[redacted]

[redacted]

Appeals Department
[redacted]

RE: External appeal for the Genetic Testing (CPT 81479) Requested by Dr. [redacted] MD
Reference #: [redacted]

Dear Appeals Coordinator,

Please consider this letter and the enclosures as the third request for appeal. Per the letter I received [redacted] I have exhausted the internal appeal process for this plan. This letter serves as a written request for an external appeal to [redacted] denial for genetic testing as referenced above. Please note that while my previous appeals have clearly demonstrated medical necessity; I have yet to be provided with a sufficient clinical rationale for why this genetic test panel was deemed not medically necessary by [redacted].

Several documents have been provided through the first two appeals as supporting evidence that the genetic testing ordered by my physician on [redacted] were indeed medically necessary. These documents are again being provided with this third appeal and are as follows:

1. First Appeal (Attachment 1)
    - Letter from Dr. [redacted] dated [redacted] noting the criticality to exclude genetic forms of bone marrow failure, including [redacted] genetic panel, [redacted] Fanconi DEB chromosomal breakage assay, and telomere length as ascertained by FlowFISH. Dr. [redacted] notes the results of these tests have significant implications on treatment decisions, such as the intensity level of pre-transplant conditioning and donor selection.
    - Scientific Article: *Clinical Applications and Utility of a Precision Medicine Approach for Patients with Unexplained Cytopenias* Mayo Clin Proc 2019:94(9):1753-1768. This article found that genomic assessment resulted in a change in clinical management in 25% of the patients, as evidenced by changes

in decisions with regards to therapeutic interventions, donor choice and/or choice of conditioning regimen for hematopoietic stem cell transplant.

2. Second Appeal (Attachment 2)
    - Letter from myself, [redacted] dated [redacted] providing additional information and rationale to address the first appeal denial decision. This letter demonstrates the decision of my doctors to request genetic testing is supported by accepted standards of medical practice based on credible scientific evidence published in peer-reviewed medical literature recognized by the relevant medical community. These accepted standards of medical practice highlight that bone marrow failure and related syndromes are rare disorders and distinguishing acquired from inherited forms of bone marrow failure is of crucial importance given differences in the risk of disease progression to other malignancies and inform the type of transplant pretreatment as well as the selection of the appropriate donor for hematopoietic stem cell transplantation.
    - Clinical Notes from Dr. [redacted], dated [redacted] which include my specific clinical history noting clinical observations dating back to my childhood, supporting a possible inherited or genetic form of bone marrow failure.
    - Letter from Dr. [redacted], dated [redacted] explaining the exclusionary diagnosis process of Aplastic Anemia used to arrive at a diagnosis of acquired idopathic aplastic anemia and confirms the necessity of this information to inform my specific treatment plan.
    - Scientific Article: *Diagnosis and management of aplastic anemia.* Hematology Am Soc Hematol Educ Program. 2011;2011:76-81. This article confirms idiopathic aplastic anemia is diagnosed through a process of exclusion, including ruling out types of inherited bone marrow failure syndromes for which genetic testing has proven to provide mutation identification.
    - Scientific Article: *Guidelines for the diagnosis and management of adult aplastic anaemia.* Br J Haematol. 2016 Jan;172(2):187-207. This article notes that patients undergoing haemopoietic stem cell transplantation should confirm the precise diagnosis as it is vital not to miss inherited bone marrow failures to avoid serious and potentially lethal toxicity from the transplant process and inappropriate selection of a sibling donor.

Per the letter (Attachment 3) I received from [redacted] on [redacted] regarding the first appeal decision noted the Medical Reviewer had reviewed the appeal information and had determined the original decision to deny coverage for the genetic testing due to testing not being medically necessary per the plan's language, continues to be upheld. The following rationale was provided in the letter:

"You have aplastic anemia and bone marrow failure syndrome. The requested test is to help with treatment decisions. Health plan guidelines and plan benefit language have been reviewed. We reviewed the information sent to us. Based on review of this information it is determined that this test is not covered under your health plan. The health plan does not cover tests and treatments that are not shown to be medically necessary for your care. The previous denial is upheld."

Per the letter (Attachment 4) I received from [redacted] on [redacted] regarding the second appeal decision noted the Medical Reviewer had reviewed the appeal information and had determined the original decision to deny coverage for the genetic testing due to testing not being medically necessary per the plan's language, continues to be upheld. The following rationale was provided in the letter:

"This case was reviewed by an external specialist Board Certified in Hematology & Oncology. This was to obtain an expert opinion. They reviewed the available medical records. They looked at the information submitted on appeal. They reviewed the health plan guidelines. They looked at the plan benefit language. The specialist said there is not enough evidence in the literature to show that this testing is helpful for treating your condition. The specialist said this test was not medically necessary for your care. The health plan does not cover tests that are not medically necessary. The prior decision is upheld."

The letter (Attachment 2) I submitted as the second level appeal included the plan language with justification for medical necessity of this genetic testing. Aplastic anemia both idiopathic and genetic forms are rare diseases as recognized by the National Organization for Rare Disorders. There are only a few facilities in the world which are trained and educated on this type of bone marrow failure. My diagnosis and treatment have been and continues to be administered at the world renown facility of [redacted] where physicians Dr. [redacted] and Dr. [redacted] have specific expertise in treating and studying this rare disease. These two physicians would be the most appropriate individuals to determine diagnosis and treatment plan for a bone marrow failure patient. I am enclosing the [redacted] Test Requisition Documentation (Attachment 5) which includes a statement of medical necessity signed by Dr. [redacted] as well as the specific Aplastic Anemia genetic panel that was requested. I ask that all information in this package be read carefully, and, in their entirety.

Furthermore, a significant amount of research supports that bone marrow failure and related syndromes are rare disorders and distinguishing acquired from inherited forms of bone marrow failure is of <u>crucial importance</u> given differences in the risk of disease progression to other malignancies as it relates to the types of transplant pretreatment regimes (i.e. chemotherapy, radiation, etc) as well as informing the selection of the appropriate donor for hematopoietic stem cell transplantation. While a full scientific literature search has not been provided in this appeal package, there is a significant amount of literature that exists which discusses the use of

genetic testing to confirm/deny inherited forms of aplastic anemia and the criticality of ensuring correct diagnosis before undergoing hematopoietic stem cell transplant. The three articles provided in these appeal letters are just a few representative pieces of the vast scientific literature confirming the medical necessity of ensuring correct diagnosis.

Finally, I would like to conclude with a personal statement. I am a certified Medical Laboratory Technician from the American Society of Clinical Pathology and medical laboratory science, especially hematology is familiar to me. The subject of this appeal is related to diagnostic testing. I question if this genetic testing had come back positive for any of the genetic subtypes of aplastic anemia it would have been covered by my health plan as a necessary test to confirm such diagnosis. However, a negative test result does not reduce the value of the testing. Rather, the results (whether negative or positive) resulted in an accurate diagnosis and most appropriate treatment plan. The negative result for this [redacted] genetic test panel ruled out several genetic subtypes of aplastic anemia. As such, my physician diagnosed me with idiopathic aplastic anemia, which can only be diagnosed through this process of exclusionary testing. Other types of diagnostic tests, such as viral assays and chromosomal assays to confirm/deny causation of my aplastic anemia have been ordered by my physicians and have been covered by my health plan. It is unclear why this genetic testing would be considered differently under my health plan. Furthermore, my transplant date could not be scheduled until the results of the [redacted] testing were concluded because my physicians needed this information to determine the type of chemotherapy/pretreatment regime they were going to administer as well as ensure there was no genetic origin for this disease given my brother was my bone marrow donor.

Based on the information provided in this letter and the previous appeals, including the expertise and medical judgement of two well-respected [redacted] physician experts as well as the many scientific journal articles on both genetic and idiopathic forms of aplastic anemia, I assert this testing was medically necessary and therefore, should be covered by [redacted]

Sincerely,

[redacted]

[redacted] MBA, MT (ASCP)

Enclosures

CC: [redacted] Benefits Team, [redacted] Human Resources

Attachments:

1. [redacted] Letter from Dr. [redacted] with scientific enclosure *Clinical Applications and Utility of a Precision Medicine Approach for Patients with Unexplained Cytopenias* Mayo Clin Proc 2019:94(9):1753-1768.
2. [redacted] Letter from [redacted] with the following enclosures:
    a. [redacted] Clinical Notes from Dr. [redacted]
    b. [redacted] Letter from Dr. [redacted]
    c. Guinan EC. *Diagnosis and management of aplastic anemia.* Hematology Am Soc Hematol Educ Program. 2011;2011:76-81.
    d. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, Hillmen P, Ireland R, Kulasekararaj A, Mufti G, Snowden JA, Samarasinghe S, Wood A, Marsh JC; British Society for Standards in Haematology. *Guidelines for the diagnosis and management of adult aplastic anaemia.* Br J Haematol. 2016 Jan;172(2):187-207.
3. [redacted] First Appeal Decision Letter
4. [redacted] Second Appeal Decision Letter
5. [redacted] Test Requisition Documentation

Attachment 1: [redacted] Letter from Dr. [redacted] with scientific enclosure *Clinical Applications and Utility of a Precision Medicine Approach for Patients with Unexplained Cytopenias* Mayo Clin Proc 2019:94(9):1753-1768.

[redacted]

[redacted]

[redacted]

Subject: Appeal for coverage of genetic testing

To Whom It May Concern:

I am writing this letter to convey the clinical necessity of genetic testing for [redacted]

Briefly, she was diagnosed with bone marrow failure. Since she was diagnosed at a relatively young age, it is critical to exclude genetic forms of bone marrow failure. These tests include the [redacted] genetic panel, [redacted] Fanconi DEB chromosomal breakage assay, and telomere length as ascertained by FlowFISH. This can have significant implications for treatment. Specifically, if a genetic form of marrow failure is identified, then bone marrow transplant has to be done in a specific way, that is, with a reduced intensity conditioning. Further, it would be important to exclude that genetic abnormality in her bone marrow or stem cell donor, that is, her siblings. These guidelines have been published by several groups [redacted](references attached). Further, certain forms of bone marrow failure have specific therapies such danazol.

Due to these reasons, we pursed genetic testing. It was absolutely critical for her clinical management.

If there are any questions, please feel free to call me at [redacted]

Sincerely,

[redacted]

Enclosures

MAYO CLINIC

ORIGINAL ARTICLE

Clinical Applications and Utility of a Precision Medicine Approach for Patients With Unexplained Cytopenias

Abhishek A. Mangaonkar, MBBS; Alejandro Ferrer, PhD; Filippo Pinto e Vairo, MD, PhD; Margot A. Cousin, PhD; Ryan J. Kunde; Naseema Gangat, MBBS; William J. Hogan, MBChB; Mark R. Litzow, MD; Timmy M. McAllister, MA; Eric W. Klee, PhD; Konstantinos N. Lazaridis, MD; A. Keith Stewart, MBChB; and Mrinal M. Patnaik, MD

Abstract

Objective: To demonstrate experience and feasibility of a precision medicine approach for patients with unexplained cytopenias, defined as low blood counts in one or more cell lineages, persistent for 6 months or longer, in the absence of known nutritional, autoimmune, infectious, toxic, and neoplastic (secondary) causes.

Patients and Methods: Patients were evaluated in our clinic between November 8, 2016, and January 12, 2018. After a thorough evaluation of known causes, family history, and appropriate clinical assays, genomic evaluation was performed in a stepwise manner, through Sanger, targeted, and/or whole-exome sequencing. Variants were analyzed and discussed in a genomics tumor board attended by clinicians, bioinformaticians, and molecular biologists.

Results: Sixty-eight patients were evaluated in our clinic. After genomic interrogation, they were classified into inherited bone marrow failure syndromes (IBMFS) (n=24, 35%), cytopenias without a known clinical syndrome which included idiopathic and clonal cytopenias of undetermined significance (CCUS) (n=30, 44%), and patients who did not fit into the above two categories ("others," n=14, 21%). A significant family history was found in only 17 (25%) patients (9 IBMFS, 2 CCUS, and 6 others), whereas gene variants were found in 43 (63%) patients (34 [79%] pathogenic including 12 IBMFS, 17 CCUS, and 5 others). Genomic assessment resulted in a change in clinical management in 17 (25%) patients, as evidenced by changes in decisions with regards to therapeutic interventions (n=8, 47%), donor choice (n=6, 35%), and/or choice of conditioning regimen for hematopoietic stem cell transplantation (n=8, 47%).

Conclusion: We show clinical utility of a real-world algorithmic precision medicine approach for unexplained cytopenias.

The advent of individualized medicine in recent years has enabled identification of genetic drivers in several human diseases, especially in cancers.[1-3] Its relevance in identification of molecular drivers is not only limited to unraveling the underlying disease biology, but also to offer novel insights into targeted therapeutic approaches.[4] At Mayo Clinic, experience with precision medicine in solid tumors was shown to have an impact on treatment decisions and clinical care in a minority of patients.[5] That being said, genomic approaches are still in their adolescence and face significant challenges such as variability in bioinformatics analyses, intratumor heterogeneity and genome variation, limited actionable mutations, access to novel therapeutic agents, and barriers to health care delivery.[6-8] However, with measured

From the Division of Hematology, [redacted]

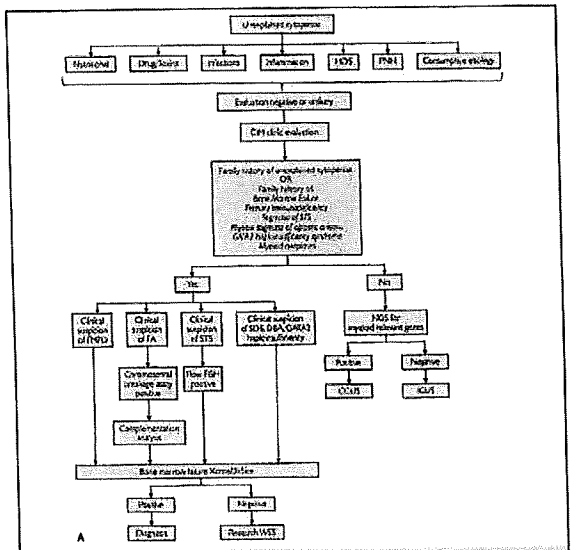

FIGURE 1. (A) Diagnostic algorithm followed by our clinic. (B) The variant decision tree adopted by our team. Nutritional causes include deficiencies of iron, B12, copper, folate, and pyridoxine. A wide array of drugs, toxins, infections, and inflammatory etiology is considered at the discretion of the referring hematologist. Consumptive etiology includes splenomegaly and various causes of intravascular and extravascular hemolysis. ACMG = American College of Medical Genetics and Genomics; CHI = Mayo Clinic Center for Individualized Medicine; CCUS = clonal cytopenia of undetermined significance; CLIA = Clinical Laboratory Improvement Amendments; COSMIC = Catalogue of Somatic Mutations in Cancer; DBA = Diamond-Blackfan anemia; ExAC = Exome Aggregation Consortium; FA = Fanconi anemia; Flow-FISH = flow cytometry/fluorescent in situ hybridization; FMPD = familial myeloid predisposition syndromes; GATA2 = GATA-binding factor 2; GnomAD = Genome Aggregation Database; HGMD = Human Gene Mutation Database; ICUS = idiopathic cytopenia of undetermined significance; M-CAP = Mendelian Clinically Applicable Pathogenicity score; MDS = myelodysplastic syndromes; NGS = next-generation sequencing; PCAN = Phenotype Consensus Analysis; PCR = polymerase chain reaction; PNH = paroxysmal nocturnal hemoglobinuria; PolyPhen = polymorphism phenotyping; PTT = Protein Panoramic Annotation Tool; RNAseq = RNA sequencing; SDS = Shwachman-Diamond syndrome; SFT = Sorting Intolerant From Tolerant; STS = short telomere syndrome; VUS = variant of uncertain significance; WES = whole-exome sequencing. Figure Continued on next page.

