REVIEW ARTICLE

blood advances

Approach to the diagnosis of aplastic anemia

Amy E. DeZern[1] and Jane E. Churpek[2]

[1]Department of Oncology, Sidney Kimmel Cancer Center at Johns Hopkins, Baltimore, MD; and [2]Department of Medicine, Division of Hematology, Oncology, and Palliative Care & Carbone Cancer Center, The University of Wisconsin–Madison, Madison, WI

Establishing a diagnosis of aplastic anemia (AA) can be challenging, but it is absolutely critical to appropriate management, especially differentiating between acquired and inherited forms of the disease. The hematology field requires updated diagnostic guidelines to ensure that appropriate clinical pathways are pursued for patients and their safety. There are increasing clinical options for patients with immunosuppressive therapy and transplant once the diagnosis is made. In a case-based format, this review emphasizes the newer data on molecular (somatic and germline) findings in AA and how they are (or are not) helpful during diagnosis. There are key details on somatic mutation profiles and stated evidence where available for prognostic and treatment indications. Germline details of newer syndromes are also outlined, which make this review modern and reflect areas of uncertainty for clinicians.

Introduction

The bone marrow failure (BMF) state of aplastic anemia (AA) is marked by cytopenias and ineffective hematopoiesis.[1] AA confers a significant risk for morbidity and death as a result of its progressivenatural history and/or complications related to suboptimal therapy.[2,3] Without definitive treatment, mortalityfrom severe AA (SAA) approaches 70% at 2 years.[4] Establishing an accurate etiology of the AA ischallenging but also absolutely critical to appropriate management, especially differentiating between acquired and inherited forms of the disease. With ever-evolving diagnostic techniques[5,6] in the field, as well as increasing recognition of genetic underpinnings of disease,[7] the standard diagnostic algorithm for AA has become more complex. Coupling this diagnostic heterogeneity with the increasingly widespreadpreference to use early related (matched and mismatched) donor bone marrow transplantation (BMT) in the field[2,8] necessitates updated guidelines to ensure that appropriate diagnostic and therapeutic pathsare pursued.

Patient 1

Patient 1 is a 48-year-old female of European ancestry with obesity, obstructive sleep apnea, and history of Hashimoto's hypothyroidism. There is a strong family history of autoimmunity with a paternal grandmother and sister with rheumatoid arthritis, as well as a father with psoriatic arthritis. Our patient called her internist because of increased bruising; she also noted a 2-month history of decreased exercise tolerance, dyspnea on exertion, and fatigue. Initial laboratory studies revealed that she had a platelet count of 5×10^9/L, hemoglobin of 6 g/dL, and a total white blood cell count of 2.9×10^9/L, with an absolute neutrophil count of 1.1×10^9/L. Initially, immune thrombocytopenia was thought to be the most likely diagnosis. In that context, she was started on prednisone, 100 mg/d for 3 days, which did not augment her counts. Further diagnostic testing was needed.

Evaluation of a patient with a suspected new diagnosis of AA. AA is a diagnosis of exclusion. Thus, there is no single test that can be used to consistently diagnose AA from the myriad other causes of BMF. Consequently, the diagnostic evaluation must assess for and exclude these alternative

Submitted 25 February 2021; accepted 25 April 2021; published online 22 June 2021.
DOI 10.1182/bloodadvances.2021004345.

etiologies. At initial AA presentation, many patients manifest with fatigue, weakness, pallor, and headaches due to anemia. Often, patients have petechiae of the skin and mucous membranes, epistaxis, and/or gum bleeding related to severe thrombocytopenia. Fever and infections can also be seen in these patients as a result of low white blood cell counts and neutropenia. AA patients identified earlier in the course of the disease by abnormalities found on routine laboratory testing may not have any physical manifestations of their disease.

Clinical presentation and classification of aplastic anemia. Acquired AA, also called idiopathic AA, represents the majority (~70%) of all newly diagnosed cases.[9,10] The incidence of acquired AA is 2 per million in Western countries and 4 to 6 per million in Asia.[9] Across the lifespan, there are 2 incidence peaks: 1 among young adults and a second in the elderly.[11] BMF as part of an underlying inherited BMF disorder (IBMFD) presumably represents the remainder of AA diagnoses. The proportion of AA cases accounted for by IBMFD-related BMF is rising as a result of the increasing recognition and the discovery of multiple new genetic syndromes in this context along with improved access to testing for these IBMFDs.[12] More than 25% of pediatric patients and ~5% to 15% of adults age 40 or younger who present with AA have an inherited etiology.[3,13,14] This proportion is less well defined for patients older than 40 years of age but likely will become increasingly common as we investigate the inherited genetic basis of this disease further.

Acquired AA (called "AA" hereafter) is a hematopoietic stem cell disorder featuring reduced bone marrow cellularity and decreased hematopoiesis. This decreased hematopoiesis may disproportionately affect 1 or 2 lineages in the early stages of disease, but AA is ultimately associated with trilineage hypoplasia.[15] The initial diagnostic work-up focuses on distinguishing AA from other diverse causes of cytopenias.[16] Diagnosis can be challenging in some patients because AA, other immune cytopenias, myelodysplastic syndrome (MDS; cellular or hypocellular), paroxysmal nocturnal hemoglobinuria (PNH), and IBMFD are all considered BMF states.[17] These disorders can have significant overlap in the clinic, as well as in an individual patient. It is not uncommon for autoimmune diseases to track in families, even if not the identical disease; therefore, a compelling family history may be suggestive of an autoimmune phenotype[18] or an inherited predisposition.[19] Patients with acquired disease are often treated with immunosuppressive therapy (IST).[2,20,21]

Patient 1 continued

Three attempts were made to obtain a marrow biopsy for patient 1 but were challenging because of her body size and resulted in insufficient sample. Ultimately, a successful aspirate and core under conscious sedation showed a markedly hypocellular (<5%) marrow consisting predominantly of fat with a few scattered areas of erythroid precursors but no signs maturing of myeloid elements. The CD34 count was <1%. A PNH red blood cell clone size of 0.09% was detected in peripheral blood. This included 0.06% type III cells and 0.03% type II cells. A PNH white blood cell clone was detected in 13.1% of granulocytes and in 12.6% of monocytes. The karyotype was 46,XX. During the 5-week assessment period, the patient received 7 platelet transfusions and 5 units of packed red blood cells; her absolute neutrophil count fell to a nadir of 0.47×10^9/L.

Basic laboratory studies. The goal in a thorough and standard work-up is to confirm the diagnosis and exclude other causes of pancytopenia in the setting of a hypocellular bone marrow. At a minimum, the laboratory tests outlined in Table 1 should be performed using peripheral blood.

