Guidelines for the diagnosis and management of adult aplastic anaemia

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Scope

Methodology

Literature review details. The guideline group was selected to be representative of UK-based aplastic anaemia (AA) medical experts. Recommendations are based on review of the literature using MEDLINE and PUBMED up to December 2014 under the heading: 'aplastic anemia'.

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria are specified in the BCSH guidance pack http://www.bcshguidelines.com/BCSH_PROCESS/EVIDENCE_LEVELS_AND_GRADES_OF_RECOMMENDATION/43_GRADE.html and the GRADE working group website http://www.gradeworkinggroup.org

The objective of this guideline is to provide healthcare professionals with clear guidance on the management of patients with AA. The guidance may not be appropriate to every patient and in all cases individual patient circumstances may dictate an alternative approach.

Working group membership. Review of the manuscript was performed by the British Committee for Standards in Haematology (BCSH) Haemato-Oncology Task Force, BCSH Executive Committee and then reviewed by a sounding board of the British Society for Haematology (BSH). This compromises 50 or more members of the BSH who have reviewed this guidance and commented on its content and applicability in the UK setting. It has also been reviewed by the Aplastic Anaemia Trust patient group but they do not necessarily approve or endorse the contents.

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Summary of key recommendations

Key recommendations for definition, severity and presentation

- **The severity of AA (AA) should be according to the Camitta criteria. Grade 1C**
- **Most cases of AA are idiopathic, nevertheless a careful drug history must be taken and any putative causative drug should be discontinued and reported to the Medicines and Healthcare products Regulatory Agency (MHRA) using the Yellow Card Scheme. Grade 1C**
- **A multidisciplinary team (MDT) meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given to seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an inherited bone marrow failure syndrome (IBMFS) is being considered.**

Key recommendations for inherited AA

- **Chromosomal breakage analysis of peripheral blood lymphocytes following exposure to diepoxybutane to test for Fanconi anaemia (FA) should be performed. Grade 1B**

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- **Comprehensive assessment should be performed, including family history, abdominal ultrasound, echocardiogram, high resolution computerized tomography scan of the chest and pulmonary function tests, and evaluation for other extra-haematopoietic abnormalities (such as cirrhosis, pulmonary fibrosis or renal anomalies); the presence of these will support a diagnosis of constitutional rather than idiopathic bone marrow failure (BMF). Grade 1B**

Key recommendations for supportive care

- **Blood transfusions should be given to improve quality of life. Grade 1A**
- **A threshold haemoglobin concentration cannot be recommended for all patients; it should be individualized according to co-morbidities. Grade 1A**
- **Phenotype (Rh and Kell) matched blood should be considered to reduce the risk of alloimmunization. Grade 1B**
- **Prophylactic platelet transfusions should be given to stable AA patients receiving active treatment. Grade 1B. A threshold (pre-transfusion) platelet count of 10×10^9/l should be used. Grade 1B**
- **In patients judged to have additional risk factors for bleeding, such as fever or sepsis, a higher prophylactic transfusion threshold of 20×10^9/l is recommended. Grade 2C**
- **Routine prophylactic platelet transfusions are not recommended for stable AA patients not on active treatment. Grade 2B**
- **Patients with chronic bleeding of World Health Organization grade 2 or above require individual management according to the severity of their symptoms and signs. Grade 2C**
- **Prior to administration of antithymocyte globulin (ATG), a daily threshold (pre-transfusion) platelet count of 20×10^9/l should be used for the duration of the ATG course. Grade 2C**
- **Only one adult platelet dose is routinely required. Grade 1A.**
- **All patients undergoing treatment with immunosuppressive therapy (ATG or Alemtuzumab) should receive irradiated blood products. Grade 1C**
- **All patients undergoing haemopoietic stem cell transplantation (HSCT) should receive irradiated blood products. Grade 1A**
- **The need for iron chelation therapy should be decided on an individual patient basis. Patients with iron overload after successful HSCT should undergo venesection. Grade 1B**
- **Aplastic anaemia patients who are severely neutropenic should be given prophylactic antibiotics and antifungal therapy according to local policies. Grade 2B**
- **Aplastic anaemia patients receiving immunosuppressive therapy (IST) should also receive prophylactic anti-viral agents, although routine prophylaxis against *Pneumocystis jirovecii* is not necessary. Grade 2C**

Key recommendations for IST

- **The current standard first line IST is horse ATG (ATG-ATGAM) combined with ciclosporin (CSA). Grade 1A**
- **Immunosuppressive therapy is recommended first line therapy for non-severe AA patients requiring treatment (see indications in text), severe or very severe AA patients who lack a matched sibling donor (MSD), and severe or very severe AA patients aged >35-50 years. Grade 1A**
- **A second course of ATG may be indicated following failure to respond to a first course [if the patient is ineligible for a matched unrelated donor (UD) HSCT] or following relapse after a first course. Grade 1A**
- **ATG is an immunosuppressive drug and should only be administered in centres familiar with its use; the drug must only be given to in-patients. Grade 1B**
- **The use of high dose or moderate dose cyclophosphamide (without stem cell support) is not recommended in AA. Grade 1A**
- **Following IST, vaccinations, including influenza, should be avoided if possible as there is a theoretical risk of disease relapse. Grade 2C**

Key recommendations for HSCT

- **All patients being considered for HSCT should be evaluated in a multi-disciplinary team setting, and consideration should be given to discussion of the case with a centre that has expertise in AA regarding the indications for HSCT and the choice of conditioning regimen. Grade 1C**
- **To inform the multi-disciplinary team decision-making regarding HSCT:**
  - **All patients who are potential HSCT candidates should undergo human leucocyte antigen (HLA) typing at diagnosis, followed by related or UD searches as appropriate to assess the availability of potential donors. Grade 1B**
  - **A careful reassessment should be made to confirm the precise diagnosis and exclude clonal evolution to myelodysplastic syndrome (MDS) or paroxysmal nocturnal haemoglobinuria (PNH), as this will influence the choice of conditioning. It is also vital not to miss constitutional AA so as to avoid (i) serious (and potentially lethal) toxicity from the transplant and (ii) inappropriate selection of a sibling donor. Grade 1C**
  - **The Haematopoietic Cell Transplant Co-morbidity Index or equivalent assessment should be documented. Grade 2B**

  - **Alternatives to HSCT, including IST, should be actively considered in the management plan. Grade 1B**

- **Up-front MSD HSCT for young and adult patients is the treatment of choice for severe AA, but patients aged between 35-50 years need to be carefully assessed for co-morbidities prior to consideration for transplantation. Grade 1B**
- **Unrelated donor HSCT in adults should be considered after lack of response to one course of IST. Grade 1B**
- **There have been recent improvements in outcomes after alternative donor HSCT for patients who lack a suitably matched donor, but these transplants are still experimental and specialist advice should be sought; only European Bone Marrow Transplantation Severe Aplastic Anaemia Working Party (SAAWP) approved protocols should be used. Grade 2B**

Key recommendations for treatment of AA in the elderly

- **Elderly patients with AA should be individually assessed and their specific wishes respected, as quality of life is paramount in this patient group. Grade 1C**
- **Immunosuppressive therapy is considered the treatment of choice. ATG and CSA result in a more rapid recovery of blood counts but, alternatively, CSA alone or oxymetholone can be considered. Grade 1B**
- **Patients unfit for, who decline or who are intolerant of IST should be offered best supportive care. Grade 1C**
- **Eltrombopag is licensed by the European Medicines Agency (EMA) for severe AA refractory to IST or patients who are heavily pre-treated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. Grade 2B**

Key recommendations for management of AA in pregnancy

- **Supportive care remains the mainstay of treatment of AA in pregnancy, aiming to maintain the platelet count above 20×10^9/l with platelet transfusions. Grade 1C**
- **CSA is safe in pregnancy if needed. Grade 2C**

Key recommendations for PNH and AA

- **All patients should be screened for PNH using flow cytometry on peripheral blood to detect deficiency of glycosylphosphatidyl-inositol (GPI) anchored proteins, such as CD14, CD16 and CD24, as well as fluorescent aerolysin (FLAER) for white blood cells, and CD55 and CD59 for red cell analysis.**
- **Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6-monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable. Grade 2C**
- **Small PNH clones can be detected in up to 50% of patients with AA, usually without evidence of haemolysis; large clones are clinically significant and may result in haemolysis as well as increased thrombotic risk ('haemolytic PNH').**
- **Presence of a small/moderate PNH clone in AA does not directly influence the choice of treatment for the underlying BMF.**
- **New PNH patients should be referred to the PNH National Service to be monitored for PNH complications and assessed for anti-complement therapy.**

Definition, disease severity and clinical presentation of AA

Aplastic anaemia is a rare and heterogeneous disorder. It is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis. To diagnose AA there must be at least two of the following (Camitta *et al*, 1975) haemoglobin concentration (Hb) <100 g/l, platelet count $<50 \times 10^9$/l, neutrophil count $<1{\cdot}5 \times 10^9$/l. The majority (70-80%) of cases are idiopathic (Marsh *et al*, 2009). The remainder mainly consist of IBMFS. The incidence is 2-3 per million per year in Europe, but higher in East Asia (Montane *et al*, 2008). There is a biphasic distribution, with peaks at 10-25 years and over 60 years.

