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REVIEW ARTICLE

Impact of clonal hematopoiesis on outcomes in patients with aplastic anemia

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Abstract

Over the years, not only have the T-cell mediated immune mechanisms of aplastic anemia (AA) involved in AA development started to become better understood, but there is now also a better understanding of the roles played by somatic mutations, cytogenetic abnormalities and defective telomerase functions and other genetically-related factors.

Somatic gene mutations suggestive of clonal hematopoiesis are detected in approximately one third of patients with AA. Recent studies have suggested that some of these may predict a better response to immunosuppressive therapy, whereas others indicate poorer outcomes with higher risks of clonal evolution to myelodysplastic syndrome or acute myeloid leukemia, and that therefore better results may be obtained based on allogeneic stem cell transplantation. Furthermore, recent advances in molecular techniques may be useful in differentiating aplastic anemia from hypocellular myelodysplastic syndrome and other clonal hematopoiesises of indeterminate potential. All of these are summarized in this review which includes further insights into treatment personalization based on the molecular pathogenesis of AA.

Key words: aplastic anemia, clonal hematopoiesis, outcomes, allogeneic stem cell transplantation

Introduction

Aplastic anemia (AA) is a rare form of bone marrow failure caused by autoimmune destruction of hematopoietic progenitor stem cells with a clinical picture dominated by pancytopenia [1, 2].

For many years, it was thought to be based solely on the response of T-cell mediated immune mechanisms to toxic agents, including cytotoxic drugs, some medications, irradiation, toxins or infections such as viruses [3, 4]. In the majority of cases, some genetic abnormalities are also relevant. In all cases, an extensive differential diagnostic work-up should be performed (Table I) to exclude other pancytopenia causes (Table II) and thus to establish the diagnosis of AA. The appropriate decisions and choices of therapy, along with an assessment of risk stratification, are based on the Camitta classification of AA (Table III) [5–7].

The incidence of AA is, on average, 2 cases per million in Europe. The incidence is roughly three times higher in Asia, which may indicate some genetic or environmental factors [8–11]. Several hypotheses have been proposed to explain why the incidence of AA is higher in Asia than in Europe and North America, but the most probable seems to be host genetics such as HLA types and nucleotide polymorphisms in some cytokine genes [12]. There is no difference in the incidence of AA between men and women, but as most cases are observed before the age of 40, a genetic predisposition to AA has been suggested. Although clonal evolution of AA to paroxysmal nocturnal hemoglobinuria (PNH), hypocellular myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) is often observed [13], co-existing somatic mutations may predispose to this process.

Irrespective of the identification of the cause of pancytopenia in the course of AA, the responses to immunosuppressive treatment confirm the thesis of autoimmune injury to hematopoietic stem cells and stem cell progenitors [14–16]. The primary role of T-cell cytotoxic lymphocytes along with the additional effect of interferon gamma and TNF on the inhibition of hematopoietic stem cell (HSCs) production together with an increasing FAS receptor expression (the first sign of apoptosis) all contribute to immune-mediated destruction of HSCs [17–22]. The Human Leukocyte Antigen (HLA) genes play key roles in mediating the immune response, especially HLA class II alleles. A Chinese study identified HLA-DRB1, DQB1 and DPB1 alleles predisposing to AA development [23]. The dysfunction of T

regulatory cells is increased NK cells and autoantibodies, which are also involved in HSC immune destruction in AA [24–28].

Table I. Proposed diagnostic procedures for aplastic anemia (AA)

Category	**Tests**
Peripheral blood testing	CBC, differential, reticulocyte count Flow cytometry for PNH
Bone marrow examination	Bone marrow smear Flow cytometry Cytogenetics Trephine biopsy
Rheumatoid disease screening	Antinuclear antibodies Rheumatoid factor
Liver function tests	ALT, AST, Bilirubin serum levels
Viral infection testing	HBV, HCV, EBV, CMV, HHV-6, HIV, Parvovirus B19
Visual imaging	CT, PET-CT, MRI, US for searching solid tumors and lymphoproliferative neoplasms

CBC — complete blood count; PNH — paroxysmal nocturnal hemoglobinuria; ALT — alanine transaminase; AST — aspartate transaminase; HBV — hepatitis B virus; HCV — hepatitis C virus; EBV — Epstein-Barr virus; CMV — cytomegalovirus; HHV-6 — human herpesvirus 6; HIV — human immunodeficiency virus; CT — computed tomography; PET-CT — positron emission tomography-computed tomography; MRI — magnetic resonance imaging; US — ultrasonography

Table II. Differential diagnosis of aplastic anemia (AA)