Detecting the presence of PNH clones is also important at diagnosis. The presence of PNH clones and a copy number–neutral loss of heterozygosity of chromosome arm 6p may suggest a diagnosis of AA.[22] Most often, a PNH clone is considered a marker of acquired disease, and its presence alone is not equivalent to a diagnosis of classical PNH. The classical PNH disease requires evidence of a hemolytic anemia or thrombotic event and often is a cellular or hypercellular marrow, distinct from AA.[23-26] The close pathophysiologic relationship between PNH and AA is explained by the lack of particular glycosylphosphatidylinositol anchor proteins on the surface of these mutated stem cells that render them relatively resistant to immune attack; thus, these cells do not have an inherent proliferative advantage but rather have a conditional advantage because they escape immune destruction.[27] Up to 70% of patients with acquired AA have a small PNH clone at diagnosis.[25,27,28] The presence of a PNH clone, irrespective of size, was a good predictor of response to IST in several series.[28-30] A PNH clone, in the setting of true marrow failure with AA, usually does not necessitate C5 blockade as primary treatment typical for a patient with more classical PNH.[31,32] However, when a clone is present, reassessing for expansion of the PNH clone over time and immediately prior to BMT is recommended because terminal complement component activation blockade (eg, C5 to C5a conversion by eculizumab) can be used to avoid thrombotic events at the start of conditioning.[33]

Bone marrow studies. A bone marrow biopsy and aspirate often showing the characteristic "empty" marrow on histology are a prerequisite for a diagnosis of AA. BMF arising from an underlying IBMFD can have distinguishing syndrome-specific features in the marrow,[12,34,35] or it can appear indistinct from acquired AA, as in cases 3 and 4 below. In the case of patchy marrow cellularity, a very low CD34 count quantified by flow cytometry of bone marrow aspirates or immunohistochemistry on the core biopsy favors a diagnosis of AA.[17,36] It is not uncommon in AA for cytogenetics to have a nondiagnostic result due to insufficient metaphases. In that case, fluorescence in situ hybridization (FISH) studies can be added to evaluate for the common aberrations (ie, chromosome 5 and 7 abnormalities) that might be diagnostic for MDS.[37-39] Most often, AA patients have normal cytogenetics; however, some cytogenetic abnormalities seen in AA are not considered adverse or indicative of MDS (in the absence of dysplasia). These include del13q, trisomy 8, and loss of heterozygosity of short arm of chromosome 6, among others.[40] Single-nucleotide polymorphism microarrays can also be used as an alternative karyotyping tool in these cases to detect most cytogenetic aberrations.[41-43]

AA is further classified clinically by the severity of the depression of the peripheral blood counts. SAA is defined by a decrease in blood counts involving ≥2 hematopoietic lineages (ie, absolute reticulocyte count $<60 \times 10^9$/L, absolute neutrophil count $<0.5 \times 10^9$/L, or platelet count $<20 \times 10^9$/L) and bone marrow hypocellularity (<25% of the normal cellularity). Very severe AA has an absolute neutrophil count $<0.2 \times 10^9$/L, whereas moderate AA is characterized by the depression of blood counts not fulfilling the definition of severe disease.[44,45] This classification system is closely tied to

Table 1. Standard work-up in the evaluation of a patient with suspected AA being considered for transplantation

Test	At diagnosis	Purpose	Notes	References[15,19]
Medical and family history assessment[61,64,68,81,*]				
Heme Long-standing cytopenia(s) or macrocytosis? Unexplained cytopenia(s) or macrocytosis, AA, MDS, or AML in 1 or more close relative(s)?	X	Could suggest IBMFD if chronic cytopenias at early age are found in the patient or cytopenias or blood cancers cluster in the family		61,68,80
Developmental Short stature, physical anomalies (especially thumb/radial ray, cardiac, or renal)?	X	FA, STS, DBA, or thrombocytopenia-absent radius		
Immunologic/infectious disease Severe, recurrent, or atypical infections (eg, mycobacterial, viral, fungal)?	X	Could suggest GATA2 or other primary immunodeficiency	If present, consider referral to immunology and immunocompromised infectious disease specialists	
Dermatologic Gray hair prior to 25? Leukoplakia or nail dysplasia? Reticulated skin pigmentation, café au lait macules?	X	Could suggest STS; Cafe au lait macules could suggest FA		
Pulmonary Pulmonary fibrosis and/or early-onset emphysema, pulmonary alveolar proteinosis, fungal or mycobacterial infection?	X	Could suggest STS or GATA2		
Abdominal Pancreatic insufficiency, liver fibrosis, renal anomaly or malplacement?	X	Could suggest SDS, STS, FA, respectively		
Neurologic Ataxia, nystagmus? Cognitive dysfunction?	X	Could suggest SAMD9L, multiple IBMFD, respectively		
Cardiac/lymphatic Cardiac anomaly, lymphedema?	X	Could suggest multiple IBMFD, GATA2, respectively		
Oncologic H&N or anogenital SCC, early-onset GI cancers or multiple cancers in patient or close relatives?	X	Could suggest STS, Li Fraumeni, other hereditary cancer syndromes		
Laboratory studies on peripheral blood				
Complete blood count with differential and blood smear review	X	Assess severity of cytopenias and for alternative etiologies	Monocytopenia should prompt consideration of GATA2-deficiency syndrome. However, many severely neutropenic SAA and VSAA patients are also monocytopenic, so it is not specific to GATA2 deficiency.	81
Reticulocyte count	X	Assess marrow response to anemia and use in AA severity assessment		44
Percentage of hemoglobin F	X	Elevated levels can indicate that an IBMFD may be present.		82
Vitamin B12, folate, copper, zinc, ferritin	X	Rule out vitamin or mineral deficiencies as cause or contributor to cytopenias		
Hepatitis A/B/C, HIV, EBV, parvovirus, and CMV serologies	X	Rule out infectious disease contributors to cytopenias and identify comanagement needs during treatment and transplant		
LDH, haptoglobin	X	Rule out a hemolysis component to anemia		

AML, acute myeloid leukemia; ANA, anti-nuclear antibodies; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GI, gastrointestinal; GPI, glycosylphosphatidylinositol; H&N, head and neck; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; NK, natural killer; SCC, squamous cell carcinoma; VSAA, very severe AA.
*These questions are intended to evaluate for signs and symptoms of the known IBMFD. This is not an all-encompassing list, but it does include the more common features that should prompt a more detailed evaluation for an IBMFD, additional specialized screening tests if indicated, and strong consideration of germline genetic testing via a panel approach per below.

Table 1. (continued)

