

# **Guidelines for the diagnosis and management of aplastic anaemia**

## **British Committee for Standards in Haematology**

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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 2004-2008 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>). 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 2003 in the British Journal of Haematology. This guideline is an update of the 2003 guideline and is to replace the 2003 guideline.

## **Summary of key recommendations**

- **Aplastic anaemia (AA) is a rare but heterogeneous disorder. The majority (70-80%) of these cases are categorized as idiopathic because their primary aetiology is unknown. In a subset of cases, a drug or infection can be identified that precipitates the BM 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 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 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 MDS
  - (iv) exclude a possible late onset inherited bone marrow failure disorder
- A 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 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 rHuEpo in aplastic anaemia is not recommended. 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 one week if there is no increase in the neutrophil count.

- (v) Prophylactic antibiotic and antifungal drugs should be given to patients with neutrophil count  $< 0.2 \times 10^9/l$ . 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  $> 1000\mu g/l$ .

- Definitive treatment

- (1) Infection or uncontrolled bleeding should be treated first before giving immunosuppressive therapy. This also applies to patients having 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 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. 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 (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. The standard immunosuppressive regimen is a combination of 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) MUD BMT may be considered when a patient has severe aplastic anaemia, has a fully matched 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 40%) of relapse of aplastic anaemia in pregnancy. Supportive care is the mainstay of treatment in pregnancy and the platelet count should be maintained > 20 x 10<sup>9</sup>/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). For these guidelines we 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; Montane et al, 2008). The incidence is 2-3 times higher in East Asia. There is a biphasic age distribution with peaks from 10-25 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 in males and 48 years in females reported by Alter, [Young and Alter, 1994]). The findings of leukoplakia, 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 and 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 and Dokal, 2006). A preceding history of jaundice, usually 2-3 months before, may indicate a post-hepatitic aplastic anaemia (Gordon-Smith, 1991; Young and 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 (Young and Alter, 1994; Kauffmann et al, 1996; Baumelou et al, 1993; Issaragrissil et al, 1997; Heimpel, 1996), (see table 1). A careful drug history should be obtained detailing all drug exposures for a period beginning 6 months and ending one month prior to presentation (Kauffmann et al, 1996; Heimpel, 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 Medicines and Healthcare products Regulatory Agency (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: [www.yellowcard.gov.uk](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 2.

## **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 (1) confirm the diagnosis (2) exclude other possible causes of pancytopenia with a hypocellular bone marrow (3) exclude inherited aplastic anaemia (4) screen for an underlying cause of aplastic anaemia and (5) document or exclude a co-existing abnormal cytogenetic clone or a paroxysmal nocturnal haemoglobinuria (PNH) clone. See table 3 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. 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 paediatric myelodysplastic syndrome (MDS) which may feature in the differential diagnosis of pancytopenia in children. It may also be useful in screening for inherited bone marrow failure disorders in adults with apparent acquired disease.

### **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 (BCSH, 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 2cm 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 and 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 (1) haemoglobin < 10g/dl (2) platelet count <  $50 \times 10^9/l$  (3) neutrophil count <  $1.5 \times 10^9/l$  (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 4 (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 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 which 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 sent 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 bone marrow transplantation (BMT) is being considered. Parvovirus causes red cell aplasia but not aplastic anaemia. 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.

## **2.6 Autoantibody screen**

The occurrence of pancytopenia in systemic lupus erythematosus may (1) be autoimmune in nature occurring with a cellular bone marrow or (2) be associated with myelofibrosis or rarely (3) occur with a hypocellular bone marrow. Blood should be sent for anti-nuclear antibody and anti-DNA antibody in all patients presenting with aplastic anaemia.

## **2.7 Tests to detect a paroxysmal nocturnal haemoglobinuria (PNH) clone**

PNH should be excluded by performing flow cytometry (Dacie and 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 quantitated 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 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 4.3.

## **2.9 Screen for inherited disorders**

Peripheral blood lymphocytes should be tested for chromosomal breakage to identify or exclude Fanconi anaemia. This should be performed in all patients who are BMT candidates. 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. 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 sent 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 (World Health Organisation Classification of Tumours, 2001; Tuzuner et al, 1995; Bennett and 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 MRC 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 which 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 (Catovsky, 2000).
- Lymphomas, either Hodgkin's disease or non-Hodgkin's 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 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 MGG stained aspirate. Some degree of fat change may also be seen in aplastic anaemia, especially early on in its evolution.

- 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 and Guinan, 2007).

A multidisciplinary team (MDT) meeting approach is recommended to collate relevant results and 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 (BCSH guidelines, 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 bone marrow transplantation (BMT) (Kaminsky et al, 1990). Other causes of platelet refractoriness should also be excluded, namely sepsis and drugs such as amphotericin and vancomycin. 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 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. The rationale for considering the use of irradiated blood products is two-fold (1) 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 (Consensus document for treating aplastic anaemia, 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. (2) Are irradiated blood products indicated during and after ATG therapy to prevent transfusion associated GVHD (TA-GVHD)? There has been only one likely case of transfusion associated GVHD reported after ATG treatment from one European centre (Per Lungman, personal communication 2007), but this occurred before the availability of leuco-depleted blood products. The recent Serious Hazards Of Transfusion (SHOT) annual report indicates that there have been no new cases of TA-GVHD in the UK since 2000-2001; 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) 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  $> 1.0 \times 10^9/l$  [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 TRALI (transfusion related acute lung injury) are well recognized complications following granulocyte transfusions. The use of irradiated granulocyte transfusions should therefore be limited to patients in whom the possible benefits outweigh the hazards (NBS 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 cells and platelet count in patients with aplastic anaemia (see reference Marsh et al, 2007 for a general review). Anecdotal use of erythropoietin (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 (rh-TPO) in aplastic anaemia. The development of anti-TPO antibodies against the truncated version of rHu-TPO, pegylated rHu-megakaryocyte and development factor (PEG-rHuMGDF) 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 at high risk of infection should be managed 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). Laminar air-flow facilities are not essential but when available, should be used. 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 *C.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 licensed, 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, which has clinically significant but manageable or avoidable interactions with other drugs, and posaconazole 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 (BCSH Guidelines on the management of invasive fungal infection during therapy for haematological malignancy, 2007)

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). Prophylaxis against PCP is essential post BMT but is not routinely given during ATG treatment (Ljungman, 2000).

For patients who are in the community and who have not recently received ATG or undergone BMT, continued mouthcare with an antiseptic mouthwash is recommended, but routine prophylactic antimicrobials are not required in all patients. For patients who are very severely neutropenic (neutrophil count  $< 0.2 \times 10^9/l$ ), 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  $0.2-0.5 \times 10^9/l$ ). 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 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  $\beta$ -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 antibiotic choice, 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 licensed 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 CXR should be included as part of the investigation of new or persistent fever, with high resolution CT 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 $\mu$ g/kg/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 one 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  $> 1000\mu\text{g/l}$ , **although the evidence base for this is lacking (Porter, 2001 and John Porter, 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 L1 (Porter, 2001), its use is not routinely recommended in patients with aplastic anaemia. The novel oral iron chelator Deferasirox (Exjade) is now licensed 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 (Viillard, 2000; Hendry, 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](mailto:aplasticanaemia@hotmail.com), website: [www.theatt.org.uk](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. The temptation to give up or try inappropriately risky procedures/drugs must be resisted, because late recovery, sometimes after a year or more, can occur not infrequently in these patients.

## **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 one week if there is no increase in the neutrophil count.**
- (vi) Prophylactic antibiotic and antifungal drugs should be given to patients with neutrophil count  $< 0.2 \times 10^9/l$ .**
- (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  $> 1000\mu g/l$ .**

#### **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 using ATG, including the recognition and management of its side effects. Level 4 care is required for related allogeneic bone marrow transplantation, providing the centre has experience in BMT for aplastic anaemia. BSBMT and 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, 1994; Marsh et al, 2007)

The recommendations for specific treatment of aplastic anaemia are summarised in figures 1 and 2.