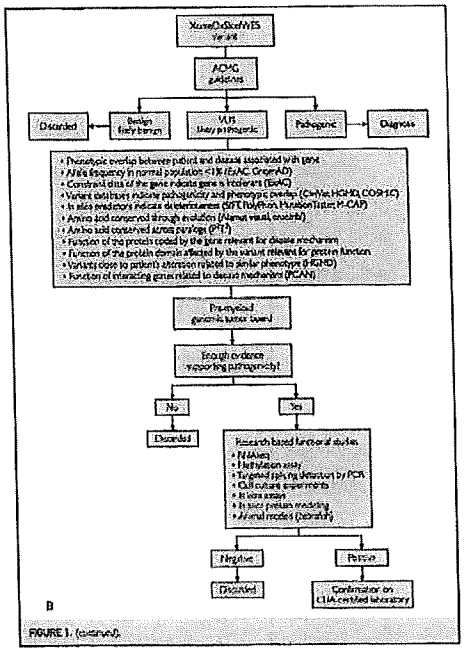

FIGURE 1. (continued).

skepticism. It is anticipated that the aforementioned approaches, with wider use and development, have the potential to significantly impact clinical care.[9-11]

In the field of neoplastic hematology, exome sequencing has enabled identification of age-related clonal hematopoiesis, which has important clinical implications such as

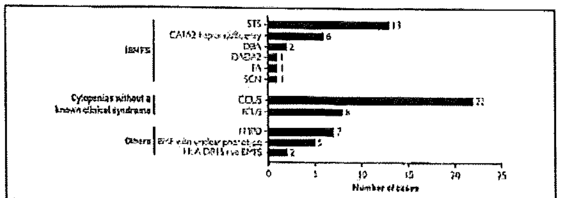

FIGURE 2. A visual description of the different diagnoses included in our cohort of patients with unexplained cytopenias. We divided 68 patients into three primary categories IBMFS (n=24, 35%), cytopenias without a known clinical syndrome (n=30, 44%) and other patients whose cases did not fit either of the two categories (n=14, 21%). BMF = bone marrow failure; BMFS = bone marrow failure syndromes; CCUS = clonal cytopenias of undetermined significance; DADA2 = deficiency of adenosine deaminase-2; DBA = Diamond-Blackfan anemia; FA = Fanconi's anemia; FMPD = familial myeloid predisposition; GATA2 = GATA-binding factor 2; IBMFS = inherited bone marrow failure syndromes; ICUS = idiopathic cytopenias of undetermined significance; SCN = severe congenital neutropenia; STS = short telomere syndromes.

elevated independent cardiovascular risk and increased propensity to develop myeloid neoplasms.[12-15] Further, precision medicine has been particularly helpful in identification of key molecular drivers associated with inherited bone marrow failure syndromes (IBMFS), such as Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), short telomere syndromes (STS), Shwachman-Diamond syndrome, *GATA2* (for expansion of gene symbols, use search tool at www.genenames.org) haploinsufficiency syndrome, thrombocytopenia absent radii, and severe congenital neutropenia (SCN) among others.[16-24] Moreover, the experience of Diagnostic Odyssey service line of the Mayo Clinic Center for Individualized Medicine (CIM) has shown that whole-exome sequencing (WES) was successful to diagnose such patients with inherited diseases including cytopenias and immunodeficiencies.[25] Despite being clinically well characterized, these inherited disorders display variable, often attenuated, clinical manifestations (forme fruste) at differing ages of presentation.[17] Variable patterns of inheritance, incomplete penetrance, and somatic reversion are some of the known factors responsible for this phenomenon.[17,26-28] Therefore in clinical practice, patients with unexplained cytopenias often suffer from delayed and/or inaccurate diagnoses, resulting in the administration of contextually inappropriate therapies such as immunosuppression, hematopoietic stem cell transplantation (HSCT) with inappropriate donors, ionizing radiation, and cytotoxic chemotherapy.[24,29] Thus, we believed that a precision medicine evaluation for these patients and their family members had the potential to not only enable timely and appropriate diagnoses, but also impact therapeutic interventions.

Through this paper, we discuss our experience with a unique service line clinic established to apply precision medicine methods to uncover and annotate gene variants in the context of appropriate clinical associations and prospectively study the natural history of patients with clonal cytopenias, including acquired and IBMFS.

METHODS

Our Pre-Myeloid and Bone Marrow Failure clinic was established through support from the Mayo Clinic CIM and the Division of Hematology, with the vision to be a unique collaborative effort between clinicians, bioinformatics specialists, and molecular biologists (Institutional Review Board

TABLE 1. Table Showing Demographic, Clinical and Genetic Characteristics of Patients Included in our Cohort[a]

Disorder subtype	No. of patients (N=68)	Median age of disease onset (range), y	No. of males (%)	Defining clinical features or diagnoses, no. of cases (%)	Progression to MDS or AML (%)	Genetic alterations identified (details in supplementary table 2)	No. of deaths (%)	Median overall survival in months (95% CI)
Inherited bone marrow failure syndromes (n=24)								
Short telomere syndromes[b]	13 (54)	53 (2-74)	9 (69)	Pancytopenia, 6 of 13 (46) Anemia, 4 of 13 (31) Leukopenia, 2 of 13 (15) (1 neutropenia, 1 lymphopenia) Thrombocytopenia, 5 of 13 (39) IIP, 5 of 13 (39) Cirrhosis, 2 of 13 (15) DKC clinical triad,[c] 2 of 13 (15)	1	TERT c.2917C>T; p.His973Tyr (n=1) c.2768C>T; p.Pro923Leu (n=1) DKC1 (details N/A, n=1)	4 (31)	141.2 (105-NR)
GATA2 haploinsufficiency syndromes	6 (25)	34 (18-50)	3 (50)	Immunodeficiency with/without fungal or viral infections (n=4, 67%) HPV-driven warts (n=1) Pancytopenia (n=1, 17%)	3 (50)	GATA2 c.1339A>C; p.S447R (n=3) c.1187G>A; p.Arg396Gln (n=1) c.1192C>T; p.Arg398Trp (n=1) Deletion (n=1)	0	Median NR
Diamond-Blackfan anemia	2 (8)	36 (31-41)	2 (100)	Anemia (n=2, 100%)	0	RPS19 c.357-1G>A; p.? (n=1) RPL5 c.90_91dup; p.Tyr31Phefs*8 (n=1)	0	Median NR
Fanconi anemia	1	41	[illegible]	Anemia Short stature Right thumb hypoplasia Bilateral hypertrophy of thenar muscles Absent scaphoid bone Horseshoe kidney Cutaneous hyper- & hypopigmentation Cafe-au-lait macules Neck webbing High-arched palate Patent ductus arteriosus	0	FANCG c.313G>T; p.Glu105* (n=1)	0	241
Deficiency of ADA2	1	17	0	Pancytopenia Cutaneous polyarteritis	0	0	0	21

Continued on next page

TABLE 1. Continued

Disorder subtype	No. of patients (N=68)	Median age of disease onset (range), y	No. of males (%)	Defining clinical features or diagnoses, no. of cases (%)	Progression to MDS or AML (%)	Genetic alterations identified (details in supplementary table 2)	No. of deaths (%)	Median overall survival in months (95% CI)
				nodosa Livedo reticularis Nodular regenerative Hyperplasia Hypogammaglobulinemia				
Cyclical neutropenia	1	23	1	Leukopenia with neutropenia	0	None	0	77
Cytopenias without a known clinical syndrome (n=30)								
CCUS	22 (73)		15 (68)	Pancytopenia (n=4, 18%) Anemia (n=12, 55%) Leukopenia (all neutropenia) (n=6, 27%) Thrombocytopenia (n=9, 41%)	6 (27)	TET2 c.210_211insC; p.Asn71Glnfs*2 (n=1) c.4045-1G>A; p.? (n=1) c.4523_4524del: Ala1508Glufs*69 (n=1) c.5642A>G; p.His1881Arg (n=1) c.3250C>T; p.Gln1084* (n=1) c.421_424dup; p.Ser142Cysfs*6 (n=1) c.3594+2dup; p.? (n=1) DNMT3A c.2645G>A; p.Arg882His (n=1) ASXL1 c.1758dup; p.Gly587Argfs*32 (n=1) c.2053C>T; p.Gly685* (n=1) IDH1 c.395G>T; p.Arg132Leu (n=1) c.394C>T; p.Arg132Cys (n=1) IDH2 c.419G>A; p.Arg140Gln (n=1) ZRSR2 c.376C>T; p.Arg126X (n=1) c.83dup; p.Lys29Glufs*26 (n=1) c.787G>T; p.Glu263X (n=1) c.558-1_558dup; p.Cys187Aspfs*52 (n=1) c.376C>T; p.Arg126* (n=1) SRSF2 c.284C>A; p.Pro95His (n=1) c.284C>G; p.Pro95Arg (n=2) U2AF1 c.470A>C; p.Gln157Pro (n=1) c.101C>T; p.Ser34Phe (n=1) SF3B1 c.2098A>G; p.Lys700Glu (n=1)	1	Median NR

Continued on next page

TABLE 1. Continued

Disorder subtype	No. of patients (N=68)	Median age of disease onset (range), y	No. of males (%)	Defining clinical features or diagnoses, no. of cases (%)	Progression to MDS or AML (%)	Genetic alterations identified (details in supplementary table 2)	No. of deaths (%)	Median overall survival in months (95% CI)
						JAK2 c.1849G>T; p.Val617Phe (n=1) SETBP1 c.2602G>A; p.Asp868Asn (n=1) BCOR c.3448dup; p.Glu1150Glyfs*18 (n=1) TP53 c.844C>T; p.Arg282Trp (n=1) c.455dup; p.Pro153Alafs*28 (n=1) c.743G>A; p.Arg248Gln (n=1) PHF6 c.941T>C; p.Ile314Thr (n=1)		
ICUS	8 (27)	45.5 (26-74)	3 (38)	Pancytopenia (n=1, 13%) Anemia (n=4, 50%) Leukopenia (n=3, 38%) Thrombocytopenia (n=1)	0	None	1	Median NR
Other (n=14)								
Familial myeloid predisposition syndromes	7 (10)	74 (6-83)	4 (57)	Pancytopenia (n=1) Thrombocytopenia (n=6, 86%)	2 (29)	RUNX1 c.710C>A; p.Arg237Lys (n=1) c.351+1G>A, p.? (n=1) ANKRD26 5'UTR exome 1 and exome 2 (n=1) c.116C>T; p.Gly39Asp (n=1)	0	Median NR
Bone marrow failure syndromes without a clear clinical phenotype	5 (7)	34 (21-65)	[illegible]	Pancytopenia (n=4, 80%) Leukopenia (both neutropenia and monocytopenia) (n=1)	0	None	0	Median NR
HLA DR15+ve bone marrow failure syndrome	2 (3)	39.5 (34-45)	0 (0)	Pancytopenia (n=2, 100%)	0	BCOR c.3448dup; p.Glu1150Glyfs*18 (n=1)	0	Median NR

[a]Range or % only provided if n>1.
[b]Ten of 13 patients were a part of a previously published cohort of 17 patients.
[c]DKC clinical triad includes dystrophic nails, lacy reticular skin pigmentation and oral leukoplakia. Variants labeled with "p.?" refer to intronic alterations close to a splicing donor/acceptor predicted to affect mRNA splicing but not confirmed with a functional test.
ADA2 = adenosine deaminase 2; AML = acute myeloid leukemia; CCUS = clonal cytopenias of undetermined significance; DKC = dyskeratosis congenita; HPV = human papilloma virus; ICUS = idiopathic cytopenias of undetermined significance; IIP = idiopathic interstitial pneumonia; MDS = myelodysplastic syndromes; N/A = not available; NR = not reached.

#16-004173 and National Clinical Trials #02958462 [www.clinicaltrials.gov]). The first patient consented to our clinic for treatment on November 8, 2016, whereas the last patient consented for treatment on January 12, 2018. Patients were evaluated after an appropriate assessment of known causes by a physician/hematologist. Based on a clinical evaluation, which included obtaining a detailed family history and physical exam, and appropriate clinical test results, genomic evaluation was performed in a stepwise manner, starting with either a targeted (next-generation sequencing [NGS]) or Sanger sequencing, and if negative, research-based WES.

Evaluation of Patients

All patients were referred by clinicians in various disciplines, in particular hematology, pulmonary medicine, gastroenterology and pediatrics, at Mayo Clinic. We defined unexplained cytopenias as low blood counts in one or more cell lineages (red or white blood cell or platelets), persistent for 6 months or longer (this time interval cutoff was chosen to definitively exclude patients with myelodysplastic syndrome [MDS] as per the World Health Organization [WHO] guidelines)[9] in the absence of known causes of cytopenias, such as, nutritional deficiencies (including deficiencies of iron, vitamin B12, folate, copper, and pyridoxine), MDS as defined by the WHO[11], effect of drugs/toxins, paroxysmal nocturnal hemoglobinuria, chronic conditions such as hepatitis C, human immunodeficiency virus, liver disease, etc. Hence, to meet eligibility, a minimum suggested evaluation at the discretion of the referring hematologist included: iron chemistries; vitamin B12, methyl-malonic acid, folate, copper and zinc levels; thyroid stimulating hormone levels; liver function tests; hepatitis B, C, and human immunodeficiency virus serologies; bone marrow aspiration and core biopsy along with cytogenetics; paroxysmal nocturnal hemoglobinuria (PNH) flow cytometry (four patients in our cohort had a minor PNH clone; however, their clinical phenotype was not consistent with PNH, and hence they were included in the unexplained cytopenias category), and an abdominal ultrasound to evaluate spleen size when clinically relevant. If a nonhematologist placed a referral, a hematologist assessed the charts to ensure adequate evaluation was pursued before genetic assessment (through an electronic consult). When necessary, an in-person hematology consult was performed and the appropriate evaluation was completed.