The modified Camitta criteria (Camitta *et al*, 1975; Bacigalupo *et al*, 1988) are used to assess severity:

- Severe AA (SAA);

  Marrow cellularity <25% (or 25–50% with <30% residual haematopoietic cells), plus at least 2 of: (i) neutrophils $<0{\cdot}5 \times 10^9$/l, (ii) platelets $<20 \times 10^9$/l (iii) reticulocyte count $<20 \times 10^9$/l (see diagnostic section for automated reticulocyte count)

- Very Severe AA (VSAA);

  As for SAA but neutrophils $<0{\cdot}2 \times 10^9$/l

- Non-severe AA (NSAA);

  AA not fulfilling the criteria for SAA or VSAA

Patients commonly present with symptoms of anaemia and thrombocytopenia. Serious infection is not a frequent symptom early in the course of the disease. A preceding history of jaundice may suggest a post-hepatitic AA. Whilst the majority of cases are idiopathic, a careful drug, occupational exposure and family history should be obtained. Any

putative drugs should be discontinued and the patient should not be re-challenged. If a possible drug association is suspected, this must be reported to the Medicines and MHRA using the Yellow Card Scheme (http://yellowcard.gov.uk). There is usually no hepatosplenomegaly or lymphadenopathy (except in infection). In young adults the presence of short stature, skin hyper/hypo pigmented areas and skeletal abnormalities, particularly affecting the thumb is suggestive of FA (Shimamura & Alter, 2010). The triad of nail dystrophy, reticular skin pigmentation and oral leucoplakia is characteristic of dyskeratosis congenita (DC) (Shimamura & Alter, 2010). The finding of peripheral lymphoedema may indicate a diagnosis of Emberger syndrome due to germline *GATA2* mutation.

Key recommendations for definition, severity and presentation

- **The severity of AA should be according to the Camitta criteria. Grade 1C**
- **Most cases of AA are idiopathic, nevertheless a careful drug history must be taken and any putative causative drug should be discontinued and reported to the MHRA using the Yellow Card Scheme. Grade 1C**
- **A MDT meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given to seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an IBMFS is being considered.**

Investigations required for the diagnosis of AA

Idiopathic AA is a diagnosis of exclusion and no single test reliably diagnoses idiopathic acquired AA. Consequently, the diagnostic evaluation must exclude assessment of alternative aetiologies of BMF. The "empty" marrow on histology of AA is characteristic and a prerequisite for the diagnosis. There is increasing recognition that IBMFS are commoner than previously thought and may present in adulthood. The following investigations (Table I) are required to confirm the diagnosis, and:

(i)exclude other causes of pancytopenia and a hypocellular bone marrow
(ii)exclude IBMFSs
(iii)screen for an underlying cause and
(iv)document co-existing abnormal cytogenetic and PNH clones.

See Table I for the summary of investigations for the diagnosis and further evaluation of AA; this table also summarizes the emerging diagnostics incorporating the latest molecular technologies that are likely to feature in the diagnosis and differential diagnosis within the next couple of years.

Both a bone marrow aspirate and trephine biopsy are required for the diagnosis of AA, and the key bone marrow findings are summarized in Table II.

The investigations in Table I should exclude non-AA causes of pancytopenia with a hypocellular bone marrow, which are listed in Table III.

A MDT meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given for seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an IBMFS is being considered.

Inherited AA

A number of inherited/genetic disorders are characterized by BMF/AA, usually in association with one or more somatic abnormality (Alter, 2007). The BMF typically presents in childhood but this can sometimes be in adulthood.

The two syndromes frequently associated with generalized BMF/AA are FA and DC (Dokal, 2011; Soulier, 2011), which can sometimes present with AA alone as their initial manifestation. These syndromes are genetically heterogeneous; 16 FA genes and 10 DC genes have been identified. The FA genes are important in DNA repair, the DC genes in telomere maintenance. Based on the DNA repair defect a diagnostic test-'chromosomal breakage test' is available for FA. Patients with DC usually have very short telomeres and this measurement [using flow cytometric fluorescence *in situ* hybridization or multiplex quantitative polymerase chain reaction (PCR)] can be useful in the assessment of DC. Genetic testing for known DC genes (representing *c.* 60% of cases) is possible in specialized centres.

In addition there are other genetic syndromes that are sometimes associated with AA/cytopenias. This includes Shwachman Diamond syndrome SDS (Dror *et al*, 2011) (mutations in *SBDS*), congenital amegakaryocytic thrombocytopenia CAMT (Ballmaier & Germeshausen, 2011) (mutations in *MPL*) and GATA2 deficiency (Emberger syndrome) (Horwitz, 2014) as well as genetically uncharacterized cases.

Some cases of inherited AA first present in adulthood and it is important to recognize these as their management differs from that of idiopathic AA. Where there are sufficient characteristic abnormalities a diagnosis may be straightforward (e.g. mucocutaneous features in DC). Where the presentation is only with AA and with minimal non-haematological abnormalities, inherited BMF should be considered and testing for known BMF syndromes should be undertaken. Investigations for inherited forms of AA should be re-appraised in patients initially classified as "idiopathic AA" and who fail to respond to anti-thymocyte globulin (ATG).

Key recommendations for inherited AA

- **Chromosomal breakage analysis of peripheral blood lymphocytes following exposure to diepoxybutane to test for FA should be performed. Grade 1B**

Table I. Summarized diagnosis and further investigation of aplastic anaemia.

Test	Key changes
1. Full blood count	Pancytopenia. Usually the haemoglobin concentration and neutrophil and platelet counts are uniformly depressed. In the early stages, isolated cytopenia, particularly thrombocytopenia, may occur. Lymphocyte counts are usually preserved. Presence of monocytopenia needs further investigation to exclude hairy cell leukaemia or inherited bone marrow failure due to *GATA2* mutation (Emberger/MonoMac syndrome, see section on inherited AA)
2. Reticulocyte count	Reticulocytopenia; automated reticulocyte counting will over-estimate the count compared with the levels set in the Camitta criteria (Camitta, 1984) for defining disease severity, which were defined on manual counts. This criterion has now been modified from manual percentages to absolute reticulocyte levels $<60 \times 10^9$/l as assessed by automated technologies (Rovo *et al*, 2013)
3. Blood film examination	Frequent macrocytosis and anisopoikilocytosis. Neutrophils may show toxic granulation. Platelets are mainly small in size. Exclude presence of dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells, such as 'hairy' cells
4. HbF%	HbF; measure pre-transfusion in children – important prognostic factor in children. Note that the level is often elevated in constitutional syndromes
5. Peripheral blood chromosomal breakage analysis: diepoxybutane test (DEB Test)	For possible FA if patient aged <50 years, but it would also be indicated to screen older patients if FA is clinically suspected. It is difficult to set an upper age limit for FA screening, as anecdotal cases have been diagnosed in the fifth decade (unpublished observations). Screen all patients who are transplant candidates and siblings of FA patients
6. Flow cytometry for GPI-anchored proteins to detect PNH clone (6-colour methodology including FLAER)	See AA and PNH section for full description
8. Vitamin B12 and folate	Documented vitamin B12 or folate deficiency should be corrected before a final diagnosis of AA is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare
9. Liver function tests	Liver function tests should be performed to detect antecedent/on-going hepatitis
10. Viral studies: hepatitis A/B/C, EBV, CMV, HIV and Parvovirus B19	AA due to hepatitis is rare, it usually occurs 2–3 months after an acute episode of hepatitis and is more common in young males (Brown *et al*, 1997). In post-hepatic AA the serology is often negative for the known hepatitis viruses. CMV should be assessed if SCT is being considered. HIV more commonly causes isolated cytopenias but is a very rare cause of AA (Wolf *et al*, 2007; Hapgood *et al*, 2013). Likewise, parvovirus B19 is more usually associated with pure red aplasia but has been reported with AA (Mishra *et al*, 2005)
11. Anti-nuclear antibody and anti-double stranded DNA	Pancytopenia in systemic lupus erythematosus may (i) be autoimmune with a cellular bone marrow (ii) associated with myelofibrosis or rarely (iii) with a hypocellular marrow
12. Chest X-ray and other radiology	Useful at presentation to exclude infection and for comparison with subsequent films. X-rays of the hands, forearms and feet may be indicated if an IBMFS is suspected. High resolution CT scan of the chest is indicated for suspected DC or constitutional *RUNX1* bone marrow failure syndrome
13. Abdominal ultrasound scan and echocardiogram	An enlarged spleen and/or 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 FA
14. Emerging diagnostic tests: the following are not currently routine diagnostic tests, but are likely to be so within the next few years	
Peripheral blood leucocyte telomere length:	Useful for disease screening for telomere gene mutations in classic DC; less specific in adult onset AA with *TERC*/*TERT* mutations; short telomeres may also occur in acquired AA with reduced stem cell reserve (Townsley *et al*, 2014)
Next generation sequencing, gene panels for:	• Telomere gene complex mutations • Other IBMFS • Acquired somatic mutations, typical of myeloid malignancies, to help distinguish AA from hypocellular MDS and for early detection of clonal evolution to MDS/AML (Kulasekararaj *et al*, 2014)
Single nucleotide polymorphism array karyotyping	Whole genome scanning to detect unbalanced chromosomal defects (Afable *et al*, 2011a)

HbF, fetal haemoglobin; GPI, glycerophosphatidylinositol; AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; FLAER, fluorescent aerolysin; EBV, Epstein Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; SCT, stem cell transplantation; IBMFS, inherited bone marrow failure syndromes; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; CT, computerized tomography; DC, dyskeratosis congenita; FA, Fanconi anaemia.