## **Recommendations**

- (i) Infection or uncontrolled bleeding should be treated first before giving immunosuppressive therapy. This also applies to patient having 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 (Bacigalupo et al, 2000a; Passweg et al, 1997; Locatelli et al, 2000; Kahl et al, 2005; Gupta et al, 2004; Ades et al, 2004; Champlin et al, 2007; Myers and Davies, 2009). Using high dose cyclophosphamide with ATG conditioning, graft failure is around 4-14%. Graft versus host disease (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 (1) have severe or very severe aplastic anaemia (see Section 2.4 and Table 4 for definitions of disease severity), (2) are younger than 40\* years and (3) have an HLA compatible sibling donor. (4) 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 (Gluckman et al, 2000) [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 50mg/kg x 4 (day -5 to -2) and ATG (Thymoglobuline, Genzyme 1.5 vials/10kg x 3 on days -5 to -3), with methylprednisolone 2mg/kg x 3 (day -5 to -3). (Methylprednisolone is not usually used for paediatric BMT). The recommended post transplant immunosuppression is (1) ciclosporin 5mg/kg/day, in two divided doses (that is, 2.5mg/kg BD), starting on day -1, and continuing for 12 months with tapering beginning at 9 months to help prevent late graft failure, and (2) short course methotrexate 15mg/m<sup>2</sup> on day +1, then 10mg/m<sup>2</sup> on days +3, +6, and +11 (Consensus document for treating aplastic anaemia, 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. 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 1200mg/m<sup>2</sup>, fludarabine 120mg/m<sup>2</sup> and either ATG or Alemtuzumab (Maury et al, 2007, Gupta et al, 2004) 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 and Guinan, 2007).

It is important to give at least  $3 \times 10^8$  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 recommended that at least  $3 \times 10^6$ /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 graft-versus-host disease 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 and 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 chimerism, as defined by >10% recipient cells or >15% increase over 3 months, using short tandem repeats by PCR analysis of mononuclear cells (McCann et al, 2007). Progressive mixed chimerism predicts a high risk of graft rejection. Stable mixed chimerism 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 350µg/l. For children, lower ciclosporin levels are often used (150-200µg/l), to avoid toxicity. Chimerism should be monitored particularly closely during the time of ciclosporin withdrawal. If there is evidence of significant mixed chimerism (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 60-80% 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  $ARC \geq 25 \times 10^9/l$  and  $ALC \geq 1.0 \times 10^9/l$ , 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 to 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 (1) patients with non-severe aplastic anaemia who are dependent on red cell and/or platelet transfusions (2) patients with non-severe aplastic anaemia who, although not transfusion dependent, may have significant neutropenia and be at risk of infection (3) patients with severe or very severe aplastic anaemia who are > 40 years of age and (4) 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**

ATG 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, 2006) and (ii) both preparations have the same immunogen (thymocytes), similar production method and they bind to similar epitopes. However, to date, there have been no reported studies using rabbit ATG as first line treatment for aplastic anaemia. 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, Michigan).

ATG is given for 5 days as a daily intravenous infusion over 12-18 hours through a central venous catheter. The daily dose of rabbit ATG is 1.5 vials/10kg body weight (one vial of rabbit ATG, Thymoglobuline, contains 25mg protein so the daily dose is 3.75mg/Kg). A test dose of 1/10<sup>th</sup> of a vial (2.5mg for rabbit ATG), diluted in 100ml normal saline and infused over one hour, 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 100ml of the first infusion very slowly over one hour.

Immediate side effects are allergic and occur commonly, including fever, rigors, rash, hypertension or hypotension and fluid retention. Each daily dose should be preceded by intravenous methylprednisolone and chlorpheniramine. 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 minutes before each daily dose of rabbit ATG at 1-2mg/kg/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 100mg 6 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.

ATG 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 5mg/kg/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 $\mu$ g/l for adults and between 100 and 150 $\mu$ g/l for children. The latter approach helps reduce drug toxicity. From a recent study in children, it was shown that there is no evidence that maintaining higher ciclosporin blood levels improves response rates further; higher blood levels increase the risk of ciclosporin toxicity. 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 25mg 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, 2006). 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 UD HSCT. The response to a second course of ATG was only 11% with a 5 year failure free survival of only 9.5% and 3 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, 2005). 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 100ml of the first infusion can be given very slowly over one hour.

#### **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 - 70 years (Tichelli, meeting presentation 2005). 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 pre-ceded 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 100-150µg/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 on 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 Tables 5a and 5b. 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 to 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.

## 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 TBI-based regimen ( Deeg et al 2006), with overall survival of between 65 and 73% at 5 years. The EBMT protocol comprises fludarabine ( $30\text{mg}/\text{m}^2 \times 4$ ), low dose cyclophosphamide ( $300\text{mg}/\text{m}^2 \times 4$ ) and ATG for 4 days, with short course of both ciclosporin and methotrexate as GVHD prophylaxis. Overall 2 year survival is 73% but graft failure is 18% (and 35% in patients > 14 years old). A similar protocol has been employed which uses Campath-1H instead of ATG (Gupta et al, 2005). The current EBMT protocol has been modified for patients > 14 years, to include the addition of 2Gy TBI, and a reduction in ATG to 7.5mg in order to reduce

the risk of EBV post transplant lymphoproliferative disorder (Bacigalupo et al, 2009). 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

MUD BMT 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)
- (3) for adults and children, have failed at least one course of ATG and ciclosporin, 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) as first line treatment in patients with constitutional aplastic anaemia and with no HLA matched sibling donor, as ATG is unlikely to be of benefit.

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 (BCSH guidelines 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 EBMT for unrelated donor BMT. Written guidelines from the UKCCSG are currently in preparation for paediatric BMT conditioning regimens.

### **7.3 Conditioning regimen**

The current regimen recommended for younger patients by the EBMT is (1) Cyclophosphamide 300mg/m<sup>2</sup> x 4 (2) fludarabine 30mg/m<sup>2</sup> x 4 (3) ATG (Thymoglobuline, rabbit, Genzyme) 1.5 vials/10kg x 4 (or Campath-1H 0.2mg/kg to maximum dose of 10mg/day x 5 pre-transplant) (4) ciclosporin commencing on day – 6 at 1mg/kg/day to day – 2 then 2mg/kg/day from day –1 to day + 20 then 8mg/kg/day orally and (5) methotrexate 10mg/m<sup>2</sup> on day + 1, then 8mg/m<sup>2</sup> on days + 3 and + 6, if using ATG instead of Campath-1H. For older patients, the addition of 200cGy TBI with reduced ATG (given for two 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 x 10<sup>8</sup> 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.**

**(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**

#### **8.11 High dose cyclophosphamide without stem cell support**

The use of high dose cyclophosphamide (45mg/kg x 4) without stem cell support has been proposed by one centre as treatment for patients with newly diagnosed aplastic anaemia (Brodsky et al, 1996; Brodsky et al, 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; Tisdale et al, 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].

#### **8.12 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 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 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

### **8.13 Alemtuzumab (Campath-1H)**

Campath-1H is currently being evaluated in 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 has been 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 virilisation, 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 (Socie et al, 2000; Marsh, 2000; Kaito et al, 1998; Ohara et al, 1997). 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. (Gluckman et al, 2002); Locascuilli et al, 2001). 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 (Gupta et al, 2006; Socie et al, 2000; Appelbaum et al, 1989). 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 (Gupta et al, 2006; Socie et al, 2000; Mikhailova et al, 1986; Piaggio et al, 1999; Geary et al, 1999; Ishiyama et al, 2002). 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 and colleagues 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 (Maciejewski et al, 2002).

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 (that is, 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 (Piaggio et al, 1999; Geary 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 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 10-15 mg. An alternative option is to consider oral ciclosporin, maintaining trough blood levels between 150-250 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; Hill et al, 2005, Hillmen et al 2006; Hillmen et al, 2007).

For patients with a significant PNH clone (>50%), it is still reasonable to consider ATG treatment. 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 2mg/kg instead of 1mg/kg prednisolone (see section 6.3)

HLA 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 (Dunn et al, 1999; DeLord et al, 1998).

