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Biomarkers for predicting clinical response to immunosuppressive therapy in aplastic anemia

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Abstract The decision to select hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy (IST) as initial therapy in acquired aplastic anemia (AA) is currently based on patient age and the availability of a human leukocyte antigen (HLA)-matched donor. Although IST is a promising treatment option, the ability to predict its long-term outcomes remains poor due to refractoriness, relapses, and the risk of clonal evolution. Several predictive biomarkers for response to IST have been posited, including age, gender, pre-treatment blood cell counts, cytokines, gene mutations, paroxysmal nocturnal hemoglobinuria (PNH), and telomere length (TL). While previous studies have provided substantial biological insights into the utility of IST, the prognostic power of the reported biomarkers is currently insufficient to contribute to clinical decision making. Recently, a large retrospective analysis proposed the combination of minor PNH clones and TL as an efficient predictor of IST response. Identification of a reliable predictor would provide a useful tool for determining the most appropriate treatment choice for AA patients, including up-front HSCT from HLA-matched unrelated donor. The present review summarizes studies evaluating the utility of biomarkers in predicting the clinical response to IST of patients with AA, and provides a baseline for prospective studies aimed at validating previously reported biomarkers.

Keywords Aplastic anemia · Immunosuppressive therapy · Paroxysmal nocturnal hemoglobinuria · Telomere length · Prognosis

Introduction

Acquired aplastic anemia (AA) is an uncommon and serious bone marrow disorder characterized by pancytopenia and hypocellular bone marrow. Although autoimmune processes are thought to underlie the pathogenesis of AA, there is a lack of data regarding inciting antigens and the mechanisms responsible for the destruction of hematopoietic stem cells by immune attack [1]. Hematopoietic stem cell transplantation (HSCT) represents a curative treatment but is substantially limited by the availability of human leukocyte antigen (HLA)-matched donors. In many cases, immunosuppressive therapy (IST) combining anti-thymocyte globulin (ATG) and cyclosporine A (CyA) is used as a first-line treatment [2]. Currently, the hematopoietic response rate of AA patients for IST is reportedly 42–74 %, with an overall long-term survival rate of approximately 90 % across several large studies in the United States, Europe, and East Asia [3–5]. On the other hand, approximately one-third of patients are not expected to respond to IST, with 20–40 % responders anticipated to relapse after initial therapy [3, 4]. Further, clonal transformation to myelodysplasia represents a serious complication of IST. The EBMT study reported a 3-year event-free survival after IST in children with AA of just 33 %, with 55 % of patients failing front-line IST and requiring rescue HSCT [6]. Therefore, the use of predictive biomarkers for identifying patients suitable for up-front

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Table 1 Summary of studies on association between biomarkers and favorable clinical response of IST in patients with aplastic anemia

Biomarkers	No. of patients	Age, median (range)	Type of ATG	Response rate	*p* value	References	
Male gender	312	8 (1–17)	Horse	62 vs 48 %	0.025	Yoshida et al. [11]	2011
Peripheral blood							
Higher ARC	194	ND	Horse	80 vs 53 %	<0.001	Scheinberg et al. [9]	2008
	125	22 (1–66)	Horse or rabbit	84 vs 49 %	<0.001	Kulagin et al. [16]	2014
Lower WBC	312	8 (1–17)	Horse	64 vs 51 %	0.009	Yoshida et al. [11]	2011
Higher ALC	194	ND	Horse	70 vs 47 %	<0.001	Scheinberg et al. [9]	2008
Cytokines							
Positive INF-γ	123	ND	ND	96 vs 32 %	<0.001	Sloland et al. [20]	2002
Wild type INF-γ SNPs	35	ND	ND	73 vs 27 %	0.041	Chang et al. [21]	2010
Lower TPO	85	9 (1–16)	Horse or rabbit	55 vs 15 %	0.001	Elmahdi et al. [23]	2016
Positive minor PNH clone	113	9.6 (1–16)	Horse or rabbit	77 vs 36 %	<0.001	Narita et al. [13]	2015
	125	22 (1–66)	Horse or rabbit	69 vs 45 %	0.010	Kulagin et al. [16]	2014
	97	22 (2–65)	Rabbit or porcine	67 vs 32 %	0.002	Zhao et al. [17]	2015
	22	9 (1–17)	ND	94 vs 33 %	0.012	Tutelman et al. [28]	2013
	171	8 (3–14)	ND	71 % vs. 55 %	0.004	Maciejewski et al. [30]	2001
	122	56 (12–83)	Horse	91 vs 48 %	<0.001	Sugimori et al. [31]	2006
	35	46 (17–83)	Horse	84 vs 65 %	0.030	Wang et al. [32]	2001
Longer telomere length	113	9.6 (1–16)	Horse or rabbit	71 vs 33 %	<0.001	Narita et al. [13]	2015

IST immunosuppressive therapy, *ATG* anti-thymocyte globulin, *ARC* absolute reticulocyte count, *WBC* white blood cell count, *ALC* absolute lymphocyte count, *INF-γ* interferon-gamma, *SNPs* single nucleotide polymorphisms, *TPO* thrombopoietin, *PNH* paroxysmal nocturnal hemoglobinuria, *ND* not described

HSCT is clinically important, not only for making treatment decisions, but also from a medical economic point of view.

There have been many efforts to identify practical and robust markers able to predict outcomes following IST (Table 1). Such candidate markers may potentially provide additional information for clinical decision making; however, none have been widely accepted into clinical implementation. The process of biomarker development and validation is a multistep process: (1) the discovery of a potential marker through hypothesis-generating preclinical or exploratory studies; (2) the establishment and first validation of the assay in clinical samples; (3) the presentation of the features in retrospective and, less frequently, prospective settings; and (4) continued assessment of the validity of the biomarker in routine clinical practice [7]. Unfortunately, most studies on biomarkers with potential utility in predicting IST response in AA have presented results from the third phase of development and were conducted retrospectively. The present review aims to provide a critical overview of biomarkers with utility in predicting response to IST in AA with respect to prognostic stratification and individual treatment selection. Further, we provide a baseline for prospective studies aimed at validating previously reported biomarkers, which may facilitate the development of new treatment algorithms for AA.

