral tranexamic acid and control of menorrhagia with norethisterone.

If a patient is a potential candidate for early or later BMT (*see Section 4.1.2.1*), it is recommended that the patient is transfused with CMV-negative blood products until the patient's CMV status is known. CMV-negative blood products should then be continued only if both the patient and donor are CMV negative (Pamphilon *et al*, 1999).

It is currently unclear whether red cell and platelet transfusions should be routinely irradiated in all aplastic anaemia patients who are potential BMT candidates and in all patients undergoing treatment with ATG (Williamson *et al*, 1996). The rationale for considering the use of irradiated blood products is twofold (i) there are animal data showing that irradiation of all red cell and platelet transfusions before BMT further reduces the risk of sensitisation to minor histocompatibility antigens (and hence reduced risk of graft rejection after allogeneic BMT) (Bean *et al*, 1994). An expert committee on aplastic anaemia previously proposed that irradiated blood products should be used routinely in all patients with aplastic anaemia who are transplant candidates (Schrezenmeier *et al*, 2000). Although this has become common practice in many centres in Europe and the USA, there is no evidence for this. It is possible that the routine use of leucodepleted blood products may have reduced the risk of alloimmunisation in aplastic anaemia patients. (ii) Are irradiated blood products indicated during and after ATG therapy to prevent transfusion-associated graft-*versus*-host disease (TA-GVHD)? There has been only one likely case of

TA-GVHD reported after ATG treatment from one European centre, but this occurred before the availability of leuco-depleted blood products (Marsh *et al*, 2009). The recent Serious Hazards of Transfusion (SHOT) annual report indicates that there have been no new cases of TA-GVHD in the UK since 2000–01; routine universal leuco-depletion was introduced in 1999 in the UK (SHOT Annual Report, 2006). However, following the recent withdrawal of horse ATG (Lymphoglobuline; Genzyme, Cambridge, MA, USA) from the market, rabbit ATG (Thymoglobuline; Genzyme) will now replace horse ATG for the initial course of immunosuppressive therapy. Rabbit ATG is more immunosuppressive than horse ATG. It results in a more prolonged period of lymphopenia, has a longer half-life and higher affinity IgG subtype to human lymphocytes than horse ATG (Thomas *et al*, 1984; Scheinberg *et al*, 2007).

In view of the lack of evidence in this area, there is conflicting practice worldwide. However, we recommend empirically the use of irradiated blood components for patients receiving immunosuppressive therapy. We cannot recommend how long this practice should continue after ATG administration; one option may be to continue until the lymphocyte count recovers to $>1{\cdot}0 \times 10^9$/l (grade C recommendation; level IV evidence). The absolute requirement for irradiated red cell and platelet transfusions from the beginning of the pre-transplant conditioning regimen applies to all patients undergoing stem cell transplantation.

Granulocyte transfusions can be used as supportive therapy in patients with life-threatening neutropenia. Despite the potential availability of this component, there is little published literature on the efficacy of buffy coat granulocyte concentrates. Adverse events, such as febrile reactions, HLA alloimmunization and transfusion-related acute lung injury (TRALI) are well-recognised complications following granulocyte transfusions. The use of irradiated granulocyte transfusions should therefore be limited to patients in whom the possible benefits outweigh the hazards (National Blood Service Clinical Guidelines 2007). The use of irradiated granulocyte transfusions from G-CSF stimulated volunteer donors is not routinely available in most centres in the UK.

3.2. Haemopoietic growth factors

There are currently no effective and safe haemopoietic growth factors to support red cell and platelet counts in patients with aplastic anaemia (see reference Marsh *et al*, 2007 for a general review). Anecdotal use of rHuEpo in aplastic anaemia has shown that it is ineffective, which is not surprising in view of the demonstration of markedly elevated serum erythropoietin levels in the majority of patients with aplastic anaemia. A concern of using rHuEpo is the potential for inducing severe and or sudden worsening of anaemia due to red cell aplasia from anti-rHuEpo antibodies (Casadevall *et al*, 2002). Furthermore, in combination with other drugs used routinely to treat aplastic anaemia, such as ciclosporin, there is the potential for toxicity, for example, hypertension. The routine use of rHuEpo in aplastic anaemia is therefore not recommended (grade C recommendation; level IV evidence). Other haemopoietic growth factors have been used in aplastic anaemia to determine whether they might stimulate thrombopoiesis. Interleukin-6 (IL-6) was evaluated in a combined German/UK pilot study, but the study was terminated early because of severe anaemia and the onset of serious haemorrhage in patients with aplastic anaemia (Schrezenmeier *et al*, 1995a). In a small study, stem cell factor was shown to stimulate trilineage haemopoiesis in some patients with aplastic anaemia (Kurzrock *et al*, 1997), but its use in a larger study with ATG, ciclosporin and stem cell factor was abandoned because of serious toxicity from anaphylaxis/anaphylactoid reactions (H. Schrezenmeier, personal communication, 2001). There have been no clinical studies of recombinant human thrombopoietin (rHu-TPO) in aplastic anaemia. The development of anti-TPO antibodies against the truncated version of rHu-TPO, pegylated rHu-megakaryocyte growth and development factor (PEG-rHu-MGDF) resulted in prolonged thrombocytopenia and discontinuation of its use in clinical trials (Vadhan-Raj, 2000). Second generation thrombopoiesis stimulating agents have not undergone clinical trials in aplastic anaemia. The use of G-CSF is discussed in further detail later (see section on *Treatment of infection*).

3.3. Prevention of infection

The risk of infection is determined by the patient's neutrophil and monocyte counts (Bodey *et al*, 1982; Keidan *et al*, 1986). The risk may also be determined on an individual basis as some patients have repeated infections whilst others may have none or very few. Patients with aplastic anaemia are at risk of bacterial and fungal infections (Ljungman, 2000). *Aspergillus* infections have a very high mortality in patients with severe aplastic anaemia because of the frequent prolonged periods of severe neutropenia (and monocytopenia).

Aplastic anaemia patients who are severely neutropenic ($<0{\cdot}5 \times 10^9$/l) should ideally be nursed in isolation when in hospital and should receive prophylactic antibiotics and antifungals, regular mouth care including an antiseptic mouthwash, such as chlorhexidine, and food of low bacterial content (Gordon-Smith, 1991; Ljungman, 2000; Gafter-Gvili *et al*, 2005). Laminar air-flow facilities are not essential but should be used when available. Prophylactic antibiotics are given to help prevent Gram-negative sepsis, either a combination of two non-absorbable antibiotics, such as neomycin and colistin, or a quinolone antibiotic, such as ciprofloxacin. However, there is concern about the emergence of quinolone-resistant bacteria, increase in Gram-positive infections, and an increased risk of *Clostridium difficile*. Also, ciprofloxacin cannot be used to treat febrile neutropenic episodes if it is used prophylactically. The choice of either non-absorbable

antibiotics or ciprofloxacin should be left to individual centres. For children, it is not standard practice to use prophylactic antibiotics; ciprofloxacin is not licenced, and non-absorbable antibiotics are very unpalatable.

Patients with aplastic anaemia are at high risk of fungal infection, including *Aspergillus*. Fluconazole provides no cover against *Aspergillus* species. The drugs of choice are itraconazole and posaconazole, the latter of which has not yet been shown to be superior in efficacy to itraconazole. Both are superior in efficacy to fluconazole. There are no data to justify the use of voriconazole for prophylaxis (Prentice *et al*, 2008).

There is no indication for routine prophylactic measures against *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*, PCP), or anti-viral prophylaxis in untreated patients with aplastic anaemia. Antiviral prophylaxis with aciclovir is essential for all transplanted patients and is commonly given during and for the first 3–4 weeks after immunosuppressive therapy with antithymocyte globulin (ATG) (Styczynski *et al*, 2008). Prophylaxis against PCP is essential post BMT for all patients regardless of diagnosis but is not routinely given during ATG treatment in Europe (Ljungman, 2000) although it is in some USA centres.

For patients who are in the community and who have not recently received ATG or undergone BMT, continued mouth-care with an antiseptic mouthwash is recommended, but routine prophylactic antimicrobials are not required in all patients. For patients who are severely neutropenic (neutrophil count $<0{\cdot}5 \times 10^9$/l), prophylactic antibiotics and antifungals should be used and foods that may be contaminated with bacteria or fungal pathogens avoided. It is less clear whether antibiotic and antifungal prophylaxis should continue for those at intermediate risk of infection (neutrophil count $0{\cdot}2–0{\cdot}5 \times 10^9$/l). The decision is best determined on an individual basis according to the frequency and severity of previous infections.

3.4. Treatment of infection

As for all neutropenic patients, fever may require immediate hospitalisation and treatment before the results of bacterial investigations are available. The local hospital guidelines for treatment of febrile neutropenia should be followed. This most frequently employs initially a synergistic combination of antibiotics, such as an aminoglycoside and a β-lactam penicillin, the exact choice depending on local hospital microbiological sensitivity/resistance patterns. The duration of neutropenia, the patient's infection history and recent antibiotics will also influence the choice of antibiotic, including the early introduction of amphotericin.