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 < 100 x 10<sup>9</sup>/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 [(Deeg et al, 1998; Sanders et al, 1996; Kahl et al, 2005).

A recently reported EBMTSAA Working Party study has evaluated 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 5 premature births and 3 abortions (one spontaneous). All infants born alive had normal post-natal 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 > 8g/dl and platelet count > 20 x 10<sup>9</sup>/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  $20 \times 10^9/l$  with platelet transfusions. This recommendation is based on the BCSH guidelines for treatment of ITP in pregnancy (BCSH guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and pregnancy, 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 (Little et al, 2000; Stanley et al, 1999; Armenti et al, 1994; McKay and 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 5mg/kg/day to maintain levels between 150 and 250 $\mu$ g/l [**grade C recommendation; level IV evidence**]. Response to ciclosporin is delayed and may take between 6-12 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 (around 40%) 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

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## Appendix

### 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	Evidence level IV

	clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality	
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## References

1. Ades, L., Mary, J. Y., Robin, M., Ferry, C., Porcher, R., Esperou, H., Ribaud, P., Devergie, A., Traineau, R., Gluckman, E., Socie, G. (2004) Long-term outcome after bone marrow transplantation for severe aplastic anaemia. *Blood* 103, 2490-2497.
2. Aitchison RGM, Marsh JCW, Hows JM, Russell NH, Gordon-Smith EC (1989). Pregnancy associated aplastic anaemia: a report of five cases and review of current management. *Br. J. Haematol* 73: 541-545.
3. Alter BP (2007). Diagnosis, genetics and management of inherited bone marrow failure syndromes. *Hematology* 29-39.
4. Appelbaum FR, Barrall J, Storb R, Ramberg R, Doney K, Sale GE, Thomas ED (1989). Clonal cytogenetic abnormalities in patients with otherwise typical aplastic anaemia. *Exp. Hematol.* 15: 1134-1139.
5. Armenti VT, Ahlswede KM, Ahlswede BA, Jarrell BE, Moritz MJ, Burke JF (1994). National Transplantation Pregnancy Registry – outcomes of 154 pregnancies in cyclosporine-treated female kidney transplant recipients. *Transplantation* 57: 502-506.
6. Bacigalupo A, Hows JM, Gluckman E, Nissen C, Marsh J, Van Lint MT, Congiu M, De Planque MM, Ernst P, McCann S (1988). Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA Working Party. *Br. J. Haematol* 70: 177-182.
7. Bacigalupo A, Chaple M, Hows J, Van Lint MT, McCann S, Milligan D, Chessells J, Goldstone AH, Ottolander J, Van't Veer ET, Korthof E, Comotti B, Coser B, Broccia G, Busi A, Locascuilli A, Catalono L, Battista R, Arcese W, Carotenuto M, Marmont AM, Gordon-Smith EC (1993). Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and

methylprednisolone (Mpred) with or without androgens: a randomised trial from the EBMT SAA Working Party. *Br. J. Haematol.* 83: 145-151.

- 8 Bacigalupo A, Brand R, Oneto R, Bruno B, Socie G, Passweg J, Locasciulli A, Van Lint MT, Tichelli A, McCann S, Marsh J, Ljungman P, Hows J, Marin P, Schrezenmeier H (2000a). Treatment of acquired severe aplastic anaemia: bone marrow transplantation compared with immunosuppressive therapy – The European Group for Blood and Marrow Transplantation experience. *Sem. Hematol.* 37: 69-80.
- 9 Bacigalupo A, Bruno B, Saracco P, Di Bona E, Locasciulli A, Gabbas A, Dufour C, Arcese W, Testi G, Broccia G, Marotenuoto M, Coser P, Barbui T, Leoni P, Ferster A (2000b) for the European Group for Blood and Bone Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midollo Osseo (GITMO). Antilymphocyte globulin, cyclosporine, prednisolone and granulocyte colony stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. *Blood* 95: 1931-1934.
- 10 Bacigalupo A, Locatelli F, Socie G, Dini G, Pession A, Locasciulli A, Prete A, Schrezenmeier H (2002a). Fludarabine, cyclophosphamide and ATG for alternative donor transplants in aplastic anaemia – A report of the SAA Working Party. *Bone Marrow Transplantation* 29 (suppl 2): 312a.
- 11 Bacigalupo, A., Locatelli, F., Lanino, E., Marsh, J., Socie, G., Maury, S., Prete, A., Locasciulli, A., Cesaro, S. Fludarabine, cyclophosphamide and ATG for alternative donor transplants in acquired severe aplastic anaemia - a report of the EBMT SAA Working Party (2005) *Bone Marrow Transplantation* 41, 45-50.
- 12 Bacigalupo A, Locatelli F, Lanino E et al (2009), Fludarabine, Cyclophosphamide with or without Low Dose TBI for Alternative Donor Transplants in Acquired Aplastic Anemia (SAA): A Report From the EBMT-SAA Working Party. *Biol. Blood Marrow Transplant.* 15, Issue 2, Page 5
- 13 Bain BJ, Clark DM, Lampert IA, Wilkins BS. *Bone marrow pathology.* Blackwell Science, 3<sup>rd</sup> Edition, 2001

- 14 Barker JN, Davies SM, DeFor T, Ramsay NKC, Weisdorf DJ, Wagner JE (2001). Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood* 97: 2957-2961.
- 15 Baumelou E, Guiguet M, Mary JY (1993), and the French Cooperative Group for Epidemiological Study of Aplastic Anaemia. Epidemiology of aplastic anaemia in France: a case control study. 1. Medical history and medication use. *Blood* 81: 1471-1478.
- 16 BCSH Guidelines for gamma irradiation of blood components for the prevention of transfusion-associated graft-versus-host disease 1996. *Transfusion Medicine* 6, 261-271.
- 17 BCSH Guidelines for the diagnosis and management of acquired aplastic anaemia (2003). *British Journal of Haematology* 123: 782-801.
- 18 BCSH Guidelines on the management of invasive fungal infection during therapy for haematological malignancy 2007. BCSH website: [www.bcshguidelines.com](http://www.bcshguidelines.com).
- 19 BCSH Guidelines for the use of platelet transfusions. *British Journal of Haematology* 2003; 122: 10-23.
- 20 Bean M, Gordon T, Appelbaum FR, Deeg HJ, Schuening F, Sale GE, Storb R (1994). Gamma irradiation of pretransplant blood transfusions from unrelated donors prevents sensitisation to minor histocompatibility antigens on dog leukocyte antigen-defined canine marrow grafts. *Transplant*. 57: 432-436.
- 21 Bennett JM, Orazi A (2009). Diagnostic criteria to distinguish hypocellular acute myeloid leukemia from hypocellular myelodysplastic syndromes and aplastic anemia: recommendations for a standardized approach. *Haematologica* 94, 264-268.
- 22 Bodey GP, Bolivar R, Fainstein V (1982). Infectious complications in leukaemic patients. *Sem. Hematol.* 19: 193.

- 23 Brodsky RA, Sensenbrenner LL, Jones RJ (1996). Complete remission in severe aplastic anemia after high dose cyclophosphamide without bone marrow transplantation. *Blood* 87: 491-494.
- 24 Brodsky RA, Sensenbrenner LL, Douglas-Smith B, Dorr D, Seaman PJ, Lee SM, Karp JE, Brodsky I, Jones RJ (2001). Durable treatment-free remission after high dose cyclophosphamide therapy for previously untreated severe aplastic anemia. *Ann. Intern. Med.* 135: 477-483
- 25 Brodsky R, Chen A, Brodsky I, Jones R. (2004) High-dose cyclophosphamide as salvage therapy for severe aplastic anaemia. *Experimental Haematology* 32, 435-440.
- 26 Brown KE, Tisdale J, Barrett AJ, Dunbar CE, Young NS (1997). Hepatitis-associated aplastic anaemia. *N. Engl. J. Med.* 336: 1059-1064.
- 27 Camitta, B.M., Rapoport, J.M., Parkman, R. & Nathan, D.G. (1975) Selection of patients for bone marrow transplantation in severe aplastic anemia. *Blood*, 45, 355–363.
- 28 Camitta BM (2000). What is the definition of cure for aplastic anemia? *Acta Haematologica* 103: 16-18.
- 29 Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian J-J, Martin-Dupont P, Michaud P, Papo T, Ugo V, Teyssandier I, Varet B, Mayeux P (2002). Pure red cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. *N. Engl. J. Med.* 346: 469-475.
- 30 Catovsky D (2000), on behalf of the British Committee for Standards in Haematology. Guidelines on diagnosis and therapy. Hairy cell leukaemia.
- 31 Champlin, R. E., Perez, W. S., Passweg, J., Klein, J. P., Camitta, B. M., Gluckman, E., Bredeson, C., Horowitz, M. M. (2007) Bone marrow transplantation for severe aplastic anemia: a randomised controlled study of conditioning regimens. *Blood* 109: 4582-4585.