Written informed consent was obtained with the help of a dedicated study coordinator. Once consent was obtained, health-related and quality of life information was collected from the medical records and by an in-person interview, survey, and clinical encounters. A detailed stepwise diagnostic algorithm for this clinic is outlined in Figure 1A.

Collection of Samples

After proper genetic counseling, all enrolled participants, at the time of a clinically indicated blood draw or bone marrow biopsy, were asked to provide 20 mL of bone marrow and/or up to 50 mL of fresh blood, from which bone marrow and peripheral blood mononuclear cells were isolated, respectively. These cells were then processed to obtain DNA, RNA, chromatin, and viably frozen cells for future use. When clinically indicated, after consent, skin biopsy specimens were obtained for fibroblast cultures so as to obtain germline DNA for assessment.

Sequencing

After an appropriate clinical evaluation, genomic sequencing was performed in a stepwise manner, beginning with Sanger or NGS sequencing and if negative, then research-based WES was performed (Figures 1A and B). Details are mentioned the Supplemental Methods and Supplemental Table 1 (available online at http://www.mayoclinicproceedings.org).

Pre-Myeloid Genomics Tumor Board

Results from this research were summarized and presented at a multidisciplinary Pre-Myeloid Genomics Tumor Board that included physicians, research scientists, pathologists, bioinformaticians, and genetic counselors

TABLE 2. Clinical and Genomic Characteristics of the Subset of Patients Who Underwent a Change in Clinical Management[a,b]

Case no.	Diagnosis	Age, y	Sex	Clinical features	Pathogenic variants	Change in clinical management[a]
36	STS	3	M	Oral leukoplakia, lacrimal duct stenosis, lacy skin pigmentation, pancytopenia	DKC1 (details unknown)	Started on danazol; subsequently underwent allogeneic RIC allogeneic PBSCT (reduced intensity conditioning chosen due to defect in telomere-related genes)
37	STS	58	M	Pancytopenia	TERC n.214G>C, noncoding variant	Underwent RIC allogeneic HSCT, RIC chosen due to STS diagnosis
38	STS	17	M	Pancytopenia	TERT c.2768C>T; p.Pro923Leu	Consideration of RIC allogeneic regimen for HSCT, related donors being screened for the same variant
39	STS	63	M	Pancytopenia, IP	None identified	Started on danazol after flow-FISH results
44	GATA2 haploinsufficiency	38	M	Pancytopenia, invasive fungal infection	GATA2 c.1339A>C (p.Ser447Arg)	Donor selection and reduced intensity conditioning for HSCT
45	GATA2 haploinsufficiency	31	F	Pancytopenia	GATA2 c.1339A>C (p.Ser447Arg)	Donor selection for HSCT
46	GATA2 haploinsufficiency	35	M	Immunodeficiency, MAC infection, NK cell dysfunction	GATA2 c.1187G>A (p.Arg396Gln)	Intensity of conditioning for HSCT
47	GATA2 haploinsufficiency	18	M	Pancytopenia, HPV-driven warts	GATA2 deletion	Intensity of conditioning for HSCT, donor selection
48	GATA2 haploinsufficiency	50	F	Pancytopenia, RSV pneumonia, aspergillus pulmonary infection	GATA2 c.1192C>T (p.Arg398Trp)	RIC regimen chosen for HSCT
50	DADA2	17	F	Pancytopenia, cutaneous polyarteritis nodosa, livedo reticularis, nodular regenerative hyperplasia, hypogammaglobulinemia	ADA2 c.37_39 del, p.Lys13del and c.1159C>A; p.Asn328Lys	Therapy with adalimumab, IVIG, donor selection for HSCT
13	CCUS	61	F	Anemia, leukopenia	TP53 c.578A>G, p.His193Arg; SRSF2 c.284C>G, p.Pro95Arg; IDH1 c.395G>T, p.Arg132Leu	No immunosuppression used due to evidence for clonal hematopoiesis, not consistent with aplastic anemia
14	CCUS	45	F	Pancytopenia	45,XX,del(13)(q12q14)[12]/46,XX[8] [no mutations identified]	Decision to proceed with immunosuppression after exclusion of somatic variants on NGS and presence of a minor PNH clone
15	CCUS	74	M	Pancytopenia	PHF6 c.941T>C p.Ile314Thr; TP53 c.743G>A; p.Arg248Gln	HMA used to abrogate cytopenias and transfusion dependency
25	ICUS	30	F	Leukopenia with neutropenia	None identified	G-CSF support after excluding myeloid relevant mutations
23	CCUS	6	M	Severe congenital neutropenia, anemia	SETBP1 c.2602G>A; p.Asp868Asn	RIC allogeneic HSCT due to clonal evolution

Continued on next page

TABLE 2. Continued

Case no.	Diagnosis	Age, y	Sex	Clinical features	Pathogenic variants	Change in clinical management[a]
58	Familial myeloid predisposition syndrome	29	F	Thrombocytopenia	RUNX1 c.710G>A; p.Arg237Lys	Donor selection for HSCT
63	HLA DR15+ BMFS	45	F	Pancytopenia	BCOR c.3448dup (p.Glu1150GlyfsTer18)	Start of immunosuppressive therapy

[a]Change in clinical management was defined as start or change of a new therapy, impact on donor selection, and/or conditioning regimen for hematopoietic stem cell transplantation as per Alder et al.[21]

[b]N=68 patients total; n=17 patients who underwent change in clinical management (25%).

AML = acute myeloid leukemia; BMFS = bone marrow failure syndrome; CCUS = clonal cytopenias of undetermined significance; DADA2 = deficiency of adenosine deaminase 2; F = female; flow-FISH = flow cytometry fluorescence in situ hybridization; GATA2 = GATA-binding factor 2; G-CSF = granulocyte-colony stimulating factor; HLA = human leukocyte antigen; HMA = hypomethylating agent; HPV = human papilloma virus; HSCT = hematopoietic stem cell transplantation; ICUS = idiopathic cytopenias of undetermined significance; IP = idiopathic interstitial pneumonia; IVIG = intravenous immunoglobulin; M = male; MAC = mycobacterium avium complex; NGS = next generation sequencing; NK = natural killer; PBSCT = peripheral blood stem cell transplantation; PNH = paroxysmal nocturnal hemoglobinuria; RIC = reduced intensity conditioning; RSV = respiratory syncytial virus; STS = short telomere syndrome.

from Mayo Clinic (Rochester, MN). Consensus among these members determined how to proceed with each patient (Figure 1D), similar to as previously described for other groups at the Mayo Clinic.[7] If a particular gene variant was not considered relevant after discussion at the tumor board, it was discarded. However, based on available data, if consensus was obtained to support a variant's role in relationship to a particular phenotype, then additional research-based tests were performed to confirm this association. These studies were specific to the affected gene and included options such as, in silico protein modeling, RNA sequencing (RNAseq), methylation assays, splicing detection by polymerase chain reaction, and/or any other in vitro, cellular, or animal model experiments that could help establish the association. Variants considered to be pathogenic were then validated in a Clinical Laboratory Improvement Amendments–certified laboratory by Sanger sequencing.

Classification of Patients

Patients were grouped into three different categories: IBFMS, cytopenias without a known clinical syndrome, and an "others" category which included patients who did not fit into either of the two categories. Patients were diagnosed with IBFMS if they had a significant family history defined as two or more first- or second-degree relatives with hematologic malignancies and/or a solid tumor with a known germline predisposition and/or longstanding cytopenias and/or bleeding history, and/or classical inherited marrow failure syndrome organ manifestations or were found to have a known IBFMS-related pathogenic variant on genomic assessment. The definition of significant family history was obtained from previously published consensus guidelines for identification of inherited myeloid malignancies.[12,13]

Impact on Clinical Management

To assess impact of our genomic assessment on clinical care, we planned for a clinician (hematologist) to retrospectively review the medical records of the patients included in our cohort. Before conducting chart review,

we defined impact on clinical management as decision to start or not to start a particular therapy, alter donor selection, and/or conditioning regimen intensity for HSCT, as described in similar recent study by Alder et al.[21] evaluating clinical utility of measuring telomere lengths using flow cytometry fluorescence in situ hybridization in hospital practice.

Charts of all patients were reviewed to assess "change in clinical management" based on genetic results and subcategorized into the following: decision to start or not to start a drug, choice of donor, and/or conditioning regimen intensity for HSCT.

RESULTS

To date, we have identified 68 patients with unexplained cytopenias and evaluated them following the algorithm outlined in Figure 1. After a genomic evaluation, 24 (35.3%) patients were diagnosed with an IBFMS (patients 31-54 in Supplemental Table 2, available online at http://www.mayoclinicproceedings.org), 30 (44%) with cytopenias without a known clinical syndrome (patients 1-30 in Supplemental Table 2), and 14 (21%) were classified into the "others" category (patients 55-68 in Supplemental Table 2) which included patients with familial myeloid predisposition syndromes (FMPD) (n=7, 50%) (patients 55-61 in Supplemental Table 2), bone marrow failure syndromes (BMFS) without a clear clinical phenotype (n=5, 36%) (patients 62-66 in Supplemental Table 2), and HLA DR15+ BMFS (n=2, 14%) (patients 67 and 68 in Supplemental Table 2) patients. See Table 1 and Figure 2 for details.

IBMFS

Of the 68 patients, 24 (35%) primary index patients were identified with IBMFS, median age 34.5 (range: 2-74) years, with 16 (67%) being males. The most common diagnoses under this category included STS (n=13, 54%) (patients 31-43 in Supplemental Table 2), GATA2 haploinsufficiency syndromes (n=6, 25%) (patients 44-49 in Supplemental Table 2, available online at http://www.mayoclinicproceedings.org), DBA (n=2, 8%) (patients 52 and 53 in Supplemental Table 2), and one patient each with FA, deficiency of adenosine deaminase 2 (ADA2) (DADA2), and SCN (patients 54, 50, and 51 in Supplemental Table 2, respectively). Ten of 13 STS patients were a part of a previously published cohort,[21] whereas the DADA2 patient (patient 50 in Supplemental Table 2) has been published previously.[22] Significant family history was found only in 9 (38%) patients (3 STS, 4 GATA2, 1 DADA2, and 1 FA), whereas 13 (54%) patients (9 STS, 1 GATA2, 1 DADA2, 1 SCN, and 1 FA) had an IBMFS-associated physical abnormality such as idiopathic interstitial pneumonia (IIP) (n=7, 53%), unexplained cirrhosis (n=4, 31%), oral leukoplakia (n=1), lacy skin pigmentation (n=1), nasolacrimal duct obstruction (n=1), human papilloma virus–driven warts (n=1), nodular regenerative hyperplasia (n=1), and short stature (n=1), resulting in a definite diagnosis in 7 (29%) patients. Genomic variants were found in 14 (58%) patients (7 via Sanger sequencing, 6 via NGS, and 1 via WES), of which 12 (50%) patients had pathogenic variants in the following genes: GATA2 (n=6, five pathogenic variants, whereas one patient had GATA2 gene deletion), telomerase reverse transcriptase (TERT) (n=2), dyskerin pseudouridine synthase 1 (DKC1) (n=1), ribosomal protein S19 (RPS19) (n=1), ribosomal protein L5 (RPL5) (n=1), and FA complementation group G (FANCG) (n=1) (details in Supplemental Tables 2 and 3), whereas variant of uncertain significance (VUS) in telomerase RNA component (TERC), colony stimulating factor 3 receptor (CSF3R), regulator of telomere elongation helicase 1 (RTEL1), ALG2 alpha 1,3/1,6 mannosyltransferase (ALG2), component of oligomeric complex 1 (COG1), and LPS responsive beige-like anchor protein (LRBA) genes were found in 3 (13%) patients. Among the 20 (83%) patients tested through gene sequencing (8 via Sanger sequencing, 11 via NGS, and 3 via WES; two patients underwent both Sanger and NGS), pathogenic variants were identified in 12 (60%) patients (7 via Sanger sequencing, 4 via NGS, and 1 via WES).

Change in Clinical Management. Definitive evidence of altered clinical management was found in 10 (42%) patients, of which, an appropriate reduced intensity conditioning HSCT regimen was selected in 7 (70%) patients; in 5 (50%) patients, donor selection was altered; and in 3 (30%) patients, decision was made to start new therapy such as danazol (n=2, both with STS diagnosis) and adalimumab plus intravenous immunoglobulin (n=1, patient with DADA2 diagnosis). Six patients had altered clinical management involving more than one of the aforementioned categories (please refer to Table 2 for details). At a median follow-up of 64 (range: 0-1416) months, there were 4 (17%) deaths, whereas 3 (13%) patients clonally evolved into MDS (two with underlying diagnosis of GATA2 haploinsufficiency, and 1 with STS) and 2 (8%) patients progressed to acute myeloid leukemia (both with germline GATA2 haploinsufficiency syndromes).

Cytopenias Without a Known Clinical Syndrome

Based on the presence or absence of a presumed clonal abnormality, two diagnoses were included in this category (n=30, 44%) (patients 1-30 in Supplemental Table 3), namely, clonal (CCUS) (n=22, 73%) and idiopathic (ICUS) (n=8, 27%) cytopenias of undetermined significance. Median age at onset of cytopenias was 63.5 (range: 6-79) years with 18 (60%) males. Significant family history of unexplained cytopenias or hematologic malignancies was identified in 2 (all CCUS) patients (1 each of aplastic anemia and polycythemia vera). Among 28 (93%) tested patients, genomic abnormalities were found in 21 (75%) patients (all through NGS-based gene panel testing), all with CCUS diagnoses, which included 17 (61%) patients with pathogenic variants (11 with more than one variant), 4 (14%) with variants of uncertain significance, and 7 (25%) with chromosomal abnormalities. One CCUS patient had both a cytogenetic abnormality (13q-) and a minor PNH clone (PNH antigen loss in 3.61% granulocytes, 0.06% red blood cells, and 4.5% monocytes) without evidence of dysplasia on bone marrow morphology. By definition, patients in whom both cytogenetic and pathogenic variants were not identified and clinical features of a BMFS/MDS were not apparent were classified as ICUS. Individual pathogenic variant details are mentioned in Table 1 and Supplemental Tables 2 and 4, whereas VUS details are outlined in Supplemental Table 3.