It is recommended that systemic antifungal therapy is introduced into the febrile neutropenia regimen early if fevers persist. Once a patient with aplastic anaemia is colonised with *Aspergillus* it may be difficult to treat successfully as the neutrophil count may not recover for a long period of time. If a patient has had previous fungal infection, or if fungal infection is proven or even suspected, systemic antifungal therapy should be used with the first line antibiotics. Early use of an appropriate lipid formulation of amphotericin or one of the newly licenced antifungal agents, such as Voriconazole or Caspofungin, should be considered in aplastic anaemia patients who may need prolonged treatment, in order to avoid serious nephrotoxicity. Pulmonary infiltrates and sinus infection should be taken as indicators of likely fungal infection in patients with severe aplastic anaemia. A chest X-ray should be included as part of the investigation of new or persistent fever, with high resolution computed tomography scanning of chest if high index of clinical suspicion.

There have been no controlled studies evaluating the use of G-CSF or other haemopoietic growth factors in the treatment of severe infection in patients with aplastic anaemia. A short course of subcutaneous G-CSF at a dose of 5 μg/kg per day may be considered for severe systemic infections that are not responding to intravenous antibiotics and antifungals (grade C recommendation; level IV evidence). G-CSF may produce a temporary neutrophil response but usually only in those patients with residual marrow granulocytic activity (that is, those with non-severe disease) (Marsh *et al*, 2007). If there is no response by 1 week, it is then reasonable to discontinue the drug. GM-CSF is not generally recommended for the treatment of severe infection in patients with aplastic anaemia as it can induce severe haemorrhage and other serious toxicity.

3.5. Iron chelation therapy

Iron overload can cause significant problems in heavily transfused patients. Subcutaneous desferrioxamine should commence when the serum ferritin is >1000 μg/l, although the evidence base for this is lacking (Porter, 2001 and John Porter, University College London, personal communication, 2008) (grade C recommendation; level IV evidence). This also needs to be assessed on an individual basis in view of the risk of local haemorrhage and infection from subcutaneous injections (Gordon-Smith, 1991). An echocardiogram should be performed prior to commencing desferrioxamine. If subcutaneous desferrioxamine is not tolerated, and the patient has an indwelling central line then intravenous desferrioxamine may be considered instead. The risk of Yersinia infection should be remembered in patients receiving desferrioxamine treatment. In view of the relatively high incidence of agranulocytosis associated with the oral iron chelator deferiprone (Porter, 2001), its use is not routinely recommended in patients with aplastic anaemia. The novel oral iron chelator deferasirox is now licenced for use in transfusion dependent anaemias in which desferal is either inadequate or contraindicated. Because of recent reports of cytopenias in a small number of patients (Maggio, 2007), its use in aplastic anaemia patients who have been treated with immunosuppressive therapy or BMT should be discussed on

an individual patient basis. For iron over-loaded patients following response to ATG or successful BMT, venesection is the standard way to remove iron.

3.6. Vaccinations

There have been anecdotal reports of vaccination producing bone marrow failure or triggering relapse of aplastic anaemia, so vaccinations, including influenza vaccination, should only be given when absolutely necessary (Viallard *et al*, 2000; Hendry *et al*, 2002) (grade C recommendation; level IV evidence). All live vaccines should be avoided after BMT and ATG treatment, indefinitely. After BMT, aplastic anaemia patients should be routinely vaccinated as recommended for all allogeneic BMT recipients.

3.7. Psychological and general support

Psychological support for the patient, family and close friends is of great importance. Aplastic anaemia is a rare disease and requires careful explanation of its nature, prognosis, as well as discussions on important issues such as pregnancy. Patients should be given the opportunity to be referred to a centre that specialises in the management of aplastic anaemia.

There is now an excellent patient support group in the UK for patients with aplastic anaemia which can be contacted at: the Aplastic Anaemia Trust, AA and MDS Support Group, 16 Sidney Rd, Borstal, Rochester, Kent ME13HF. Telephone: 0870-487 0099, email: aplasticanaemia@hotmail.com, website: http://www.theatt.org.uk.

The chronic nature and slow response to treatment should be stressed early in the disease. The morale of the patient (family and close friends) and staff may sag when recovery has not occurred at 6 months or longer.

Recommendations

- **(i) Prophylactic platelet transfusions should be given when the platelet count is $<10 \times 10^9$/l (or $<20 \times 10^9$/l in the presence of fever).**
- **(ii) Irradiated blood products should be given routinely to all patients having ATG treatment.**
- **(iii) Transfusion of irradiated granulocyte transfusions may be considered in patients with life-threatening neutropenic sepsis.**
- **(iv) The routine use of rHuEpo in aplastic anaemia is not recommended.**
- **(v) A short course of G-CSF may be considered for severe systemic infection that is not responding to intravenous antibiotics and anti-fungal drugs, but should be discontinued after 1 week if there is no increase in the neutrophil count.**
- **(vi) Prophylactic antibiotic and antifungal drugs should be given to patients with neutrophil count $<0{\cdot}2 \times 10^9$/l.**
- **(vii) Systemic antifungal therapy should be introduced into the febrile neutropenia regimen early if fevers persist.**
- **(viii) Iron chelation therapy should be considered when the serum ferritin is >1000 μg/l.**

4. Specific treatment of aplastic anaemia: general comments

The standard specific treatment for a newly diagnosed patient with aplastic anaemia is either allogeneic stem cell transplantation from an HLA-identical sibling donor or immunosuppressive therapy with a combination of ATG and ciclosporin. The results of transplantation for aplastic anaemia from a matched unrelated donor have recently been improved by using a reduced intensity conditioning regimen, and this procedure may be considered in young patients with severe disease who do not respond to treatment with ATG and ciclosporin.

It is essential that before specific treatment is given, the patient is stabilised clinically in terms of controlling bleeding and treating infection. It is dangerous to give immunosuppressive therapy in the presence of infection or uncontrolled bleeding (grade C recommendation; level IV evidence). The presence of infection is an adverse factor for outcome after stem cell transplantation (grade B recommendation; level IIa evidence). However, it may sometimes be necessary to proceed with BMT in the presence of active infection, particularly fungal infection, as the transplant offers the best chance of early neutrophil recovery, and delaying the transplant may risk progression of the fungal infection.

Because aplastic anaemia is a rare disease, the haematologist responsible for the patient should contact a centre/specialist with expertise in aplastic anaemia soon after presentation to discuss a management plan for the patient. Care should be shared with the local hospital if possible.

Hospitals providing general haematology care at Level 2 (as defined by the Clinical Haematology Task Force for BCSH, 2000) should be capable of the safe treatment of a patient with severe aplastic anaemia with ATG, providing medical and nursing staff have experience of using ATG, including the recognition and management of its side effects. Level 4 care is required for related allogeneic BMT, providing the centre has experience in BMT for aplastic anaemia. British Society of Blood and Marrow Transplantation and European Group for Blood and Marrow Transplantation (EBMT) accreditation is required for centres to perform unrelated donor BMT.

How long should one wait after presentation before starting treatment for the disease? Early spontaneous recovery occurs infrequently and, in practical terms, by the time the patient has been stabilised clinically, the disease confirmed, its disease severity assessed, potential sibling donor(s) HLA tissue typed and a management plan discussed in collaboration with an expert specialist centre, specific treatment should not be delayed much beyond this time.

Patients with aplastic anaemia should be followed up indefinitely to monitor for relapse and later clonal disorders, such as MDS, leukaemia, PNH and solid tumours. When children approach adult age, arrangements should be made for their subsequent transfer to an adult unit for continued follow up.

Prednisolone should not be used to treat patients with aplastic anaemia (grade C recommendation; level IV evidence). Corticosteroids are ineffective; they encourage bacterial and fungal colonisation, and can precipitate serious gastrointestinal haemorrhage in the presence of severe thrombocytopenia. Similarly, haemopoietic growth factors, such as G-CSF and rHuEpo, should not be used on their own in newly diagnosed patients in the mistaken belief that they may cure the disease (grade C recommendation; level IV evidence). The use of haemopoietic growth factors in this way would lead to delay in giving specific treatment, during which time the patient may become infected or allo-immunised (Marsh *et al*, 1994a; Marsh *et al*, 2007). The recommendations for specific treatment of aplastic anaemia are summarised in Figs 1 and 2.

Recommendations

(i) Infection or uncontrolled bleeding should be treated first before giving immunosuppressive therapy. This also applies to patients scheduled for BMT, although it may sometimes be necessary to proceed straight to BMT in the presence of severe infection as a BMT may offer the best chance of early neutrophil recovery.