- 32 Chessells JM (2001) Pitfalls in the diagnosis of childhood leukaemia. *Br. J. Haematol* 114: 5-6-511.
- 33 Clark AD, Butt N (1997). Ecstasy-induced very severe aplastic anaemia complicated by invasive pulmonary mucormycosis treated with allogeneic peripheral blood progenitor cell transplant. *Clin. Lab. Haematol.* 19: 279-281.
- 34 Consensus document for treating aplastic anaemia. Consensus document of a group of international experts. In: *Aplastic anaemia, pathophysiology and treatment*. Eds H. Schrezenmeier and A. Bacigalupo. Cambridge University Press, 2000.
- 35 Dacie and Lewis *Practical Haematology*. Eds SM Lewis, BJ Bain, Bates I. 9<sup>th</sup> edition, Churchill Livingstone 2001, p. 219-225.
- 36 Davies JK and Guinan EC (2007). An update on the management of severe idiopathic aplastic anaemia in children. *British Journal of Haematology* 136, 549-564.
- 37 Deeg HJ, Leisenring W, Storb R, Nims J, Flowers ME, Witherspoon RP, Sanders J, Sullivan KM (1998). Long term outcome after marrow transplantation for severe aplastic anaemia. *Blood* 91: 3637-3645.
- 38 Deeg H J, O'Donnell M, Tolar J et al. Optimisation of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood* 2006, in press.
- 39 Delord C, Tooze JA, Saso R, Marsh JCW, Gordon-Smith EC (1998). Deficiency of glycosylphosphatidylinositol-anchored proteins in patients with aplastic anaemia does not affect response to immunosuppressive therapy. *British Journal of Haematology* 101: 90-93.
- 40 Di Bona E, Rodeghiero F, Bruno B, Gabbas A, Foa P, Locasciulli A, Rosanelli C, Camba L, Saracco P, Lippi A, Jori A P, Porta F, De R V, Comotti B, Iacopino P, Dufour C, Bacigalupo A. (1999). Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a

first course of intensive immunosuppressive therapy. *British Journal of Haematology* 107, 330-334.

- 41 Dokal I (2000). Dyskeratosis congenita in all its forms. *Br. J. Haematol.* 110: 768-779.
- 42 Dunn DE, Tanawattanacharoen P, Boccuni P, Nagakurs S, Green SW, Kirby MR, Kumar MS, Rosenfeld S, Young NS (1999). Paroxysmal nocturnal haemoglobinuria cells in patients with bone marrow failure syndromes. *Ann. Int. Med.* 131: 401-408.
- 43 Elebute MO, Ball SE, Gordon-Smith EC, Sage D, Marsh JCW (2002). Autologous recovery following non-myeloablative unrelated donor bone marrow transplantation for severe aplastic anaemia. *Annals Haematol.* 81: 378-381.
- 44 Fleming LE, Timmeny W (1993). Aplastic anaemia and pesticides. An etiologic association ? *J. Occup. Med.* 35: 1106-1116.
- 45 Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H. (2003) for the German Aplastic Anaemia Study Group. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomised trial comparing treatments of aplastic anaemia. *Blood*; 101: 1236-1242.
- 46 Geary CG, Harrison CJ, Philpott NJ, Hows JM, Gordon-Smith EC, Marsh JCW (1999). Abnormal cytogenetic clones in patients with aplastic anaemia: response to immunosuppressive therapy. *Br. J. Haematol.* 104: 271-274.
- 47 Gluckman E, Rocah V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, Ortega J, Souillet G, Ferreira E, Laporte J-P, Fernandez M, Chastang C (1997), for the Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. Outcome of cord-blood transplantation from related and unrelated donors. *N. Engl. J. Med.* 337: 373-381.
- 48 Gluckman E, Rokicka-Milewska R, Hann I, Nikiforakis E, Tavakoli F, Cohen-Scali S, Bacigalupo A (2002). Results and follow-up of a phase III randomised study of recombinant

human-granulocyte stimulating factor as support for immunosuppressive therapy in patients with severe aplastic anaemia. *Br. J. Haematol.* 119: 1075-1082.

49 Gordon-Smith EC. Acquired aplastic anaemia. In: *Hematology, basic principles and practice*. Eds R. Hoffman, EJ Benz, SJ Shattil, B. Furie, HJ Cohen. Churchill Livingstone 1991.

50 Gordon-Smith EC, Marsh JCW, Geary CG (1995). Is it time to stop using chloramphenicol on the eye? Prospective study of aplastic anaemia should give definitive answer. *British Medical Journal* 311: 450-451.

51 Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and pregnancy. British Committee for Standards in Haematology, General Haematology Task Force, 2003; 120: 574-596.

52 Gupta, V., Ball, S., Yi, Q., Sage, D., McCann, S., Lawler, M., Ortin, M., Freires, M., Hale, G., Waldmann, H., Gordon-Smith, E., Marsh, J. (2004) Favorable effect on acute and chronic graft-versus-host disease with cyclophosphamide and in vivo anti-CD52 monoclonal antibodies for marrow transplantation from HLA-identical sibling donors for acquired aplastic anemia. *Biology of Blood & Marrow Transplantation* 7: 867-876.

53 Gupta, V., Ball, S., Sage, D., Ortin, M., Freires, M., Gordon-Smith, E., Marsh, J (2005) Marrow transplants from matched unrelated donors for aplastic anaemia using alemtuzumab, fludarabine and cyclophosphamide based conditioning. *Bone Marrow Transplantation* 35, 467-471.

54 Gupta, V., Gordon-Smith, E., Cook, G., Parker, A., Duguid, J., Wilson, K., Yi, Q., Marsh, J. A third course of anti-thymocyte globulin in aplastic anaemia is only beneficial in previous responders (2005). *British Journal of Haematology* 128, 110-117. Gupta V, Brooker C, Tooze JA et al. Clinical relevance of cytogenetics abnormalities in adult patients with acquired aplastic anaemia. *Brit. J. Haematol.* 2006; 134: 95-99.

55 Hall C, Richards S, Hillmen P (2003). Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal haemoglobinuria (PNH). *Blood* 102: 3587-3591.

- 56 Heimpel H (1996). When should the clinician suspect a drug-induced blood dyscrasia, and how should he proceed? *European Journal of Haematology*. 57 (suppl): 11-15.
- 57 Heimpel H. Epidemiology and aetiology of aplastic anaemia. In: *Aplastic anaemia: Pathophysiology and treatment*. Eds H. Schrezenmeier and A. Bacigalupo. Cambridge University Press 2000.
- 58 Hendry CL, Marsh JCW, Gordon-Smith EC, Sivakumaran M (2002). Relapse of severe aplastic anaemia after influenza immunisation. *Br. J. Haematol.* 119: 283-284
- 59 Hill, A., Hillmen, P., Richards, S., Elebute, D., Marsh, J., Chan, J., Mojcik, C., Rother, R (2005). Sustained response and long term safety of eculizumab in paroxysmal nocturnal haemoglobinuria. *Blood* 106, 2559-2565.
- 60 Hillmen, P., Hall, C., Marsh, J. C. W., Elebute, M., Bombara, M. P., Petro, B. E., Cullen, M. J., Richards, S. J., Rollins, S. A., Mojcik, C. F, Rother, R. P. (2004) Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria. *New England Journal of Medicine*, 350: 552-559.
- 61 Hillmen P, Young NS, Schubert J, Brodsky RA, Socié G, Muus P, Röth A, Szer J, Elebute MO, Nakamura R, Browne R, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik, Rother RP, Luzzatto L (2006). The Complement Inhibitor Eculizumab in Paroxysmal Nocturnal Hemoglobinuria. *New England Journal of Medicine* 355, 1233-1243.
- 62 Hillmen P, Muus P, Dührsen U, Risitano AM, MD, Schubert J, Luzzatto L, Schrezenmeier H, Szer J, Brodsky RA, Hill A, Socié G, Bessler M, Rollins SA, Bell L, Rother RP, Young NS,(2007) Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood* 110: 4123 - 4128.