Change in Clinical Management. Evidence of altered clinical management due to our precision genomics approach was documented in 5 (17%) patients. This included starting specific therapies in 3 patients such as hypomethylating agent therapy (n=1, after discovery of a myeloid-relevant pathogenic variant), and immunosuppressive therapy (IST) or granulocyte-colony stimulating factor support (n=2, after exclusion of myeloid-relevant pathogenic variants). On the other hand, in 1 patient we decided to avoid the use of IST due to the detection of myeloid clonal evolution, as evidenced by the presence of myeloid-relevant pathogenic variants (in isocitrate dehydrogenase [NADP(+)] 1, cytosolic [IDH1], serine and arginine rich splicing factor 2 [SRSF2] and tumor protein p53 [TP53] genes) and finally, 1 patient underwent reduced intensity conditioning HSCT due to concern for myeloid clonal evolution with a pathogenic variant in the SET binding protein 1 (SETBP1) gene (additional details in Table 2). At a median follow-up of 25 (range: 2-290) months, there were 2 (7%) deaths and 6 (20%) patients (all with CCUS) who underwent myeloid clonal evolution (5 MDS and 1 chronic myelomonocytic leukemia).

Others Category

Patients who did not fit into either of the two aforementioned categories were classified into the "others" category (n=14, 21%), with the most frequent diagnoses being FMPD (n=7, 50%; with variants identified in the following genes: DEAD box

helicase 41 [DDX41] [n=1], RUNX family transcription factor 1 [RUNX1] [n=2], ANKRD26 5' UTR [n=1], ANKRD26 [n=1]; in 3 patients variants were not identified despite a highly suggestive family history) (patients 55-61 in Supplemental Table 3), BMFS without a clear clinical phenotype (n=5, 36%) (patients 62-66 in Supplemental Table 3), and HLA DR15+ BMFS (n=2, 14%) (patients 67 and 68 in Supplemental Table 3). Family history was significant in 6 (43%) patients (all FMPD). A PNH clone was identified through peripheral blood flow cytometric immunophenotyping in 3 (21%) patients (all BMFS without a clear clinical phenotype) with median partial antigen loss in 0.01% (range: 0.01%-0.13%) erythrocytes, median complete antigen loss in 2.14% (range: 0.03%-3.23%) erythrocytes, 31.41% (range: 2.35%-62.55%) granulocytes, and 10.57% (range: 2.45%-65.96%) monocytes; however, other clinical/laboratory criteria in these patients were insufficient for a diagnosis of PNH. Among tested patients (n=13 [93%], 3 via Sanger sequencing, 9 via NGS, and 2 via WES; 1 patient underwent both Sanger and NGS sequencing), genetic abnormalities were identified in 8 (62%) patients (3 via Sanger sequencing, 3 via NGS, and 2 via WES). 5 (63%) pathogenic variants [RUNX1 (n=2), ANKRD26 5'UTR (n=1), ANKRD26 (n=1), and BCL6 corepressor [BCOR] [n=1]]. One patient each of RUNX-1–mutated and ANKRD26-associated thrombocytopenia has been previously published.[16,17] Details on variants are mentioned in Supplemental Tables 2,3 and 4.

Change in Clinical Management. Altered clinical management was seen in 2 (14%) patients, of which in one we chose to treat with equine antithymocyte globulin plus cyclosporine and prednisone in a HLA DR15+ BMFS patient with a minor PNH clone (after exclusion of myeloid-relevant pathogenic variants) (details in Table 2), whereas to the other patient, as we had identified an FMPD, an appropriate sibling donor was identified after making sure that the donor did not have the same variant. At a median follow-up of 35 (range: 7-598) months, there were no deaths and 2 transformations to myeloid neoplasms (1 MDS, 1 chronic myelomonocytic leukemia both FMPD with alterations in ANKRD26 5'UTR exon 1/2 and ANKRD26 coding sequence, respectively [c.116C>T]).

In summary, 68 patients were evaluated in our clinic and, after genomic interrogation, they were classified into IBMFS, (n=24, 35%), cytopenias without a known clinical syndrome which included both CCUS and ICUS (n=30, 44%) patients, and patients that did not fit into the above two categories (n=14, 21%). Significant family history defined as per the aforementioned definition was ascertained in only 17 (25%) patients (9 IBMFS, 2 CCUS, and 6 other patients), whereas gene variants were found in 43 (63%) patients (34 [79%] pathogenic: 12 IBMFS, 17 CCUS, and 5 other). A retrospective chart review was conducted and concluded a change in clinical management in 17 (25%) patients after a genomic assessment, as evidenced by changes in decisions with regards to therapeutic interventions (n=8, 47%), donor choice (n=6, 35%), and/or choice of conditioning regimen for HSCT (n=8, 47%).

DISCUSSION

Precision medicine is playing a major role in our understanding of various malignant and nonmalignant disorders. BMFS are often caused by inherited or somatic genomic alterations, with several variants already well established as pathogenic.[[illegible]] As reiterated in previous studies, our study confirms the clinical and genetic heterogeneity of IBMFS.[[illegible]] Although germline nature is established by the presence of genetic variants in nonhematopoietic cells such as skin fibroblasts and screening of affected and unaffected family members, family history may be noticeably absent, as shown in our cohort and other studies.[16,18] Further, some of these conditions may have attenuated clinical presentations, well into adulthood.[[illegible],17] Hence, a high index of clinical suspicion, with use of latest sequencing technologies, would not only enable timely

diagnosis, but also avoid subjecting patients to inadvertent therapies such as immunosuppression, myeloablative doses of chemotherapy, alkylating agents, and ionizing radiation. Even when a definite diagnosis was not reached, based on the risk of developing a malignancy, patients received a tailored follow-up plan (e.g., CCUS). Moreover, in patients for whom there is a clinical need for allogeneic HSCT, screening family members for the same gene variants is important to choose appropriate donors.[[illegible]] On the other hand, molecular characterization of marrow failure states can help direct personalized therapies. One such example is the role of NGS in acquired BMFS/aplastic anemia, where the detection of clonal hematopoiesis in the form of mutations involving BCOR, BCORL1 and phosphatidylinositol glycan anchor biosynthesis class A (PIG-A) predict response to IST, whereas the presence of mutations involving DNA methyltransferase 3 alpha (DNMT3A), ASXL transcriptional regulator 1(ASXL1) and tet methylcytosine dioxygenase 2 (TET2) predict incomplete responses/refractoriness to IST.[[illegible]] Additional targeted/specific therapies available once an appropriate diagnosis is made include, tumor necrosis factor–alpha inhibitors for patients with DADA2 syndromes,[22] danazol for STS patients with BMFS,[[illegible]] and granulocyte-colony stimulating factor therapy for heterozygous variants in gene encoding for elastase, neutrophil expressed (ELANE) in patients with SCN.[[illegible]] Besides therapy for cytopenias, prophylactic therapies to prevent infections, such as azithromycin to prevent mycobacterium avium complex infections in patients with GATA2 haploinsufficiency syndromes, are also supported by consensus guidelines.[[illegible]]

Significant challenges for this approach include difficulty in associating clinical signs and symptoms with variants (especially, if not previously described) and the possibility that not all causal genes for IBMFS have hitherto been discovered. Analyzing WES and RNAseq data from patients with a striking clinical presentation, yet negative results on targeted sequencing, is expected to uncover additional genomic associations, especially with novel approaches such as pathway analysis or studying paralog genes from other organisms.[[illegible]] We are already pursuing further research-based options in our clinic performing functional studies on several VUS uncovered using this approach that will help us conclude the clinical relevance of these variants. This is a work in progress that will need to also be evaluated in the future for its applicability. However, as shown in our cohort, this methodology is not foolproof and fails to identify candidate genomic drivers in all patients despite a suggestive clinical phenotype. This is likely due to inherent technical limitations of WES such as failure to pick up copy number variations,[[illegible]] variations in promoter regions,[[illegible]] and abnormalities in the epigenome. Hence, in addition to sequencing, incorporation and clinical validation of novel molecular biology techniques such as reduced representative bisulfite sequencing to analyze methylation patterns, chromatin immunoprecipitation sequencing to study chromatin dynamics, novel sequencing technologies for enhanced detection of deletions, duplications, and subtelomeric region abnormalities is currently underway at our institution.

CONCLUSION

A precision genomics approach for patients with unexplained cytopenias has the potential to alter clinical management, at least in a subset of patients. Despite limitations, these novel technologies are expected to enable personalized care and direct therapies. Future studies such as ours would enable adoption of this streamlined genomics approach for patients with unexplained cytopenias with an anticipated positive impact on clinical management.

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SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: BMFS = bone marrow failure syndrome; CCUS = clonal cytopenia of undetermined significance; CIM = Center for Individualized Medicine; DADA2 = deficiency of adenosine deaminase 2; DBA = Diamond Blackfan anemia; FA = Fanconi's anemia; FMPD = familial myeloid predisposition; HSCT = hematopoietic stem cell transplantation; IBMFS = inherited bone marrow failure syndromes; ICUS = idiopathic cytopenia of undetermined significance; IST = immunosuppressive therapy; MDS = myelodysplastic syndrome; NGS = next-generation sequencing; PNH = paroxysmal nocturnal hemoglobinuria; SCN = severe congenital neutropenia; STS = short telomere syndrome; VUS = variant of uncertain significance; WES = whole exome sequencing

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How I treat acquired aplastic anemia

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Acquired severe aplastic anemia (SAA) is a rare hematologic disease associated with significant morbidity and mortality. Immune destruction of hemopoietic stem cells plays an important role in pathogenesis, as shown by successful treatment with immunosuppressive agents, leading to transfusion independence or complete recovery of peripheral blood counts in a proportion of patients. Growth factors can be combined with immunosuppressive therapy (IST) and may improve response rates, as recently shown with thrombopoietin analogs. Anabolic steroids may still play a role in combination with IST. The problem with IST is failure to respond and the development of late clonal disorders. Bone marrow transplantation (BMT) is the other therapeutic option: a matched sibling donor remains the best choice. For patients lacking a matched family donor, unrelated donors can be readily found, although mostly for patients of Caucasian origin. Other BMT options include unrelated cord blood or mismatched family donors. Acute and chronic graft-versus-host disease remain important complications of BMT. Patient age is a strong predictor of outcome for both IST and BMT, and must be considered when designing therapeutic strategies. Early diagnosis and treatment, as well as long-term monitoring, remain crucial steps for successful treatment of SAA. (Blood 2017;129(11):1428-1436)

Clinical presentation

Pathogenesis

Acquired SAA is regarded as the result of an immune mediated destruction of hemopoietic cells, at least in a proportion of patients.[1] The emergence of late clonal disorders in 10% to 20% of patients after immunosuppressive therapy (IST)[2] raises the question of whether some patients with SAA actually have a premalignant disease and whether IST is just postponing the inevitable.[3] Support for this view has come from the identification of somatic mutations involving telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT)[4] and, more recently, involving myeloid cancer candidate genes in a significant proportion of patients.[5-7]

Diagnosis and early intervention

The diagnosis of acquired SAA is based on the exclusion of other disorders that can cause pancytopenia and on the well-known Camitta criteria[8] (Figure 1). A BM biopsy is mandatory (preceded by platelet transfusions if the platelet count is below 20 × 10⁹/L) and will confirm an empty marrow; it should also exclude a MDS or leukemia, as well as marrow metastasis from solid tumors (Figure 1). A BM aspirate will be used for cytogenetics and/or FISH analysis to determine chromosomal abnormalities. Whether the identification of chromosomal abnormalities is compatible with the diagnosis of SAA is debated[9]; clearly, some abnormalities carry a poor prognosis (such as deletion of chromosome 7), whereas others (such as +Y and 13) are more benign and may not affect the therapeutic strategy.[9] Identification of a paroxysmal nocturnal hemoglobinuria (PNH) clone by flow cytometry will help exclude an inherited form of marrow failure and may suggest this is not a hypoplastic MDS (Figure 1); a negative diepoxybutane test will exclude FA. Determination of telomere length is not mandatory, but will help exclude telomeropathies.[4]

Severity can be determined by neutrophil counts: patients with 0 to 0.2, 0.21 to 0.5, and >0.5 polymorphonuclear cells (PMN) × 10⁹/L are classified, respectively, as very severe, severe, and nonsevere aplastic anemia,[10] and severity has been a strong predictor of survival in patients receiving IST.[11] Patients with SAA require early diagnosis and intervention, whether IST or BMT, because the interval between diagnosis and treatment is another strong predictor of survival.[11] Transfusion policies are important in the early days of diagnosis, and guidelines for supportive care have been published.[12] In approximately 5% of patients, SAA will follow an episode of elevated transaminase and hyperbilirubinemia,[14] although the search for hepatitis A, B, and C virus (HAV, HBV, and HCV) is typically negative, as well as the search for other viruses. Abnormal liver function test and/or elevated bilirubin levels should not stop therapeutic strategies.

Initially, one should concentrate on the diagnosis,[15] with some key tests, as outlined in Figure 1. The BM biopsy is the diagnostic procedure with the highest level of accuracy. In the meantime, the patient will be transfused according to guidelines.[12] Once the diagnosis has been ascertained, HLA typing of the patient and his/her family should be one of the first interventions in a patient with SAA, certainly in patients younger than 60 years of age.

HLA identical sibling transplantation

BMT or IST

If an HLA-matched family donor is identified, marrow transplantation should be the first-line therapy in patients younger than 40 years (Figure 2); this is based on studies comparing HLA-identical BMT vs first-line IST.[15,16] However, the advantage in failure-free survival for a young patient with a low neutrophil count declines with increasing age[11] as a result of higher mortality after HLA-identical BMT in patients aged 21 to 40 years or older than 40 years.

The age effect

In patients grafted from matched siblings, there is a very strong age effect, with survival of 82%, 72%, and 53% for patients aged 1 to 20,

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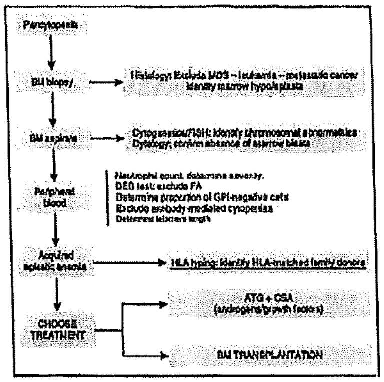
Figure 1. Diagnostic procedures in patients with pancytopenia. ATG, antithymocyte globulin; BM, bone marrow; CsA, cyclosporine A; DEB, diepoxybutane; FA, Fanconi's anemia; FISH, fluorescent in situ hybridization; GPI, glycosyl phosphatidyl inositol; MDS, myelodysplastic syndrome.