Infectious diseases	**Cancers**	**Other**
HBV, HCV EBV, CMV HHV-6 HIV Parvovirus B19	MDS, AML, Myelofibrosis ALL NHL	Megaloblastic anemia PNH HLH

Mycobacterial infections	HCL Solid tumor metastases	

HBV — hepatitis B virus; HCV — hepatitis C virus, EBV — Epstein-Barr virus; CMV — cytomegalovirus; HHV-6 — human herpesvirus 6; HIV — human immunodeficiency virus; MDS — myelodysplastic syndrome; AML — acute myeloid leukemia; ALL — acute lymphoblastic leukemia; NHL — non-Hodgkin's lymphoma; HCL — hairy cell leukemia; PNH — paroxysmal nocturnal hemoglobinuria; HLH — hemophagocytic lymphohistiocytosis

Table III. Camitta criteria for aplastic anemia (AA) stratification

Stage	**Criteria**
Severe aplastic anemia (SAA)	Bone marrow cellularity <25% (or 25–50% with <30% residual hematopoietic cells), plus at least two of the following peripheral blood findings: Neutrophils $<0.5 \times 10^9$ Platelets $<20 \times 10^9$/L Reticulocytes $<20 \times 10^9$/L
Very severe aplastic anemia (VSAA)	As SAA, but neutrophils less than 0.2×10^9/L
Non-severe aplastic anemia (NSAA)	Criteria for SAA or VSAA not fulfilled and decreased bone marrow cellularity, plus at least two of the following peripheral blood findings: Neutrophils $<1.5 \times 10^9$ Platelets $<100 \times 10^9$/L Hemoglobin <10g/dl

Inherited bone marrow failure syndromes

Several genetic disorders including Schwachman-Diamond syndrome (which leads to a reduction in hematopoietic stem cells' ability to repair DNA because of genetic lesions),

congenital amegakaryocytic thrombocytopenia (MPL gene), Diamond Blackfan anemia (SBDF gene), Fanconi anemia, some GATA2 spectrum disorders, congenital keratosis, SRP72, and congenital pure red cell aplasia have all been identified as familiar cases of AA [29–34]. Careful history-taking and physical examinations may be helpful in the identification of germ-like genetic bone marrow failure disorders associated with AA and included in differential diagnostics in children, adolescents and young adults (Table IV) [6, 35]. Next-generation sequencing technologies have facilitated the discovery of mutations that cause pancytopenia and lead to aplastic anemia. All of them carry a high risk of MDS/AML, and some of them are associated with an especially high risk of a range of solid tumors. Thus a tailored stem cell transplantation regimen, such as reduced intensity conditioning, may be the optimal treatment. This is especially true for Fanconi anemia, dyskeratosis congenita, Diamond Blackfan anemia, and Shwachman-Diamond syndrome, not only because of the high risk of clonal evolution, but also due to the high risk of morbidity and mortality [36–38].

Table IV. Selected anomalies in physical examination indicative of inherited AA

Anomaly	**Disease or mutation**
Short stature	FA, DKC, DBA, SDS, SAMD9
Microcephaly	FA, DKC
Café-au-lait skin lesions	FA
Abnormal skin pigmentation, dystrophic nail and oral leucoplakia	DC
Skeletal anomalies	SDS
Erythema nodosum, warts and molluscum	GATA2
Absent radii	TARS
Abnormal thumbs	FA, DBA
Hypertelorism, epicanthal folds	DBA
Cerebellar ataxia	SAMD9L

FA — Fanconi anemia; DBA — Diamond Blackfan anemia; SDS — Shwachman-Diamond syndrome; DC — dyskeratosis congenita; CAMT — congenital amegakaryocytic thrombocytopenia; TARS — thrombocytopenia-absentradii syndrome

Somatic mutations in AA

Recurrent mutations and variants have been detected in up to 50% of patients with AA using targeted next generation sequencing hematopoiesis [39–42]. Although some of these mutations are limited to AA, such as PIGA [43] and BCOR/BCORL1 mutations, others are frequently found in myeloid malignancies, including ASXL1 and DNMT3A. Moreover, DNMT3A-mutated and ASXL1-mutated clones tend to increase in size over time, whereas BCOR- and BCORL1-mutated and PIGA-mutated clones decrease or remain stable [44].