- 63 Hows JM (2001). Status of umbilical cord blood transplantation in the year 2001. *J. Clin. Path.* 54: 428-434.
- 64 International Agranulocytosis and Aplastic Anaemia Study (1987). Incidence of aplastic anaemia: relevance of diagnosis criteria. *Blood* 70: 1718-1721.
- 65 Ishiyama K, Karasawa M, Miyawaki S, Ueda Y, Noda M, Wakita A, Sawanobori M, Nagai H, Nakao S (2002). Aplastic anaemia with 13q-: a benign subset of bone marrow failure responsive to immunosuppressive therapy. *Br. J. Haematol.* 117: 747-750.
- 66 Issaragrissil S, Kauffmann DW, Anderson T, Chansung K, Thamprasit T, Sinjurachai J, Piankijagum A, Porakkham Y, Vannasaeng S, Leaverton PE, Shapiro S, Young NS (1997) and The Aplastic Anaemia Study Group. Low drug attributability of aplastic anaemia in Thailand. *Blood* 89: 4034-4039.
- 67 Issaragrisil, S., Kaufman, D., Anderson, T., Chansung, K., Leaverton, P., Shapiro, S., Young, N (2006) and the Aplastic Anaemia Study Group. The epidemiology of aplastic anaemia in Thailand. *Blood* 107, 1299 - 1307.
- 68 Kahl, C., Leisenring, W., Deeg, H. J., Chauncey, T., Flowers, M., Martin, P., Sanders, J., & Storb, R (2005) Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long term follow up. *Brit. J. Haematol.* 130, 747-751.
- 69 Kaito K, Kobayashi M, Katayama T (1998). Long term administration of G-CSF for aplastic anaemia is closely related to the early evolution of monosomy 7 MDS in adults. *Br. J. Haematol.* 103: 297-303.
- 70 Kaminsky ER, Hows JM, Goldman JM, Batchelor JR (1990). Pretransfused patients with severe aplastic anaemia exhibit high numbers of cytotoxic T lymphocyte precursors probable directed at non-HLA antigens. *Br. J. Haematol.* 76: 401-405.

- 71 Kao SY, Xu W, Brandwein JM et al (2008). Outcomes of older patients ( $\geq 60$  years) with acquired aplastic anaemia treated with immunosuppressive therapy. *Brit. J. Haematol.* 143, 738-743.
- 72 Kauffmann DW, Kelly JP, Jurgelon JM, Anderson T, Issaragrisil S, Wilhom BE, Young NS, Leaverton P, Levy M, Shapiro S (1996). Drugs in the aetiology of agranulocytosis and aplastic anaemia. *Eur. J. Haematol.* 57 (suppl): 23-30.
- 73 Keidan AJ, Tsatalas C, Cohen J, Cousins S, Gordon-Smith EC (1986). Infective complications of aplastic anaemia. *Br. J. Haematol.* 63: 503-508.
- 74 Killick SB, Win N, Marsh JC, Kaye T, Yandle A, Humphries C, Knowles SM, Gordon-Smith EC (1997). Pilot study of HLA alloimmunisation after transfusion with pre-storage leucodepleted blood products in aplastic anaemia. *Br. J. Haematol.* 97: 677-684.
- 75 Kobayashi R, Hiromasa Y, Hara J, Morimoto A, Tsuchid M, Mugishima H, Akira O, Ohara H, Tsukimoto I, Kato H, Kigasawa H, Tabuchi K, Nakahata T, Ohga S, Kojima S, for the Japan Childhood Aplastic Anaemia Study Group (2006) Preceding immunosuppressive therapy with antithymocyte globulin and ciclosporin increases the incidence of graft rejection in children with aplastic anaemia who underwent allogeneic bone marrow transplantation from HLA-identical siblings. *Brit. J. Haematol.* 135, 693-696.
- 76 Kodaera Y, Morishima Y, Kato S, Akiyama Y, Sao H, Matsuyama T, Kawa K, Sakamaki H, Nakagawa S, Hirabayashi N, Dohi H, Okamoto S, Hiraoka A, Gondo H, Tsuchida M, Harada M, Asano S, Juji T, Sasazuki T, Takaku F (1999). Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukaemia and aplastic anaemia. *Bone Marrow Transpl.* 24: 995-1003.
- 77 Kosaka Y, Yagasaki H, Sano K et al (2008), on behalf of the Japan Childhood Aplastic Anemia Study Group. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. *Blood* 111; 1054 - 1059

- 78 Kurzrock R, Paquette R, Gratwohl A (1997). Use of stem cell factor (Stemgen, SCF) and filgrastim (G-CSF) in aplastic anaemia patients who have failed ATG/ALG therapy. *Blood* 90 (suppl 1): 173a.
- 79 Kwon JY, Lee Y, Shin JC, Lee JW, Rha JG, Kim SP. Supportive management of pregnancy-associated aplastic anemia (2006) *International Journal of Gynaecology and Obstetrics* 95, 115-120.
- 80 Lancaster T, Swart AM, Jick H (1998). Risk of serious haematological toxicity with use of chloramphenicol eye drops in a British general practice database. *British Medical Journal* 316: 667.
- 81 Lawler M, McCann SR, Marsh JC, Ljungman P, Hows J, Vandenberghe E, O'Riordan J, Locasciulli A, Socié G, Kelly A, Schrezenmeier H, Marin P, Tichelli A, Passweg JR, Dickenson A, Ryan J, Bacigalupo A (2009) from the Severe Aplastic Anaemia Working Party of the European Blood and Marrow Transplant Group. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Brit. J. Haematol.* 2009 Jan 9. [Epub ahead of print]
- 82 Leleu X, Terriou L, Duhamel A, Moreau A-S, Andrieux J, Dupire S, Coiteux V, Berthou C, Micol J-B, Guieze, R, Facon, T, Bauters F (2006). Long-term outcome in acquired aplastic anemia treated with an intensified dose schedule of horse antilymphocyte globulin in combination with androgens. *Annals of Hematology.* 85, 711-716.
- 83 Little MA, Abraham KA, Kavanagh J, Connolly G, Byrne P, Walshe JJ (2000). Pregnancy in Irish renal transplant recipients in the cyclosporine era. *Ir. J. Med. Sci.* 169: 19-21.
- 84 Ljungman P. Supportive treatment of patients with severe aplastic anaemia. In: *Aplastic anaemia, pathophysiology and treatment*. Eds. H. Schrezenmeier and A. Bacigalupo, Cambridge University Press, 2000, pp 137-153.