Test	At diagnosis	Purpose	Notes	References[15,19]
PNH clone	X	Assess presence or absence of GPI-anchored protein expression	Clone sizes vary (in monocytes and granulocytes) in acquired SAA, but larger clone sizes (>10%) are often associated with response to IST.	26,29,83
ANA with reflex to anti–double-stranded DNA if positive	X	Assess for systemic lupus erythematosus		
Immunoglobulins A, G, and M quantification	X	Assess for additional immune deficits	Severe deficits and/or combination with B-, T-, or NK cell deficits may warrant work-up for GATA2-deficiency syndrome and/or autoimmunity/primary immunodeficiency disorders. Consider referral to immunologist.	81,84,85
Flow cytometry to assess B, T, and NK cell numbers	X	Assess for additional immune deficits	Severe or combination B-, T-, and NK cell deficits may warrant work-up for GATA2 deficiency syndrome and/or autoimmunity/primary immunodeficiency disorders. Consider referral to immunologist.	81,84,85
Telomere length measurement of peripheral blood lymphocytes by Flow-FISH	X (age ≤40 y or those proceeding to BMT)	Determine lymphocyte telomere length by Flow-FISH; if less than first percentile, patient may have an STS and be at risk for increased transplant-related toxicity with standard preparative regimens, and an STS-specific regimen should be considered.	Lymphocyte telomere lengths less than first percentile are highly sensitive and specific for an STS diagnosis in young patients with AA. **Caveats:** Other IBMFDs can have lengths less than or equal to the first percentile; individuals with pathogenic telomere gene mutations can have lengths in the normal range, usually between the first and tenth percentiles; and short telomeres can be seen in acquired AA with reduced stem cell reserve.	66-68,86
Chromosome breakage analysis on peripheral blood	X (age ≤40 y or those proceeding to BMT)	Evaluate for FA; if test is positive and consistent with a diagnosis of FA, patient is at increased risk for transplant-related toxicity, and an FA-specific regimen should be used.	If results are normal but clinical suspicion remains high, this test can be performed on cultured skin fibroblasts to rule out a false negative in the peripheral blood.	87
Bone marrow aspirate and biopsy	X	Assess cellularity, iron stores, and reticulin fibrosis and rule out other marrow pathologies.		
Conventional karyotyping	X		Most often AA patients have normal cytogenetics but there are some cytogenetic abnormalities seen in AA that are not considered adverse or indicative of MDS (in the absence of dysplasia). These include del13q, trisomy 8, loss of heterozygosity of short arm of chromosome 6, among others. If <20 metaphases are obtained, perform an MDS FISH panel; a microarray can be considered as an alternative in these cases. Monosomy 7, especially in young patients, increases suspicion for an IBMFD.	40,88
HLA typing	X	Determine HLA profile for stem cell donor search.		

AML, acute myeloid leukemia; ANA, anti-nuclear antibodies; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GI, gastrointestinal; GPI, glycosylphosphatidylinositol; H&N, head and neck; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; NK, natural killer; SCC, squamous cell carcinoma; VSAA, very severe AA.

*These questions are intended to evaluate for signs and symptoms of the known IBMFD. This is not an all-encompassing list, but it does include the more common features that should prompt a more detailed evaluation for an IBMFD, additional specialized screening tests if indicated, and strong consideration of germline genetic testing via a panel approach per below.

Table 1. (continued)

Test	At diagnosis	Purpose	Notes	References[15,19]
Myeloid malignancy gene sequencing from peripheral blood or bone marrow	Strongly consider if any concern for possible hypoplastic MDS	Evaluate for mutations in genes recurrently mutated in AA (eg, *PIGA*, *BCOR*, *BCORL1*) and/or MDS (eg, epigenetic mutations, *TP53*).	Identification of mutations should not necessarily be used as a discriminating tool between AA and hypoplastic MDS, because most MDS-associated mutations (including *BCOR*, *BCORL1*, and epigenetic mutations, such as *DNMT3A*, *TET2*, *ASXL1*, and others) are seen in AA, MDS, and aging-related clonal hematopoiesis and have poor discriminating power for AA or MDS in this context. Acquired mutation panel may identify a subset of AA likely to progress to MDS/AML. A portion of the genes on these acquired panels overlap with inherited marrow failure gene panels but they should not be considered adequate testing for IBMFD as a stand-alone test.	52,56,89
Inherited BMF gene panel sequencing	Patients aged ≤40 y or if clinical picture or screening tests warrant	Evaluate for multiple IBMFDs at once. Overlapping phenotypes and lack of physical features and family history in a substantial subset of those with IBMFD makes universal testing of young patients warranted. Tissue source for this testing should ideally be cultured skin fibroblasts (see text for discussion).	Yield in adult patients with AA aged 18-40 y is 5-15%. Yield increases with the presence of phenotypic features or family history or in cases where hypoplastic MDS is a consideration (eg, monosomy 7).	13,14
Erythrocyte adenosine deaminase	If clinical picture warrants	Screen for Diamond-Blackfan anemia		90
Serum pancreatic isoamylase (age > 3 y)	If clinical picture warrants	Screen for pancreatic insufficiency suggestive of Shwachman-Diamond syndrome		91
Fecal elastase	If clinical picture warrants	Screen for pancreatic insufficiency suggestive of Shwachman-Diamond syndrome		91

AML, acute myeloid leukemia; ANA, anti-nuclear antibodies; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GI, gastrointestinal; GPI, glycosylphosphatidylinositol; H&N, head and neck; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; NK, natural killer; SCC, squamous cell carcinoma; VSAA, very severe AA.

*These questions are intended to evaluate for signs and symptoms of the known IBMFD. This is not an all-encompassing list, but it does include the more common features that should prompt a more detailed evaluation for an IBMFD, additional specialized screening tests if indicated, and strong consideration of germline genetic testing via a panel approach per below.

prognosis and is often used to triage patients meeting Camitta criteria for severe or very severe disease to a therapeutic path including early BMT.[2]

In patients with cytopenias, it is no longer standard practice to do bilateral biopsies for initial AA diagnosis. However, repeat marrow examinations over time may be necessary to establish the diagnosis. The interval between marrows should be informed by the severity of cytopenias in patients meeting criteria for moderate disease at the time of diagnosis, any major changes in clinical status or blood counts, and whether the etiology is AA or BMF due to an underlying IBMFD.

Patient 1 was ultimately diagnosed with acquired SAA in the setting of compelling personal/family autoimmune history and presence of the PNH clone. She went on to receive IST with antithymocyte globulin and cyclosporine. She did have immune-responsive disease with improvement in blood counts and transfusion independence by 5 months posttreatment, which has lasted for >4 years and allowed for weaning of her cyclosporine over time.

Patient 2

A 63-year-old male has only a history of type 2 diabetes, hypercholesterolemia, and hypertension. He suffered nosebleeds on 3 consecutive days and went to the emergency department where he presented with a platelet count of 9×10^9/L, hemoglobin of 7.9 g/dL, total white blood cell count of 1.6×10^9/L, and an absolute neutrophil count of 0.9×10^9/L. A bone marrow biopsy was hypocellular at 5% cellularity overall with scattered clusters of fat cells with rare myeloid cells. Karyotype was 46,XY, and a targeted next-generation sequencing panel test revealed a pathogenic BCOR variant at a variant allele frequency of 5.3% and a DNMT3A mutation detected below the lower limit of standard reporting (<2%). His local hematologist wondered whether this BCOR variant or the DNMT3A raised concern for a diagnosis of hypocellular MDS vs AA.