Impact of somatic mutations on outcomes

Several reports have evaluated the clinical significance of somatic mutations in AA. Firstly, it has been shown that the response to immunosuppressive therapy is better in patients with PIGA, BCOR and BCORL1 mutations [45]. In the study by Hosokawa et al., the presence of increased glycosylphosphatidylinositol-anchored protein-deficient cells correlated with a positive response to immunosuppressive therapy and prognosis, and thus was found helpful in choosing the optimal treatment for trisomy +8 patients with AA or low-risk MDS [45]. Although the natural history of AA patients with PNH clones has been studied, no impact on progression to symptomatic PNH or transformation to AML/MDS has been observed [46]. Furthermore, higher rates of overall and progression-free survival have been found in these subgroups of mutations [44]. However, other somatic mutations such as DNMT3A and ASXL1 are associated with worse outcomes. Recently, a study into mutation status and the differences between severe and non-severe AA by Patel et al. [47] detected at least one mutation in 19% of patients with AA at the time of diagnosis, independent of the severity of the AA. However, patients with severe AA had a higher mutation rate compared to moderate AA (56% vs. 19%), which corresponds to the unstable hematopoietic clones and higher risk of clonal evolution [47].

Finally, the effect of somatic mutations on a higher risk of progression to MDS/AML was revealed by Kulasekararaj et al. [42]. Furthermore, other specific mutations are likely predictors of secondary MDS [48]. The effect of the therapy applied also influences the mutational status, and *BCOR/BCORL1* mutations may expand during the course of IST [48]. Negoro et al. demonstrated that, in serial samples of AA without evolution to MDS, clones with *GATA2*, *PHF6*, *RUNX1*, *SMC3*, *TET2* and *BCORL1* mutations decreased in size during the course of AA, whereas *ASXL1*, *CALR*, *CUX1*, *ETV6*, *EZH2*, *G3BP1*, *RIT1*, *U2AF1*, and *ZRSR2* expanded. In contrast, *DNMT3A*, *BCOR*, and *CEBPA* clones showed individually

variable behavior with regard to clonal dynamics [48]. Lastly, Negoro et al. also demonstrated the clinical impact of MDS-driver mutations found in AA at presentation, which transformed to MDS and had a shorter median progression-free survival and overall survival compared to cases without such somatic alterations [48]. Other researchers have postulated that clonal dynamics might be highly variable and may not predict response to therapy in individual patients.

Telomerases abnormalities

Telomere shortening is found in up to 35% of patients with AA [49, 50]. It is known that this can result in chromosomal instability and may lead to evolution to MDS/AML [51]. To resist the attrition, germ-like cells utilize telomerase reverse transcriptase (TERT), telomerase RNA component (TERC) telomerase genes, and the stabilizing protein dyskerin (DKC1) to assemble the telomerase complex and maintain telomere length [52]. It has been found that several mutations in TERT, TERC-DKC1 (stabilizing protein dyskerin) and *RTEL1* (regulator of telomere elongation helicase 1) are associated with telomere shortening in AA patients [53, 54].

Shortened telomere length at diagnosis in patients with AA has been shown to correlate with poorer outcomes [55–57], particularly due to an inadequate response to immunosuppressive therapy. Moreover, some mutations like TERT or TERC mutations [54, 58] are associated with transformation to MDS/AML [51, 55, 59, 60]. Sex hormones or other pharmacological agents have been shown to be effective in up-regulating telomere length and reducing the risk of clonal evolution to AML [61]. A frequency of up to 38% of clonal patterns of X-chromosome inactivation in female patients with AA has been observed [62].

Cytogenetic abnormalities

The most common cytogenetic abnormality is monosomy 7 (-7), occurring in up to 13% of AA cases. Overall, this is associated with a poorer prognosis and a high risk of progression to MDS or AML [63, 64]. Evaluation of the karyotypes in patients with MDS secondary to AA revealed the presence of chromosomes 6, 7 and 8 abnormalities [64] which suggests that these cytogenetic abnormalities, at the initial diagnosis or developed later in patients with AA, can promote progression to MDS/AML. Some cytogenetic abnormalities such as trisomy 8 or del(13q) are associated with a favorable response to immunosuppressive therapy [65–67].

Although they are commonly found in other myeloid malignancies, they are related to a low risk of transformation to MDS or AML [57, 63, 68]. There are many cytogenetic abnormalities whose clinical impact on outcomes remains to be established [69].

Circulating exosomal microRNAs

MicroRNAs (miRNAs) can regulate T cell differentiation and plasticity by targeting their corresponding message RNAs (mRNAs), which play important roles in many autoimmune diseases and also AA [70–73].