21 to 40, and older than 40 years,[17] as a result of a higher incidence of graft failure and graft-versus-host disease (GVHD).[17] European Group for Blood and Marrow Transplantation (EBMT) data for patients grafted from matched siblings in the decade from 2001 to 2010 show the same age effect (Figure 2): 86%, 76%, and 55% survival at 10 years. For this reason, IST should be first line therapy in older patients, as

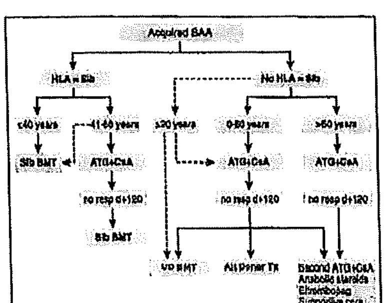


Figure 3. Treatment strategy in patients with acquired aplastic anemia.

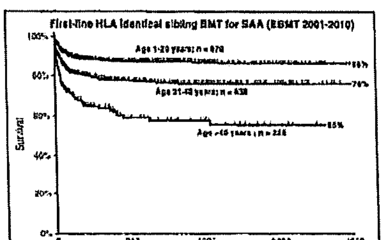


Figure 2. A strong age effect in patients with aplastic anemia, after transplantation from an HLA identical sibling. Data from the EBMT registry.

shown in Figure 2, and BMT should be carefully considered for selected cases with good performance status and severe disease (Figure 2, dashed arrow).

The conditioning regimen

The standard conditioning regimen for matched sibling transplants is cyclophosphamide 200 mg/kg (CY 200) and ATG, as originally described.[18] Although a randomized study failed to show an advantage for ATG,[19] the same survival difference proved significant in a larger retrospective study.[20] This regimen is excellent for young patients, but CY 200 may be too toxic in older patients, although attempts have been made to reduce toxicity with fludarabine (FLU)-based regimens and lower doses of CY.[21-23] Recent EBMT data (A.B., EBMT database, unpublished data) suggest that survival of patients older than 40 years can be significantly improved with a FLU-based regimen, in addition to ATG or alemtuzumab (CAMPATH), and is comparable to survival of patients in the 21- to 40-year-old age group (74% vs 75%). Current guidelines from EBMT[21] and the British Society for Standards in Haematology[25] call for a combination of FLU-CY with ATG (FCA) or alemtuzumab (CAMPATH) (FCC) for patients with SAA who are older than 30 years and receiving a matched sibling donor transplant. The CY dose to be combined with FLU is a matter of discussion, ranging from 40 to 120 mg/kg.

BM or peripheral blood

Two registry-based studies have shown that BM results in superior outcome as compared with peripheral blood (PB) in matched sibling transplants[26,27] because of less acute and chronic GVHD with BM and comparable risk for rejection (25% for PB and 15% for BM). The recent British guidelines call for BM as a stem cell source in ATG-based conditioning.[25] The evidence we currently have suggests BM should be the only acceptable stem cell source for transplants from HLA-identical siblings in SAA.

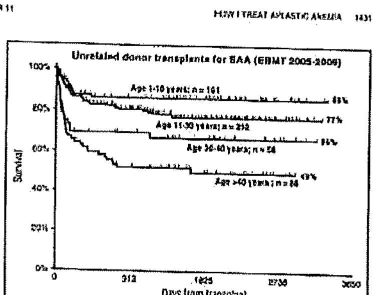
Alternative donor transplantation

Case report

A 23-year-old woman was referred to us in December 2011, having failed 2 courses of ATG and CsA. The patient had developed an invasive fungal infection after the IST, with lung lesions and a left pneumothorax. She was admitted on December 20, 2010, with drainage in her left pleural cavity, high temperature, and absolute pancytopenia. In addition, the patient had developed polyneuritis with almost complete tetraplegia. Her performance status was extremely poor. She received a conditioning regimen including FLU 120 mg/m², CY 120 mg/kg, ATG (thymoglobulin; Sanofi France) 3.75 mg/kg², and a BMT from an 8/8 mated UD. Prophylaxis of GVHD consisted of CsA and short-course methotrexate; rituximab 200 mg was given on day +5 to prevent Epstein-Barr virus (EBV) reactivation. Treatment with voriconazole was continued. Engraftment was rapid and complete, and the pleural drainage was removed 1 month later. There was no GVHD and no EBV reactivation, but neurologic rehabilitation was slow, and the patient remained admitted in the transplant unit for several months. Antifungal treatment was continued, and liposomal amphotericin (AmBisome) was also introduced. Two years later, the patient underwent upper left lobectomy to remove her aspergillus lesions because of their proximity to large vessels. The patient is alive and well and off immunosuppression 4 and a half years posttransplant.

This case exemplifies the infectious complications of prolonged neutropenia and immune suppression in aplastic anemia, as well as showing that an UD BMT can be performed successfully, even in very sick patients with marrow failure, in keeping with the reported improved outcome of UD grafts.[28,29] This case also suggests that in the presence of a matched UD, perhaps second-line therapy should be a UD transplant, rather than a second course of ATG (Figure 3). In the absence of a matched sibling donor, I usually start a search for an UD as early as possible, certainly when the patient is younger than 50 years of age.

Figure 4. The age effect in UD transplants: best outcome is seen for very young patients, for whom first-line UD BMT may be considered. Data from the EBMT registry.



UDs and stem cell source. In addition, for UD grafts, BM provides a survival advantage over PB; this has been shown in studies from EBMT and from the Center for International Blood and Marrow Transplant Research.[34,35] In the recent EBMT analysis, PB was the strongest negative predictor of survival in multivariate analysis.[34] The British guidelines[26] suggest PB can be used as a stem cell source with CAMPATH as GVHD prophylaxis, despite significant inferior survival of PB vs BM transplants ($P = .03$) in the British report on patients grafted with the FCC conditioning regimen.[36] I personally think the data strongly suggest we should always use BM as a stem cell source; an exception could be a second transplant because of graft rejection.

UD BMT and conditioning regimens. Several studies have explored the combination of low-dose total body irradiation (TBI) with FLU-CY[32,37-39]; the dosage of both TBI and cyclophosphamide has been the topic of prospective studies.[40,41] The current recommendation is CY at a dose between 30 and 100 mg/kg (total dose), TBI between 2 and 4 Gy, and FLU 100-150 mg/m².[26] Registry data (EBMT) for UD transplants show survival of 85%, 77%, 66%, and 49% for patients aged 1 to 10, 11 to 30, 31 to 40, and older than 40 years (Figure 4). The donor's age appears to be an additional prognostic factor.[42]

The ideal UD is a male, HLA-matched donor at the A,B,C,DRB1 loci, younger than 30 years, cytomegalovirus matched with the recipient; the conditioning regimen would include FCA and low-dose TBI or FCC.

Unrelated cord blood transplants. Unrelated CB transplants have been reported in 71 patients who received a single or double unrelated CB transplant,[43] with 45% survival for patients receiving a total nucleated cell dose greater than 3.9×10^7/kg. The conditioning regimen for CB transplants is identical to the regimen proposed for UD transplants, although methotrexate is omitted from GVHD prophylaxis

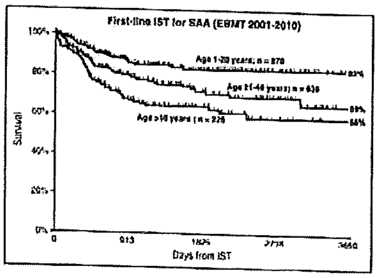


Figure 5. The age effect in patients receiving first-line IST. Data from the EBMT registry.

of CB grafts; 1 dose rituximab 150 mg is recommended on day +5 to prevent EBV lymphoproliferative disorders.[44] Patients who do not have a suitable UD could be eligible for a CB graft if the nucleated cell dose is adequate (Figure 2).

Haploidentical transplants. Young patients who do not have a matched UD or a suitable CB unit and who have failed at least 1 course of IST or have rejected a previous UD or CB graft are eligible for a transplant from HLA haploidentical family donors (HAPLO). Small series of patients have been reported with different conditioning regimens, many using high-dose posttransplant CY.[45-47] A total of 84 patients were reported in these studies, and the average 1-year survival was 74%, which is very encouraging. Nevertheless, HAPLO grafts are still in the experimental stage and should be considered only after having failed at least 1 course of IST in the absence of a suitable UD (Figure 2, identified as Alt Donor Tx).

EBV lymphoproliferative disorders. Patients with SAA undergoing alternative donor transplants, especially with an ATG-based regimen, are at high risk of developing EBV-related lymphoproliferative disorders. We have been using a small dose of rituximab (200 mg, fixed dose) on day +5 after transplantation[48] for all patients receiving an alternative donor graft and ATG in the conditioning regimen, and this has eliminated the issue of EBV-related lymphoproliferative disorders.[49]

Rejection and autologous recovery. Rejection is seen in 5% to 15% of patients with SAA undergoing a BMT and calls for immediate action with the attempt of rescuing the patient with a second transplant. The same donor can be used, usually collecting granulocyte colony-stimulating factor (G-CSF)-mobilized PB cells, but haploidentical grafts have also been shown to be effective in a proportion of patients.[50-52] Measures to completely prevent rejection in SAA have not been identified; however, the risk will be reduced with the use of ATG, low-dose radiation, and FLU for UD grafts and a marrow cell dose greater than 2×10^8/kg. Some patients rejecting their graft can undergo autologous hematologic recovery[53]; this is a rare event, with an overall rate of 4.2%, but when it occurs, survival is excellent (84%).

Nontransplant therapy

ATG+CsA

The standard regimen for first-line IST remains ATG and CsA, with hematological recovery in 50% to 70% of cases and excellent long-term survival among responders.[13,55-58] First-line therapy is recommended for all patients without a matched sibling donor (Figure 2), but also for patients with a matched sibling who are older than 40 years.[26] There is a strong age effect also after IST, as shown in Figure 5, with survival of 82% and 56% for patients younger than 20 or older than 40 years (A.B., EBMT database, unpublished data); this figure outlines survival after first-line IST and includes salvage therapy with a stem cell transplant for nonresponders. Failure-free or transplant-free survival would be clearly inferior[59] and would favor transplantation, but only in younger patients and only when compared with transplants from matched sibling donors.[11] For this reason, current guidelines recommend IST first-line therapy for all patients without a matched sibling donor.[26] After failing a first course of IST, patients with a matched UD should undergo a transplant if clinically fit; the choice of a transplant vs a second course of IST will need to be patient oriented.

Duration of CsA therapy

CsA should be given for a minimum of 6 months, but I tend to prolong CsA for more than a year, although at low doses. Once a response has been obtained, discontinuation should be very, very slow, with frequent evaluation of peripheral blood counts to identify early signs of relapse.[51] I tend to reduce CsA until I give it 2 to 3 times/week, rather than daily. We are treating an autoimmune disorder, and these patients are really never cured of the disease.

Source of ATG

Horse ATG (ATGAM; Pfizer) has been compared with rabbit ATG (rATG) (Thymoglobulin; Sanofi France) in a prospective randomized trial and has been shown to be superior in terms of response at 6 months (68% vs 37%) and 3-year survival (96% vs 76%).[60] Similar results have been obtained in a matched-pair analysis of the EBMT[61] and in a retrospective pediatric Japanese study.[62] One hypothesis to explain this difference has been a stronger immunosuppressive effect of rATG, leading to more early infections in patients receiving this product.[60,61] Other studies, although not prospective, have failed to show significant differences between horse ATG and rATG.[57,63,64] A large study on more than 800 patients receiving first-line therapy with rATG (Thymoglobulin) is currently being completed.

G-CSF

The combination of ATG+CsA with G-CSF has shown very good response rates and survival.[65] However, 3 randomized studies comparing ATG+CsA with or without G-CSF have failed to show a survival advantage for patients receiving G-CSF.[66-68] In the most recent study,[66] the neutrophil count on day +30 predicted response and survival in G-CSF patients. This is in keeping with our initial study,[65] suggesting ATG+CsA+G-CSF identifies early nonresponders, and perhaps may indicate an urgent transplant approach. The 3 randomized studies also confirmed that there was no increased incidence of malignant clonal disorders for G-CSF patients, disproving the reported increased risk for patients receiving prolonged G-CSF treatment shown in retrospective studies.[69] I personally believe ATG+CsA+G-CSF remains an interesting first-line therapy because it allows for a rapid neutrophil recovery and identifies early nonresponders. There is no place for G-CSF as single-drug therapy.

Eltrombopag

The new player in the field of nontransplant therapy for SAA is EPAG. After initial encouraging results in refractory patients,[70] EPAG has been used together with ATG+CsA as initial treatment in 88 patients.[71] The overall response at 6 months was 85%, with survival on the order of 90%. Cytogenetic abnormalities and clonal evolution to myelodysplasia occurred at a similar frequency compared with standard IST, although follow-up is still short.[71] The EBMT Working Party for Severe Aplastic Anemia is running 2 prospective randomized studies: 1 compares ATG+CsA with or without EPAG in SAA, and the other compares CsA with or without EPAG in non-SAA.

Case report

A 62-year-old man presented in October 2012 with severe pancytopenia (hemoglobin, 8.3 g/dL after transfusions; white blood cells, 0.8×10^9/L; PMNs, 0.3×10^9/L; platelets, 8×10^9/L), which had gradually developed over the course of a year.

The patient's medical history revealed a liver transplant at the age of 50 for HCV⁺ HBV⁺ cirrhosis, and treatment with CsA since then. In 2011, with declining peripheral blood counts, a BM aspirate showed an empty marrow with trisomy 8 and −Y. The patient was diagnosed with aplastic anemia and received a first course of horse ATG in December 2011. Because of a lack of response, the patient received a second

course of ATG (rATG) in July 2012. Four months later, a BM aspirate was unsuccessful, and a marrow biopsy showed complete aplasia. The patient continued to be cytopenic and dependent on red blood cells and platelet transfusions. An unrelated transplant was ruled out because of age and the lack of an 8/8 matched donor. The patient was continued on CsA; testosterone 40 mg by mouth was added 3 times a week, together with EPAG 50 mg every other day. The dose was kept low because of potential liver toxicity, and G-CSF was also given 3 times a week. PB counts improved gradually, and the patient was transfusion-independent after 3 months. In 2013, his counts were as follows: hemoglobin, 11.3 g/dL; white blood cells, 4.8×10^9/L; PMNs, 3.7×10^9/L; platelets, 71×10^9/L. The patient was started on sofosbuvir and simeprevir for his HCV, and CsA was changed to tacrolimus. He was able to complete his anti-HCV therapy and is now 5 years post-ATG, HCV-negative, receiving low-dose tacrolimus, EPAG 25 mg thrice weekly, low-dose testosterone, and deferasirox for iron overload, with ferritin reduced from 13,000 to 409 ng/mL. He has developed a small PNH clone (16% CD14, 3% CD16, 0.4% CD59).