Molecular diagnostic studies in AA diagnosis. For the patient and their treating clinicians, it is important to use available

techniques to ensure adequate explanation of the biology of the marrow failure process. This includes thorough attempts to differentiate AA from hypoplastic MDS and unique subtypes of IBMFD where feasible, because phenotypic presentations of these diseases might be indiscernible.[46] To help with this diagnostic challenge, the work-up of pancytopenia (even suspected AA) now often incorporates next-generation sequencing of targeted panels of genes commonly acquiring pathogenic variants in various myeloid malignancies.[47] Multiple studies have applied these techniques to investigate the mutational spectrum in AA; the frequencies of somatic mutations range from 5% to >70% in some series.[48-53] Mutations in *DNMT3A*, *BCOR*, *BCORL1*, and *ASXL1* are seen in AA, MDS, aging populations, and inherited marrow failure disorders.[54] There is considerable overlap between the mutational spectrum in AA and MDS, with mutations in *BCOR/BCORL1*, *DNMT3A*, and *ASXL1* common to both disease states[52] at similar variable allele frequencies (VAFs). For example, the prevalence of *BCOR* and *BCORL1* mutations is estimated at 5% in MDS[55] vs 4%[49] or 7% to 10% in AA.[52] Thus, mutations in these genes do not have the discriminating power to help with the diagnosis of hypoplastic MDS vs AA. However, mutational profiles more typical of AA included smaller clonal populations represented by VAFs < 10%.[52] Mutations in *DNMT3A* and *ASXL1* are frequent in AA and MDS as well and may increase in clonal burden over in time.[56] A baseline panel at diagnosis also allows assessment of new mutations acquired over tim with changes in disease status (eg, relapse).

Currently, the prognostic implications of somatic mutations in AA are also unclear, because no prospective study has evaluated the outcomes of newly presenting AA patients with adequate patient numbers and follow-up. However, retrospective[49,50] and prospective studies[52,54] suggest that detection of certain somatic mutations by next-generation sequencing (ie, *ASXL1*, *DNMT3A*, and *BCOR*) can enable risk stratification of patients with AA at high risk for progressing to a myeloid neoplasm. Yoshizato et al[52] did not find any significant difference in overall survival and progression-free survival (evolution to MDS) between all patients with somatic mutations and those without mutations. When analyzed by individual genes, only *BCOR/BCORL1* were independently associated with an improved therapeutic response. In the same study, the investigators also found (by exploratory artificial learning analysis) an improved prognosis for AA patients with a favorable set of mutations (*BCOR/BCORL1* and *PIGA*); another aggregated unfavorable set of mutations was associated with worse outcomes. Notably, none of the individual genes (including *DNMT3A* and *ASXL1*) was found to be linked to poor outcomes on their own. Other series have also attempted to explain risk in AA, based on various mutations, without clear therapeutic recommendation or necessary action.[56-59] Detection of a less favorable mutation profile at diagnosis may push certain patients toward upfront related or alternative donor BMT over IST where feasible; however, it is important to note that detection of a somatic mutation by itself is not a sufficient indication for BMT. A variety of other parameters, such as peripheral blood counts, VAF, and karyotype, are crucial to take into account when considering the preferred treatment approach.

Patient 2 ultimately went on to receive a related donor transplant for his treatment-naive SAA on a clinical protocol and has a normal hemogram and full donor chimerism without further evidence of clonal hematopoiesis 2 years post-BMT.

Patient 3

Patient 3 is a 16 year-old male with no significant health history who was brought to the pediatrician by his mother. She had noted he was more listless and less engaged over the prior few weeks. On examination, he was pale with ecchymoses throughout his lower extremities. His physical examination was otherwise unremarkable, with appropriate stature for age and no facial or limb abnormalities. His total white blood cell count was 0.4×10^9/L with 0% neutrophils, his hemoglobin was 7.7 g/dL, and his platelet count was 8×10^9/L at presentation. A peripheral blood smear showed a paucity of cells and no circulating malignant cells. The pediatrician noted "irregular bloodwork" a few times in his youth. He had not seen a provider since age 11 years. There was no significant family history.

Patient 4

Patient 4 is 19 year-old otherwise healthy male who was seen in the emergency room for intense migraines, fatigue, and several weeks of gingival bleeding. He had had a significant upper respiratory infection 6 to 8 weeks prior. He had wet purpura in his mouth, thrush, and ecchymoses throughout his lower extremities. His physical examination was otherwise unremarkable. His total white blood cell count was 1.2×10^9/L with only 24% neutrophils, his hemoglobin was 5.7 g/dL, and his platelet count was 2×10^9/L at presentation. A peripheral blood smear showed a paucity of cells and no circulating malignant cells. His family history was unrevealing.

Patients 3 and 4 were referred to hematology/oncology for management. Both had urgent bone marrow evaluations that showed marked hypocellularity (<5%) and no evidence of infiltrating tumor. Karyotype was 46,XY in patient 4 and noninformative in patient 3, but FISH was negative for common MDS abnormalities in both. Patient 3 had an elevated fetal hemoglobin level at 9%; patient 4 did not have this checked.

Clinical and family history. The clinical and family history can be useful for differentiating AA from other BMF states and to identify those with an IBMFD. This is imperative in patients of all ages but perhaps is more acute in pediatric and young adult assessments. Table 1 outlines medical and family history questions that should be asked to screen for the more frequent signs and symptoms of the known IBMFD. Positive history and physical combinations that are suggestive of an IBMFD should warrant a dedicated work-up by an expert clinician or genetic counselor. For example, the combination of AA with a personal or family history of pulmonary fibrosis is strongly suggestive of an underlying short telomere syndrome (STS) with an expected positive yield of genetic evaluation.[60,61] In contrast, a personal history of extensive cutaneous or anogenital warts and lymphedema in a patient with AA would greatly increase suspicion for GATA2 deficiency syndrome.[62] Several excellent reviews summarize patterns of medical and family history in the known IBMFD.[13,19,63-65] However, it is important to recognize that the usual signs, symptoms, and family history of an IBMFD are absent in up to 40% of cases.[13] Thus, a high index of suspicion is necessary. Further, given that the expected frequencies of an IBMFD among children and young adults aged ≤40 years with AA are 25% and 5% to 15%, respectively, and syndrome-specific treatment and BMT regimens are available for many of these disorders, screening for these disorders per the Laboratory and Molecular Diagnostics Studies sections in Table 1 is now warranted for all patients with AA aged 40 years and younger. An upper age cutoff remains challenging,

because IBMFDs have been diagnosed in individuals into their sixth decade of life. Clinical judgment is necessary until data exist regarding IBMFD frequency in older adults without other syndromic features. Patients with a high suspicion for or with a documented IBMFD likely do not warrant treatment with IST; thus, early diagnosis is imperative for management decisions.

Careful attention to findings that may suggest the presence of an IBMFD, such as severe monocytopenia (eg, GATA2 deficiency, although not specific) or elevated hemoglobin F (seen in multiple IBMFDs) is necessary. Screening for the presence of an STS via peripheral blood lymphocyte telomere length measurement by Clinical Laboratory Improvement Amendments–certified flow cytometry and FISH (Flow-FISH) and for Fanconi anemia (FA) by chromosome breakage studies on peripheral blood lymphocytes is recommended for all adults with AA aged 40 years and younger or any adults with history findings that are suggestive of either of these disorders. Lymphocyte telomere length shorter than the first percentile in the context of AA is highly sensitive and specific for a diagnosis of STS[66]; however, it is possible for individuals with an STS to have lymphocyte telomere lengths within the low normal range (most often in the first to tenth percentile), especially those older than age 40 years.[66-68] Rarely, other IBMFDs have lymphocyte telomere lengths at or just below the first percentile.[66] Thus, all patients with a lymphocyte telomere length at or below the first percentile and those with a personal and family history suggestive of an STS, regardless of the telomere length, should have a genetic evaluation as suggested in Figure 1. Polymerase chain reaction–based quantification of telomere length is not a reliable measure and should not be used in clinical settings.[68,69] There are reports of patients with acquired AA with short telomeres; however, these may not have used Flow-FISH methodology and may not be less than the first percentile.[68,70,71] Patients with radial chromosomes or increased chromosome breaks on diepoxybutane (DEB) and/or mitomycin C exposure in standard chromosome-breakage assays that are within the range typical for FA warrant genetic evaluation for a molecular diagnosis. In these scenarios, it is critical that a genetic evaluation is completed prior to donor and preparative regimen selection in patients considering BMT to ensure donor and recipient safety.