(ii) Haemopoietic growth factors, such as rHuEpo or G-CSF, should not be used on their own in newly diagnosed patients in an attempt to 'treat' the aplastic anaemia.

(iii) Prednisolone should not be used to treat patients with aplastic anaemia because it is ineffective and encourages bacterial and fungal infection.

5. HLA identical sibling donor transplantation

5.1. Results

Transplantation for severe aplastic anaemia from an HLA identical sibling donor is now very successful with a 75–90% chance of long term cure (Passweg *et al*, 1997; Bacigalupo *et al*, 2000a; Locatelli *et al*, 2000; Ades *et al*, 2004; Gupta *et al*, 2004; Kahl *et al*, 2005; Champlin *et al*, 2007; Myers & Davies, 2009). Using high dose cyclophosphamide with ATG conditioning, graft failure is around 4–14%. GVHD remains a problem. Although acute GVHD grade III–IV 12–30%) appears to occur less commonly now, chronic GVHD still occurs in 30–40% of patients, (unless a Campath-1H based regimen is used which reduces the risk to <5% (Gupta *et al*, 2004). Prior treatment with immunosuppressive therapy is associated with a worse outcome and increased graft rejection (Ades *et al*, 2004; Kobayashi *et al*, 2006).

5.2. Indications for HLA-identical sibling BMT

Allogeneic BMT from an HLA-identical sibling donor is the initial treatment of choice for newly diagnosed patients with aplastic anaemia if they (i) have severe or very severe aplastic anaemia (*see Section 2.4* and Table IV for definitions of disease severity), (ii) are younger than 40 years (although there is controversy concerning the upper age limit for BMT; see

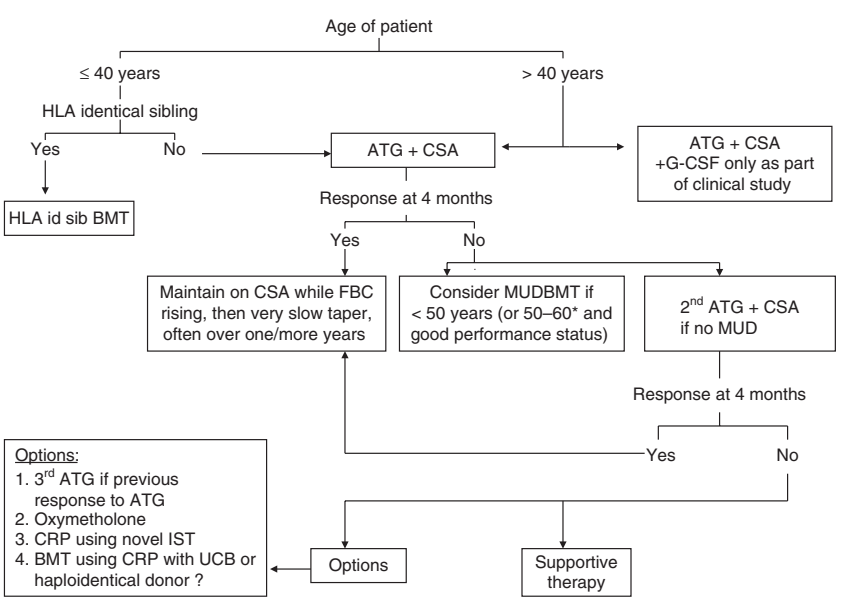

Fig 1. Treatment of acquired severe aplastic anaemia. FBC, full blood count; CRP, clinical research protocol; IST, immunosuppressive therapy; UCB, umbilical cord blood; MUD, matched unrelated donor; ATG, antithymocyte globulin; CSA, ciclosporin; G-CSF, granulocyte colony-stimulating factor; BMT, bone marrow transplantation; HLA id sib, human leucocyte antigen-identical sibling. *For patients older than 60 years, there is currently insufficient data on the role of HSCT in severe AA although data for MDS suggests that this may be a future option (see text).

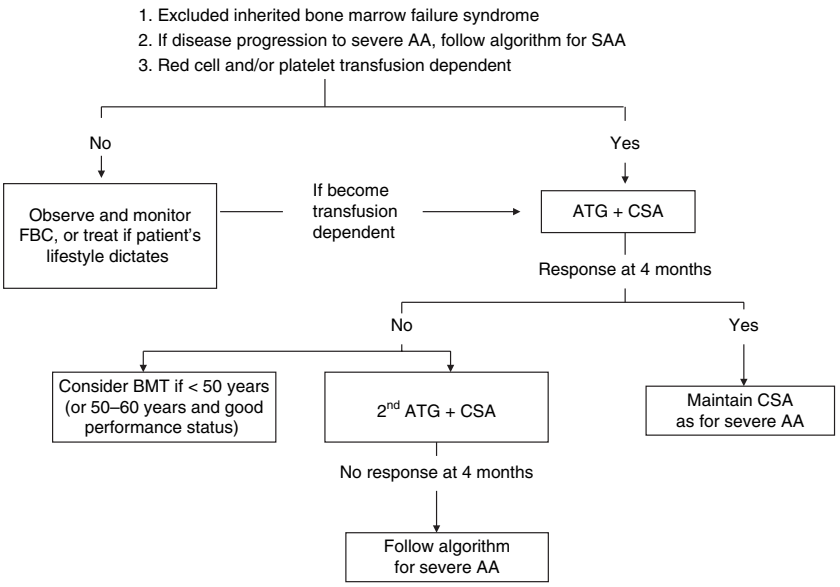

Fig 2. Treatment of non-severe acquired aplastic anaemia in adults. SAA, severe aplastic anaemia; FBC, full blood count; ATG, antithymocyte globulin; CSA, ciclosporin; BMT, bone marrow transplantation.

below) and (iii) have an HLA compatible sibling donor. (iv) For children who have non-severe aplastic anaemia and in whom treatment is indicated, then HLA matched sibling donor transplant should be the first choice, (Grade B recommendation, level IIb evidence).

There is controversy concerning the upper age limit for BMT. Results of BMT using an HLA identical sibling donor are worse in patients >30 years of age compared with patients <30 years of age (Bacigalupo *et al*, 2000a), and particularly >40 years. The decision whether to treat patients aged 30–40 years with ATG and ciclosporin or to transplant upfront should take into account the patient's general medical condition. For patients >40 years who have failed immunosuppressive therapy with ATG and ciclosporin, who have an HLA compatible donor and who are in good medical condition, BMT may be considered. A reduced intensity conditioning regimen may be preferable in such patients, as proposed by the EBMT Severe Aplastic Anaemia Working Party (Maury *et al*, 2007) in view of the high transplant-related mortality using high dose cyclophosphamide (Grade B recommendation, level IIb evidence). A similar conditioning regimen may be indicated for patients between 30 and 40 years of age, although there is currently no published data to support this approach.

5.3. Conditioning and GVHD prophylaxis regimen

The conditioning regimens and GVHD prophylaxis described below refer specifically to patients with acquired aplastic anaemia. Patients with Fanconi anaemia and other types of inherited aplastic anaemia need special consideration and should not follow these pathways, as the conditioning regimen and GVHD prophylaxis are completely different (Rosenberg *et al*, 2005) (grade B recommendation; level IIa evidence).

(a) Conditioning regimen for patients aged <30 years. The preparation used is a non-myeloablative and highly immunosuppressive regimen to help prevent graft rejection and GVHD. The current standard regimen used is high dose cyclophosphamide 50 mg/kg × 4 (day −5 to −2) and ATG (Thymoglobuline, Genzyme 1·5 vials/10 kg × 3 on days −5 to −3), with methylprednisolone 2 mg/kg × 3 (day −5 to −3). (Methylprednisolone is not usually used for paediatric BMT). The recommended post-transplant immunosuppression is (i) ciclosporin 5 mg/kg per day, in two divided doses (that is, 2·5 mg/kg BD), starting on day −1, and continuing for 12 months with tapering beginning at 9 months to help prevent late graft failure, and (ii) short course methotrexate 15 mg/m^2 on day +1, then 10 mg/m^2 on days +3, +6, and +11 (Schrezenmeier *et al*, 2000). The potential benefit of using ATG with cyclophosphamide is unclear as a recently published prospective randomised study from the Centre for Blood and Marrow Transplant Research (CIBMTR) showed no significant benefit in terms of graft rejection, GVHD and survival rates using the combination of cyclophosphamide and ATG compared with cyclophosphamide alone (Champlin *et al*, 2007). The study was underpowered to show differences between the two groups, but the addition of ATG did not significantly improve outcome (Champlin *et al*, 2007).

(b) Conditioning regimens for patients aged >30 years. For patients between the ages of 30 and 50 years, who are potential transplant candidates, the best conditioning regimen is not known. Patients who are >40 years of age and who are medically fit enough for BMT (see above), may receive a reduced intensity

conditioning regimen, using cyclophosphamide 1200 mg/m^2, fludarabine 120 mg/m^2 and either ATG or Alemtuzumab (Gupta *et al*, 2004, Maury *et al*, 2007). A similar approach may be considered for patients aged 30–40 years.