Androgens

This patient is an example of an older adult with severe marrow failure, cytogenetic abnormalities, and comorbidities in whom combined treatment with growth factors, CsA, and androgens was successful. I often use this combination in frail patients. Androgens have shown encouraging results both in the animal model[72] and in patients with marrow failure.[73] A first randomized trial comparing ATG with or without androgens failed to show improved response and survival in the ATG+androgens group.[14] However, in a second randomized trial, women receiving ATG+androgens had significantly superior response rates, although comparable survival, as with ATG without androgens.[75] Intensified IST with ATG and androgens produced a 77% response rate and 78% survival at 5 years in a single-center study.[76] We have shown a 59% response rate with testosterone 40 mg/day (5 days/week) in patients with persistent cytopenia after IST.[77]

Recently, sex hormones have been shown to induce telomere elongation in vitro,[78] and also in vivo.[79] Taken altogether, these studies point to a significant effect of androgens in patients with marrow failure, including increased telomerase activity. Perhaps we should reconsider their role in nontransplant strategies with ATG, CsA, and EPAG.

High-dose cyclophosphamide

The Baltimore group has reported encouraging results with the use of high-dose CY instead of ATG+CsA in 67 patients, with a response rate of 77% and a 10-year survival of 88%[80]; nevertheless, a prospective study has failed to show an advantage of CY over ATG and has highlighted an increased risk for infections.[81] For these reasons, high-dose CY cannot be recommended as a nontransplant therapy outside a clinical trial.

Problems with IST

Patients treated with IST are not cured of their disease and are at risk for 3 major complications: no response, relapse, and clonal evolution. Patients not responding to a first course of ATG should be considered for an UD transplant if younger than 40 years (Figure 2); above the age of 40 years, a transplant from an unrelated or alternative donor is one possibility, but a second course of IST, or the addition of EPAG, are valid alternative options (Figure 2), and the choice should be made on the basis of the age of the patients, the clinical conditions, and the severity of the disease. Relapse is the second problem: it occurs in approximately 30% of responders, but can be successfully treated in the majority of patients with a second course of ATG or transplantation if a suitable donor is available. Clonal evolution is the third problem[1,3]; the emergence, or existence from diagnosis, of a small GPI negative clone, as determined by flow cytometry, may be involved in the pathogenesis[82] and may be a favorable prognostic indicator of response to IST[83,84]; a large GPI negative erythrocyte clone may lead to hemolysis and clinical PNH.[85] The most worrisome problem is cytogenetic evolution or a myelodysplastic syndrome, which has been reported to occur in 18% of patients[86] and calls for a transplant strategy for eligible patients.[87] For this reason, morphologic and cytogenetic examination of marrow cells should be performed in patients with SAA after immunosuppressive treatment, possibly at yearly intervals.[12,26]

Treatment of patient older than 60 years

Transplantation is rarely offered to patients older than 60 years because of high transplant-related mortality. Patients between 60 and 70 years of age should be treated with conventional ATG+CsA, and I also use G-CSF because of faster neutrophil recovery. In a study looking at elderly patients, the EBMT has shown that response to IST also can be achieved in older patients.[88] Above the age of 70 or 80 years, patients can be rather frail, and I often use CsA plus androgens and reserve CsA+ATG for fit patients, as suggested also by Scheinberg and Young.[14]

Conclusions

Acquired aplastic anemia remains a difficult disease, with problems of diagnosis and treatment, and patients should be treated, preferentially, in experienced centers, and best in the context of clinical trials. Significant progress has been made in understanding the pathogenesis of the disease, and new treatment options are now available. Improved outcome of UD transplants has produced survival comparable to that of matched sibling donor grafts, although with increased risk for GVHD. Haploidentical transplants should be considered experimental, and are being used in severe cases lacking a suitable matched donor or CB unit; early results seem promising. EPAG could become a relevant actor in the field of nontransplant therapy and may change our treatment strategies if response and survival rates, recently reported, are confirmed. I often use androgens in patients who fail to achieve hematopoietic recovery after IST, and have seen a significant number of responses. In the seventies, the mortality of acquired SAA was on the order of 80% to 90%; we are currently looking at 80% to 90% survival, which is a fantastic result of worldwide research in this field.

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Authorship

Contribution: A.B. wrote this review.

Conflict-of-interest disclosure: A.B. reports being on the speakers bureau for Sanofi, Pierre Fabre, Therakos, Miltenyi, ADIENNE, Gilead, and Novartis.

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Attachment 2: [redacted] Letter from [redacted] with the following enclosures:

a. [redacted] Clinical Notes from Dr. [redacted]
b. [redacted] Letter from Dr. [redacted]
c. Guinan EC. *Diagnosis and management of aplastic anemia*. Hematology Am Soc Hematol Educ Program. 2011;2011:76-81.
d. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, Hillmen P, Ireland R, Kulasekararaj A, Mufti G, Snowden JA, Samarasinghe S, Wood A, Marsh JC; British Society for Standards in Haematology. *Guidelines for the diagnosis and management of adult aplastic anaemia*. Br J Haematol. 2016 Jan;172(2):187-207.

[redacted]

[redacted]

[redacted]

Appeals Department

[redacted]

RE: Appeal for the Genetic Testing (CPT 81479) Requested by Dr. [redacted], MD
Reference #: [redacted]

On [redacted] I received a letter regarding the appeal for genetic testing (CPT 81479) requested by Dr. [redacted], MD, reference #: [redacted]. The letter noted the Medical Reviewer had reviewed the appeal information and had determined the original decision to deny coverage for the genetic testing due to testing not being medically necessary per the plan's language, continues to be upheld. The following rationale was provided in the letter:

> "You have aplastic anemia and bone marrow failure syndrome. The requested test is to help with treatment decisions. Health plan guidelines and plan benefit language have been reviewed. We reviewed the information sent to us. Based on review of this information it is determined that this test is not covered under your health plan. The health plan does not cover tests and treatments that are not shown to be medically necessary for your care. The previous denial is upheld."

As per the plan of [redacted], Medically Necessary / Medical Necessity means health care services provided for the purpose of preventing, evaluating, diagnosing, or treating an illness, injury, mental illness, substance use disorder, condition, or disease or its symptoms, that generally meet the following criteria:

- In accordance with Generally Accepted Standards of Medical Practice (meaning standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community, relying primarily on controlled clinical trials, or, if not available, observational studies from more than one institution that suggest a causal relationship between the service or treatment and health outcomes); and

- Clinically appropriate, in terms of type, frequency, extent, site, and duration, and considered effective for your illness, injury, mental illness, substance use disorder, or disease or its symptoms; and
- Not mainly for your convenience or that of your doctor or other health care provider;
- Is the most appropriate, most cost-efficient level of service(s), supply, or drug that can be safely provided to the member and that at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of your illness, injury, disease, or symptoms

I am writing to request a second appeal and provide additional information supporting that the genetic testing was indeed medically necessary to determine my diagnosis and subsequent treatment plan. In addition to Dr. [redacted] letter dated [redacted] provided in the first round of appeal, please consider this additional information supporting that CPT 81479 genetic testing was medically necessary to determine my diagnosis and treatment plan.

The genetic testing ordered by Dr. [redacted], MD was ordered to confirm a diagnosis of acquired versus inherited aplastic anemia bone marrow failure. The attached clinical notes (Attachment 1) from [redacted] from Dr. [redacted] include my specific clinical history including observations dating back to my childhood. A subsequent letter dated [redacted] (Attachment 2) from Dr. [redacted] explains the exclusionary diagnosis process used to arrive at a diagnosis of acquired aplastic anemia and confirms the use of this information for my specific treatment plan.

The decision of my doctors to request genetic testing is supported by generally accepted standards of medical practice based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community. These generally accepted standards of medical practice highlight that bone marrow failure and related syndromes are rare disorders and although many are associated with characteristic clinical features, advances in medical care have shown a more complicated picture with a spectrum of broad and overlapping phenotypes and imperfect genotype-phenotype correlations where clinical presentation can present into adulthood. These medical advances also include necessitating genetic testing in the diagnosis of bone marrow failure syndromes. Whereas distinguishing acquired from inherited forms of bone marrow failure is of crucial importance given differences in the risk of disease progression to other malignancies which would inform the type of transplant pretreatment as well as inform the selection of the appropriate donor for hematopoietic stem cell transplantation.

The American Society of Hematology (Guinan, 2011 and Attachment 3) confirms idiopathic aplastic anemia diagnosis is diagnosed through a process of exclusion. This includes ruling out types of inherited bone marrow failure syndromes for which genetic testing has proven fruitful in providing mutation identification.

Furthermore, the Guidelines for the Diagnosis and Management of Adult Aplastic Anaemia from the British Journal of Haematology (Killick et al, 2016 and Attachment 4) note that patients undergoing haemopoietic stem cell transplantation should confirm the precise diagnosis as it is vital not to miss inherited bone marrow failures to avoid serious and potentially lethal toxicity from the transplant process and inappropriate selection of a sibling donor.

As evidenced by the clarifying information provided in this letter and specific to my clinical presentation and rare disease condition, the genetic testing requested by Dr. [redacted] MD was clinically appropriate and medically necessary to confirm a diagnosis of acquired aplastic anemia versus an inherited type of bone marrow failure. Furthermore, obtaining a more definitive diagnosis using these diagnostic tests was critical to inform the type of pretreatment and donor type for hematopoietic stem cell transplantation. Without utilizing genetic testing to further confirm precise diagnosis, incremental health risks with serious and potentially lethal toxicity or treatment relapse would have been incurred.

Please consider this letter and the attachments as part of reference #: [redacted] in the continued appeal in coverage for genetic testing (CPT 81479) requested by Dr. [redacted] MD.

Sincerely,

[redacted]

Attachments:

1. [redacted] Clinical Notes from Dr. [redacted]
2. [redacted] Letter from Dr. [redacted]
3. Guinan EC. *Diagnosis and management of aplastic anemia.* Hematology Am Soc Hematol Educ Program. 2011;2011:76-81.
4. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, Hillmen P, Ireland R, Kulasekararaj A, Mufti G, Snowden JA, Samarasinghe S, Wood A, Marsh JC; British Society for Standards in Haematology. *Guidelines for the diagnosis and management of adult aplastic anaemia.* Br J Haematol. 2016 Jan;172(2):187-207.

Attachment 1: [redacted] Clinical Notes from Dr. [redacted]

Redacted

Attachment 2: [redacted] Letter from Dr. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Subject: Second appeal for genetic testing
To Whom It May Concern:

This is to write a letter to convey the medical necessity of the genetic testing ordered for [redacted]

We ordered this genetic testing to arrive at the correct diagnosis for [redacted] Aplastic anemia is a diagnosis of exclusion and it is critical to perform genetic testing for germline or inherited forms of bone marrow failure prior to arriving at this diagnosis. Treatment of genetic forms of bone marrow failure, including bone marrow transplant methods are different. Without this testing, we would not have been able to proceed with bone marrow transplant for her in an expeditious manner.

In summary, I wish to convey that this testing was medically necessary and I sincerely urge you to re-consider your decision.

If there are any questions, please feel free to call me at [redacted]

Sincerely,

[redacted]

[redacted]

[redacted]

Attachment 3: Guinan EC. *Diagnosis and management of aplastic anemia*. Hematology Am Soc Hematol Educ Program. 2011;2011:76-81.

Diagnosis and Management of Aplastic Anemia

Eva C. Guinan[1]

[1]*Dana-Farber Cancer Institute, Boston, MA*

Aplastic anemia remains a diagnosis of exclusion. Our ability to reliably diagnose, and therefore exclude, a variety of inherited or acquired diseases with similar phenotypes has improved markedly. An efficient diagnostic plan is important because time from diagnosis to treatment is related to outcome regardless of the therapeutic option chosen. HSCT remains the mainstay of therapy for those with matched sibling donors, and results have improved even further in recent years. For those without a sibling donor, the high response and overall survival rates of combined immunosuppressive therapy (IST) have proven robust. Nonetheless, incomplete response, relapse, and progression to myelodysplasia/leukemia have more clearly emerged as significant long-term issues. Improvements in outcome of alternative donor transplantation and the use of established and novel immunosuppressive agents provide multiple alternatives for treating refractory or relapsed patients. Best practices in this regard are not yet clearly established and may vary by a variety of demographic and treatment-specific factors. Regardless of the type of therapeutic approach, patients require ongoing monitoring for occurrence of disease and/or therapy-related side effects.

Introduction

Aplastic anemia remains a disorder confined by conventions that specify a combination of low peripheral blood counts with specific appearances of the BM itself. The most common conventions, modified by severity criteria, are shown in Table 1. Whereas it has always been thought that BM failure meeting these criteria could arise as a consequence of diverse pathophysiologic mechanisms, the details surrounding some of these mechanisms—both their commonalities and their singularities—are becoming better understood. The intention of this review is to use specific examples of new observations, ways in which existing information is being used in new ways, and some current fields of investigation to illustrate areas of progress or controversy that either are influencing or may come to influence the diagnosis and management of aplastic anemia patients in the near term.

Diagnosis

Despite the precision of its diagnostic criteria, aplastic anemia has always been a diagnosis of exclusion. No single test allows us to reliably diagnose idiopathic aplastic anemia, but the field has advanced considerably in terms of awareness of and diagnosis of other disorders resulting in a similar or indistinguishable hematologic phenotype.[1-4] Consequently, the diagnostic evaluation has become increasingly detail driven in its attempt to exclude a list of potential alternative etiologies of BM failure. Some of these investigations are quite novel, whereas others are variations on old themes illuminated by new detail. As practitioners, we need to remain alert to emerging changes in diagnostic tools and practices, and to that end, examples of several such diagnostic practice changes are highlighted below.

Whereas the reasons for obtaining a complete blood count may be very diverse, the differential diagnosis of aplastic anemia usually arises from the observation of pancytopenia (Table 2). Although many sophisticated tests are now available, it remains essential to obtain a thorough history and perform a detailed examination. One goal of history taking is to elicit evidence of any drug or toxin exposures that have been associated with BM aplasia. A quick perusal of any online drug database discloses the ubiquity of BM suppression and, albeit less frequently, BM failure as potential adverse events seen after the administration of drugs from virtually every class. The recognition of these associations reflects the circumstances and extent of usage of/exposure to any particular drug or toxin. Therefore, potential exposures should be explored for plausibility in up-to-date databases. Unfortunately, no tests permit ascertainment of causal relationships between any specific exposure and subsequent BM failure. From the perspective of the individual patient, any associations therefore remain presumptive, and the utility is simply in removing any ongoing exposure. From a population perspective, however, aggregated information may inform post-marketing decisions governing drug labeling. Moreover, Web-based communication platforms make the resulting information readily available for use in treatment decisions. Accordingly, physicians should be aware of and use national programs to report such associations. In the United States, one such resource is the Food and Drug Administration's MedWatch program (http://www.fda.gov/Safety/MedWatch/HowToReport/ucm085568.htm).