- 85 Locasciulli A, Arcese W, Locatelli F, Di Bona E, Bacigalupo A and Italian Aplastic Anaemia Study Group (2001). Treatment of aplastic anaemia with granulocyte-colony stimulating factor and risk of malignancy. *Lancet* 357: 43-44.
- 86 Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A, Schrezenemeir, H, Passweg J, Fuhrer M. (2007) Outcome of patients with acquired aplastic anaemia given first line bone marrow transplantation or immunosuppression treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica* 91, 11-18.
- 87 Locatelli, F., Bruno, B., Zecca, M., Van Lint, M. T., McCann, S., Arcese, W., Dallorso, S., Di Bartolomeo, P., Fagioli, F., Locasciulli, A., Lawler, M., Bacigalupo, A. (2000) Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: Results of a GITMO/EBMT randomized trial. *Blood* 96: 1690-1697.
- 88 Maggio A (2007) Light and shadows in the iron chelation treatment of haematological diseases. *Brit. J. Haematol.* 138: 407-421.
- 89 Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS (2002). Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anaemia. *Blood* 99: 3129-3135.
- 90 Mao P, Zhu Z, Wang H, Wang S, Mo W, Ying Y, Li Q, Xu Y. (2005) Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur. J. Haematol.* 75, 430-435
- 91 Margolis D, Camitta BM, Pietoyga D, Keever-Taylor C, Baxter-Lowe LA, Pierce K, Kupst MJ, French J, Truitt R, Lawton C, Murray K, Garbrecht F, Flomberg N, Casper J (1996). Unrelated donor bone marrow transplantation to treat severe aplastic anaemia in children and young adults. *Br. J. Haematol.* 94: 65-72.

- 92 Marin P. Clinical presentation, natural course and prognostic factors. In *Aplastic anaemia: pathophysiology and treatment*. Eds. H. Schrezenmeier and A. Bacigalupo. Cambridge University Press 2000, pp 117-136.
- 93 Marsh JCW, Abboudi ZH, Gibson FM, Scopes J, Daly S, O'Shaunnessy DF, Baughan ASJ, Gordon-Smith EC (1994). Aplastic anaemia following exposure to 3,4-methylenedioxymethamphetamine (Ecstasy). *Br. J. Haematol.* 88: 281-285.
- 94 Marsh JCW, Socie G, Schrezenmeier H, Tichelli A, Gluckman E, Ljungman P, McCann SR, Raghavachar A, Marin P, Hows JM, Bacigalupo A (1994), for the European Bone Marrow Transplant Working Party for Severe Aplastic Anaemia. Haemopoietic growth factors in aplastic anaemia: a cautionary note. *Lancet* 344: 172-173.
- 95 Marsh J, Schrezenmeier H, Marin P, Ilhan O, Ljungman P, McCann S, Socie G, Tichelli A, Passweg J, Hows J, Raghavachar A, Locascuilli A, Bacigalupo A (1999). A prospective randomised multicentre study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with non-severe aplastic anaemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. *Blood* 93: 2191-2195.
- 96 Marsh JCW, Ganser A, Stadler M. (2007) Hematopoietic growth factors in the treatment of acquired bone marrow failure states. *Semin. Hematol.* 44, 138-147.
- 97 Maury S, Viollier R, Oneto R, Anderlini P, Aljurf M, Garban F, Cordonnier C, Marsh J, Bacigalupo A, Passweg J on behalf of the EBMT-SAA Working Party (2007) Overcoming the negative impact of age using fludarabine based conditioning regimens for HLA-identical sibling HSCT in patients with severe aplastic anaemia. *Bone Marrow Transplantation* 39, S1, (abstract 327).
- 98 McCann SR, Passweg J, Storb R, Deeg HJ. HLA identical sibling bone marrow transplantation to treat severe aplastic anaemia. In: *Aplastic anaemia, pathophysiology and treatment*. Cambridge University Press, 2000, pp 230-257.

- 99 McCann S, Passweg J, Bacigalupo<sup>3</sup> A, Locasciulli A, Locatelli A, Ryan J, Schrezenmeier<sup>6</sup> H, Lawler M (2007). The influence of cyclosporin alone, or cyclosporin and methotrexate, on the incidence of mixed haematopoietic chimaerism following allogeneic sibling bone marrow transplantation for severe aplastic anaemia. *Bone Marrow Transplantation* 39: 109-114.
- 100 Montané E, Ibáñez L, Vidal X et al (2008) for the Catalan Group for the Study of Agranulocytosis and Aplastic Anemia. Epidemiology of aplastic anemia: a prospective multicenter study. *Haematologica* 93, 518 - 523.
- 101
- 102 McKay D, Josephson M. Pregnancy in recipients of solid organs - effects on mother and child. *N. Engl. J. Med* 2006; 354, 1281-1293.
- 103 Mikhailova N, Sessarego M, Fugazza G, Cuima A, De Filippi S, Van Lint MT, Bregante S, Valeriamai A, Mordini N, Lamparelli T, Gualandi F, Occhini D, Bacigalupo A (1986). Cytogenetic abnormalities in patients with aplastic anaemia. *Hematologica* 81: 418-422.
- 104 Muir KR, Chilvers CED, Harriss C, Coulson L, Grainge M, Darbyshire P, Geary C, Hows J, Marsh J, Rutherford T, Taylor M, Gordon-Smith EC (2003). The role of occupational and environmental exposures in the aetiology of acquired severe aplastic anaemia: a case control investigation. *Br. J. Haematol*, 123: 906-914.
- 105 Murphy MF, Brozovic B, Murphy W, Ouwehand W, Waters AH (1992), Working Party of the Blood Transfusion Task Force. Guidelines for platelet transfusions. *Transf. Med.* 2: 311-318.
- 106 Myers KC, Davies SM (2009). Haemopoietic stem cell transplantation for bone marrow failure syndromes in children. *Biol. Blood Marrow Transplant.* 15: 279-292.
- 107 National Blood Service (NBS) (2007) Clinical Guidelines for the use of Granulocyte Transfusions. Information document INF/MED/MA/006/02. Website: [www.blood.co.uk/hospitals](http://www.blood.co.uk/hospitals)

- 108 Niederwieser N, Pepe M, Storb R, Loughran TP Jr, Longton G (1988), for the Seattle Marrow Transplant Team. Improvement in rejection, engraftment rate and survival without increase in graft versus host disease by high marrow cell dose in patients transplanted for aplastic anaemia. *Br. J. Haematol.* 69: 23-28.
- 109 Nisbet-Brown, E., Olivieri, N. F., Giardina, P. J., Grady, R. W., Neufeld, E. J., Sechaud, R., Krebs-Brown, A. J., Anderson, J. R., Alberti, D., Sizer, K. C., Nathan, D. G. (2003) Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial, *Lancet*, 361: 1597-1602.
- 110 Norfolk DR, Ancliffe PJ, Contreras M, Hunt BJ, Machin SJ, Murphy WG (1998). Consensus conference on platelet transfusion, Royal College of Physicians of Edinburgh. *Br. J. Haematol.* 101: 609-617.
- 111 Ohara A, Kojima S, Hamajima N (1997). Myelodysplasia and acute leukaemia as a late clonal complication in children with acquired aplastic anaemia. *Blood* 90: 1009-1013.
- 112 Oosterkamp HM, Brand A, Kluin-Nelemans JC, Vandenbroucke JP (1998). Pregnancy and severe aplastic anaemia: causal relation or coincidence? *Br. J. Haematol.* 103: 315-316.
- 113 Packman CH (1998). Pathogenesis and management of paroxysmal nocturnal haemoglobinuria. *Blood Reviews* 12: 1-11.
- 114 Pamphilon DH, Rider JR, Barbara JAJ, Williamson LM (1999). Prevention of transfusion-transmitted cytomegalovirus infection. *Transf. Med.* 9: 115-123.
- 115 Parker, C., Omine, M., Richards, S., Nishimura, J., Bessler, M., Ware, R., Hillmen, P., Luzzatto, L., Young, N., Kinoshita, T., Rosse, W., Socie, G. for the International PNH Interest Group (2005). Diagnosis and management of paroxysmal nocturnal haemoglobinuria. *Blood* 106: 3699-3709.