Table II. Bone marrow features of aplastic anaemia.

Bone marrow aspirate	Can be performed without platelet support, providing adequate surface pressure is applied (Kelsey, 2003), even in severe thrombocytopenia. Difficulty obtaining fragments may indicate marrow fibrosis or infiltration and should raise the suspicion of a diagnosis other than AA. In AA, fragments and trails are hypocellular with prominent fat spaces and variable numbers of residual haemopoietic cells. Erythropoiesis is reduced or absent; dyserythropoiesis is very common, often marked and does not distinguish MDS from AA. Megakaryocytes and granulocytic cells are markedly reduced or absent. Dysplastic megakaryocytes and granulocytic cells are not seen in AA. Lymphocytes, macrophages, plasma cells and mast cells often appear prominent. In the early stages of disease, there may be increased macrophages with some haemophagocytosis and background eosinophilic staining representing interstitial oedema
Cytogenetic and FISH analysis	Karyotyping may fail in very hypocellular marrows with there being insufficient metaphases. In this situation perform FISH analysis for chromosomes 5, 7, 8 and 13 It was previously assumed that the presence of an abnormal cytogenetic clone indicated a diagnosis of MDS and not AA. However it is now evident that abnormal cytogenetic clones [such as del(13q), trisomy 8 and others], which may be transient, are present in up to 12% of patients with otherwise typical AA at diagnosis (Gupta *et al*, 2006; Afable *et al*, 2011b). Although monosomy 7 may indicate the likelihood of MDS in children, in adults monosomy 7 can also be seen in AA. Abnormal cytogenetic clones may arise during the course of the disease and the appearance of a new cytogenetic abnormality may provide evidence of clonal evolution (Maciejewski *et al*, 2002)
Bone marrow trephine biopsy	A good quality trephine biopsy of at least 2 cm is essential to assess overall cellularity and morphology of residual haemopoietic cells, and to exclude an abnormal infiltrate. Care should be taken to avoid tangential biopsies because subcortical marrow is normally hypocellular In most cases the biopsy specimen is hypocellular throughout; sometimes hypocellularity is patchy with both hypocellular and residual cellular areas. Focal hyperplasia of erythroid or granulocytic cells at a similar stage of maturation may be observed. Small lymphoid aggregates may occur, particularly in the acute phase of the disease or when AA is associated with systemic autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus. Increased reticulin staining, dysplastic megakaryocytes (best assessed by immunohistochemistry) and blasts are not seen in AA; their presence either indicates a hypoplastic MDS or evolution to leukaemia (Bennett & Orazi, 2009)

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; FISH, fluorescence *in situ* hybridization; MDS, myelodysplastic syndrome.

- **Comprehensive assessment should be performed, including family history, abdominal ultrasound, echocardiogram, high resolution computerized tomography scan of the chest and pulmonary function tests, and evaluation for other extra-haematopoietic abnormalities (such as cirrhosis, pulmonary fibrosis or renal anomalies); the presence of these will support a diagnosis of constitutional rather than idiopathic BMF. Grade 1B**

Supportive care

Blood product support

Transfusion of red blood cells. For most patients with AA, transfusion with red blood cells (RBC) is essential to maintain a safe blood count, improve symptoms of anaemia and maintain quality of life. The decision to transfuse RBC should be based on clinical symptoms (signs of anaemia), taking into consideration the patient's age and co-morbidities (cardiac, pulmonary or vascular). Although no specific pre-transfusion haemoglobin concentration (Hb) trigger can be recommended, it is important to maintain quality of life and avoid symptoms. A higher trigger may be needed for elderly patients and those with co-morbidities. Optimal use of RBC transfusion involves administration of enough red cells to maximize clinical outcome whilst avoiding unnecessary transfusions (Carson *et al*, 2012).

Alloimmunization against red cell antigens and iron overload are the commonest risks associated with regular transfusion therapy. Provision of phenotype-matched blood (for Rh and Kell) should be considered to reduce the risk of alloimmunization.

Transfusion of platelets. Regular platelet transfusion support may be required for AA patients. With the exception of one publication (Sagmeister *et al*, 1999), literature specific to platelet transfusion support in AA is lacking, and evidence is taken from studies addressing the need for platelet transfusion support in patients with reversible thrombocytopenia (Estcourt *et al*, 2012; Stanworth *et al*, 2013; Killick *et al*, 2014). It is recommended that prophylactic platelet transfusions should be given to stable AA patients on active therapy (where the treatment aims to reverse the severe thrombocytopenia) with a platelet count $<10 \times 10^9$/l. For patients with sepsis, the platelet count should be kept $>20 \times 10^9$/l. For thrombocytopenic patients requiring invasive procedures, platelet transfusions must be administered, aiming to achieve a platelet count in line with BCSH guidelines for the relevant procedures (British Committee for Standards in Haematology, 2003), and a pre-procedure platelet count should be checked.

During treatment with ATG, worsening thrombocytopenia can occur. This is due to increased platelet consumption in the presence of cross-reacting antibodies in ATG binding to

Table III. Other causes of pancytopenia and a hypocellular bone marrow.

Associated with PNH (AA/PNH)	Variable cellularity depending on the phase of disease and transition from PNH to AA. Test peripheral blood immunophenotyping for GPI-linked molecules on red and white cell populations
Hypoplastic MDS/AML	Sometimes difficult to distinguish from AA. The following features of MDS are not found in AA: dysplastic cells of the granulocytic and megakaryocytic lineages, blasts in the blood, marrow aspirate or trephine biopsy specimen (Bennett & Orazi, 2009). In trephine biopsy specimens, increased reticulin, increased $CD34^+$ cells and residual areas of haemopoiesis suggests hypoplastic MDS rather than AA. The presence of ALIPs is more indicative of MDS than AA, though small collections of immature granulocytic cells may be seen in the bone marrow in AA when regeneration occurs. ALIPs must not be confused with dysplastic proerythroblast islands, and can be easily differentiated on immunohistochemistry. Dyserythropoiesis is very common in AA and does not distinguish MDS from AA
Hodgkin lymphoma or non-Hodgkin lymphoma	Can present with pancytopenia and a patchy hypocellular bone marrow with limited areas of lymphoid infiltration that can easily be missed in small samples. The bone marrow biopsy should be examined carefully for foci of lymphoma cells or fibrosis, which may be seen in only a small part of the specimen. Lymphocytes are often prominent in AA and immunophenotypic marker studies and gene rearrangement studies will help to exclude a diagnosis of lymphoma. Additional features, such as splenomegaly, make AA very unlikely
Primary myelofibrosis	Primary myelofibrosis is usually accompanied by abnormal blood film (teardrop poikilocytosis, leucoerythroblastic) changes and splenomegaly. The absence of an enlarged spleen in the presence of marrow fibrosis suggests a secondary malignancy
Mycobacterial infections	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 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 and 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, gelatinous transformation (serous degeneration/atrophy), loss of fat cells as well as haemopoietic cells, and increased background substance which stains a pale pink on haematoxylin/eosin stain (Bain *et al*, 2001). The pink background substance may also be seen on a May–Grünwald–Giemsa stained aspirate
ITP	Occasionally AA presents with an isolated thrombocytopenia, and pancytopenia develops later. Such patients can initially be misdiagnosed as ITP but bone marrow examination in AA shows hypocellularity with reduced or absent megakaryocytes, which is not commonly seen in ITP, although rarely ITP is associated with reduced megakaryocytes
AA in children	A recent comprehensive review discusses in more detail conditions that may present with pancytopenia and a hypocellular bone marrow in children (Davies & Guinan, 2007)
GATA2 deficiency – MonoMac	This diagnosis maybe considered in hypoplastic marrows with absent peripheral blood monocytes or severe monocytopenia (Spinner *et al*, 2014)

GPI, glycerophosphatidylinositol; AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; ALIPs, abnormal localization of immature precursors; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; AAFB, acid/alcohol fast bacilli; ITP, immune thrombocytopenia; MonoMac, monocytopenia with susceptibility to mycobacteria.

platelets. Although there are no studies to support the exact threshold for platelet transfusion support prior to ATG, most authors use a threshold of 20×10^9/l (Scheinberg *et al*, 2011; Scheinberg & Young, 2012).

Regular support with RBC and platelet transfusions increases the risk of HLA and non-HLA (minor histocompatibility) alloimmunization, leading to poor platelet increments and increased risk of graft rejection after HSCT. Leucodepletion of cellular blood components may reduce, but not eliminate, alloimmunization (Killick *et al*, 1997; Desmarets *et al*, 2009). The possibility of HLA alloimmunization and provision of HLA-selected platelets should be considered for patients refractory to platelet transfusion, provided other causes of refractoriness have been excluded. In the absence of HLA antibodies and for patients failing to increment with HLA-matched platelets, investigation and matching for human platelet antigen antibodies should be considered.