The importance of a detailed physical examination has also not declined. However, there are substantive limitations of the examination in firmly excluding alternative diagnoses previously felt to have pathognomonic findings.[5,6] Fortunately, highly specific diagnostic tests have emerged for multiple disorders that can present as aplastic anemia. Genetic testing has proven fruitful in several regards, particularly in providing genetic (mutation identification) tests for inherited BM failure syndromes (IBMFSs). These tests and their application will be discussed in more detail by Dr Niemeyer (see pages 84-89).[7] The generalizable message in regard to using these tests to exclude diagnoses other than idiopathic aplastic anemia is that they provide incomplete information. We do not yet know all of the inherited genetic variants that can result in a clinical BM failure phenotype, or in a specific IBMFS phenotype.[8,9] Classical mutations can be found in individuals without physical findings of an IBMFS and the same mutation can be associated with very diverse clinical presentations.[5,9] Whereas better diagnostic algorithms using phenotypic, clinical laboratory, and genetic data are evolving rapidly and new genetic variants continue to be discovered, current testing does result in improved but not absolute exclusion of a specific IBMFS.[7]

Table 1. Definition of aplastic anemia with severity criteria*

Classification	Criteria
Severe	BM cellularity < 25% (or < 50% if < 30% of BM is hematopoietic cells) AND ≥ 2 of the following: • Peripheral blood neutrophil count $< 0.5 \times 10^9$/L • Peripheral blood platelet count $< 20 \times 10^9$/L • Peripheral blood reticulocyte count $< 20 \times 10^9$/L
Very severe	As above, but peripheral blood neutrophil count must be $< 0.2 \times 10^9$/L
Nonsevere	Hypocellular BM with peripheral blood values not meeting criteria for severe aplastic anemia

Adapted with permission from: Davies JK, Guinan EC.[50]

A different example of the expanded tool kit provided by genetic testing is a recent report in which an infant presented with pancytopenia and BM failure without megaloblastic changes (albeit with some dysplasia) and normal B12 and folate levels. Eventual evaluation of metabolic status led to the finding of a novel transcobalamin mutation resulting in B12-responsive BM failure.[9]

Routine cytogenetic testing has further revealed that approximately 10% of patients with apparent aplastic anemia by all other criteria may have clonal chromosomal abnormalities. The complex relationship of clonality and aplasia is presented in great detail by Dr Maciejewski (see pages 90-95).[11] Additional data based on cell-surface and intracellular markers such as p53, Hgb F and telomere length are also being evaluated for their utility in the diagnostic quagmire of differentiating hypoplastic myelodysplasia and idiopathic aplastic anemia.[12,13]

Innovations in established diagnostic algorithms have become commonplace. One paradigm is investigation of paroxysmal nocturnal hemoglobinuria (PNH) as a cause of aplasia. Testing for PNH has evolved significantly from function-based biochemical assays such as the sucrose hemolysis and Ham tests to flow cytometric analysis.[14] Somewhat confusingly from a diagnostic point of view, both healthy individuals and those with aplastic anemia can have clones of cells with a PNH phenotype, and these clones can wax and wane in absolute and relative frequency.[14] Therefore, accurate biomarker identification in conjunction with accurate quantitation has proven requisite. Both goals have been met by flow cytometric detection and quantification of PNH clones by use of the glycophosphatidylinositol-anchor binding, fluorescently labeled inactive toxin aerolysin, which is more sensitive than antibody binding to CD59, another glycophosphatidylinositol-anchor-binding cell-surface molecule.[14]

Mutation analysis is a highly specific but incomplete mechanism for establishing an alternate IBMFS diagnosis. However, testing for a common functional or structural phenotype can be highly synergistic and facilitative. Just as abnormal sister-chromatid exchange became the diagnostic test for virtually every Fanconi anemia patient, determination of telomere length has become a valuable adjunct to diagnosis of dyskeratosis congenita in particular, but to other IBMFSs as well.[15,16] Using several different techniques, nomograms of telomere length by age and by cell of origin are becoming sufficiently refined to provide significant diagnostic assistance. Differences in cell-cycle markers may also prove useful.[17] As additional biological correlates of IBMFS molecular findings become better understood, such "functional" screening should become both more robust and accessible.

Management

Whereas there has been no significant shift in the management strategy for aplastic anemia over the last several years, there are some emergent data that can support and direct the practitioner in managing such patients.

Supportive care

Medical care continues to depend upon meticulous attention to issues of infectious and hemorrhagic diatheses, expectant management of regimen-related toxicities, and provision of information and psychological support.[1-4] Potentially useful data drawn from related

Table 2. Differential diagnosis of peripheral pancytopenia

Condition	BM appearance	Possible diagnostic investigations
Idiopathic aplastic anemia	Hypocellular	Exclusion
Associated with IBMFS	Hypocellular	Mutation analysis; functional testing
Associated with drug or toxin	Hypocellular	Careful history
Associated with pregnancy	Hypocellular	β-hCG
Viral-associated (may include CMV, EBV, HIV, HHV-6, hepatitis non-A, B, or C, other)	Hypocellular (or variable)	Serology; PCR for viral DNA; specific tests for antigens; tetramer analysis
PNH	Variable	Peripheral blood immunophenotyping for PIG-linked molecules; Ham/sucrose lysis test
Myelodysplasia	Hyper- or hypocellular	Variable by presentation but may include: BM morphology by aspirate; trephine biopsy; immunocyto-/histochemistry; immunophenotyping; cytogenetics including FISH; molecular analysis
Acute myelogenous leukemia	Hypercellular (rarely hypocellular)	
Acute lymphoblastic leukemia	Hyper- or hypocellular	
Hodgkin disease	Infiltrated or may be hypocellular	
Solid tumors	Infiltrated	
Myelofibrosis	Reticulin fibrosis	
Histiocytic disorders	Hypocelluar, hemophagocytosis	
Osteopetrosis	Increased bony trabeculae	Trephine biopsy
Storage disorders	Hypercellular, infiltrated	Trephine biopsy
Anorexia nervosa	Hypocellular ± fat necrosis	Careful history; physical examination; psychiatric evaluation
Acquired nutritional deficiency	Hypercellular	B12/folate levels (pretransfusion); metabolic pathway analysis

hCG indicates human chorionic gonadotrophin; HHV-6, human herpes virus-6; PIG, XXXX.
Adapted with permission from Davies and Guinan.[50]

patient populations or those with similar issues emerge routinely. For example, increased scrutiny of platelet transfusion triggers in diverse populations, few of whom have aplasia with its attendant protracted platelet production failure, has been undertaken but should be interpreted cautiously for this population.[18] Similarly, the approach to potential infection in neutropenic, febrile patients is frequently updated and provides important algorithms, but its applicability is limited by the persistent pancytopenia of aplastic anemia patients compared with other populations. However, the spectrum of infections in aplastic patients specifically has been reviewed recently and treatment recommendations have been provided.[19]

Emerging data on the use of iron chelation in patients with aplastic anemia

Iron-related mortality, especially related to hepatic and cardiac dysfunction, has not surprisingly emerged as an issue in aggregated BM failure cohorts, including some individuals with aplasia.[20,21] Daily chelation with oral deferasirox has been studied prospectively in a large number of aplastic anemia patients with iron overload. Treatment was well tolerated and effective in decreasing serum ferritin and transaminases.[21] Expectant, cautious management was urged in regard to renal impairment, especially if there was concomitant use of renal toxic immunosuppressive drugs.[21] In addition to producing desired improvements in organ function,[20,21] in a few cases, chelation with either deferasirox or deferoxamine has also intriguingly been associated with significant hematologic improvement.[22,23]

Matched family member HSCT

Mainstays for treatment for aplastic anemia remain HSCT, the only curative therapy to date, and immunosuppressive therapy (IST). HSCT continues to be the recommended first-line therapy for individuals with severe or very severe aplastic anemia who have a matched sibling donor.[1-4] The upper limit of age for this recommendation has been 40 years, although there is increasing variation in this regard, especially with use of less-aggressive conditioning regimens. Results of matched sibling HSCT have improved over time. A large, recent retrospective review found a significantly inferior overall survival rate of 73% (n = 614) in those patients receiving transplantations between 1991 and 1996 compared with 80% (n = 550) in those receiving transplantations between 1997 and 2002.[24] Survival of children in the latter cohort was even higher at 91%. Indeed, younger age, year of transplantation, and decreased interval from diagnosis to transplantation all contributed to improved outcome in this European registry report. Conditioning regimens for matched sibling HSCT have historically been limited in their reliance on radiation, a trend that has become more pronounced. For example, 24% compared with 8% of matched sibling HSCT incorporated radiation into the conditioning regimen in the above 2 time periods, respectively, and irradiation was inversely correlated with survival.[24] The mainstay of conditioning has remained cyclophosphamide with or without additional agents.

Immunosuppressive therapy

For individuals lacking a matched sibling donor or above the age at which sibling HSCT is felt to represent the best opportunity for good outcome, IST is indicated.[1-4] The multiagent regimen of antithymocyte globulin (ATG) and cyclosporine (generally accompanied by a brief course of corticosteroids) has proven very robust.[25] Response to IST generally ranges from 50%-80% and, in contrast to HSCT, the response rate of patients to IST has not changed in recent years.[1,2,24,26-28] IST regimens are somewhat variable, particularly with respect to source and administration schedules of ATG as detailed in the cited reports and reviews. Most studies have used horse ATG, although for various reasons more practical than theoretical, rabbit ATG is currently also in use. No substantial data as to the relative efficacy of the preparations have yet emerged. Modifications to the conventional IST regimen, including addition of danazol,[29] mycophenolate mofetil,[30] sirolimus,[27] or hematopoietic growth factors,[31] have not significantly improved response or decreased relapse rates. Such agents currently have no place in primary therapy, although a few studies suggest that the addition of danazol[29] or growth factors[29,31] has altered relapse rates. Very little information is available about the substitution of tacrolimus for cyclosporine.[32] Alternative immunosuppressive regimens, such as cyclophosphamide[33,34] or alemtuzumab with or without cyclosporine,[35] also show promise. Most IST modifications have been studied in very limited cohorts and have not yet been subjected to prospective, randomized comparisons with the standard of care.

With current improvements in alternative donor HSCT, the desire to predict response to IST has grown. Several recent reports have suggested that younger age overall is associated with greater likelihood of response.[1,24,36] Among affected children only, younger age has inconsistently been associated with greater response rate.[1,2] Interval from diagnosis to treatment, gender, absolute neutrophil count (ie, very severe aplastic anemia vs severe aplastic anemia), and reticulocyte and lymphocyte counts have also, but more variably, been correlated with response.[1,2,24,28] Telomere length has not been shown to be correlated with response.[13]

Scrutiny of the definition of response chosen, which varies between investigators and programs, is important in interpreting the results of IST studies; the widespread adoption of consensus criteria should facilitate comparison of outcomes. In addition to varied degree of improvement, IST responders can follow highly variable clinical paths. Some are stable after discontinuation of treatment, whereas others (15%-25%) have significant cyclosporine dependence.[26] Slower weaning off of cyclosporine (over approximately 1 year) has been associated with decreased relapse.[26] Some patients remain stable or slowly improve their hematologic status, whereas others may relapse (apparently) spontaneously or with "provocations" such as pregnancy, and still others go on to develop persistent clonal cytogenetic findings and myelodysplasia or acute myelocytic leukemia. This latter topic is reviewed and referenced in detail elsewhere in this book, and is an important component of both managing and triaging treatment in aplastic anemia patients. For patients who are refractory to IST or who relapse after successful IST, treatment with an additional course of ATG-based IST is possible.[3,4,29,37,38] Response rates in recent reports range from 11%-65%,[29,37,38] with rates in previous responders generally more favorable than in initially refractory patients. More experimental modalities, including alternative IST (eg, rituximab[39] or cyclophosphamide[34]) may also be considered. In addition to an understanding of the diverse courses of patients after IST, which range from uninterrupted hematologic stability to either relapse or evolution of myelodysplasia/leukemia (both at very unpredictable times after treatment), these choices should be determined by factors including patient age, performance status, transfusion requirement, comorbid conditions, and availability of alternative donors for HSCT. Intriguingly, shorter age-adjusted telomere length has been shown recently to be associated with likelihood of relapse, clonal evolution, and overall mortality.[13]

Alternative donor HSCT

The above choice is heavily influenced by the recent significant improvement in the outcome of alternative donor transplantation, using either matched or mismatched unrelated donors (URDs) or mismatched related donors. In part because of the underlying historical tendency of both alternative donor and aplastic anemia patients to have increased graft failure/rejection, alternative donor transplantations for aplasia were initially carried out with aggressive, immunosuppressive, and myeloablative regimens, markedly different from those used for matched sibling HSCT. Moreover, the aplastic anemia patients who first came to URD were often heavily treated patients who had failed numerous therapies, had repeated episodes of febrile neutropenia or infection, and had received extensive transfusion support. In a multivariate analysis of the European registry data, in which actuarial survival after alternative donor HSCT improved from 38% to 65% in the periods 1991-1996 and 1997-2002, respectively, only year of transplantation was associated with increased survival.[24] It is likely that progressive changes in dimensions such as improved performance status, decreased prior transfusion, decreased interval from diagnosis to transplantation, improved supportive care, better donor-recipient matching, and use of less-intensive (particularly low-dose radiation or radiation-free) regimens contributed to this association and to the improved results in other recent studies.[24,38,40-44] Five-year survival ranges from approximately 35%-85% in these reports, depending upon approach, match, and recipient age. More reliable data on unrelated umbilical cord blood HSCT for aplastic anemia have also started to emerge, although this approach remains used largely in pediatric patients.[45,46]

Overall, younger, better-matched patients have superior outcomes after alternative donor HSCT.[24,38,40-44] However, degree of match or choice of alternative donor has not significantly affected various outcome measures in some studies.[42] Attention has recently turned to additional pretransplantation characteristics that might influence or predict outcomes. One example would be consideration of the effects of iron overload at time of HSCT. Because patients with matched sibling donors most often move rapidly to HSCT, these studies are largely referable to alternative donor HSCT. Once again, inferences are drawn from studies in which patients with various diagnoses are aggregated rather than studies in which the diagnosis is confined to those with aplastic anemia. Nonetheless, the majority of aplastic patients in these studies tended to have high ferritin levels or high aggregate "iron scores" at a level the reports associated with increased 100-day mortality, acute GVHD occurrence, bacteremia/infection, and decreased overall survival.[47,48] However, the associations between iron overload and adverse outcomes observed may be skewed by the prevailing myelodysplasia diagnosis in the study cohorts, and this and other confounding factors may contribute to these observed outcomes. There is as yet no data in aplastic anemia that directly address the value of chelation-related decreases in iron compared with the natural presentation to HSCT with less iron-loaded status. Whereas it is likely fair to take the issue of iron-related toxicity as another incentive among many to proceeding with treatment quite expeditiously, overall, the consideration of the costs, benefits, and potential toxicities of iron chelation must continue to be considered largely on a patient-by-patient basis.