- 116 Passweg JR, Socie G, Hinterberger W, Bacigalupo A, Biggs JC, Camitta BM, Champlin RE, Gale RP, Gluckman E, Gordon-Smith EC, Hows JM, Klein JP, Nugent ML, Pasquini R, Rowlings PA, Speck B, Tichelli A, Zhang MJ, Horowitz MM, Bortin MM (1997). Bone marrow transplantation for severe aplastic anaemia: has outcome improved ? *Blood* 90: 858-864.
- 117 Passweg J, Perez W, Eapen M, Camitta B, Gluckman E, Hinterberger W, Hows J, Marsh J, Pasquini R, Schrezenmeier H, Socie G, Zhang MJ, Bredeson C. (2006) Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anaemia. *Bone Marrow Transplantation* 37, 641-649.
- 118 Piaggio G, Podesta M, Pitto A, Sessavego M, Figari O, Fugazza G, Benvenuto F, Bruno B, Van Lint MT, Truini M, Frassoni F, Bacigalupo A (1999). Coexistence of normal and clonal haemopoiesis in aplastic anaemia patients treated with immunosuppressive therapy (IST). *Br. J. Haematol.* 107: 505-511.
- 119 Porter JB (2001). Practical management of iron overload. *Br. J. Haematol.* 115: 239-252.
- 120 Przepiorka D, Anderlini P, Saliba R, Cleary K, Mehra R, Khouri I, Huh YO, Giralt S, Braunschweig I, van Besien K, Champlin R (2001). Chronic graft-versus-host-disease after allogeneic blood stem cell transplantation. *Blood* 98: 1695-1700.
- 121 Roberts HJ (1997). Effect of pentachlorophenol exposure. *Lancet* 19: 279-281.
- 122 Russell NH, Gratwohl A, Schmitz N (1998). Developments in allogeneic peripheral blood progenitor cell transplantation. *Br. J. Haematol.* 103: 594-600.
- 123 Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, Doney K, Storb R, Sullivan K, Witherspoon R, Appelbaum FR (1996). Pregnancies following high dose cyclophosphamide with or without high dose busulphan or total body irradiation and bone marrow transplantation. *Blood* 87: 3045-3052.

- 124 Saracco P, Quarello P, Iori AP, Zecca M, Longoni D, Svahn J, Varotto S, Vecchio GC, Dufour C, Ramenghi U, Bacigalupo A, Locasciulli A (2008). Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow up. *British Journal of Haematology* 140: 197-205.
- 125 Saso R, Marsh J, Cevreska L, Szer J, Gale RP, Rowlings PA, Passweg JR, Nugent ML, Luzzatto L, Horowitz MM, Gordon-Smith EC (1999). Bone marrow transplants for paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* 104: 392-396.
- 126 Scheinberg P, Nunez O, Young N (2006a). Re-treatment with rabbit antithymocyte globulin and ciclosporin for patients with relapsed or refractory severe aplastic anaemia. *Brit. J. Haematol.* 133: 622-627.
- 127 Scheinberg P, Nunez O, Wu C, Young N (2006b). Treatment of severe aplastic anaemia with combined immunosuppression: antithymocyte globulin, ciclosporin and mycophenolate mofetil. *Brit. J. Haematol.* 133: 606-611.
- 128 Scheinberg P, Fischer SH, Nunez O, Wu CO, Sloand EM, Cohen JI, Young NS, Barrett, AJ (2007). Distinct EBV and CMV reactivation patterns following antibody-based immunosuppressive regimens in patients with severe aplastic anaemia. *Blood* 109: 3219-3224.
- 129 Scheinberg P, Wu CO, Nunez O, Young NS (2009). Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. *Brit. J. Haematol.* 144, 206-216.
- 130
- 131 Schrezenmeier H, Marin P, Raghavachar A, McCann SR, Hows J, Gluckman E, Nissen C, Van't Veer Korthof ET, Ljungman P, Hinterberger W (1993). Relapse of aplastic anaemia after immunosuppressive treatment: a report from the European Bone Marrow Transplantation Group SAA Working Party. *Br. J. Haematol.* 85: 371-377.

- 132 Schrezenmeier H, Marsh JCW, Stromyer P, Heimpel H, Gordon-Smith EC, Raghavachar A (1995a). A phase I/II trial of recombinant human interleukin-6 in patients with aplastic anaemia. *Br. J. Haematol.* 90: 283-292.
- 133 Schrezenmeier H, Hertenstein B, Wagner B, Raghavachar A, Heimpel H (1995b). A pathogenetic link between aplastic anemia and paroxysmal nocturnal hemoglobinuria is suggested by a high frequency of aplastic anemia patients with a deficiency of phosphatidylinositol glycan anchored proteins – rapid communication. *Exp. Hematol.* 23: 81-87.
- 134 Schrezenmeier H, Passweg JR, Marsh JCW et al (2007). Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. *Blood* 110, 1397 – 1400.
- 135 Schrezenmeier, H., Bacigalupo, A., Dohner, H., Ramenghi, A., Locascuilli, A., Blundell, E., Platzebecker, U., Marsh, J., Raghavachar, A., Reinold, H., Weh, E., Thiel, E., Passweg, J (2003) Mycophenolate mofetil as treatment of patients with acquired aplastic anaemia failing to durably respond to standard immunosuppressive treatment. *Bone Marrow Transplantation* 31 (suppl 1), 0363a.
- 136 Serious Hazards of Transfusion (SHOT) Annual Report 2006. Website: [www.shotuk.org](http://www.shotuk.org).
- 137 Smith MT (1996). Overview of benzene-induced aplastic anaemia. *European Journal of Haematology* 57 (suppl): 107-111.
- 138 Socie G, Henry-Amar M, Bacigalupo A et al (1993). Malignant tumors occurring after treatment of aplastic anemia. *N. Eng. J. Med.* 329, 1152-1157.
- 139 Socie G, Rosenfeld S, Frickhofen N, Gluckman E, Tichelli A (2000). Late clonal diseases of treated aplastic anaemia. *Semin. Hematol.* 37: 91-101.

- 140 Socie G, Mary J-Y, Schrezenmeier H, Marsh J, Bacigalupo A, Locasciulli A, Fuhrer M, Bekassy A, Tichelli A, Passweg (2007). Granulocyte colony stimulating factor for severe aplastic anaemia: A survey by the European Group for Blood and Marrow Transplantation. *Blood*, 109: 2794-2796.
- 141 Stanley CW, Gottlieb R, Zager R, Eisenberg J, Richmond R, Moritz MJ (1999). Developmental well-being in offspring of women receiving cyclosporine post-renal transplant. *Transplant. Proc.* 31: 241-242.
- 142 Stern M, Passweg J, Locasciulli A., Socie G, Schrezenemeier H, Bekassy A, Fuehrer , Korthof E, McCann S, Tichelli A, Zoumbos N, Marsh JC, Bacigalupo A, Gratwohl, A (2006). Influence of donor/recipient sex matching on outcome of allogeneic hematopoietic stem cell transplantation for aplastic anemia. *Transplantation* 82, 218-226.
- 143 Sugimori C, Chuhjo T, Feng X, Yamazaki H, Takami A, Teramura M, Mizoguchi H, Omine M, Nakao, S. (2005) Minor populations of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anaemia. *Blood* 107, 1308-1314.
- 144 Teramura M, Kimura A, Iwase S, Yonemura Y, Nakao S, Urabe A, Omine M<sup>7</sup>, and Mizoguchi H (2007) Treatment of severe aplastic anemia with antithymocyte globulin and cyclosporin A with or without G-CSF in adults: a multicenter randomized study in Japan. *Blood* 110: 1756-1761.
- 145 Thomas FT, Griesedieck G, Thomas J. (1984) Differential effects of horse ATG and rabbit ATG on T cell and T cell subset levels measured by monoclonal antibodies. *Transplantation Proceedings* 16: 1561-1563.
- 146 Tichelli A, Gratwohl A, Nissen C, Signer E, Stebler Gysi C, Speck B (1992). Morphology in patients with severe aplastic anaemia treated with antilymphocyte globulin. *Blood* 80:337-345.
- 147 Tichelli A, Socie G, Marsh J, McCann S, Hows J, Schrezenmeier H, Marin P, Hinterberger W, Ljungman P, Ragavachar A, Vant-Veer Korthof E, Gratwohl A, Bacigalupo