Granulocyte transfusions. The use of irradiated granulocytes should be considered in patients with life-threatening infection related to severe neutropenia (Quillen *et al*, 2009), and anecdotally may be life saving. Data about the effectiveness of granulocyte concentrates are limited and usage is linked with a number of adverse events, such as transfusion-related acute lung injury, alloimmunization and febrile reactions.

Use of irradiated cellular blood components for AA patients. Irradiation of cellular blood components prevents transfusion-associated graft-*versus*-host disease (TA-GVHD). This is a rare complication of blood transfusion with 100%

mortality. Irradiation may also reduce the risk of alloimmunization in AA, as reported from animal data (Bean *et al*, 1994).

- AA patients undergoing HSCT must be transfused with irradiated blood components in line with BCSH guidelines (Treleaven *et al*, 2011).
- All granulocyte concentrates and HLA-matched platelets must be irradiated.
- The risk of development of TA-GVHD following treatment with ATG, although appearing to be low, remains unclear. In view of the seriousness of the condition, and in line with previous BCSH guidelines and European Group for Blood and Marrow Transplantation (EBMT) recommendations (Marsh *et al*, 2010; Hochsmann *et al*, 2013), irradiated blood components are currently recommended for patients receiving ATG. It is not known how long the use of irradiated blood products following ATG treatment should be continued, but it may be reasonable to continue while patients are still taking CSA following ATG therapy.
- Patients treated with alemtuzumab must also receive irradiated blood components according to the BCSH guidelines (Treleaven *et al*, 2011).

CMV tested blood products. Following universal leucodepletion in the UK, the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) no longer recommends the use of cytomegalovirus (CMV)-negative blood components (if they have been leucodepleted) for patients with immunodeficiency (unless pregnant) and those undergoing HSCT (SaBTO Annual Report, 2011/12), although PCR monitoring should be considered (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/215126/dh_132966.pdf). To date, there has not been a statement from the British Society of Bone Marrow Transplantation regarding blood products and CMV status.

CMV-negative granulocyte components should be provided for CMV-negative recipients.

Iron chelation therapy. Aplastic anaemia patients on regular RBC transfusion support will develop tissue iron overload, but there remains debate on the clinical impact of transfusional iron overload. In the setting of HSCT, a raised serum ferritin is an adverse predictor of outcome in myeloablative stem cell transplantation (Armand *et al*, 2007). Although unreliable, serum ferritin remains the most widely quoted parameter for assessment of iron overload. Magnetic resonance imaging (T2* or R2) can quantitate cardiac and liver iron, and is a useful adjunct although its utility in AA has not been published.

There are few published data regarding iron chelation therapy in AA. A large study was the 1-year Evaluation of Patients' Iron Chelation with Exjade study (Lee *et al*, 2010). This confirmed that chelation with deferasirox can be administered safely in patients with AA (no drug-induced cytopenias were noted), and can reduce the serum ferritin. However, dose adjustments are required to adequately chelate those who are heavily transfusion dependent. Impaired renal function is observed with deferasirox, and the drug should be used with caution in AA patients who are taking CSA. Deferasirox is licensed for use in transfusion-dependent anaemia, but only as second line therapy when desferrioxamine is inadequate or contra-indicated. Deferiprone is efficacious but not recommended in neutropenic patients (Cermak *et al*, 2011).

For those responding to immunosuppression, or after a successful HSCT, venesection is recommended for iron overload.

Infection is the major cause of death in AA: prevention and treatment options

Infections remain the major cause of death in AA (Marsh & Kulasekararaj, 2013). In contrast to cancer patients undergoing chemotherapy, in SAA neutropenia is prolonged and persistent, resulting in a higher incidence of invasive fungal infection (IFI) and severe bacterial sepsis. Survival of non-responders to ATG in the last two decades has markedly improved and this has occurred in conjunction with decreased infection-related mortality and decreased frequency of IFIs (Valdez *et al*, 2011).

Prevention of infections. Aplastic anaemia patients who are severely neutropenic should ideally be nursed in isolation when in hospital. In the UK it is common practice to give prophylactic antibiotics and antifungals, regular mouth care including an antiseptic mouthwash (such as chlorhexidine or saline) and food of low bacterial content (Hochsmann *et al*, 2013). Prophylactic antibiotics, either two non-absorbables (e.g. colistin and neomycin) or quinolones (e.g. ciprofloxacin), may be initiated but the preference should be according to local policy. A mould (aspergillus) active azole, preferably itraconazole or posaconazole, should be used as prophylaxis. In the UK, prophylaxis against *Pneumocystis jirovecii* is not routinely given. Anti-viral prophylaxis in untreated patients with AA is not routinely given. Antiviral prophylaxis with aciclovir or valaciclovir should be used during and after ATG therapy. During ATG therapy, sub-clinical reactivation of CMV and Epstein–Barr virus (EBV) is common but self-limiting, and therefore does not need antiviral treatment; EBV-related post-transplant lymphoproliferative disease has only very rarely been reported after ATG, most often after rabbit ATG. It is not UK practice to give *Pneumocystis jirovecii* prophylaxis with ATG.

Treatment of infections. Protocols and guidelines for the management of febrile neutropenia, including the assessment and management of fungal infections, are well developed and clinicians should follow local hospital and National Institute for Health and Care Excellence guidance (Phillips *et al*, 2012). Empirical anti-fungal therapy, as per local guidelines,

should be initiated early for patients with clinically suspected IFIs, as these patients have persistent neutropenia. Granulocyte transfusions may be potentially life saving in severe sepsis, such as invasive fungal disease, particularly for patients due to proceed to HSCT (Quillen *et al*, 2009).

Haemopoietic growth factors. Haemopoietic growth factors, such as erythropoiesis-stimulating agents and granulocyte colony-stimulating factor (G-CSF), are usually ineffective in supporting blood counts in AA patients (Marsh *et al*, 2007), although encouraging preliminary results are reported with the thrombopoietin-mimetic, eltrombopag (Desmond *et al*, 2014); see also section on Treatment of AA in the Elderly.

Key recommendations for supportive care

- **Blood transfusions should be given to improve quality of life. Grade 1A**
- **A threshold haemoglobin concentration cannot be recommended for all patients; it should be individualized according to co-morbidities. Grade 1A**
- **Phenotype (Rh and Kell) matched blood should be considered to reduce the risk of alloimmunization. Grade 1B**
- **Prophylactic platelet transfusions should be given to stable AA patients receiving active treatment. Grade 1B. A threshold (pre-transfusion) platelet count of 10×10^9/l should be used. Grade 1B**
- **In patients judged to have additional risk factors for bleeding, such as fever or sepsis, a higher prophylactic transfusion threshold is recommended of 20×10^9/l. Grade 2C**
- **Routine prophylactic platelet transfusions are not recommended for stable AA patients not on active treatment. Grade 2B**
- **Patients with chronic bleeding of World Health Organization grade 2 or above require individual management according to the severity of their symptoms and signs. Grade 2C**
- **Prior to administration of ATG, a daily threshold (pre-transfusion) platelet count of 20×10^9/l should be used for the duration of the ATG course. Grade 2C**
- **Only one adult platelet dose is routinely required. Grade 1A.**
- **All patients undergoing treatment with IST (ATG or Alemtuzumab) should receive irradiated blood products. Grade 1C**
- **All patients undergoing HSCT should receive irradiated blood products. Grade 1A**
- **The need for iron chelation therapy should be decided on an individual patient basis. Patients with iron overload after a successful HSCT should undergo venesection. Grade 1B**
- **Aplastic anaemia patients who are severely neutropenic should be given prophylactic antibiotics and antifungal therapy according to local policies. Grade 2B**
- **Aplastic anaemia patients receiving IST should also receive prophylactic anti-viral agents, although routine prophylaxis against *Pneumocystis jirovecii* is not necessary. Grade 2C**

Immunosuppressive therapy

Current standard first line IST

Standard first line IST is the combination of horse ATG (ATGAM; Pfizer, New York, NY, USA) and CSA. Lymphoglobuline horse ATG is no longer available (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012). A prospective randomized study from the National Institutes of Health (NIH) and a prospective EBMT study showed significantly better response at 3 and 6 months, and survival with horse ATG compared to rabbit ATG for first line IST (Scheinberg *et al*, 2011; Marsh *et al*, 2012). There is no indication for routine use of G-CSF with ATG + CSA (Tichelli *et al*, 2011). Prednisolone is used with ATG for the sole purpose of prevention of side effects of ATG.

Indications

ATG + CSA is indicated as first line therapy for:

- NSAA patients who are transfusion dependent, bleeding, encountering infections or for lifestyle (activities).
- SAA)/VSAA patients in the absence of an HLA-matched sibling.
- SAA/VSAA patients >35-50 years of age (see Fig 1).

There is no upper age limit for ATG, but there is increased mortality in patients aged >60 years treated with ATG (Tichelli *et al*, 1999, 2011) (see later section on Treatment of AA in the Elderly). A second course of ATG may be indicated for failure to respond or relapse after a first course or if the patient is ineligible for UD HSCT (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012) (see Fig 2). For a second course, rabbit ATG may be given. A second course of horse ATG is an alternative option, but this may be associated with more immediate and late (serum sickness) side effects (Marsh *et al*, 2012). Compared to horse ATG, rabbit ATG produces more profound and prolonged lymphodepletion and, in some recent studies, more infections. It is therefore important to ensure that patients receive adequate prophylactic antimicrobial support when using rabbit ATG.