There is no indication for using irradiation-based regimens in HLA-identical sibling BMT for aplastic anaemia (Schrezenmeier *et al*, 2000) (Grade B recommendation, level IIa evidence). Although irradiation reduces the risk of rejection, it confers no benefit on survival and its use is associated with an increased risk of later solid tumours and inevitable infertility, as well as impaired growth and development in children.

5.4. Source and dose of stem cells

It is recommended that bone marrow stem cells, and not G-CSF mobilised peripheral blood stem cells (PBSC), should be used (Schrezenmeier *et al*, 2007) (Grade B recommendation, level IIb evidence). In a retrospective combined CIBMTR and EBMT study, earlier engraftment occurred with PBSC although there was no difference in probability of neutrophil or platelet engraftment by day +30, and no difference in graft rejection compared with bone marrow transplants. Of major concern was significantly worse survival for all patients, and more chronic GVHD in younger patients, using PBSC compared with bone marrow grafts (Schrezenmeier *et al*, 2007). There are other important reasons for using bone marrow in children. Because most of the sibling donors will also be children, it may be much easier to obtain bone marrow than PBSC. In addition, the collection of bone marrow stem cells avoids the exposure of G-CSF (Davies & Guinan, 2007).

It is important to give at least 3×10^8 nucleated marrow cells/kg because at lower doses the risk of graft rejection increases significantly (Niederwieser *et al*, 1988). There are no data on the minimum dose of CD34$^+$ marrow cells to give in aplastic anaemia but it is recommend that at least 3×10^6/kg should be given (Russell *et al*, 1998).

The effect of sex-mis-match between donor and recipient has recently been evaluated in a large retrospective study from the EBMT of patients undergoing HLA-identical sibling or HLA-identical unrelated donor BMT for aplastic anaemia. Survival was significantly better in patients with donors from the same sex. Male patients with female donors had an increased risk of severe GVHD compared to recipients of sex-matched grafts. In contrast, female patients with male donors had an increased risk of graft rejection. These negative effects of donor/recipient sex-mismatching were abrogated by the use of ATG in the conditioning regimen (Stern *et al*, 2006).

Umbilical cord blood as an alternative source of stem cells for transplantation has been used in a small number of patients with aplastic anaemia (Gluckman *et al*, 1997; Barker *et al*, 2001). Its use, however, is limited to small recipients because of the low number of haemopoietic cells that can be obtained from a donation, despite their higher proliferative potential compared with bone marrow cells (Hows, 2001). Compared with bone marrow transplants, umbilical cord blood transplants are associated with a lower risk of acute and chronic GVHD (Gluckman *et al*, 1997; Barker *et al*, 2001). Umbilical cord blood transplantation may also be considered in children who lack an HLA-identical sibling donor or a fully matched unrelated adult donor. The role of double umbilical cord blood transplants in adults with aplastic anaemia is currently being explored (Mao *et al*, 2005; Myers & Davies, 2009), but the major problem anticipated is failure of engraftment.

5.5. Post-transplant management

There is a significant risk of late graft failure in aplastic anaemia following allogeneic BMT which is most commonly associated with discontinuing ciclosporin too early or low ciclosporin blood levels, and in the presence of progressive mixed chimaerism, as defined by >10% recipient cells or >15% increase over 3 months, using short tandem repeats by polymerase chain reaction (PCR) analysis of mononuclear cells (McCann *et al*, 2007). Progressive mixed chimaerism predicts a high risk of graft rejection. Stable mixed chimaerism is associated with excellent survival and a low risk of GVHD (Lawler *et al*, 2009). Therapeutic ciclosporin should be continued for at least 9 months before gradually reducing the dose to zero over the following 3 months. For adults, ciclosporin trough blood levels should be maintained between 250 and 350 μg/l. For children, lower ciclosporin levels are often used (150–200 μg/l), to avoid toxicity. Chimaerism should be monitored particularly closely during the time of ciclosporin withdrawal. If there is evidence of significant mixed chimaerism (see above) or a rising proportion of recipient cells, as assessed with sensitive techniques such as PCR of short tandem repeats, there is a high risk of late rejection, and ciclosporin should not be reduced or withdrawn at that time (McCann *et al*, 2000; Lawler *et al*, 2009).

Fertility is usually well preserved or near normal after BMT for aplastic anaemia using high dose cyclophosphamide and where irradiation is not used (Sanders *et al*, 1996; Deeg *et al*, 1998). It is not necessary to arrange for sperm (or oocyte) cryopreservation pre-transplant, and it is very important that all patients receive appropriate counselling regarding contraception following their transplant (Grade B recommendation, level IIb evidence). For older patients receiving a fludarabine-based regimen (see above), because there is currently insufficient data on fertility post transplant, cryopreservation of sperm or oocyte should be planned.

Patients can be advised that because irradiation is not given, the risk of second tumours is very low (Witherspoon *et al*, 1991; Socie *et al*, 1993; Ades *et al*, 2004).

Recommendations

(i) Allogeneic BMT from an HLA-identical sibling donor is the initial treatment of choice for newly diagnosed patients if they have severe or very severe aplastic

anaemia, are < 40 years old and have an HLA-compatible sibling donor.

(ii) Patients with Fanconi anaemia and other types of inherited aplastic anaemia need special consideration and should not follow recommendations made in this guideline.

(iii) There is no indication for using irradiation-based conditioning regimens for patients undergoing HLA identical sibling BMT for aplastic anaemia.

(iv) The recommended source of stem cells for transplantation in aplastic anaemia is bone marrow.

(v) Fertility is well preserved after high dose cyclophosphamide conditioning in BMT for aplastic anaemia, and patients should be given appropriate contraceptive advice to prevent unwanted pregnancy. Until longer term data is available in patients receiving fludarabine-based regimens, cryopreservation of sperm and oocytes should be planned.

6. Immunosuppressive therapy: antithymocyte globulin (ATG) and ciclosporin

6.1. Results of treatment

Immunosuppressive therapy using the combination of ATG and ciclosporin is associated with response rates of between 60% and 80% with current 5 year survival rates of around 75–85% (Bacigalupo *et al*, 2000a; Bacigalupo *et al*, 2000b, Fuhrer *et al*, 2005; Locasciulli *et al*, 2007). A recent study has shown that on multivariate analysis of response at 6 months, only younger age, absolute reticulocyte count (ARC) and absolute lymphocyte count (ALC), correlate with response to ATG. The lack of association with the absolute neutrophil response reflected a high number of early deaths in patients with very severe neutropenia. For patients with both ARC $\geq 25 \times 0^9$/l and ALC $\geq 1{\cdot}0 \times 10^9$/l, the response was 83% compared with 41% for those with lower counts (Scheinberg *et al*, 2009). For severe aplastic anaemia, the event-free survival and response rate to ATG alone is significantly less than with the combination of ATG and ciclosporin (Bacigalupo *et al*, 2000a; Frickhofen *et al*, 2003), and for patients with non-severe aplastic anaemia the response to the combination of ATG and ciclosporin is significantly greater than with ciclosporin alone (Marsh *et al*, 1999). Response to ATG and ciclosporin is delayed and response usually does not start much before 3–4 months. This means that patients need to continue with regular red cell and platelet transfusional support and will remain neutropenic during this time period. Relapse may occur after immunosuppressive therapy. This was previously reported to be around 30% (Schrezenmeier *et al*, 1993) but with longer use and slower tailing of ciclosporin the rate is closer to 10% (Bacigalupo *et al*, 2000b). Patients are at risk of later clonal disease, 8% for MDS/AML, 10% for haemolytic PNH and 11% for solid tumours at 11 years (Frickhofen *et al*, 2003).

6.2. Indications

Immunosuppressive therapy is indicated for patients who are not eligible for sibling donor BMT. This includes (i) patients with non-severe aplastic anaemia who are dependent on red cell and/or platelet transfusions (ii) patients with non-severe aplastic anaemia who, although not transfusion-dependent, may have significant neutropenia and be at risk of infection (iii) patients with severe or very severe aplastic anaemia who are >40 years of age and (iv) younger patients with severe or very severe disease who lack an HLA-compatible sibling donor (Grade B recommendation; level IIb evidence). Children with non-severe aplastic anaemia with an HLA-identical sibling donor and who are transfusion-dependent, and particularly if the blood count is falling, may be considered for BMT.

For those patients with non-severe aplastic anaemia 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 instigating immunosuppressive therapy. The decision whether and when to start treatment is usually determined by the pattern of the blood counts, the individual patient's life-style and choice, and older age (*see Section 6.4*).

6.3. Administration

Antithymocyte globulin is a powerful immunosuppressive drug and its use in severely neutropenic patients requires very careful monitoring, prophylaxis and treatment of fevers and infections, as well as adequate (and sometimes intensive) platelet transfusional support (grade A recommendation; level Ib evidence).