Approach to evaluation of patients with AA for an IBMFD. Our practice is to use a systematic approach to evaluate a patient with AA (Figure 1). Some investigators advocate a stepwise methodology that allows for initial results to come in prior to initiation of a more extensive work-up. However, this approach can be less efficient, even if resource conscience, and may miss those without a classical IBMFD clinical presentation or family history, as is expected for 40% of cases ultimately diagnosed with an IBMFD. It should be noted that estimates of IBMFD prevalence are highly dependent on the specific patient populations studied. The prevalence of IBMFD in young patients referred for genetic testing for clinical suspicion of IBMFD is higher compared with the prevalence in older adults with previously normal blood counts and newly diagnosed AA.[9,12,63]

Using the approach outlined in Figure 1, all patients with AA would undergo the same clinical/family history, basic laboratory tests, and bone marrow studies outlined in Table 1. Patients aged ≤40 years

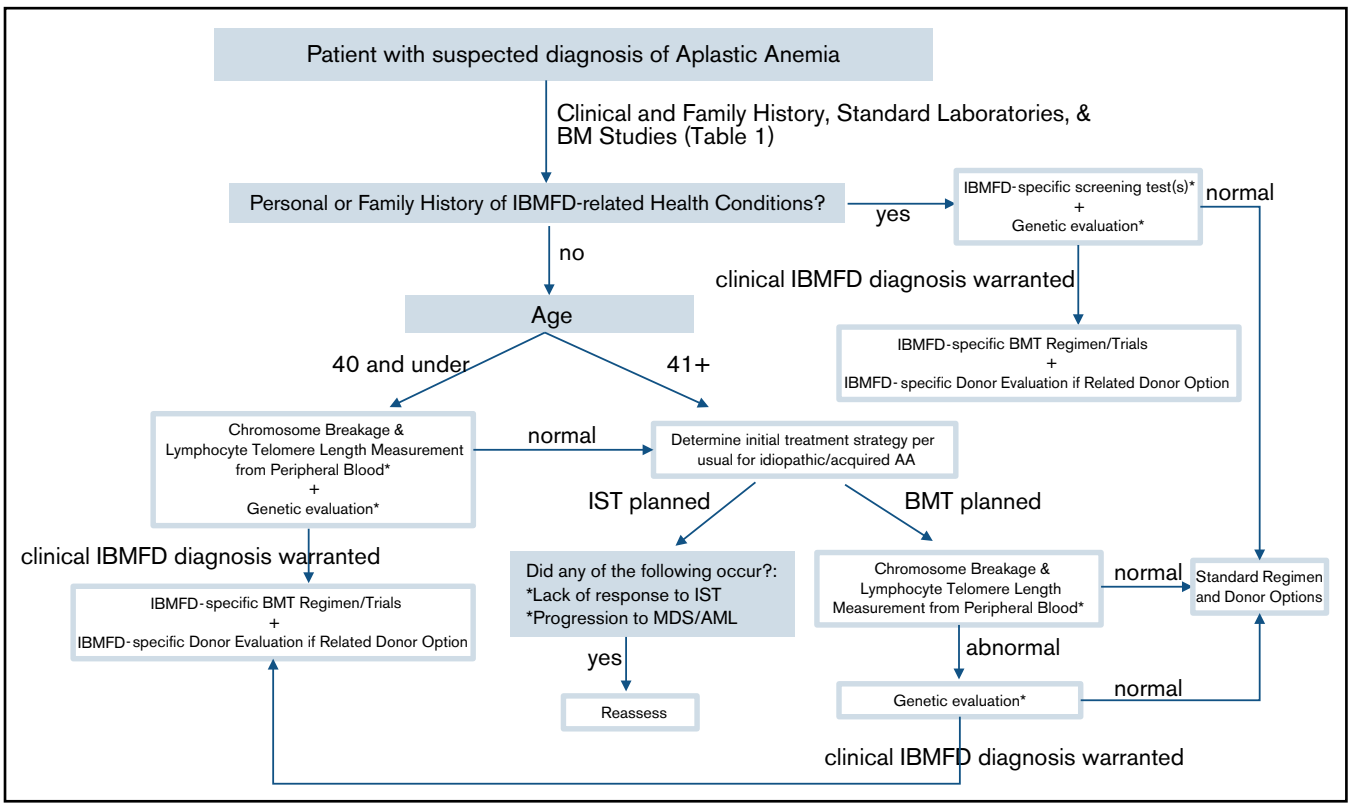

Figure 1. Workflow for AA evaluation. *These tests can be requested urgently with turnaround times ≤2 weeks if needed for the clinical scenario. If there is urgency, an all-at-once approach with screening tests + genetic testing is preferred. AML, acute myeloid leukemia; BM, bone marrow.

or those proceeding to BMT as initial therapy would also have peripheral blood lymphocyte telomere length measurement by Flow-FISH[68] and chromosome breakage with DEB to rule out STS and FA, respectively. If the clinical scenario warrants, these tests should be requested immediately with expected 10- to 14-day turnaround times. The benefit of this approach is that it quickly identifies patients at risk for STS and FA who are at high risk for increased toxicity, as described above. However, it is important to note that, although a lymphocyte telomere length shorter than the first percentile is highly sensitive and specific for a STS diagnosis in young patients with AA, other IBMFDs can be associated with lengths falling within this range, making a broad genetic panel recommended over genes of telomere maintenance alone. Further, lymphocyte telomere lengths in adults, especially those older than age 40 years with a genetically confirmed STS, can be well within the normal range, usually between the first and tenth percentiles for age. These caveats can make this a challenging stand-alone screening test, especially for older adults.

If a patient has findings on any of these tests or screening questions that suggest an IBMFD, an IBMFD-specific genetic evaluation at a pace that is appropriate for the clinical scenario should be undertaken. For example, if the patient has very severe AA and needs treatment initiation immediately, genetic evaluation and its associated testing should be requested urgently. This evaluation may be performed by a geneticist, genetic counselor, hematologist, or other clinician who has experience identifying IBMFD-related signs and symptoms in the clinical history and examination, can aid in family member evaluations if needed, and is able to help coordinate and interpret genetic findings from IBMFD screening and genetic tests. Telemedicine consultations may help if access is limited locally.