Administration of ATG

Antithymocyte globulin must be given as an in-patient. ATG is a powerful immunosuppressive agent; it should only be used in centres that are familiar with using the drug and with its side effects. Prior to starting ATG:

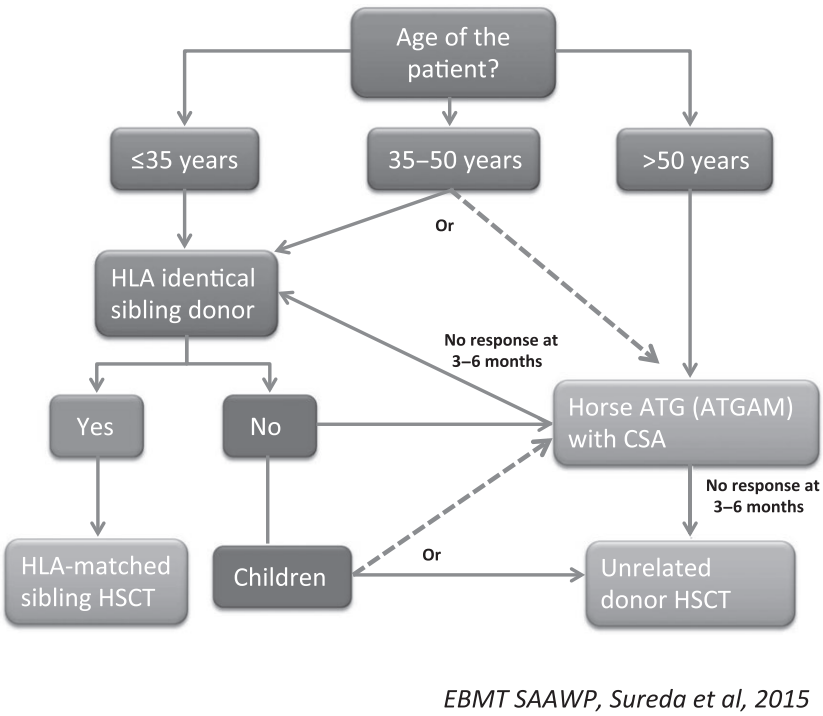

Fig 1. Treatment of acquired severe aplastic anaemia. HSCT may be considered, using a matched sibling donor or a suitably matched unrelated donor if no matched sibling donor is available, for patients aged 35–50 or >50 years who fail to respond to first line immunosuppressive therapy (Sureda *et al*, 2015). ATG, antithymocyte globulin; HLA, human leucocyte antigen; HSCT, haemopoietic stem cell transplantation; CSA, ciclopsporin.

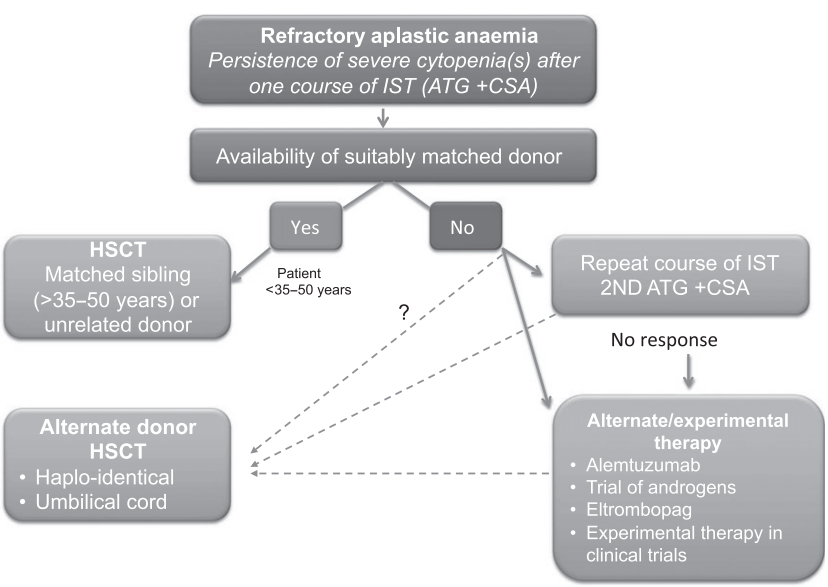

Fig 2. Treatment of adult refractory severe aplastic anaemia. ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplantation; IST, immunosuppressive therapy. Modified from Marsh, J.C. & Kulasekararaj, A.G. 2013.

- The patient should be clinically stable and ideally afebrile.
- Platelet count increment studies should be performed to exclude platelet refractoriness.
- Prophylactic antiviral, antibiotic and antifungal drugs should be administered according to local policy.
- For patients aged >60 years, careful assessment of co-morbidities is necessary to determine medical fitness prior to consideration for ATG, because there is increased mortality from infection and bleeding after ATG in this age group.

The dose of horse ATG (ATGAM) is 40 mg/kg/d for 4 d. It is given as an intravenous infusion over 12-18 h. Due to the risk of anaphylaxis, a 'test' dose must be given. Current practice is to use an intravenous infusion test dose (recent survey of the EBMT SAA Working Party, unpublished data May 2012), whereby the first 100 ml of the first day infusion is given over 1 h. ATG should be given through a double lumen Hickman or other central venous catheter, as it is sclerosing to peripheral veins, and also for ease of administration of other drugs and blood products. Each dose of ATG should be preceded with intravenous methyl prednisolone 1 mg/kg, chlorphenamine, and platelet transfusions aiming to keep the platelet count

>20–30 × 10^9/l (Marsh *et al*, 2009; Scheinberg & Young, 2012). Broad-spectrum intravenous antibiotics according to local policy should be given for febrile episodes irrespective of the neutrophil count. Fluid retention occurs commonly during ATG treatment, especially in older patients; careful attention to fluid balance is important. Prednisolone is started on the day after ATG is completed at a dose of 1 mg/kg/d for 2 weeks, followed by rapid tapering over the 2 weeks.

Ciclosporin should be commenced as the prednisolone dose is tapered, at a dose of 5 mg/kg/d to achieve trough blood levels of 100–200 μg/l. CSA should be continued whilst the blood count continues to rise. A slow tapering of the drug (25 mg every 2 3 months) can be started after at least a further 12 months of therapy, to reduce the risk of later relapse (Dufour *et al*, 2013).

Side effects of ATG are (i) early reactions, including fever, rash, rigors, hypo/hypertension, fluid retention, rarely acute pulmonary oedema/adult respiratory distress syndrome and anaphylaxis and (ii) later, serum sickness occurring days 7 14 from the start of ATG, most commonly with arthralgia, myalgia, rash and fever.

Serum sickness is treated with intravenous hydrocortisone 100 mg four times a day (QDS) and adequate analgesia; it usually requires a few days of treatment. Extra platelet transfusions are often needed during the period of serum sickness due to platelet consumption.

There is no indication for using G-CSF with ATG + CSA, as prospective randomized studies have shown that daily G-CSF given for 3 months after ATG does not improve response or overall survival (OS) (Tichelli *et al*, 2011).

Table IV. Criteria for response to IST in AA (Marsh *et al*, 2009).

(a) Response criteria following IST in severe AA	
None	Still fulfil severe disease criteria
Partial	Transfusion independent No longer meet criteria for severe disease
Complete	Haemoglobin concentration normal for age and gender Neutrophil count >1·5 × 10^9/l Platelet count >150 × 10^9/l
(b) Response criteria following IST for non-severe AA	
None	Blood counts are worse, or do not meet criteria below
Partial	Transfusion independence (if previously dependent) *or* doubling or normalization of at least one cell line *or* increase of baseline • haemoglobin concentration of >30 g/l (if initially <60) • neutrophils of >0·5 × 10^9/l (if initially <0·5) • platelets of >20 × 10^9/l (if initially <20)
Complete	Same criteria as for severe disease

AA, aplastic anaemia; IST, immunosuppressive therapy.

Outcomes

Response to ATG (as defined in Table IVa,b) is delayed, starting after an average of 3 4 months. The 6-month response rate to a first course of horse ATG is around 70%. Five-year OS is age-dependent: 100% for age <20 years, 92% for 20–40 years, 71% for 40–60 years and 56% for >60 years (Tichelli *et al*, 2011). In comparison, the response to a first course of rabbit ATG is around only 35 45%, with significantly worse OS (Scheinberg *et al*, 2011; Marsh *et al*, 2012; Scheinberg & Young, 2012). For NSAA, ATG + CSA results in significantly higher response rates, 74% *versus* 46% (and better event-free survival), compared to CSA alone (Marsh *et al*, 1999). Relapse after ATG occurs in up to 35% of patients; the risk of later clonal evolution to MDS/acute myeloid leukaemia is 15%, and haemolytic PNH in 10% (Rosenfeld *et al*, 2003; Scheinberg & Young, 2012).

Response to a second course of ATG from most studies is around 35% for refractory AA and 55–60% for relapsed AA (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012). Factors predicting for response are summarized in Table V.

Other immune suppressive drugs that have been used in AA

It is recommended that expert advice be sought when considering the use of other immunosuppressive drugs. Mycophenolate mofetil, sirolimus, corticosteroids and

Table V. Factors predicting response to ATG.