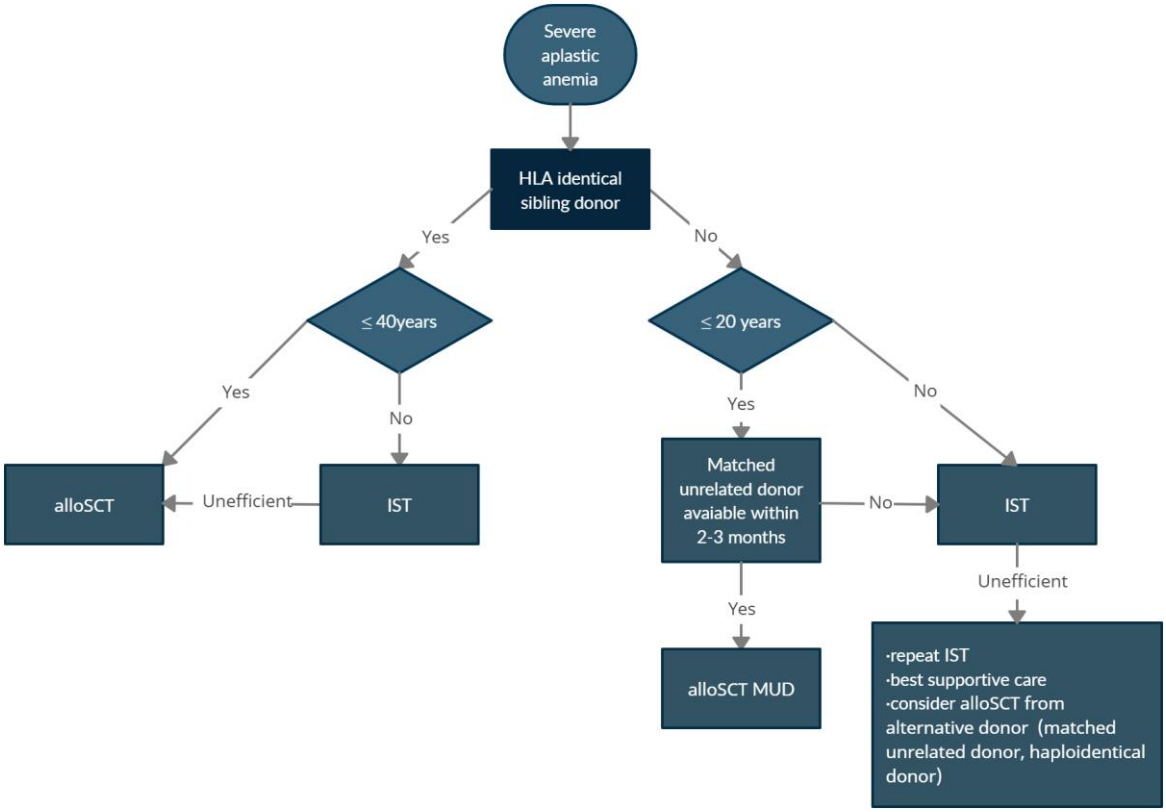
Among several specific miRNAs which regulate RNA silencing and post-transcriptional regulation of gene expression to have been studied in AA and MDS, Guidice et al. identified 25 exosomal microRNAs uniquely or frequently present in AA and/or MDS [74]. One of these, mir-126-5p, with its higher expression at diagnosis in patients with AA, was associated with a shorter progression-free survival and a poorer response to therapy. In another study by Hosokawa, two miRNAs were identified: miR-150-5p which regulated the induction of T-cell differentiation, and miR-146b-5p which was involved in innate immune response. Both of these increased in AA patients, whereas miR-1 was decreased in AA [75]. Moreover, the elevated expression of miR-150-5p was significantly reduced after successful immunosuppressive therapy but did not change in non-responders, indicating the clinical utility of miR-150-5p for disease monitoring [75].

Management of patients with aplastic anemia

Prior to initiating treatment for AA, other causes of pancytopenia should be excluded, particularly inherited bone marrow failure syndrome (IBMFS), hypoplastic MDS and some others transient causes of pancytopenia including drugs or infections. As AA may be associated with PNH, detection of the PNH clone is more indicative for AA than any other cause of pancytopenia and bone marrow failure. Although allo-HSCT is considered to be the only curative procedure for patients with SAA, it is recommended that younger patients, particularly children, undergo careful evaluation of concomitant illnesses and performance status to determine unfit or frail patients before intensive therapies, including allo-HSCT or IST (ATG or CsA), due to treatment-related mortality and morbidity [76–78]. Figure 1 shows a practical therapeutic algorithm in SAA [EBMT algorithm for SAA in 2019, modified] [5]. In cases of the detection of clonal hematopoiesis, especially monosomy 7 (-7) or other

abnormalities related to high-risk MDS or insufficient response to IST in patients with SAA below the age of 60, if these patients are assessed as eligible for transplant but have no identical sibling donor, an alternative donor should be sought.