In the UK, most of Europe and many other countries, the standard preparation of ATG has until recently been horse ATG (Lymphoglobuline; Genzyme). The rabbit preparation (Thymoglobuline; Genzyme) was usually reserved for second or subsequent courses. From June 2007, supply of horse ATG (Lymphoglobuline) was withdrawn due to manufacturing difficulties maintaining quality control. Rabbit ATG (Thymoglobuline) is therefore now recommended as first line treatment. Response rates to rabbit ATG are anticipated to be similar to horse ATG, based on (i) response rates when rabbit ATG is used for a second course (Di Bona *et al*, 1999; Scheinberg *et al*, 2006a) and (ii) both preparations have the same immunogen (thymocytes), similar production method and they bind to similar epitopes. To date, there have been only two studies using rabbit ATG as first line treatment for aplastic anaemia. In a small single centre phase II study of 13 patients with aplastic anaemia and 12 with low risk MDS, among the patients with aplastic anaemia, there were five complete responses and seven partial responses (Garg *et al*, 2009). Preliminary results from a Spanish retrospective multicentre study of 72 patients, reported an overall response rate of 46% (Vallejo *et al*, 2009). For a second course of ATG, options include giving rabbit ATG again or using an alternative

preparation of horse ATG, such as ATGAM (Pharmacia and Upjohn Company, Kalamazoo, MI, USA).

Antithymocyte globulin is given for 5 days as a daily intravenous infusion over 12–18 h through a central venous catheter. The daily dose of rabbit ATG is 1·5 vials/10 kg body weight (one vial of rabbit ATG, Thymoglobuline, contains 25 mg protein so the daily dose is 3·75 mg/kg). A test dose of 1/10th of a vial (2·5 mg for rabbit ATG), diluted in 100 ml normal saline and infused over 1 h, is often given beforehand and, if a severe systemic reaction or anaphylaxis occurs, further doses of that preparation of ATG must not be given. Instead of giving a separate test dose, some centres give the first 100 ml of the first infusion very slowly over 1 h. A patient should not be given ATG from the same animal source after anaphylaxis or a severe systemic reaction.

Immediate side effects are allergic and occur commonly, including fever, rigours, rash, hypertension or hypotension and fluid retention. Each daily dose should be preceded by intravenous methylprednisolone and chlorphenamine. Platelet transfusions should be given to maintain a safe platelet count (ideally $>30 \times 10^9$/l), but should not be given concurrently with ATG administration because of the anti-platelet activity of ATG. Prior to starting ATG, patients should be assessed to ensure adequate platelet increments with random donor platelets. Poor platelet increments should be investigated beforehand, as previously described (*see Transfusional support*). Patients are often nursed in isolation with reverse barrier nursing. All fevers, even if suspected to be due to the ATG, should be treated with broad spectrum antibiotics. Intravenous methylprednisolone (or oral prednisolone) and paracetamol are given at least 30 min before each daily dose of rabbit ATG at 1–2 mg/kg per day (depending on individual study preference) and then orally, reducing the dose by half every 5 days, to help prevent serum sickness, in line with current EBMT studies. Serum sickness typically occurs between day 7 and 14 from the start of ATG treatment. If serum sickness occurs, intravenous hydrocortisone 100 mg six hourly should be commenced. The common symptoms of serum sickness include arthralgia, myalgia, rash, fever, mild proteinuria and platelet consumption often necessitating increased platelet transfusion support.

Antithymocyte globulin must not be given as an out-patient. The patient should remain hospitalised from the start of ATG through the period when serum sickness occurs. If there is immediate access to in-patient or day care facilities for treatment of later complications, such as serum sickness, infection or bleeding, then admission for the 5 days of ATG treatment alone can be considered (grade C recommendation; level IV evidence).

Oral ciclosporin at 5 mg/kg per day may be started either on the first day of ATG (in line with EBMT studies), or after prednisolone has been discontinued, aiming to keep the trough ciclosporin blood level between 150 and 250 μg/l for adults and between 100 and 150 μg/l for children. The latter approach helps reduce drug toxicity. A recent study in children showed no evidence that maintaining higher ciclosporin blood levels improves response rates further; higher blood levels increase the risk of ciclosporin toxicity (Saracco *et al*, 2008). In addition, there was a significant risk of relapse with rapid tapering of ciclosporin and the authors recommend that ciclosporin should be continued for at least 12 months after a maximal response before starting to taper the drug (Saracco *et al*, 2008). A very slow taper is recommended, for example, by 25 mg every 3 months. A similar approach in adults would seem prudent. Blood pressure, renal and liver function tests should also be monitored regularly while on ciclosporin.

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 3 months after the first course because it usually takes around 3 months before a response occurs. There is a 30–60% chance of response to a second course (Tichelli *et al*, 1996; Scheinberg *et al*, 2006a); these figures reflect treatment with either two courses of horse ATG (Tichelli *et al*, 1996) or treatment with rabbit ATG after non-response to horse ATG (Scheinberg *et al*, 2006a). When rabbit ATG is given for the second course following an initial course of horse ATG, the response rate was only 30% for non-responders and 65% for relapsing patients. A recent study from Japan has examined prospectively the outcome of 52 children who have failed one course of IST, and who went on to either receive a second course of ATG or an unrelated donor HSCT. The response to a second course of ATG was only 11% with a 5-year failure-free survival of only 9·5% and three children had anaphylaxis to ATG (Kosaka *et al*, 2008). There are currently no data on re-treatment with rabbit ATG. When horse ATG was still available, it was possible to consider giving a third course of ATG. Those patients most likely to respond were those who have shown response to previous ATG course(s). If patients had shown no response to the first or second courses, then the chance of responding to a third course was low (Gupta *et al*, 2005a). As when giving a first or second course of ATG, a test dose must always be given beforehand, and if there is no severe reaction one can then proceed with the full dose. Instead of giving a separate test dose, as discussed above, the first 100 ml of the first infusion can be given very slowly over 1 h.

6.4. ATG treatment in older patients

The decision whether to use ATG in older patients can be difficult and requires careful assessment and discussion of the risks with the patient. For older patients, the response rate and survival rate are lower compared with younger patients. The response rate for patients aged >60, 50–59 and <50 years is 37%, 49% and 57%, and 5-year survival is 50%, 57% and 72%, respectively (Tichelli *et al*, 1999). For patients aged >70 years, the 10-year survival is 33% compared with 60% for those aged between 50 and 70 years (A. Tichelli, unpublished observations). Older patients (aged >60 years) also have a higher risk of serious cardiac events after ATG (Kao *et al*, 2008). Although there is no upper age limit for ATG treatment, consideration for treatment should be preceded by medical assessment to

exclude significant co-morbidities and bone marrow examination, including trephine and cytogenetics (and/or FISH) to exclude hypocellular MDS. Discussion with the patient should include the increased risk of mortality from bleeding, infection and cardiac events associated with ATG treatment. For older patients who are not candidates for ATG, optimal supportive care should be provided. Ciclosporin may be considered, but because of the increased risk of significant renal toxicity and hypertension in older patients, a lower trough ciclosporin blood level or 100–150 μg/l is suggested. Oxymetholone may be useful in men but often causes unacceptable masculinisation in women. The risk of cardiac failure, liver toxicity, high serum cholesterol, impaired glucose tolerance and prostatism warrant further caution when used in older patients.

6.5. Definition of response

There has previously been no agreement regarding the measurement of response to immunosuppressive therapy, with the result that it has been difficult to compare response rates. New criteria for response have recently been accepted by an expert committee on aplastic anaemia, and these are summarised in Table Va, b (Camitta, 2000). Responses should be confirmed by two or more blood counts at least 4 weeks apart, and should ideally be measured in patients who are not receiving haemopoietic growth factors (Camitta, 2000).

6.6. Follow up of patients post ATG

Following treatment with ATG and ciclosporin, patients should be monitored carefully with regular FBC for evidence of relapse, and also for later clonal disorders such as PNH, MDS and AML. At 3–4 months post-ATG, a screen for PNH should be performed. Further bone marrow examinations with cytogenetics are indicated if there is evidence of relapse or other change in the blood count or blood film. A careful review of the blood film is important to monitor for evidence of MDS. It is suggested that a PNH screen is performed annually in all patients.