1	Young age
2	Less severe disease
3	Absolute reticulocyte count >25 × 10^9/l and absolute lymphocyte count >1·0 × 10^9/l (Scheinberg *et al*, 2009)
4	The finding of either of the chromosomal abnormalities trisomy 8 or del(13q) in the context of AA predicts for good response to ATG (Maciejewski *et al*, 2002; Holbro *et al*, 2013)
5	The presence of a PNH clone is predictive of response in some but not all studies
6	Telomere length is not predictive of response, but longer telomeres identify a sub-group who show excellent overall survival after IST (Scheinberg *et al*, 2010)
7	Response to a second course of ATG from most studies is around 35% for refractory AA and 55–60% for relapsed AA (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012)

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; ATG, antithymocyte globulin; IST, immunosuppressive therapy.

Table VI. Other immunosuppressive drugs that have been used in AA.

Alemtuzumab	Effective in around 35% and 55% of patients with refractory and relapsed AA, respectively Not recommended as first line IST, as a response rate of only 19% was reported from the prospective NIH study (Scheinberg *et al*, 2012). May be considered as an option for refractory/relapsed AA (i) when a second course of ATG is not possible, or (ii) in the presence of renal impairment, as it is effective as monotherapy without addition of CSA or (iii) if the patient is ineligible for HSCT Given as a total dose of 100 mg; given as a subcutaneous dose of 10, 30, 30 and 30 mg over 4 d. Relapses are frequent although patients may respond again to a further course. All patients should receive adequate prophylaxis including against *Pneumocystis jirovecii* Patients being considered for alemtuzumab should be referred to a tertiary centre, be treated as part of the established EBMT protocol and reported to EBMT registry
Mycophenolate mofetil and sirolimus	There is no indication for the addition of other immunosuppressive drugs, such as mycophenolate mofetil or sirolimus, either in addition to ATG or in isolation, as there is no evidence that they are effective in AA In combination with ATG + CSA they do not increase the response rate, survival or reduce relapse, compared to ATG + CSA (Scheinberg & Young, 2012)
Cyclophosphamide	The use of high dose or even so called 'moderate' dose cyclophosphamide as treatment for AA is not recommended. Although response occurs in around 50% of patients with refractory AA, its predictable prolonged duration of neutropenia results in a high incidence of severe fungal infections and mortality (Tisdale *et al*, 2000; Marsh *et al*, 2009; Samarasinghe & Webb, 2012; Scheinberg & Young, 2012; Scheinberg *et al*, 2014)

AA, aplastic anaemia; IST, immunosuppressive therapy; ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplantation; EBMT, European Group for Bone Marrow Transplantation.

cyclophosphamide are not recommended in the treatment of AA (see Table VI).

Vaccinations in non-transplanted patients

There is a potential relapse risk of AA following vaccinations in those patients who have responded to IST. The evidence base is limited and based on anecdotal case reports, as well as an appreciation that a viral insult is likely to be an important trigger in the pathogenesis of AA (Viallard *et al*, 2000; Hendry *et al*, 2002). Vaccinations, including influenza vaccination, should be avoided if possible, except following HSCT, when AA patients should be routinely vaccinated as recommended for all allogeneic bone marrow transplantation recipients (see HSCT section).

Key recommendations for IST

- **The current standard first line IST is horse ATG (ATG-ATGAM) combined with CSA. Grade 1A**
- **Immunosuppressive therapy is recommended first line therapy for non-severe AA patients requiring treatment (see indications in text), severe or very severe AA patients who lack a MSD, and severe or very severe AA patients aged >35-50 years. Grade 1A**
- **A second course of ATG may be indicated following failure to respond to a first course (if the patient is ineligible for a matched UD HSCT) or following relapse after a first course. Grade 1A**
- **ATG is an immunosuppressive drug and should only be administered in centres familiar with its use; the drug must only be given to in-patients. Grade 1B**
- **The use of high dose or moderate dose cyclophosphamide (without stem cell support) is not recommended in AA. Grade 1A**
- **Following IST, vaccinations, including influenza, should be avoided if possible as there is a theoretical risk of disease relapse. Grade 2C**

Haemopoietic stem cell transplant in AA

Current indications for HSCT in adults

The current indications for HSCT are based on the EBMT SAAWP guidelines (Sureda *et al*, 2015). Patients should be managed in JACIE [Joint Accreditation Committee-International Society for Cellular Therapy (ISCT) and EBMT]-accredited centres.

HLA identical sibling donor. Up-front HSCT from a MSD is indicated for SAA in young and adult patients who have a MSD. EBMT data show similar outcomes for patients aged 40-50 to those aged 30-40 years (Sureda *et al*, 2015). However, co-morbidities should be carefully assessed to determine fitness for up-front transplantation instead of IST for patients aged 35-50 years.

Unrelated donor. Unrelated donor HSCT is indicated for SAA after failure to respond to one course of IST. There is no strict upper age limit but this should be discussed on an individual patient basis and according to co-morbidities at the respective transplant centre. The donor should be 10/10- or 9/10-matched based on HLA high resolution typing for class I (HLA-A, -B, -C) and II (HLA-DRB1, -DQB1) antigens.

Alternative donor: cord blood and haploidentical. Alternative donor HSCT using either cord blood, a haploidentical family donor or a 9/10-matched UD may be considered, among other treatment options, after failure to respond to IST and in the absence of a MSD and a suitably matched UD (Samarasinghe *et al*, 2012; Passweg & Aljurf, 2013). All donors should be screened for donor-directed HLA antibodies, the presence of which is associated with a very high risk of graft rejection. There is less clear guidance on the exact indication for alternative donor HSCT as this is less successful than MSD or UD HSCT, but new approaches to alternative donor HSCT are being evaluated using uniform EBMT protocols.

Syngeneic donor. In the rare situation where there is a syngeneic donor available, HSCT should be considered in all patients regardless of age as long term OS exceeds 90% (Marsh & Kulasekararaj, 2013).

Timing of donor search/availability

For all newly diagnosed AA patients who may be potential transplant candidates, HLA tissue typing should be performed at time of diagnosis, so that (i) MSD HSCT can proceed as soon as possible, and ideally before the patient becomes sensitized, not only to HLA but also to minor histocompatibility antigens, and (ii) the potential availability of UDs is established, so that if there is no response to a course of ATG and CSA, the patient can then proceed to UD HSCT (or earlier if the patient's condition is of concern with severe and/or recurrent infections). Assessment for response to IST is usually made at 3 6 months.

Pre-transplant work up

An MDT approach is essential for the pre-transplant work up. The aims of the work up are to (i) confirm the diagnosis and exclude/document clonal evolution (ii) assess co-morbidities (iii) select the donor, conditioning regimen, stem cell dose and source, (iv) address fertility issues and (v) inform the transfusion laboratory of the potential transplant and review of transfusion requirements (Table VII).

Conditioning regimens

The choice of conditioning regimens to use depends on (i) patient age (ii) type of donor (iii) centre preference for choice of antibody, whether ATG (Bacigalupo *et al*, 2010; Sanders *et al*, 2011) or alemtuzumab (Marsh *et al*, 2011; Bacigalupo *et al*, 2012). See Table VIII.

How successful is HSCT for AA?

For adult MSD HSCT, the survival is age-dependent, but OS is 70-85% between the ages of 30 and 50 years. A recent EBMT analysis has shown that outcomes after UD HSCT are no longer inferior to MSD HSCT, in that UD is not a negative predictor of survival (Bacigalupo *et al*, 2013; Marsh *et al*, 2014).

Specific issues relating to AA HSCT regarding early post-transplant management and management of late effects are summarized in Table IX.

Key recommendations for haemopoietic stem cell transplantation

- **All patients being considered for HSCT should be evaluated in a multi-disciplinary team setting, and consideration should be given to discussion of the case with a centre that has expertise in AA regarding the indications for HSCT and the choice of conditioning regimen. Grade 1C**
- **To inform the multi-disciplinary team decision making regarding HSCT:**
  - **All patients who are potential HSCT candidates should undergo HLA typing at diagnosis, followed by related or UD searches as appropriate to assess the availability of potential donors. Grade 1B**
  - **A careful reassessment should be made to confirm the precise diagnosis and exclude clonal evolution to MDS or PNH, as this will influence the choice of conditioning. It is also vital not to miss constitutional AA so as to avoid (i) serious (and potentially lethal) toxicity from the transplant and (ii) inappropriate selection of a sibling donor. Grade 1C**
  - **The Haematopoietic Cell Transplant Co-morbidity Index or equivalent assessment should be documented. Grade 2B**
  - **Alternatives to HSCT, including IST, should be actively considered in the management plan. Grade 1B**
- **Up-front MSD HSCT for young and adult patients is the treatment of choice for severe AA, but patients aged between 35 50 years need to be carefully assessed for co-morbidities prior to consideration for transplantation. Grade 1B**
- **Unrelated donor HSCT in adults should be considered after lack of response to one course of IST. Grade 1B**
- **There have been recent improvements in outcomes after alternative donor HSCT for patients who lack a suitably matched donor, but these transplants are still experimental and specialist advice should be sought; only European Bone Marrow Transplantation SAAWP approved protocols should be used. Grade 2B**

Treatment of AA in the elderly

The treatment of elderly patients (aged >60 years) with AA is more complex than in younger patients. In addition, the

Table VII. Pre-transplant work up.