Genetic testing in patients with AA requires consideration of the tissue to be used for testing, as well as the genes to be tested. There has not been a head-to-head comparison of the use of peripheral blood vs cultured skin fibroblasts for genetic testing in this scenario. However, in many expert centers, cultured skin fibroblasts are preferred because it is known that acquired events can obscure detection of the germline pathogenic variant(s) in patients with IBMFD.[63] For example, a somatic reversion event occurring in a population of hematopoietic precursors can completely correct 1 of the 2 pathogenic germline variants in patients with FA, masking the diagnosis if testing is done using peripheral blood.[72-74] SAMD9/SAMD9L, in which the germline mutation–containing allele is lost as part of acquired monosomy 7, is relevant as well.[75] In addition, the finding of somatic variants suggestive of clonal hematopoiesis (see *Molecular diagnostic studies in AA diagnosis*) in the blood of patients with AA can lead to confusion if they overlap with genes causative of IBMFD (eg, *RUNX1*). With multiple commercial laboratories now offering to ship the appropriate media to the clinic, culture the skin, and perform the genetic testing all as part of a single test, lack of access should be less of an issue. Advanced planning to have the correct supplies and someone to do the skin biopsy when the patient is going to be present in the clinic is necessary. Timely referral to an academic center may aid in access.

Except for rare situations in which there is a known pathogenic variant running in a patient's family or the patient's clinical picture is highly suggestive of a genetic disorder for which there is a single causative gene (eg, GATA2 deficiency syndrome), a gene panel–based approach will be necessary. The number of genes known in the field to cause BMF in the setting of an IBMFD continues to increase. Even within each specific IBMFD, new genes are increasingly identified to explain the pathobiology of disease, and we continue to search for more in patients in whom a genetic cause remained elusive.[63] IBMFDs that have been identified in patients with AA include STS, FA, Schwachman-Diamond syndrome, Diamond-Blackfan anemia, GATA2-deficiency syndrome, as well as newer syndromes. Other hereditary syndromes less commonly thought of as contributors to BMF, such as Li-Fraumeni syndrome (*TP53*) and familial platelet disorder (*RUNX1*), have also been observed.[13,63] Therefore using a panel specifically designed for detection of an IBMFD (through an experienced laboratory) as well as assurances of dedicated testing for any genes of particular relevance to your patient remains of key importance. Attention to the details of the testing ordered is vital because false reassurance could come from a test that does not cover the relevant genes for the inherited syndrome; consultation with a genetic counselor, IBMFD expert, or molecular pathologist prior to testing should be considered to ensure selection of the ideal test, as well as after results are received to aid appropriate interpretation.

Rationale for screening to differentiate AA from BMF due to an IBMFD. The rapid pace of discovery and lack of uniform consensus guidelines on when and how to test for IBMFDs in adult AA populations have led to controversy and confusion about the need for screening. We believe that there are many benefits of real-time differentiation of AA from a BMF due to an IBMFD. First and foremost are the lack of expected benefit and the potential for harm from the use of IST in patients with an IBMFD, thus making a thorough diagnosis imperative. Additionally, some IBMFDs are characterized by extrahematopoietic manifestations that can contribute to significant morbidity and for which screening can change management (Table 1). Recognition of the possibility of these multiorgan system implications and treatment interactions is only the first step. Subspecialist referrals and engagement of additional resources should follow.[76] Second, knowledge of the etiology provides insight into the expected natural history of the disorder and can impact interpretation of bone marrow findings. For example, dysplasia may be part of the underlying IBMFD-altered hematopoiesis, making differentiation of BMF and hypocellular MDS in this context syndrome specific. Third and most importantly, diagnosis of an IBMFD can facilitate more appropriate therapy in an affected individual. For example, awareness of the potential for severe systemic toxicity in FA and STS alters selection of the BMT preparative regimen. Potential lack of responsiveness to immunosuppression and other noncurative therapies for BMF due to an IBMFD should prompt earlier BMT evaluation. Lastly, prompt recognition of an IBMFD in the patient with BMF allows cascade testing of at-risk relatives who may be willing to serve as a donor for matched sibling or haploidentical BMT.

Given these benefits, at a minimum, screening for STS and FA is needed in patients younger than age 40 years or those proceeding to BMT, because they are at risk for harm from the conditioning for BMT if undiagnosed.[77,78] Notably, these tests include peripheral blood lymphocyte telomere length measurement by Flow-FISH[68] and chromosome breakage with DEB, as detailed above.[79] The significant phenotypic overlap and the challenge of somatic events that may mask IBMFD diagnoses using these peripheral blood–based tests alone suggest that all AA patients aged ≤40 years and those proceeding to transplant should have a germline IBMFD genetic panel test. In several academic centers where access to this testing is readily available, this strategy is already in place.

Patient 3 was ultimately diagnosed with acquired SAA in the setting of negative DEB testing, normal telomere lengths by Flow-FISH, and a skin biopsy negative for known genes associated with IBMFDs. He underwent a matched sibling donor bone marrow transplant and is well, with normal blood counts at 5 years postprocedure.

Patient 4 had negative DEB testing and normal telomere lengths by Flow-FISH; however, a skin biopsy grown for fibroblast culture for testing of known genes associated with an IBMFD revealed biallelic mutation of the MPL gene as the presumed etiology for his SAA. He underwent an unrelated donor BMT and is well, with normal blood counts at 3 years postprocedure.

Conclusions

Efficient and accurate diagnosis of AA are critical to proper management of this disease. The severity of the illness also warrants a clear path from accurate diagnosis to the most appropriate treatment option. Germline predisposition to AA, even in teenagers and adults, is more common than previously recognized and important to diagnosis in real time prior to treatment and, especially, prior to BMT. Consistent work-up of all patients with AA, as outlined here, will facilitate high-quality care and foster research.

Authorship

Contribution: A.E.D. and J.E.C. wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Amy E. DeZern, 1650 Orleans St, CRBI Room 3M87, Baltimore, MD 21287; e-mail: adezern1@jhmi.edu.