Table V. Criteria for response to immunosuppressive therapy in aplastic anaemia.

a. Response criteria for severe aplastic anaemia	
None	Still severe
Partial	Transfusion independent
	No longer meeting criteria for severe disease
Complete	Haemoglobin normal for age
	Neutrophil count $>1{\cdot}5 \times 10^9$/l
	Platelet count $>150 \times 10^9$/l
b. Response criteria for non-severe aplastic anaemia	
None	Worse or not meeting criteria below
Partial	Transfusion independence (if previously dependent)
	or doubling or normalisation of at least one cell line
	or increase of baseline haemoglobin of >30 g/l (if initially <6)
	or increase of baseline neutrophils of $>0{\cdot}5 \times 10^9$/l (if initially <0·5)
	or increase of baseline platelets of $>20 \times 10^9$/l (if initially <20)
Complete	Same criteria as for severe disease

Recommendations

(i) Immunosuppressive therapy is recommended for (1) patients with non-severe aplastic anaemia who are transfusion-dependent (2) patients with severe or very severe disease who are >40 years old and (3) younger patients with severe or very severe disease who do not have an HLA-identical sibling donor.

(ii) ATG is a powerful immunosuppressive drug and its use in severely neutropenic patients requires very careful monitoring, prophylaxis and treatment of fevers, and adequate (and sometimes intensive) platelet transfusional support.

(iii) ATG must only be given as an in-patient.

(iv) Ciclosporin should be continued for at least 12 months after achieving maximal haematological response, followed by a very slow tapering, to reduce the risk of relapse.

7. Matched unrelated donor bone marrow transplantation (MUD BMT)

7.1. Results

The role of MUD BMT in the treatment of severe aplastic anaemia is now clearer in view of recent improvements in morbidity and mortality. Up until the late 1990s, long term survival was around only 30% with a high incidence of graft rejection, GVHD and severe infections (Passweg *et al*, 2006), although more encouraging results had been reported from Milwaukee (Margolis *et al*, 1996) and from Japan (Kodera *et al*, 1999) in children and young adults. In many cases a myeloablative regimen incorporating, most frequently, irradiation had been employed. More recent data has shown improved results using either a non-irradiation, fludarabine-based regimen, as reported by the EBMT (Bacigalupo *et al*, 2005), or a low dose total body irradiation (TBI)-based regimen (Deeg *et al*, 2006), with overall survival of between 65% and 73% at 5 years. The EBMT protocol comprises fludarabine ($30\ mg/m^2 \times 4$), low dose cyclophosphamide ($300\ mg/m^2 \times 4$) and ATG for 4 days, with short course of both ciclosporin and methotrexate as GVHD prophylaxis. The overall 2-year survival is 73% but graft failure is 18% (and 35% in patients >14 years old). A similar protocol has been employed that uses Campath-1H instead of ATG (Gupta *et al*, 2005b). The current EBMT protocol has been modified for patients >14 years, to include the addition of 2 Gy TBI, and a reduction in ATG to 7·5 mg in order to reduce the risk of EBV post-transplant lymphoproliferative disorder (Bacigalupo *et al*,

2009). The EBMT Severe Aplastic Anaemia Working Party view is to avoid irradiation in children and young adults, even at low doses, and to use fludarabine instead. For older patients, the addition of low dose irradiation may be of benefit in reducing graft rejection (Bacigalupo *et al*, 2005; Deeg *et al*, 2006) (grade B recommendation; level III evidence).

7.2. Indications

Matched unrelated donor bone marrow transplantation may be considered when patients fulfil all the following criteria. They should:

(1) have a fully matched (at DNA level for both class I and II antigens) donor
(2) be <50 years old (although patients aged 50–60 years may be considered if good performance status). There are currently insufficient data available on the use with HSCT in SAA patients >60 years of age (Gupta *et al*, 2008). However, for MDS patients transplanted using a reduced intensity conditioned HSCT, multivariate analysis of factors for overall survival, showed no significant difference for patients aged >60 compared with 50–60 years (Lim *et al*, 2009)
(3) have failed at least one course of ATG and ciclosporin (for both adults and children), although in adults a second course of ATG may be preferred if there are particular reasons not to proceed to MUD BMT after one failed course, based on individual patient circumstances
(4) have severe or very severe aplastic anaemia and
(5) have no evidence of active infection and or acute bleeding at time of BMT (Grade B recommendation, level IV evidence). However, see comments under Psychological and general support
(6) be used as first line treatment in patients with constitutional aplastic anaemia and with no HLA matched sibling donor.

Even then, the decision to proceed with MUD BMT or a second course of ATG is not always straightforward, especially when the patient may be clinically well. Full discussions about other treatment options and the natural history of aplastic anaemia should take place with the patient and family who must be appropriately informed about the risks of MUD BMT in aplastic anaemia. Because results of MUD BMT for acquired aplastic anaemia have improved significantly over the last 5–10 years (Viollier *et al*, 2007), MUD BMT should no longer be considered as a last resort after failing two courses of ATG, as previously recommended in these guidelines (Marsh *et al*, 2003). The disadvantages of continuing with ATG is that the patient's condition may continue to deteriorate with continuing sepsis and increasing iron overload, thus reducing the chance of a successful outcome at time of transplantation. Because aplastic anaemia is a rare disease and because of the particular risks of MUD BMT in this condition, such a procedure should only be done at centres with experience in transplantation for aplastic anaemia and accredited by the European Blood and Marrow Transplant Registry for unrelated donor BMT. Written guidelines from the United Kingdom Children's Cancer Study group are currently in preparation for paediatric BMT conditioning regimens.

7.3. Conditioning regimen

The current regimen recommended for younger patients by the EBMT is (i) Cyclophosphamide 300 mg/m^2 × 4 (ii) fludarabine 30 mg/m^2 × 4 (iii) ATG (Thymoglobuline, rabbit; Genzyme) 1·5 vials/10 kg × 4 (or Campath-1H 0·2 mg/kg to maximum dose of 10 mg/day × 5 pre-transplant) (iv) ciclosporin commencing on day −6 at 1 mg/kg per day to day −2, then 2 mg/kg per day from day −1 to +20, then 8 mg/kg per day orally and (v) methotrexate 10 mg/m^2 on day +1, then 8 mg/m^2 on days +3 and +6, if using ATG instead of Campath-1H. For older patients, the addition of 200 cGy TBI with reduced ATG (given for 2 days instead of four) may be considered (grade B recommendation; level III evidence).

It is acceptable to use either ATG or Campath-1H depending on the patient's previous exposure to ATG and individual centre preference. Other approaches are valid within the setting of prospective clinical trials. Patients should receive bone marrow and not PBSC as the source of stem cells and at least 3 × 10^8 nucleated cells/kg should be infused. (*see Section 4.1.2.1*).

7.4. Timing of unrelated donor BMT search

An unrelated donor marrow search should be performed in patients with severe aplastic anaemia who may be eligible for unrelated donor transplantation and who do not have an HLA-identical sibling donor, at the time of the first course of ATG, so that at the time of assessment of response to ATG (about 3 months), further information regarding the possible availability of a high resolution matched donor can be sought. For both children and adults, an unrelated donor transplant may be considered if there is no response to a first course of ATG, although in adults a second course of ATG may be preferred if there are particular reasons not to proceed to MUD BMT after one failed course, based on individual patient circumstances.

Recommendations

(i) MUD BMT may be considered when a patient has a fully matched donor, they are <50 years old (or 50–60 years old with good performance status), and have failed at least one course of ATG and ciclosporin, have severe aplastic anaemia. There is currently insufficient data on outcome for patients >60 years of age.
(ii) The optimal conditioning regimen for MUD BMT is uncertain, but currently a fludarabine, non-irradiation-based regimen is favoured for younger patients.

8. Trial therapy or clinical research protocols

8.1. Other immunosuppressive drugs

8.1.1. High dose cyclophosphamide without stem cell support. The use of high dose cyclophosphamide (45 mg/kg × 4) without stem cell support has been proposed by one centre as treatment for patients with newly diagnosed aplastic anaemia (Brodsky *et al*, 1996, 2001). However, a prospective randomised study comparing its use in combination with ciclosporin against the gold standard of ATG and ciclosporin was terminated prematurely because of an excess of early deaths and systemic fungal infections in the cyclophosphamide arm. The use of cyclophosphamide was associated with profound and very prolonged pancytopenia resulting in a significant increase in use of blood and platelet transfusions, days of intravenous antibiotics and amphotericin and in-patient days in hospital (Tisdale *et al*, 2000a, 2002). For patients refractory to ATG, high dose cyclophosphamide induces a response in 70% of patients but does not eradicate PNH clones in all patients and later MDS has been reported (Brodsky *et al*, 2004).

Therefore, the use of high dose cyclophosphamide without stem cell support cannot be recommended in either newly diagnosed patients or patients who have failed ATG and ciclosporin in view of its serious toxicity and high mortality (Grade A recommendation, level Ib recommendation).