Confirm diagnosis and exclude/document clonal evolution	Perform a reassessment BM aspirate, trephine biopsy, cytogenetic analysis (and FISH for chromosomes 5, 7, 8 and13 if cytogenetic analysis fails) to confirm the diagnosis is still AA, and to exclude other causes of pancytopenia, such as hypocellular MDS (see diagnostic section)
	Repeat flow cytometry to document whether there is a PNH clone
	Exclude a constitutional form of AA (see diagnostic section, emerging diagnostics), for example FA or DC not only in children but also adults. Late onset FA or DC may present without the classical somatic abnormalities, and instead may be associated with, for example, pulmonary fibrosis or cirrhosis, which may both impact on transplant outcomes (Gerull *et al*, 2013). Conditioning regimens are different from those used in acquired AA, which are likely to be fatal in undiagnosed constitutional AA. Avoid using a MSD with an unsuspected constitutional AA
	Consider referral for opinion/advice to a centre with AA expertise and access to integrated diagnostic laboratories, including molecular genetic techniques to help differentiate AA from MDS and to exclude constitutional AA
Assess co-morbidities	Follow standard guidelines as for all patients undergoing allogeneic HSCT and document the Hematopoietic Cell Transplant Co-morbidity Index or equivalent
	As AA patients are likely to be multi-transfused at the time of HSCT, assess for iron overload with serum ferritin and, if available, T2* MRI scan for assessment of cardiac and liver iron can be considered (see section Blood Product Support for patients with AA)
	Perform serum HLA antibody screen to assess for HLA antibodies. This is to (i) ensure adequate platelet count increments and (ii) select the appropriate donor for patients being considered for mis-matched HSCT, whether using cord blood, haploidentical or a 9/10-matched unrelated donor
Select donor, conditioning regimen, stem cell source and dose	Choice of donor and type of conditioning regimen is usually straightforward but not always, so consider discussion with a centre with AA expertise
	Compared to HSCT for haematological malignancies, a higher stem cell dose is required, in order to reduce the risk of graft failure. For MSD and UD HSCT, a minimum of 3×10^6 CD34-positive cells/kg (or 3×10^8 TNC/kg) is required. For cord blood HSCT, a minimum of 4×10^7 TNC/kg is recommended, thus usually necessitating a double cord infusion (Passweg & Aljurf, 2013). There is no consensus on cell dose for haploidentical HSCT, but a proposed algorithm for donor selection to optimize the cell dose includes using, if possible, a young and male family donor (Parikh & Bessler, 2012)
	For ATG-based conditioning regimens, BM is the preferred stem cell source (http://ebmtonline.forumservice.net; Bacigalupo *et al*, 2010). For alemtuzumab-based regimens, either BM or PBSC may be used. The use of PBSC to increase the stem cell dose is being explored in the EBMT SAAWP protocol for haploidentical HSCT (Clay *et al*, 2014)
Address fertility issues	AA patients receiving high dose cyclophosphamide as part of the conditioning regimen are likely to retain their fertility post-HSCT (Ciurea & Champlin, 2013). Less long term data are available using fludarabine with lower dose cyclophosphamide regimens, although cases of successful pregnancy have been reported. The effect of low dose TBI (2 Gy) is another factor
	For patients of childbearing age, referral to an assisted conception unit for discussions on fertility should be offered. Men should be offered sperm storage. Women should have the opportunity to discuss with an assisted conception unit specialist the latest results of egg/embryo cryopreservation so they can decide if they wish to proceed with this
	However, if the patient has on-going systemic sepsis and needs an urgent HSCT, the procedure of gonadal hyperstimulation may be too dangerous. In addition, in the presence of a significant PNH clone, the risk of venous thrombosis is further increased by the state of gonadal hyperstimulation, and in this situation expert advice from one of the two national UK PNH centres should be sought regarding the use of eculizumab

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; IBMFS, inherited bone marrow failure syndrome; MDS, myelodysplastic syndrome; ATG, antithymocyte globulin; CSA, ciclosporin; FA, Fanconi anaemia; DC, dyskeratosis congenital; HSCT, haemopoietic stem cell transplantation; MSD, matched sibling donor; UD, unrelated donor; EBMT, European Group for Bone Marrow Transplantation; SAAWP, Severe Aplastic Anaemia Working Party; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; MRI, magnetic resonance imaging; HLA, human leucocyte antigen; TNC, total nucleated cells.

outcome is worse due to inferior tolerability of the treatment. Therefore patients should be individually assessed for co-morbidities and their specific wishes should be respected, as quality of life is an important outcome in this group. With regard to diagnosis, it is important to exclude hypoplastic MDS, as MDS is far more common than AA in this age group (see diagnostic section).

Older age *per se*, is not a reason to withhold treatment even in the very elderly. Immunosuppression is considered the treatment of choice. There is no place for allogeneic HSCT as first line therapy in patients aged >60 years, although HSCT can be considered in selected patients with a syngeneic donor. Ideally, the least toxic and most convenient treatment should be given. However, another consideration

Table VIII. Conditioning regimens used in HSCT for severe AA.

Matched sibling donor	For patients aged <30 years, high dose CY (200 mg/kg) with ATG or alemtuzumab. Post-graft immune suppression with CSA and 'short' course MTX if using ATG, or CSA alone if using alemtuzumab For patients aged >30 years, fludarabine 30 mg/m^2 × 4, CY 300 mg/m^2 × 4 and ATG ('FCATG') or alemtuzumab ('FCC'). Post-graft immune suppression as for patients aged <30 years Post-graft CSA is usually continued for 9 months with tapering of dose over 3 months, to reduce late graft failure There is no indication for using radiation as part of the conditioning regimen
Unrelated donor	For 10/10-matched UD HSCT, for adults, the choice is either (i) the EBMT protocol of FCATG with 2 Gy TBI or (ii) FCC without TBI For 9/10-matched UD HSCT, either FCATG + 2 Gy TBI or FCC + 2 Gy TBI
Cord blood	There is no consensus but it is recommended that the EBMT-adopted French protocol be followed, using fludarabine, CY 120 mg/kg, ATG, TBI 2 Gy, with one dose of rituximab on day +5, total nucleated cell dose infused >4 × 10^7/kg and not less than 4 out of 6 HLA mis-matched cord units (Passweg & Aljurf, 2013)
Haploidentical family	There is no consensus (Passweg & Aljurf, 2013) but it is recommended that the current EBMT SAAWP protocol be followed, using non-myeloablative conditioning (CY 14·5 mg/kg × 2, fludarabine 30 mg/m^2 × 4, TBI 2 Gy) with post-graft high dose CY (50 mg/kg on days +3 and +4) with tacrolimus and MMF post-graft. Either BM or PBSC can be used, but a high stem cell dose is essential (Clay *et al*, 2014)
Syngeneic	Conditioning prior to stem cell infusion is recommended, using high dose CY and probably also ATG. There may be good rational for using PBSC in preference to BM, as the use of PBSC is associated with a lower risk of graft failure in the setting of syngeneic HSCT (Marsh & Kulasekararaj, 2013)

AA, aplastic anaemia; ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplant; MSD, matched sibling donor; UD, unrelated donor; CY, cyclophosphamide; MTX, methotrexate; FCC, fludarabine, cyclophosphamide, alemtuzumab (Campath); EBMT, European Group for Bone Marrow Transplantation; SAAWP, Severe Aplastic Anaemia Working Party; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; MMF, mycophenolate mofetil.

Table IX. Management of early issues and late complications post-HSCT for severe AA.

Early post-transplant management	• Post-graft CSA is continued for 9 months followed by tapering to 12 months, to reduce the risk of late graft failure • Blood CSA trough levels need to be maintained at higher levels than used in haematological malignancies, between 300 and 350 μg/l. If renal function is compromised, a 'half dose' CSA and 'half dose' MMF regimen can be used instead • Regular monitoring of unfractionated and lineage-specific CD3 (T-cell) chimaerism in peripheral blood and bone marrow is recommended to detect early graft failure. Progressive mixed chimaerism predicts a high risk of graft rejection. Stable mixed T-cell chimaerism in the presence of full donor myeloid chimaerism is common when using the FCC regimen (Marsh *et al*, 2011; Bacigalupo *et al*, 2012)
Management of late effects	• Late effects monitoring should follow international guidelines, and these include routine surveillance for secondary malignancy, endocrine, metabolic, bone (including avascular necrosis) and cardiovascular risks (Majhail *et al*, 2012) • The risk of second malignancy in AA HSCT is reduced by avoiding irradiation and by the absence of chronic GVHD • Iron overload is common and is most easily addressed by regular venesections once patients are fully engrafted post-transplant • In transplanted AA patients, re-vaccination should proceed as per standard allogeneic HSCT practice

AA, aplastic anaemia; ATG, antithymocyte globulin; CSA, ciclosporin; MMF, mycophenolate mofetil; HSCT, haemopoietic stem cell transplantation; FCC, fludarabine, cyclophosphamide, alemtuzumab (Campath); GVHD, graft-*versus*-host disease.

is how quickly a response is required, such that those with life threatening cytopenias (neutrophil count <0·2 × 10^9/l) or having suffered a severe infection requiring hospitalization should be treated more intensely than those with less severe disease.