References

1. Young NS. Aplastic anaemia. *Lancet*. 1995;346(8969):228-232.
2. Bacigalupo A. How I treat acquired aplastic anemia. *Blood*. 2017;129(11):1428-1436.
3. Alter BP. Diagnosis, genetics, and management of inherited bone marrow failure syndromes. *Hematology Am Soc Hematol Educ Program*. 2007;2007:29-39.
4. Tichelli A, Peffault de Latour R, Passweg J, et al. Long-term outcome of a randomized controlled study in patients with newly diagnosed severe aplastic anemia treated with antithymocyte globulin and cyclosporine, with or without granulocyte colony-stimulating factor: a Severe Aplastic Anemia Working Party Trial from the European Group of Blood and Marrow Transplantation. *Haematologica*. 2020;105(5):1223-1231.
5. Killick SB, Bown N, Cavenagh J, et al; British Society for Standards in Haematology. Guidelines for the diagnosis and management of adult aplastic anaemia. *Br J Haematol*. 2016;172(2):187-207.
6. Brümmendorf TH, Rufer N, Holyoake TL, et al. Telomere length dynamics in normal individuals and in patients with hematopoietic stem cell-associated disorders. *Ann N Y Acad Sci*. 2001;938(1):293-303, discussion 303-304.
7. Muramatsu H, Okuno Y, Yoshida K, et al. Clinical utility of next-generation sequencing for inherited bone marrow failure syndromes. *Genet Med*. 2017;19(7):796-802.
8. DeZern AE, Zahurak ML, Symons HJ, et al. Haploidentical BMT for severe aplastic anemia with intensive GVHD prophylaxis including posttransplant cyclophosphamide. *Blood Adv*. 2020;4(8):1770-1779.
9. Ahmed P, Chaudhry QUN, Satti TM, et al. Epidemiology of aplastic anemia: a study of 1324 cases. *Hematology*. 2020;25(1):48-54.
10. Mukherjee S, Sekeres MA. What's all the fuss about? Facts and figures about bone marrow failure and conditions. *Curr Hematol Malig Rep*. 2012;7(4):300-309.
11. Montané E, Ibáñez L, Vidal X, et al; Catalan Group for Study of Agranulocytosis and Aplastic Anemia. Epidemiology of aplastic anemia: a prospective multicenter study. *Haematologica*. 2008;93(4):518-523.
12. Kallen ME, Dulau-Florea A, Wang W, Calvo KR. Acquired and germline predisposition to bone marrow failure: diagnostic features and clinical implications. *Semin Hematol*. 2019;56(1):69-82.
13. Keel SB, Scott A, Sanchez-Bonilla M, et al. Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients. *Haematologica*. 2016;101(11):1343-1350.
14. Feurstein S, Churpek JE, Walsh T, et al. Germline variants drive myelodysplastic syndrome in young adults [published online ahead of print 28 Jan 2021]. *Leukemia*.
15. Brodsky RA, Jones RJ. Aplastic anaemia. *Lancet*. 2005;365(9471):1647-1656.
16. Gondek LP, DeZern AE. I walk the line: how to tell MDS from other bone marrow failure conditions. *Curr Hematol Malig Rep*. 2014;9(4):389-399.
17. DeZern AE, Sekeres MA. The challenging world of cytopenias: distinguishing myelodysplastic syndromes from other disorders of marrow failure. *Oncologist*. 2014;19(7):735-745.
18. Davidson A, Diamond B. Autoimmune diseases. *N Engl J Med*. 2001;345(5):340-350.
19. West AH, Churpek JE. Old and new tools in the clinical diagnosis of inherited bone marrow failure syndromes. *Hematology Am Soc Hematol Educ Program*. 2017;2017:79-87.
20. Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag added to standard immunosuppression for aplastic anemia. *N Engl J Med*. 2017;376(16):1540-1550.

21. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med*. 2011;365(5):430-438.
22. Peslak SA, Olson T, Babushok DV. Diagnosis and treatment of aplastic anemia. *Curr Treat Options Oncol*. 2017;18(12):70.
23. Hill A, DeZern AE, Kinoshita T, Brodsky RA. Paroxysmal nocturnal haemoglobinuria. *Nat Rev Dis Primers*. 2017;3(1):17028.
24. Parker CJ. Management of paroxysmal nocturnal hemoglobinuria in the era of complement inhibitory therapy. *Hematology Am Soc Hematol Educ Program*. 2011;2011:21-29.
25. Dezern AE, Borowitz MJ. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 1 - clinical utility. *Cytometry B Clin Cytom*. 2018;94(1):16-22.
26. DeZern AE, Symons HJ, Resar LS, Borowitz MJ, Armanios MY, Brodsky RA. Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes. *Eur J Haematol*. 2014;92(6):467-470.
27. Young NS, Abkowitz JL, Luzzatto L. New insights into the pathophysiology of acquired cytopenias. *Hematology Am Soc Hematol Educ Program*. 2000;2000:18-38.
28. Nakao S, Sugimori C, Yamazaki H. Clinical significance of a small population of paroxysmal nocturnal hemoglobinuria-type cells in the management of bone marrow failure. *Int J Hematol*. 2006;84(2):118-122.
29. Fattizzo B, Ireland R, Dunlop A, et al. Clinical and prognostic significance of small paroxysmal nocturnal hemoglobinuria clones in myelodysplastic syndrome and aplastic anemia [published online ahead of print 4 March 2021]. *Leukemia*.
30. Sugimori C, Chuhjo T, Feng X, et al. Minor population of CD55−CD59− blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. *Blood*. 2006;107(4):1308-1314.
31. Brodsky RA. How I treat paroxysmal nocturnal hemoglobinuria. *Blood*. 2021;137(10):1304-1309.
32. DeZern AE, Dorr D, Brodsky RA. Predictors of hemoglobin response to eculizumab therapy in paroxysmal nocturnal hemoglobinuria. *Eur J Haematol*. 2013;90(1):16-24.
33. DeZern AE, Jones RJ, Brodsky RA. Eculizumab bridging before bone marrow transplant for marrow failure disorders is safe and does not limit engraftment. *Biol Blood Marrow Transplant*. 2018;24(12):e26-e30.
34. Calvo KR, Vinh DC, Maric I, et al. Myelodysplasia in autosomal dominant and sporadic monocytopenia immunodeficiency syndrome: diagnostic features and clinical implications. *Haematologica*. 2011;96(8):1221-1225.
35. Proytcheva, M, ed. Diagnostic Pediatric Hematopathology. Cambridge, United Kingdom: Cambridge University Press, 2011:I-VI.
36. Matsui WH, Brodsky RA, Smith BD, Borowitz MJ, Jones RJ. Quantitative analysis of bone marrow CD34 cells in aplastic anemia and hypoplastic myelodysplastic syndromes. *Leukemia*. 2006;20(3):458-462.
37. Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. *Blood*. 2017;130(4):424-432.
38. Kennedy AL, Shimamura A. Genetic predisposition to MDS: clinical features and clonal evolution. *Blood*. 2019;133(10):1071-1085.
39. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia [published correction appears in *Blood*. 2016;128(3):462–463]. *Blood*. 2016;127(20):2391-2405.
40. Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. *Blood*. 2002;99(9):3129-3135.
41. Medeiros BC, Othus M, Estey EH, Fang M, Appelbaum FR. Unsuccessful diagnostic cytogenetic analysis is a poor prognostic feature in acute myeloid leukaemia. *Br J Haematol*. 2014;164(2):245-250.
42. Tiu RV, Gondek LP, O'Keefe CL, et al. New lesions detected by single nucleotide polymorphism array-based chromosomal analysis have important clinical impact in acute myeloid leukemia. *J Clin Oncol*. 2009;27(31):5219-5226.
43. Dougherty MJ, Wilmoth DM, Tooke LS, et al. Implementation of high resolution single nucleotide polymorphism array analysis as a clinical test for patients with hematologic malignancies. *Cancer Genet*. 2011;204(1):26-38.
44. Camitta BM, Rappeport JM, Parkman R, Nathan DG. Selection of patients for bone marrow transplantation in severe aplastic anemia. *Blood*. 1975;45(3):355-363.
45. Bacigalupo A, Hows J, Gluckman E, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. *Br J Haematol*. 1988;70(2):177-182.
46. Stanley N, Olson TS, Babushok DV. Recent advances in understanding clonal haematopoiesis in aplastic anaemia. *Br J Haematol*. 2017;177(4):509-525.
47. Steensma DP. How I use molecular genetic tests to evaluate patients who have or may have myelodysplastic syndromes[published correction appears in *Blood*. 2018;132(22):2419]. *Blood*. 2018;132(16):1657-1663.
48. Lane AA, Odejide O, Kopp N, et al. Low frequency clonal mutations recoverable by deep sequencing in patients with aplastic anemia. *Leukemia*. 2013;27(4):968-971.
49. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood*. 2014;124(17):2698-2704.
50. Babushok DV, Perdigones N, Perin JC, et al. Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. *Cancer Genet*. 2015;208(4):115-128.