8.1.2. Mycophenolate mofetil. Mycophenolate mofetil (MMF) inhibits the proliferation of B and T-lymphocytes and has been used in the treatment and prevention of rejection in organ transplantation as well as in the treatment of autoimmune disorders, such as ulcerative colitis, rheumatoid arthritis and multiple sclerosis. Its use in the treatment of refractory aplastic anaemia is anecdotal and there are no reported series of patients treated with the drug (Tisdale *et al*, 2000b). The EBMT SAA Working Party has recently performed a pilot study of 17 patients (Schrezenmeier *et al*, 2003) to assess its safety and efficacy in patients who are ineligible for BMT and refractory to standard immunosuppressive therapy. However, no responses were observed (Schrezenmeier *et al*, 2003). A retrospective study from the US National Institutes of Health (NIH) showed no improvement in response or reduction in relapse after ATG and ciclosporin when MMF was added (Scheinberg *et al*, 2006b). MMF appears to be ineffective in the treatment of patients with refractory aplastic anaemia.

8.1.3. Alemtuzumab (Campath-1H). Campath-1H is currently under evaluation for the treatment of refractory aplastic anaemia in prospective trials at NIH in USA, and retrospectively by the EBMT, following reports of its efficacy in patients with autoimmune cytopenias, particularly autoimmune neutropenia (Willis *et al*, 2001).

8.2. Oxymetholone

Oxymetholone was used extensively in the treatment of aplastic anaemia for many decades before the availability of ATG and ciclosporin. In some patients, oxymetholone can stimulate erythropoiesis in particular but sometimes can produce a trilineage response. Response to androgens, particularly if no PNH clone is present, raises the possibility of a congenital cause for the marrow failure. In combination with ATG, it increases the response compared to ATG alone (Bacigalupo *et al*, 1993; Leleu *et al*, 2006). Oxymetholone is hepatotoxic and can cause liver dysfunction, clinical jaundice, hepatomas and peliosis hepatis. It must therefore be used with caution, with regular monitoring of liver function tests and liver ultrasound. Because the drug causes virilization, it is often unacceptable to women. The drug is available on a named patient basis and is still useful as an option for those patients who have failed several courses of ATG and ciclosporin, or in certain patients where standard immunosuppressive treatment may not be possible.

8.3. Should haemopoietic growth factors, such as G-CSF, be used with ATG and ciclosporin?

The rationale for using G-CSF after ATG and ciclosporin was to attempt to reduce the risk of infection during the 3 months before haematological, particularly neutrophil, response is expected, and also to improve response (trilineage) to immunosuppressive therapy, as G-CSF may work in combination with other endogenous haemopoietic growth factors to stimulate haemopoietic stem cells. However, there are concerns about the cost of using G-CSF long term and at high dose and the potential increase in later clonal disorders, particularly from studies in Japan, which can only be fully appreciated with follow up of at least 10 years (Ohara *et al*, 1997; Kaito *et al*, 1998; Marsh, 2000; Socie *et al*, 2000). A pilot study of 100 patients treated with ATG and ciclosporin and 3 months of G-CSF was associated with low mortality, a response rate of almost 80% and actuarial survival at 5 years of 87% (Bacigalupo *et al*, 2000a). However, a relatively small, prospective randomised study comparing ATG, ciclosporin and G-CSF with ATG and ciclosporin alone demonstrated no difference in response and survival between the two groups. Although there was no obvious increase in clonal disorders, the follow up of this study was too short to evaluate this properly. (Locascuilli *et al*, 2001; Gluckman *et al*, 2002). A recent prospective randomised study of 101 adult patients from Japan comparing ATG and ciclosporin with or without G-CSF showed a higher response rate at 6 months and a lower relapse rate in the G-CSF arm, but no difference in survival between the two arms. Although there was no difference in the incidence of MDS and AML at 4 years, the follow up was too short to evaluate this adequately (Teramura *et al*, 2007). This question is also currently being evaluated further in a larger prospective randomised multicentre EBMT study, which has just closed and is awaiting

analysis. The EBMT Severe Aplastic Anaemia Working Party has recently reported results of a large retrospective study of 840 patients treated with ATG and ciclosporin, of whom 43% also received G-CSF. The incidence of MDS/AML was 10·9% with G-CSF and 5·8% without G-CSF (Socie *et al*, 2007). The routine use of long term G-CSF after ATG and ciclosporin is not currently recommended outside prospective clinical trials (grade A recommendation; level Ib evidence).

A large prospective randomised study from China showed no benefit in using both GM-CSF and erythropoietin with ATG and ciclosporin, as well as confirming that the combination of ATG and ciclosporin is superior to ATG alone in terms of response and survival (Zheng *et al*, 2006).

Recommendations

(i) The use of high dose cyclophosphamide without stem cell support is not recommended in the treatment of aplastic anaemia.
(ii) MMF does not appear to be effective in the treatment of aplastic anaemia.
(iii) The routine use of long term G-CSF, or other haemopoietic growth factors, after ATG and ciclosporin is not recommended outside the setting of prospective clinical trials.

9. Management of aplastic anaemia in the presence of an abnormal cytogenetic clone

As discussed previously (*see section 2.8*) an abnormal cytogenetic clone can be detected in up to 12% of patients with aplastic anaemia at diagnosis (Appelbaum *et al*, 1989; Socie *et al*, 2000; Gupta *et al*, 2006). The most frequently observed abnormalities include trisomy 8, trisomy 6, 5q- and anomalies of chromosomes 7 and 13. Often the abnormal clone is small, comprising only a small proportion of total metaphases, and not infrequently, it may be transient and disappear spontaneously or after haematological response to immunosuppressive therapy (Mikhailova *et al*, 1986; Geary *et al*, 1999; Piaggio *et al*, 1999; Socie *et al*, 2000; Ishiyama *et al*, 2002; Gupta *et al*, 2006). From small reported series, the response rates to immunosuppressive therapy appear to be similar to patients with aplastic anaemia who lack an abnormal cytogenetic clone (Mikhailova *et al*, 1986; Geary *et al*, 1999; Ishiyama *et al*, 2002), with good response seen particularly in those patients with trisomies (Gupta *et al*, 2006). Maciejewski *et al* (2002) also reported a good response to immunosuppressive treatment in patients who acquired a trisomy 8 after treatment compared with a worse prognosis and high risk of leukaemic transformation for patients with monosomy 7.

In the absence of morphological evidence of MDS or AML after thorough review of blood and bone marrow slides (*see Sections 2.1, 2.2 and 2.11*) a diagnosis of aplastic anaemia rather than hypocellular MDS/AML can usually be made confidently. The presence of monosomy 7, however, is often more sinister with a high risk of transformation to MDS or acute leukaemia. Monosomy 7 in children should be notified to the Paediatric MDS Registry and treated as MDS.

Patients with aplastic anaemia and an abnormal cytogenetic clone (except monosomy 7) who are not BMT candidates should be managed in the same way as patients who lack an abnormal cytogenetic clone (grade C recommendation; level IV evidence). These patients should be treated with immunosuppressive therapy (ATG and ciclosporin) and should not receive chemotherapy as this will result in predictable worsening of pancytopenia with the likelihood of irreversible marrow failure. If the patient fulfils the criteria for sibling BMT (i.e., they have severe aplastic anaemia, an HLA-identical sibling donor and are <30–40 years of age), they should be transplanted. For patients with monosomy 7, for example, a myeloablative regimen may be preferable. For other chromosome abnormalities, however, there is no data to support this approach as the disease appears to follow a course similar to that of aplastic anaemia (Geary *et al*, 1999; Piaggio *et al*, 1999; Ishiyama *et al*, 2002; Maciejewski *et al*, 2002). In this situation, the standard immunosuppressive conditioning regimen for transplantation for aplastic anaemia should be used (grade C recommendation; level IV evidence). The presence of an abnormal cytogenetic clone alone (except perhaps monosomy 7) is not an indication for BMT if the patient does not have severe aplastic anaemia. The conditioning regimen for children with an abnormal cytogenetic clone should be discussed with the Paediatric MDS Registry.

For patients with an abnormal cytogenetic clone, bone marrow examination with cytogenetic analysis should be repeated every 6–12 months. If there is any evidence of dysplasia or if blasts are seen, the patient can be considered for early BMT. A rising proportion of abnormal metaphases should also alert one to the possibility of transformation.

Recommendations

(i) The presence of an abnormal cytogenetic clone in the presence of a otherwise typical aplastic anaemia, does not necessarily equate with a diagnosis of MDS or AML, as abnormal cytogenetic clones occur in up to 12% of patients with aplastic anaemia.
(ii) The presence of monosomy 7 is more often sinister with a high risk of transformation to MDS or AML.