Treatment with ATG and CSA results in a more rapid and complete response than CSA alone in patients with NSAA (Marsh *et al*, 1999). However, patients require hospitalization and have a higher risk of acute and delayed toxicity than younger patients, so the risks and benefits of treatment should be weighed up for each individual patient. Patients must be assessed carefully before treatment, as the risk of infection, bleeding, heart failure and arrhythmias with ATG is higher in the elderly. Older patients have an inferior survival after ATG compared to younger patients (Tichelli *et al*, 1999).

Alternative treatments include CSA alone, oxymetholone (or danazol) or alemtuzumab. Although the response rate of CSA alone is inferior to the combination of ATG and CSA in NSAA, OS is not inferior as CSA-refractory patients may

respond to second line therapy with ATG and CSA (Marsh *et al*, 1999). CSA alone has the convenience of being outpatient-based but patients must be carefully monitored for nephrotoxicity and hypertension. Alemtuzumab may be used as a single agent in refractory/relapsed AA, but medical fitness needs very careful assessment in older patients prior to considering this agent as a possible option (Scheinberg *et al*, 2012).

Oxymetholone or danazol can be considered in men intolerant or unresponsive to CSA (Allen *et al*, 1968; Jaime-Perez *et al*, 2011). Danazol has fewer masculinizing side effects than oxymetholone so may be a better alternative for women. Careful monitoring of oxymetholone is required as it can cause nephrotoxicity, hepatic tumours, mood changes, cardiac failure, prostatic enlargement and raised blood lipids.

Patients who are intolerant of, or who decline IST should be offered best supportive care.

Eltrombopag

Eltrombopag is a peptide, small molecule, oral thrombopoietin receptor agonist. In an extension of an earlier phase II study at NIH, 43 patients with refractory SAA were treated with eltrombopag (Desmond *et al*, 2014). Haematological responses, including trilineage response, were observed in 40% of patients. The drug was well tolerated in most patients. Elevated transaminase levels may occur and there are particular concerns about clonal evolution, including monosomy 7, which requires further evaluation. Eltrombopag has been approved by the Food and Drug Administration in the USA for treatment of SAA refractory to IST. It has recently, as of August 2015, been licensed by the EMA for SAA refractory to IST or patients who are heavily pretreated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. It is advised that a repeat bone marrow is performed prior to starting treatment to exclude an abnormal cytogenetic clone typical of MDS/AA, particularly monosomy 7.

Key recommendations for treatment of AA in the elderly

- **Elderly patients with AA should be individually assessed and their specific wishes respected, as quality of life is paramount in this patient group. Grade 1C**
- **Immunosuppressive therapy is considered the treatment of choice. ATG and CSA result in a more rapid recovery of blood counts but, alternatively, CSA alone or oxymetholone can be considered. Grade 1B**
- **Patients unfit for, who decline or who are intolerant of IST should be offered best supportive care. Grade 1C**
- **Eltrombopag is licensed by the EMA for SAA refractory to IST or patients who are heavily pre-treated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. Grade 2B**

Management of AA in pregnancy

Although the relationship, either casual or coincidental, between AA and pregnancy is controversial, it remains a serious condition, challenging to manage and with a variable clinical outcome. AA can be diagnosed for the first time during pregnancy, in early or late gestation. Cytopenia(s) often progresses during pregnancy, but the disease may remit spontaneously, after abortion (spontaneous or therapeutic) or after delivery (Aitchison *et al*, 1989). Relapse is common during pregnancy in AA patients who have previously responded to ATG, especially those with partial response (Tichelli *et al*, 2002). Pregnancy does not trigger relapse of the disease in patients who had undergone successful HSCT.

Tichelli *et al* (2002) evaluated outcomes among 36 pregnancies in women previously treated with immunosuppression for AA. They reported almost half involved a complication in the mother (three abortions, two cases each of eclampsia and maternal deaths) and/or baby (five premature deaths). Relapse of AA occurred in 19% and a further 14% needed transfusion during delivery. Normal blood counts before conception did not guarantee freedom from relapse of AA during pregnancy.

Better supportive care in recent years, particularly in supply of blood products, has led to improvements in maternal and fetal outcome (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 (Deka *et al*, 2003). It is essential that the patient be monitored frequently throughout pregnancy, initially monthly but later more frequently and according to disease severity, and with very close liaison with the obstetric team and haematologist. Presence of a PNH clone should warrant discussion with a specialist centre. The mode of delivery should be determined on obstetric grounds.

Supportive care is the mainstay of treatment of AA in pregnancy and the platelet count should, if possible, be maintained above 20×10^9/l with platelet transfusions. The high risk of alloimmunization and platelet refractoriness needs to be considered. CSA is safe during pregnancy (McKay & Josephson, 2006) and is recommended for those needing transfusions. ATG, allogeneic HSCT or androgens for AA during pregnancy are not recommended.

Key recommendations for management of AA in pregnancy

- **Supportive care remains the mainstay of treatment of AA in pregnancy, aiming to maintain the platelet count above 20×10^9/l with platelet transfusions. Grade 1C**
- **CSA is safe in pregnancy if needed. Grade 2C**

Paroxysmal nocturnal haemoglobinuria and AA

Tests to detect a PNH clone

Paroxysmal nocturnal haemoglobinuria should be excluded by performing flow cytometry (Parker *et al*, 2005; Borowitz *et al*, 2010). Analysis of GPI-anchored proteins is a sensitive and quantitative test for PNH, enabling the detection of small PNH clones which occur in up to 50% of AA patients, the proportion depending on the sensitivity of the flow cytometric analysis used (Dunn *et al*, 1999; Sugimori *et al*, 2006). Such small clones are most easily identified in the neutrophil and monocyte lineages in AA and will be detected by flow cytometry. If the patient has had a recent blood transfusion, a population of GPI-deficient red cells may still be detected by flow cytometry in the granulocyte and monocyte population. However, the clinical significance of a small PNH clone in AA as detected by flow cytometry remains uncertain. Such clones can remain stable, diminish in size, disappear or increase, hence the need for monitoring the clone. What is clinically important is the presence of a significant PNH clone often associated with clinical or laboratory evidence of haemolysis. Urine should be examined for haemosiderin as this is a constant feature of haemolytic PNH even when the patient does not have macroscopic haemoglobinuria. Evidence of haemolysis associated with PNH should be quantitated with the reticulocyte count, serum bilirubin, serum haptoglobin and lactate dehydrogenase. Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6 monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable.

The presence of a PNH clone in the setting of AA does not directly influence the choice of therapy for the underlying BMF. There is some evidence that the finding of a PNH clone predicts a better response to IST but this is not universal in all published reports. Patients with a significant PNH clone receiving IST, especially ATG, should be actively monitored for signs of haemolysis. Conversely, AA may later emerge in PNH patients in the presence of significant haemolysis.

New PNH patients should be referred to one of the two specialized nationally commissioned PNH centres, St James's University Hospital, Leeds, and King's College Hospital, London, to be assessed for PNH complications and for consideration for anti-complement therapy, following formal PNH National Service MDT review. Patients will be seen in either of the two national centres or in one of 10 Outreach clinics.

Data from the French Registry compared to the EBMT outcomes demonstrates that allogeneic stem cell transplant has an inferior outcome in haemolytic and thrombotic PNH compared to best supportive care including eculizumab when indicated (Peffault de Latour *et al*, 2012). Therefore the finding of a PNH clone does not affect positively or negatively on the decision to transplant.

Key recommendations for PNH and AA

- **All patients should be screened for PNH using flow cytometry on peripheral blood to detect deficiency of GPI anchored proteins, such as CD14, CD16, CD24 as well as FLAER for white blood cells, and CD55 and CD59 for red cell analysis.**
- **Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6 monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable. Grade 2C**
- **Small PNH clones can be detected in up to 50% of patients with AA, usually without evidence of haemolysis; large clones are clinically significant and may result in haemolysis as well as increased thrombotic risk ('haemolytic PNH').**
- **Presence of a small/moderate PNH clone in AA does not directly influence the choice of treatment for the underlying BMF.**
- **New PNH patients should be referred to the PNH National Service to be monitored for PNH complications and assessed for anti-complement therapy.**

Disclaimer

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Committee for Standards in Haematology (BCSH) nor the publishers accept any legal responsibility for the content of these guidelines. These guidelines are only applicable to adult patients with AA.

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Author contributions

SBK chaired the guidelines group. JCWM was the senior author. All authors were involved in formulation, writing and approval of the guidelines. All authors approved the final version of the manuscript.

Conflict of interest

All authors have made a full declaration of interests to the BCSH and Task Force Chairs, which may be reviewed on request. In summary the following authors have declared the following conflicts of interest: SBK has received payment from Celgene for speaking at education meetings and from Novartis for speaking at education meetings and advisory work; TF has received payment from Alexion; ID has received payment from Life Length for lecturing; AK has received funding from Alexion for speaking at educational meetings; JM has received funding from Pfizer, Sanofi, Novartis and Alexion; GM has received funding from Celgene; JS has received funding from Merck Sharp and Dohme, Celgene, Orthobiotec and Pfzier. PH, AH have received payment from Alexion for lecturing. The rest of the authors have no declarations of interest.

Review process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BCSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BCSH guidelines website at www.bcshguidelines.com. If minor changes are required due to changes in level of evidence or significant additional evidence supporting current recommendations a new version of the current guidance will be issued on the BCSH website.

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