51. Huang J, Ge M, Lu S, et al. Mutations of ASXL1 and TET2 in aplastic anemia. *Haematologica*. 2015;100(5):e172-e175.
52. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. *N Engl J Med*. 2015;373(1):35-47.
53. Heuser M, Schlarmann C, Dobbernack V, et al. Genetic characterization of acquired aplastic anemia by targeted sequencing. *Haematologica*. 2014;99(9):e165-e167.
54. Malcovati L, Gallì A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. *Blood*. 2017;129(25):3371-3378.
55. Damm F, Chesnais V, Nagata Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. *Blood*. 2013;122(18):3169-3177.
56. Patel BA, Ghannam J, Groarke EM, et al. Detectable mutations precede late myeloid neoplasia in aplastic anemia. *Haematologica*. 2021;106(2):647-650.
57. Dumitriu B, Feng X, Townsley DM, et al. Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. *Blood*. 2015;125(4):706-709.
58. Negoro E, Nagata Y, Clemente MJ, et al. Origins of myelodysplastic syndromes after aplastic anemia. *Blood*. 2017;130(17):1953-1957.
59. Sun L, Babushok DV. Secondary myelodysplastic syndrome and leukemia in acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria. *Blood*. 2020;136(1):36-49.
60. Parry EM, Alder JK, Qi X, Chen JJ, Armanios M. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase [published correction appears in *Blood*. 2016;127(14):1837]. *Blood*. 2011;117(21):5607-5611.
61. Feurstein S, Adegunsoye A, Mojsilovic D, et al. Telomere biology disorder prevalence and phenotypes in adults with familial hematologic and/or pulmonary presentations. *Blood Adv*. 2020;4(19):4873-4886.
62. Hsu AP, Johnson KD, Falcone EL, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood*. 2013;121(19):3830-3837, S1-S7.
63. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood*. 2018;131(7):717-732.
64. DiNardo CD, Bannon SA, Routbort M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the Hereditary Hematologic Malignancy Clinic (HHMC). *Clin Lymphoma Myeloma Leuk*. 2016;16(7):417-428.e2.
65. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood*. 2015;126(22):2484-2490.
66. Alter BP, Giri N, Savage SA, Rosenberg PS. Telomere length in inherited bone marrow failure syndromes. *Haematologica*. 2015;100(1):49-54.
67. Alter BP, Rosenberg PS, Giri N, Baerlocher GM, Lansdorp PM, Savage SA. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. *Haematologica*. 2012;97(3):353-359.
68. Alder JK, Hanumanthu VS, Strong MA, et al. Diagnostic utility of telomere length testing in a hospital-based setting [published correction appears in *Proc Natl Acad Sci USA*. 2018;115(18):E4312]. *Proc Natl Acad Sci USA*. 2018;115(10):E2358-E2365.
69. Martin-Ruiz CM, Baird D, Roger L, et al. Reproducibility of telomere length assessment–an international collaborative study. *Int J Epidemiol*. 2015;44(5):1749-1754.
70. Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC. Progressive telomere shortening in aplastic anemia. *Blood*. 1998;91(10):3582-3592.
71. Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT, Young NS. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. *JAMA*. 2010;304(12):1358-1364.
72. Jongmans MC, Verwiel ET, Heijdra Y, et al. Revertant somatic mosaicism by mitotic recombination in dyskeratosis congenita. *Am J Hum Genet*. 2012;90(3):426-433.
73. Jongmans MCJ, Diets IJ, Quarello P, Garelli E, Kuiper RP, Pfundt R. Somatic reversion events point towards *RPL4* as a novel disease gene in a condition resembling Diamond-Blackfan anemia. *Haematologica*. 2018;103(12):e607-e609.
74. Soulier J, Leblanc T, Larghero J, et al. Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. *Blood*. 2005;105(3):1329-1336.
75. Noy-Lotan S, Krasnov T, Dgany O, et al. Incorporation of somatic panels for the detection of haematopoietic transformation in children and young adults with leukaemia predisposition syndromes and with acquired cytopenias. *Br J Haematol*. 2021;193(3):570-580.
76. Clifford M, Bannon S, Bednar EM, et al. Clinical applicability of proposed algorithm for identifying individuals at risk for hereditary hematologic malignancies. *Leuk Lymphoma*. 2019;60(12):3020-3027.
77. Agarwal S. Evaluation and management of hematopoietic failure in dyskeratosis congenita. *Hematol Oncol Clin North Am*. 2018;32(4):669-685.
78. Wang Y, McReynolds LJ, Dagnall C, et al. Pre-transplant short telomeres are associated with high mortality risk after unrelated donor haematopoietic cell transplant for severe aplastic anaemia. *Br J Haematol*. 2020;188(2):309-316.
79. Auerbach AD. Diagnosis of Fanconi anemia by diepoxybutane analysis. *Curr Protoc Hum Genet*. 2003;Jul; Chapter 8:Unit 8.7.
80. Schratz KE, Haley L, Danoff SK, et al. Cancer spectrum and outcomes in the Mendelian short telomere syndromes. *Blood*. 2020;135(22):1946-1956.

81. Ganapathi KA, Townsley DM, Hsu AP, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood*. 2015;125(1):56-70.

82. Alter BP, Rosenberg PS, Day T, et al. Genetic regulation of fetal haemoglobin in inherited bone marrow failure syndromes. *Br J Haematol*. 2013;162(4):542-546.

83. Pu JJ, Mukhina G, Wang H, Savage WJ, Brodsky RA. Natural history of paroxysmal nocturnal hemoglobinuria clones in patients presenting as aplastic anemia. *Eur J Haematol*. 2011;87(1):37-45.

84. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood*. 2014;123(6):809-821.

85. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. *Br J Haematol*. 2015;169(2):173-187.

86. Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. *Blood*. 2014;124(18):2775-2783.

87. Fargo JH, Rochowski A, Giri N, Savage SA, Olson SB, Alter BP. Comparison of chromosome breakage in non-mosaic and mosaic patients with Fanconi anemia, relatives, and patients with other inherited bone marrow failure syndromes. *Cytogenet Genome Res*. 2014;144(1):15-27.

88. Afable MG II, Wlodarski M, Makishima H, et al. SNP array-based karyotyping: differences and similarities between aplastic anemia and hypocellular myelodysplastic syndromes. *Blood*. 2011;117(25):6876-6884.

89. Bono E, McLornan D, Travaglino E, et al. Clinical, histopathological and molecular characterization of hypoplastic myelodysplastic syndrome. *Leukemia*. 2019;33(10):2495-2505.

90. Vlachos A, Ball S, Dahl N, et al; Participants of Sixth Annual Daniella Maria Arturi International Consensus Conference. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol*. 2008;142(6):859-876.

91. Burroughs L, Woolfrey A, Shimamura A. Shwachman-Diamond syndrome: a review of the clinical presentation, molecular pathogenesis, diagnosis, and treatment. *Hematol Oncol Clin North Am*. 2009;23(2):233-248.