Figure 1. Therapeutic algorithm in severe aplastic anemia (SAA)



Clonal hematopoiesis and supportive therapy

All patients with AA require ongoing supportive care to alleviate symptoms and reduce the adverse effects related to pancytopenia. Most studies have reported that infections were the predominant cause of death; therefore recommendations for infection prevention are included in several guidelines, independent of the intensity of AA treatment, both for transplant- or IST-eligible patients and for less fit patients on ongoing supportive care [6, 76, 79–81].

G-CSF

Hematopoietic growth factor, granulocyte colony stimulating factor (G-CSF) stimulates granulocyte progenitors as well as stem cells for proliferation and differentiation. A randomized prospective trial on patients with newly diagnosed severe AA (n =192), receiving antithymocyte globulin and cyclosporine, with and without G-CSF, did not demonstrate any impact of G-CSF on the outcome of severe AA, independent of cytogenetic abnormalities. Overall survival and progression-free survival was comparable in both groups, as well as the risk of clonal abnormalities and myeloid neoplasm development [82]. Moreover, the results of a metanalysis of four studies confirm that the usage of G-CSF in IST is not associated with a higher occurrence of clonal evolution into malignant neoplasm and PNH in SAA patients [83]. On the other hand, a rapid granulocyte recovery in patients treated with IST with G-CSF addiction may identify early non-responders, and perhaps indicate the need for urgent transplantation [84, 85].

Eltrombopag

Eltrombopag (EPAG), an oral thrombopoietin (TPO) receptor agonist used in immune thrombocytopenia treatment, is a new therapeutic option in transplant-ineligible SAA patients. The role of TPO in hematopoiesis is not limited only to thrombopoiesis: a TPO receptor c-Mpl is present on hematopoietic stem and progenitor cells (HSPCs), and its lack in murine models leads to HSPC deficiency [86]. EPAG is efficient at SAA refractory to IST and in some patients it restores trilineage hematopoiesis with a sustained response even after discontinuation of the treatment [87–89]. Nevertheless, a risk of clonal evolution during this treatment remains an area of concern. Two prospective studies of EPAG usage in treatment naïve and second in refractory/relapsed SAA have not shown a higher risk of clonal evolution or myeloid neoplasm development compared to historical data [87, 88]. On the other hand, in phase 1 / 2 EPAG in R/R SAA (18%) have developed new cytogenetic abnormalities, most of these (87%) within six months of beginning treatment. However, some were unstable and disappeared after EPAG withdrawal. Chromosome 7 abnormalities were observed in 8% (7/83) of patients, and four of them had persistent aberration in control cytogenetic testing one month after drug discontinuation. Nevertheless, none of them progressed to MDS/AML [88].

The impact of EPAG on the overall risk of cytogenetic progression, clonal evolution, and/or clinical progression to MDS/AML in patients with SAA requires further investigation. Due to an insufficient response to IST, patients who are platelet transfusion-dependent may

receive EPAG as secondary SAA therapy, but its high costs limit the widespread application of this treatment option in many countries [79, 90].

Survival after hematopoietic stem cell transplantation (HSCT)

A recent study demonstrated that in some situations, despite the identification of certain genetic abnormalities of germline monoallelic deleterious variants in the Fanconi anemia gene in patients with idiopathic AA (21 variants in 730 patients), the abnormalities do not influence the outcome of hematopoietic cell transplantation [91].

Generally, although allogeneic HSCT has shown an improvement in survival rates, particularly for HLA-matched unrelated donor transplants, haploidentical transplantation has been proposed as the effective treatment for severe aplastic anemia and it is increasingly being used [15]. The optimal choice of haploidentical donor has also been the subject of research [92]. Furthermore, a recent meta-analysis of 5,336 patients comparing front-line treatments for AA showed significantly longer survival among AA patients undergoing first-line allo-HSCT compared to IST. On the other hand, one of the most important complications after allo-HSCT is graft-versus-host disease, and this needs to be carefully balanced against the concerns of IST [93].

It has to be emphasized that the choice of initial treatment for patients with newly diagnosed AA still requires a comprehensive evaluation of donor availability, patient age, expected quality of life, and the risk of disease relapse or clonal evolution after IST [94].

Conclusions

There are difficulties in differentiating between AA and MDS due to the high prevalence of clonal hematopoiesis in AA with genetic abnormalities overlapping with MDS. Furthermore, a better understanding of the pathogenesis of AA with respect to somatic mutations, cytogenetic abnormalities and defective telomerase functions, and their impacts on the response to IST, along with a balancing of the risk of clonal progression to MDS/AML, may in future allow for treatment personalization with precise indications for upfront allo-HSCT.

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