Despite increased data on the outcome of patients treated in diverse ways, there is as yet no absolute algorithm in regard to treatment for patients relapsed after or refractory to IST. A recent prospective, multicenter study in Japan evaluated the outcome of pediatric patients with either severe or very severe aplastic anemia who failed to respond to IST at 6 months. The patients underwent HSCT if they had a serologically matched URD, an HLA-one antigen–mismatched family donor, or an HLA-matched or HLA-one antigen–mismatched umbilical cord blood donor at the time of evaluation or they received a second IST course identical to the first.[38] The failure-free survival (ie, survival with hematologic response) at 5 years was 83.9% in those receiving HSCT, significantly better than 9.5% in those given a second round of IST. With the caveat that the epidemiology of aplastic anemia and HSCT in Japan has been somewhat different from that in other geographic areas, these are nevertheless important and provocative data. Results that can be inferred from prior, retrospective reports are somewhat less divergent than this, albeit not in direct contrast. Additional analyses based on patients treated over the last decade will help to inform better decision making in regard to secondary treatment.

Long-term toxicity

The long-term complications of HSCT (somewhat independent of underlying disease) are increasingly well appreciated,[49] and long term survivors of IST[1,29] are also at risk for a multiplicity of complications. Some of these complications become obvious only with prolonged followup. Moreover, these data are imperfect because both the characteristics of specific patient cohorts and regimens influence outcome. One might expect that as patients with different diseases, particularly IBMFS, are removed from the "aplastic anemia" cohort and as regimens alter (certainly toward less radiation and potentially to alternative IST), the challenges faced by survivors will also change.

Summary

Incremental gains have been made in both the diagnosis and management of aplastic anemia. The issues of predicting treatment response and clinical course remain unresolved, although some intriguing data have started to emerge. Greater understanding of regimen-related toxicities, either acute or delayed and potentially chronic, provides an impetus for the improvement of therapeutic strategies.

Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests. Off-label drug use: Very few of the drugs used to treat aplastic anemia are approved for that purpose (eg, cyclosporine, cyclophosphamide).

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bjh guideline

Guidelines for the diagnosis and management of adult aplastic anaemia

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Keywords: aplastic anaemia, paroxysmal nocturnal haemoglobinuria, anti-thymocyte globulin, haemopoietic stem cell transplantation.

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:

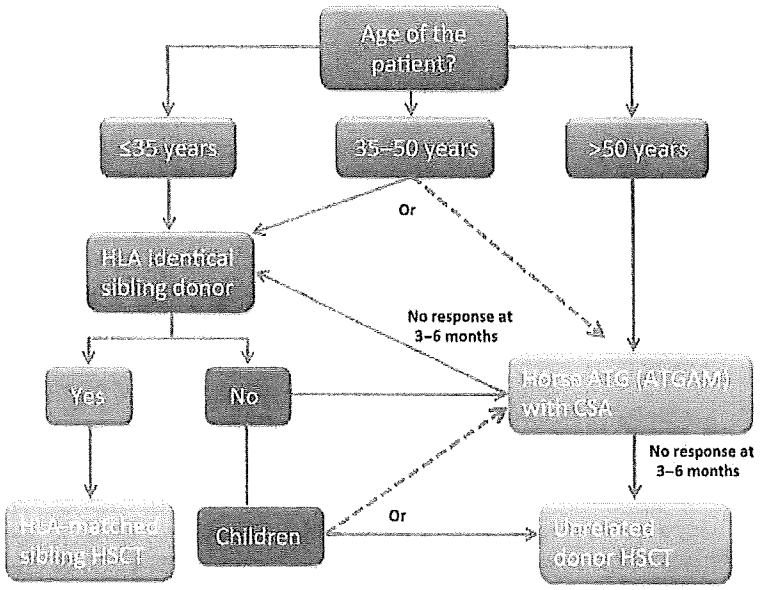

Fig 1. Treatment of acquired severe aplastic anaemia. HSCT may be considered, using a matched sibling donor or a suitably matched unrelated donor if no matched sibling donor is available, for patients aged 35–50 or >50 years who fail to respond to first line immunosuppressive therapy (Sureda *et al*,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

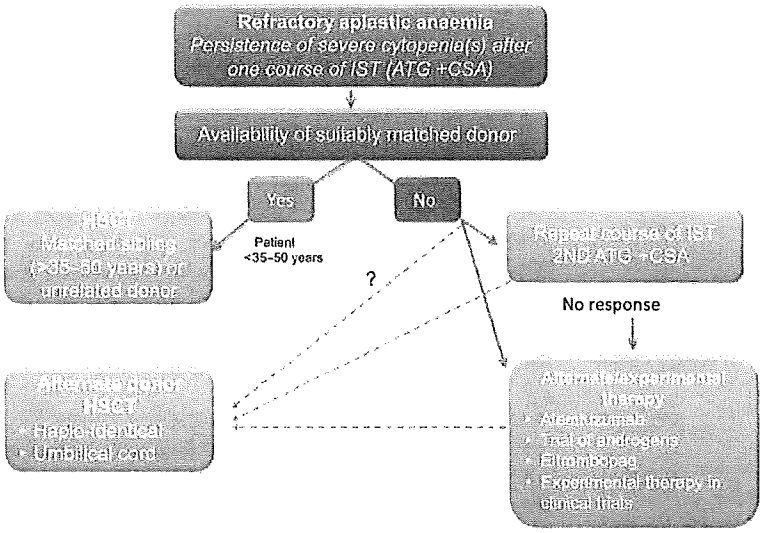

Fig 2. Treatment of adult refractory severe aplastic anaemia. ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplantation; IST, immunosuppressive therapy. Modified from Marsh, J.C. & Kulasekararaj, A.G. 2013.

- 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 pre-treated 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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Attachment 3: [redacted] First Appeal Decision Letter

[redacted]

[redacted]

RE: Appeal for the Genetic Testing (CPT 81479) Requested by Dr. [redacted]
Reference #: [redacted]

Dear [redacted]

We received your appeal regarding the genetic testing (CPT 81479) requested by Dr. [redacted]. The appeal letter, along with all submitted clinical information, was forwarded to a Medical Reviewer for consideration. We upheld the original decision, as the genetic testing (CPT 81479) was determined to be not medically necessary per the Plan's language. The following is the rationale:

"You have aplastic anemia and bone marrow failure syndrome. The requested test is to help with treatment decisions. Health plan guidelines and plan benefit language have been reviewed. We reviewed the information sent to us. Based on review of this information it is determined that this test is not covered under your health plan. The health plan does not cover tests and treatments that are not shown to be medically necessary for your care. The previous denial is upheld."

Therefore, we are unable, according to the summary plan description language, to certify the genetic testing (CPT 81479) as a covered benefit under the plan.

Please be advised that the [redacted] health benefit plan excludes coverage for services that are not medically necessary. You may refer to page 90 of the plan document (copy attached) which outlines this topic.

Clinical rationale utilized in making the appeal decision will be provided in writing upon request by calling the Care Coordinators. You are entitled to receive, upon request and free of charge, reasonable access to and copies of all documents, records and other information relevant to the claim.

Your Plan provides for a second appeal level. You may submit a second appeal within 60 days from the receipt of this letter to:

[redacted]

If you require additional information regarding your appeal rights, you can contact the [redacted] You have the right to bring a civil action under ERISA § 502(a) if you file an appeal and your request for coverage or benefits is denied following review. Any such action must be filed not later than two years after the completion of the Plan's claims review process.

If we can provide any information or assistance regarding your healthcare needs, please contact the Care Coordinators at [redacted]

Sincerely,

[redacted]
Appeals Coordinator
[redacted]

cc: [redacted]

Other Services the Plan Does Not Cover

- Acupuncture
- Care you receive before the **effective date** or after the termination date of your coverage
- Benefits you receive from other plans or services (other than personal coverage policies)
- Benefits you are eligible to receive from other sources, such as through government programs, including the Veterans' Administration, for an **illness** or **injury** connected to military service (This exclusion does not apply to Medicaid or Medicare.)
- Whole blood, packed red blood cells, blood donor fees, and blood storage fees
- Cosmetic services and procedures, except as specifically included in the Plan
- Dental care, even when the dental condition is related to or caused by a medical condition or medical treatment unless specified in this SPD
- Exams or treatment required by a third party
- Fees associated with missed appointments
- Injection of varicose veins
- Services that do not conform with UMR's medical policy guidelines
- Services or supplies that you received when you were not enrolled in the Plan
- Services not expressly identified in this SPD as covered services and supplies
- Services related to an injury or illness caused or contributed by international armed conflict, hostile acts of foreign enemies, invasion, war or acts of war, whether declared or undeclared.
- Alternative/Complementary treatment including treatment, services or supplies for holistic or homeopathic medicine, hypnosis or other alternate treatment that is not accepted medical practice as determined by the Plan
- Biofeedback Services
- Claims received later than 12 months from the date of service.
- Any treatment or therapy that is court-ordered, or that is ordered as a condition of parole, probation, or custody or visitation, unless such treatment or therapy is otherwise listed as a covered benefit.
- Services related to an injury or illness caused or resulting from taking part in the commission of an assault or battery (or a similar crime against a person) for which the individual is charged or a felony for which the individual is charged.
- Charges in excess of the allowed charge (usual and customary)
- Extended care facility services that exceed the appropriate level of skill required for treatment as determined by the Plan
- Growth hormones
- Private duty nursing services
- Services related to an illness or injury related to Hazardous Recreational Activities, unless the injuries or illness are caused primarily as the result of another medical condition not related to Hazardous Recreational Activities or to domestic violence
- Exams, evaluations, or services that are performed solely for educational or developmental purposes, unless covered under this Early Intervention Services provision
- Services and supplies furnished by providers that are not covered by the Plan
- Any service or supply furnished along with a non-covered service
- Services and supplies that UMR, in its discretion, determines are not medically necessary
- Phototherapy or devices used in connection with Seasonal Affective Disorder (SAD)
- Services that are furnished to someone other than the patient, except as described in this SPD for hospice services and the harvesting of a donor's organ or bone marrow when the recipient is covered under this Plan
- Services that are furnished to all patients due to a

Attachment 4: [redacted] Second Appeal Decision Letter

[redacted]

[redacted]

RE: Second Level Appeal for the Genetic Testing (CPT 81479) Requested by Dr. [redacted]
Reference #: [redacted]

Dear [redacted]

We received your second level appeal regarding the genetic testing (CPT 81479) requested by Dr. [redacted]. The appeal letter, along with all submitted clinical information, was forwarded to a Medical Reviewer for consideration. We upheld the original decision, as the genetic testing (CPT 81479) was determined to be not medically necessary per the Plan's language. The following is the rationale:

"This case was reviewed by an external specialist Board Certified in Hematology & Oncology. This was to obtain an expert opinion. They reviewed the available medical records. They looked at the information submitted on appeal. They reviewed the health plan guidelines. They looked at the plan benefit language. The specialist said there is not enough evidence in the literature to show that this testing is helpful for treating your condition. The specialist said this test was not medically necessary for your care. The health plan does not cover tests that are not medically necessary. The prior decision is upheld."

Therefore, we are unable, according to the summary plan description language, to certify the genetic testing (CPT 81479) as a covered benefit under the plan.

Please be advised that the [redacted] health benefit plan excludes coverage for services that are not medically necessary. You may refer to page 90 of the plan document (copy attached) which outlines this topic.

Clinical rationale utilized in making the appeal decision will be provided in writing upon request by calling the Care Coordinators. You are entitled to receive, upon request and free of charge, reasonable access to and copies of all documents, records and other information relevant to the claim.

You have exhausted the internal appeal process for this plan. You have a right to file a request for an external appeal within four (4) months from the date of this letter. The written request for an external appeal must be submitted to the following address:

[redacted]

[redacted]

If you require additional information regarding your appeal rights, you can contact the [redacted] You have the right to bring a civil action under ERISA § 502(a) if you file an appeal and your request for coverage or benefits is denied following review. Any such action must be filed not later than two years after the completion of the Plan's claims review process.

If we can provide any information or assistance regarding your healthcare needs, please contact the Care Coordinators at [redacted]

Sincerely,

[redacted]

cc: [redacted]

Other Services the Plan Does Not Cover

- Acupuncture
- Care you receive before the **effective date** or after the termination date of your coverage
- Benefits you receive from other plans or services (other than personal coverage policies)
- Benefits you are eligible to receive from other sources, such as through government programs, including the Veterans' Administration, for an **illness** or **injury** connected to military service (This exclusion does not apply to Medicaid or Medicare.)
- Whole blood, packed red blood cells, blood donor fees, and blood storage fees
- Cosmetic services and procedures, except as specifically included in the Plan
- Dental care, even when the dental condition is related to or caused by a medical condition or medical treatment unless specified in this SPD
- Exams or treatment required by a third party
- Fees associated with missed appointments
- Injection of varicose veins
- Services that do not conform with UMR's medical policy guidelines
- Services or supplies that you received when you were not enrolled in the Plan
- Services not expressly identified in this SPD as covered services and supplies
- Services related to an injury or illness caused or contributed by international armed conflict, hostile acts of foreign enemies, invasion, war or acts of war, whether declared or undeclared.
- Alternative/Complementary treatment including treatment, services or supplies for holistic or homeopathic medicine, hypnosis or other alternate treatment that is not accepted medical practice as determined by the Plan
- Biofeedback Services
- Claims received later than 12 months from the date of service.
- Any treatment or therapy that is court-ordered, or that is ordered as a condition of parole, probation, or custody or visitation, unless such treatment or therapy is otherwise listed as a covered benefit.
- Services related to an injury or illness caused or resulting from taking part in the commission of an assault or battery (or a similar crime against a person) for which the individual is charged or a felony for which the individual is charged.
- Charges in excess of the allowed charge (usual and customary)
- Extended care facility services that exceed the appropriate level of skill required for treatment as determined by the Plan
- Growth hormones
- Private duty nursing services
- Services related to an illness or injury related to Hazardous Recreational Activities, unless the injuries or illness are caused primarily as the result of another medical condition not related to Hazardous Recreational Activities or to domestic violence
- Exams, evaluations, or services that are performed solely for educational or developmental purposes, unless covered under this Early Intervention Services provision
- Services and supplies furnished by providers that are not covered by the Plan
- Any service or supply furnished along with a non-covered service
- Services and supplies that UMR, in its discretion, determines are not medically necessary
- Phototherapy or devices used in connection with Seasonal Affective Disorder (SAD)
- Services that are furnished to someone other than the patient, except as described in this SPD for hospice services and the harvesting of a donor's organ or bone marrow when the recipient is covered under this Plan
- Services that are furnished to all patients due to a

Attachment 5: [redacted] Test Requisition Documentation

Redacted