- A (1996). Cytogenetic abnormalities in aplastic anaemia. *Bone Marrow Transpl.* 7 (suppl 1): 268a.
- 148 Tichelli A, Passweg J, Nissen C, Bargetzi M, Hoffman T, Wodnar-Filipowicz A, Signer E, Speck B, Gratwohl A (1998). Repeated treatment with horse antilymphocyte globulin for severe aplastic anaemia. *Br. J. Haematol.* 100: 393-400.
- 149 Tichelli A, Socie G, Marsh J, Barge R, Frickhofen N, McCann S, Bacigalupo A, Hows J, Marin P, Nachbaur D, Symeonidis A, Passweg J, Schrezenmeier H (2002), on behalf of the European Group for Blood and Marrow Transplantation (EBMT) Severe Aplastic Anemia Working Party. Outcome of pregnancy and disease outcome among women with aplastic anemia treated with immunosuppression. *Ann. Int. Med.* 137: 164-172.
- 150 Tisdale JF, Dunn DE, Geller N, Plante M, Nunez O, Dunbar CE, Barrett AJ, Walsh TJ, Rosenfeld SJ, Young NS (2000a). High dose cyclophosphamide in severe aplastic anaemia: a randomised trial. *Lancet* 356: 1554-1557.
- 151 Tisdale, J. F., Maciejewski, J. P., Nunez, O., Rosenfeld, S. J., Young, N. S. (2002) Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial. *Blood.* 100: 4668-4670.
- 152 Tisdale JF, Dunn DE, Majewski J (2000b). Cyclophosphamide and other new agents for the treatment of severe aplastic anemia. *Semin. Hematol.* 37: 102-109.
- 153 Tuzuner N, Bennett JM (1994). Reference standards for bone marrow cellularity. *Leuk Res.* 18: 645-647.
- 154 Tuzuner N, Cox C, Rowe JM, Watrous D, Bennett JM (1995). Hypocellular myelodysplastic syndromes (MDS): new proposal. *Br. J. Haematol.* 91: 612-617.
- 155 Vadhan-Raj S (2000). Clinical experience with recombinant human thrombopoietin in chemotherapy-induced thrombocytopenia. *Sem. Hematol.* 36: 28-34.

- 156 Van Besien K, Tricot G, Golichowski A, Padilla L, Hoffman R (1991). Pregnancy associated aplastic anaemia-report of 3 cases. *Eur. J. Haematol.* 47: 253-256.
- 157 Van Kamp H, van Imhoff GW, de Wolf JT, Smit JW, Halie MR, Vellenga E (1995). The effect of cyclosporine on haematological parameters in patients with paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* 89: 79-82.
- 158 Viallard JF, Boiron JM, Pawens M, Moreau JF, Randu V, Reiffers J, Leng B, Pellegrin JL (2000). Severe pancytopenia triggered by recombinant hepatitis B vaccine. *Br. J. Haematol.* 110: 230-233
- 159 Viollier R, Socié G, Tichelli A, Bacigalupo A, Korthof E, Marsh J, Cornish J, Ljungman P, Oneto R, Bekassy A, Fuehrer M, Maury S, Schrezenmeier H, van Lint MT, Wojcik D, Locasciulli A, Passweg JR for the working party aplastic anaemia (WPSAA) of the European Group for Blood and Marrow Transplantation (EBMT) Recent improvement in outcome of unrelated donor transplantation for aplastic anaemia (2007) *Bone Marrow Transplant.* 41, 45 – 50.
- 160 Vulliamy T, Marrone A, Dokal I, Mason PJ (2002). Association between aplastic anaemia and mutations in telomerase RNA. *Lancet.* 359: 2168-2170.
- 161 Vulliamy, T., Walne, A., Baskaradas, A., Mason, P., Marrone, A., Dokal, I (2005). Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. *Blood Cells, Molecules and Diseases.* 34: 257-263.
- 162 Vulliamy T and Dokal I (2006). Dyskeratosis congenita. *Seminars in Hematology,* 43: 157-166.
- 163 Walne AJ, Dokal I (2009). Advances in the understanding of dyskeratosis congenita. *Brit. J. Haematol.* In press.

- 164 Wilholm B-E, Kelly JP, Kaufmann D, Issaragrissil S, Levy M, Anderson T, Shapiro S (1998). Relation of aplastic anaemia to use of chloramphenicol eye drops in two international case-control studies. *British Medical Journal* 316: 666.
- 165 Willis, F., Marsh, J., Bevan, D., Killick, S., Lucas, G., Griffiths, R., Ouwehand, W., Hale, G., Waldmann, H., Gordon-Smith, E (2001). The effect of treatment with Campath-1H in patients with autoimmune cytopenias. *British Journal of Haematology* 114, 891-898.
- 166 Witherspoon RB, Storb R, Pepe M, Longton G, Sullivan KM (1991). Cumulative incidence of secondary solid malignant tumors after conditioning with chemotherapy alone. *Blood* 79: 289-291.
- 167 World Health Organisation Classification of Tumours. Pathology and Genetics. Myelodysplastic syndromes. In: Tumours of haematopoietic and lymphoid tissues. Eds. ES Jaffe, NL Harris, H. Stein, JW Vardiman. IARC Press Lyon, 2001.
- 168 Yamaguchi, H., Calado, R., Ly, H et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl. J. Med* 2005; 352, 1413-1424.
- 169 Yin SN, Li Y, Tian F, Du C, Jin C (1987). Occupational exposure to benzene in China. *British Journal of Industrial Medicine* 44: 192-195.
- 170 Yin S-N, Hayes RB, Linet MS, Li G-L, Dosemeci M, Travis LB, Zhang Z-N, Li D-G, Chow W-H, Wacholder S, Blot WJ and the Benzene Study Group (1996). An expanded cohort study of cancer among benzene-exposed workers in China. *Environmental Health Perspectives*. 104 (suppl 6): 1339-1341.
- 171 Young NS and Alter BP eds. *Aplastic anaemia: acquired and congenital*. WB Saunders 1994.
- 172 Young N S, Calado R T, Scheinberg P. 2006 Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood* 108, 2509-2519.

173 Zheng Y, Liu Y, Chu Y. Immunosuppressive therapy for acquired severe aplastic anemia (SAA): A prospective comparison of four different regimens (2006). *Experimental Hematology* 34:826-831.

**Table 1: Currently licensed drugs which have been reported as a rare association with aplastic anaemia.**

**Evidence based on case reports or uncontrolled series (Young and Alter, 1994) or case control studies (Kauffmann et al, 1996; Baumelou et al, 1993; Issaragrissil et al, 1997 and 2005)**

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 (Issaragrissil et al, 2005). There is no evidence for an association between chloramphenicol eye drops and aplastic anaemia (Gordon-Smith et al, 1995; Wilholm et al, 1998; Lancaster et al, 1998).

# More likely to cause neutropenia

\$ From epidemiological study in Thailand (Issaragrissil et al, 2005)

**Table 2: 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, 2005])
Agricultural pesticides: Organochlorines eg Lindane, Organophosphates, Pentachlorophenol [Muir et al, 2003 (case control study), Fleming and Timmeny, 1993; Roberts, 1997 (literature reviews of case reports)], DDT and Carbamates (Issaragrisil et al, 2005)
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, 2005)
Recreational drugs: methylenedioxy-methamphetamine, MDMA, Ecstasy, (evidence based on case reports, [Marsh et al, 1994a; Clark and Butt, 1997])

**Table 3 : Summary of investigations required for the diagnosis of aplastic anaemia**

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

\* GPI = glycerophosphatidylinositol

# 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)

**Table 4: Definition of severity of aplastic anaemia**

Severe AA (Camitta et al, 1975)	<ul style="list-style-type: none"><li>• BM cellularity &lt;25%, or 25-50% with &lt;30% residual haemopoietic cells *</li><li>• 2/3 of the following:<ol style="list-style-type: none"><li>1. neutrophils &lt;0.5 x 10<sup>9</sup>/l</li><li>2. platelets &lt; 20 x 10<sup>9</sup>/l</li><li>3. reticulocytes &lt; 20 x 10<sup>9</sup>/l</li></ol></li></ul>
Very severe AA (Bacigalupo et al, 1988)	As for severe AA but neutrophils <0.2 x 10 <sup>9</sup> /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 and Bennett, 1994).



Figures 1 and 2: see in separate powerpoint file