10. Management of patients with aplastic anaemia who have a significant PNH clone, resulting in clinical and/or laboratory evidence of haemolysis

Patients with aplastic anaemia may later develop haemolytic PNH and conversely patients with haemolytic PNH can later progress to aplastic anaemia (Socie *et al*, 2000). A comprehensive overview of the diagnosis and management of PNH has recently been drawn up by the International PNH Interest

Group (Parker *et al*, 2005). Evolution to haemolytic PNH may be associated with worsening anaemia and reticulocytosis (or sometimes a rise in haemoglobin level) or recurrent pancytopenia. Abdominal pain and jaundice should alert one to the possible diagnosis of hepatic vein thrombosis (Budd Chiari syndrome) and should be further investigated with Doppler ultrasound. The bone marrow in PNH is hypercellular with erythroid hyperplasia. Regular or intermittent blood transfusions may be required and the now standard pre-storage leucocyte-depleted red cells are safe to use. There is rarely the need to use washed red cells to prevent acute haemolysis, except when this very occasionally occurs after transfusion with leucocyte-depleted blood in PNH patients. All patients should receive regular folic acid supplementation 5 mg/day. If the patient becomes iron deficient due to intravascular haemolysis, oral iron supplementation should be given, but cautiously, as it can trigger acute haemolysis. It is recommended to start with a low dose, for example 200 mg on alternate days and then increase to 200 mg daily if no acute haemolysis occurs (Packman, 1998).

For patients with severe and/or frequent episodes of acute intravascular haemolysis, prednisolone usually helps to reduce the degree of haemolysis, but continued use of high doses is usually limited by their side effects, necessitating an alternate day regimen at low dose of between 10 and 15 mg. An alternative option is to consider oral ciclosporin, maintaining trough blood levels between 150 and 250 μg/l, which may improve the haemoglobin level as well as the neutrophil and platelet counts (Van Kamp *et al*, 1995). Eculizumab (Alexion), a complement C5 blocking monoclonal antibody, has recently been shown to be effective at reducing haemolytic paroxysms and blood transfusion requirements, and reducing the risk of thrombosis, in patients with haemolytic PNH (Hillmen *et al*, 2004, 2006, 2007; Hill *et al*, 2005).

It is still reasonable to consider ATG treatment for patients with a significant PNH clone (>50%). An increased risk of haemolysis during treatment and the period of serum sickness is anticipated but this may be reduced by commencing prednisolone on the first day of ATG and by using 2 mg/kg instead of 1 mg/kg prednisolone (*see Section 6.3*).

Human leucocyte antigen-identical sibling BMT for haemolytic PNH is only indicated for those patients who later develop severe aplastic anaemia or patients with multiple and life-threatening venous thromboses (Saso *et al*, 1999; Parker *et al*, 2005), although the effectiveness of Eculizumab in reducing venous thromboses may obviate the indication for BMT in patients with severe thromboses (Hillmen *et al*, 2007).

In patients with aplastic anaemia one can commonly detect a small PNH clone by flow cytometry in the absence of haemolysis and in the presence of a hypocellular bone marrow (Dunn *et al*, 1999; Socie *et al*, 2000). Most often the monocyte and neutrophil series are affected alone and usually the affected clone comprises only a small proportion of the cells. The PNH clone may vary, either increasing or decreasing in size or it may remain stable. It is recommended that these patients are treated in exactly the same way as for patients with aplastic anaemia who lack a PNH clone. Although one series reported a lower response rate to ATG (Schrezenmeier *et al*, 1995b), two subsequent studies have shown similar response to ATG regardless of whether there is a small PNH clone present or not (Delord *et al*, 1998; Dunn *et al*, 1999).

The decision to start anticoagulation in aplastic anaemia patients with a significant PNH clone (>50% in granulocytes, Hall *et al*, 2003) is controversial: some centres routinely anticoagulate all patients, but others only start anticoagulation (i) after one episode of venous thrombosis or (ii) if there is reduced flow through the hepatic veins on Doppler ultrasound scan or (iii) in the presence of recurrent abdominal pain. Routine anticoagulation is contraindicated in aplastic anaemia patients with a platelet count $<50 \times 10^9$/l.

Recommendations

(i) Small PNH clones, in the absence of evidence of haemolysis, occur in up to 50% of patients with aplastic anaemia.

(ii) ATG is not recommended if there is a history of PNH-associated thrombosis (grade C recommendation; level IV evidence).

(iii) All patients who are not transplanted should be screened for PNH by flow cytometry every 12 months.

11. Management of aplastic anaemia in pregnancy

This is a difficult area. Aplastic anaemia can present in pregnancy although this may be due to chance and other possible causes should always be sought. The disease may remit spontaneously after termination, whether spontaneous or therapeutic, and after delivery, but not in all cases and much support may be needed. The disease often progresses during pregnancy and there is a significant risk of relapse in pregnancy in patients who have previously responded to immunosuppressive therapy (Aitchison *et al*, 1989; Van Besien *et al*, 1991; Oosterkamp *et al*, 1998; Tichelli *et al*, 2002; Kwon *et al*, 2006). In contrast, after successful allogeneic BMT, pregnancy does not appear to trigger relapse of the disease (Sanders *et al*, 1996; Deeg *et al*, 1998; Kahl *et al*, 2005).

A recently reported EBMT SAA Working Party study evaluated the outcome of pregnancy and disease course among 36 pregnancies in women previously treated with immunosuppression (Tichelli *et al*, 2002). Almost half the pregnancies involved a complication in the mother and/or baby. There were five premature births and three abortions (one spontaneous). All infants born alive had normal postnatal development. There were two cases of eclampsia and two maternal deaths after delivery. Relapse of aplastic anaemia occurred in 19% and a further 14% needed transfusion during delivery.

Most patients with relapsed aplastic anaemia or progressive thrombocytopenia during pregnancy were delivered by caesarian section. During pregnancy, blood counts changed significantly – haemoglobin levels and platelet counts decreased while neutrophil counts increased. However, the counts tended to return to pre-pregnancy values within 1–6 months of delivery. Normal blood counts before conception did not guarantee freedom from relapse of aplastic anaemia during pregnancy.

It is possible for a patient with aplastic anaemia to go through pregnancy safely. The prognosis is better than it was several decades ago, largely because of better supportive care particularly in supply of blood products. From a recently reported single centre experience of 14 patients, treated with transfusional support alone to maintain Hb >80 g/l and platelet count $>20 \times 10^9$/l, there were no maternal deaths (Kwon *et al*, 2006). However, it is important to discuss with the patient and family the potentially serious risks to both the mother and baby. The final decision whether to proceed with the pregnancy or have a therapeutic abortion lies with the patient after being fully informed of the risks.

Supportive care is the mainstay of treatment of aplastic anaemia in pregnancy and the platelet count should, if possible, be maintained above 20×10^9/l with platelet transfusions. This recommendation is based on the BCSH guidelines for treatment of ITP in pregnancy (Provan *et al*, 2003). There is an increased risk of alloimmunisation to red cell and platelet transfusions during normal pregnancy and this risk is increased further in aplastic anaemia (*see Section 4.2.1*). ATG is too hazardous to give during pregnancy, although there is one reported case of its use in late pregnancy in a patient with very severe aplastic anaemia who delivered a normal healthy baby (Aitchison *et al*, 1989). One can consider the use of ciclosporin in pregnancy. Data from renal transplant recipients shows that ciclosporin seems to be safe and does not increase the risk of malformations above the risk for the general population (Armenti *et al*, 1994; Stanley *et al*, 1999; Little *et al*, 2000; McKay & Josephson, 2006). If a patient needs transfusions or if the blood counts are falling towards levels that will soon require transfusional support, it is recommended to start oral ciclosporin 5 mg/kg per day to maintain levels between 150 and 250 μg/l (grade C recommendation; level IV evidence). Response to ciclosporin is delayed and may take between 6 and 12 weeks.

Finally, it is essential that the patient and their blood counts are monitored frequently throughout pregnancy, initially monthly but later more frequently and according to disease severity, and very close liaison with the obstetric team and general practitioner is essential. The mode of delivery should be determined on obstetric grounds.

Recommendations

(i) There is a high risk (33%) of relapse of aplastic anaemia in pregnancy.

(ii) Supportive care is the mainstay of treatment in pregnancy and the platelet count should be maintained $>20 \times 10^9$/l, if possible.

(iii) It is safe to use ciclosporin in pregnancy.

Suggested topics for audit

1. The use of irradiated blood products in aplastic anaemia patients.
2. The effectiveness and safety of iron chelation therapies in patients with transfusion-dependent aplastic anaemia.
3. Comparison of infectious complications in aplastic anaemia patients transplanted with ATG- or Alemtuzumab-conditioning regimens.

Disclaimer

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Keywords: aplastic anaemia, antithymocyte globulin, stem cell transplantation.

Appendix I

Classification of evidence levels

Ia	Evidence obtained from meta-analysis of randomised controlled trials
Ib	Evidence obtained from at least one randomised controlled trial
IIa	Evidence obtained from at least one well-designed controlled study without randomisation
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study*
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlated studies and case studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities

*Refers to a situation in which implementation is outwith the control of the investigators, but an opportunity exists to evaluate its effect.

Classification of grades of recommendation

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing specific recommendation	Evidence levels Ia, Ib
B	Requires the availability of well-conducted clinical studies but no randomised clinical trials on the topic of recommendation	Evidence levels IIa, IIb, III
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality	Evidence level IV