Biomarkers of treatment response in aplastic anemia

Age and gender

Age has been demonstrated as predictor of IST outcomes in many previous studies. In general, the response rate is higher in children than in older patients following IST [4, 8–10]. Pediatric patients reportedly have an approximately 70–80 % response rate; younger adult patients, 60–70 %; and adult patients >40–50 years of age, 50–60 % [11]. Yoshida et al. [12] demonstrated men display better responses than women. This relationship was also observed in a European study in which a young female cohort demonstrated delayed recovery of bone marrow function following IST [13].

Pre-treatment peripheral blood counts

A number of studies have shown that pre-treatment laboratory variables are correlated with good response to IST. Scheinberg et al. [10] demonstrated that absolute reticulocyte count (ARC) and absolute lymphocyte count (ALC) were predictive of response and survival in SAA patients treated with IST. When the two predictive parameters of ARC and ALC were combined, patients with higher baseline ANC and ARC had a response rate 40 % higher than

those with lower baseline ANC and ARC (83 vs 41 %, $p < 0.0001$). The utility of ARC in predicting IST outcomes has been confirmed in a proportion of reports, but not in others [12, 14–17]. Higher absolute neutrophil count (ANC) appears to be correlated with a better response rate of IST [15, 18]; however, this result was not confirmed in Japanese and German children [12, 19, 20]. Further, the study of Japanese children demonstrated that lower white blood cell (WBC) count was indicative of a better response to IST rather than higher ANC [12].

Cytokines

AA is associated with the overproduction of multiple cytokines. Research laboratory findings that reflect the pathophysiology of AA include an increased ratio of activated T cells, and increased interferon gamma (IFN-γ) expression in bone marrow and peripheral T cells. Sloland et al. [21] reported a higher response rate to IST in patients with circulating IFN-γ containing T cells than those without. Interestingly, Chang et al. [22] demonstrated an association between the IFN-γ single nucleotide polymorphism +874 (T/A), which can directly affect the expression of the IFN-γ gene, and response to IST in patients with AA.

Thrombopoietin (TPO) levels are markedly increased in AA, and these abnormal levels correlate with disease severity [23]. Elmahdi et al. [24] demonstrated significantly higher TPO plasma levels in non-responders to IST than in responders.

Gene mutations

Yoshizato et al. [25] performed next-generation sequencing in 439 patients with AA and observed no apparent relationship between the presence of mutations and the response to IST. However, when mutated genes were assessed individually, mutations in *BCOR* and *BCORL1* were found to correlate with a better response rate. Moreover, mutations in *PIGA*, *BCOR,* and *BCORL1* were associated with longer and a higher rate of overall and progression-free survival, while mutations in a subgroup of genes that included *DNMT3A* and *ASXL1* were associated with worse outcomes. These results indicate close monitoring of clonal hematopoiesis by means of deep sequencing will need to be combined with clinical evaluation to estimate prognosis and to guide treatment of patients with AA.

Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is an acquired disorder associated with episodic intravascular hemolysis of red blood cells that are deficient in cell surface glycophosphatidylinositol (GPI)-anchored proteins [26, 27]. High sensitive flow cytometry analysis has indicated that 20–70 % of pediatric AA patients possess minor PNH populations at the time of diagnosis [17, 28–30]. In several studies, investigators have attempted to reveal the clinical significance of such PNH-type cells in patients with bone marrow failure. However, the reliability of results obtained from minor PNH populations remains controversial. For example, several studies conducted on adults and/or children with AA reported that the presence of minor PNH populations was associated with a favorable response to IST [14, 17, 18, 29, 31–33]. In contrast, a retrospective National Institutes of Health (NIH) study that included adults and children did not find differences between AA patients who did or did not respond to IST [10].

Telomere length

Short telomeres have been proposed as a marker of the aging process as they have shown to shorten with each cell division, thereby reflecting cell turnover [34]. In AA patients, significant telomere shortening in lymphocytes is presumed to be secondary to hematopoietic stress [35]. Telomere erosion reduces the replication of hematopoietic stem cells and progenitor cells, although there is debate regarding the value of telomere length (TL) in predicting the response to IST. An NIH study reported that baseline TL was associated with risk of relapse, clonal transformation, and overall survival, but not related to hematologic response in adult AA patients [36]. In contrast, Tutelman et al. [29] demonstrated shorter granulocyte telomeres in a childhood AA cohort than in age-matched healthy controls. Among patients treated with IST, very short granulocyte TL was correlated with inferior IST outcomes [29]. Sakaguchi et al. [37] also posited that lymphocyte TL at the time of diagnosis was significantly associated with the response to IST in children. These conflicting results may be explained by differences between adult and children. As age is an important factor for the interpretation of TL and telomeres have been shown to shorten with age, differences in TL between patients and healthy individuals may be smaller in adults than in children.

Combination PNH and TL

We previously studied 113 children diagnosed with acquired AA to determine the utility of TL as a reliable predictor of response to IST and prognosis [14]. PNH^+ patients had a significant better response rate to IST at 6 months than did PNH^- patients (77 vs 36 %, respectively, $p < 0.001$). Responses in the longer TL group (71 %) were significantly better than those in the shorter TL group (33 %; $p < 0.001$). In multivariate logistic regression analysis, lower ARC at diagnosis (OR, 3.43; 95 % CI 1.19–9.82;

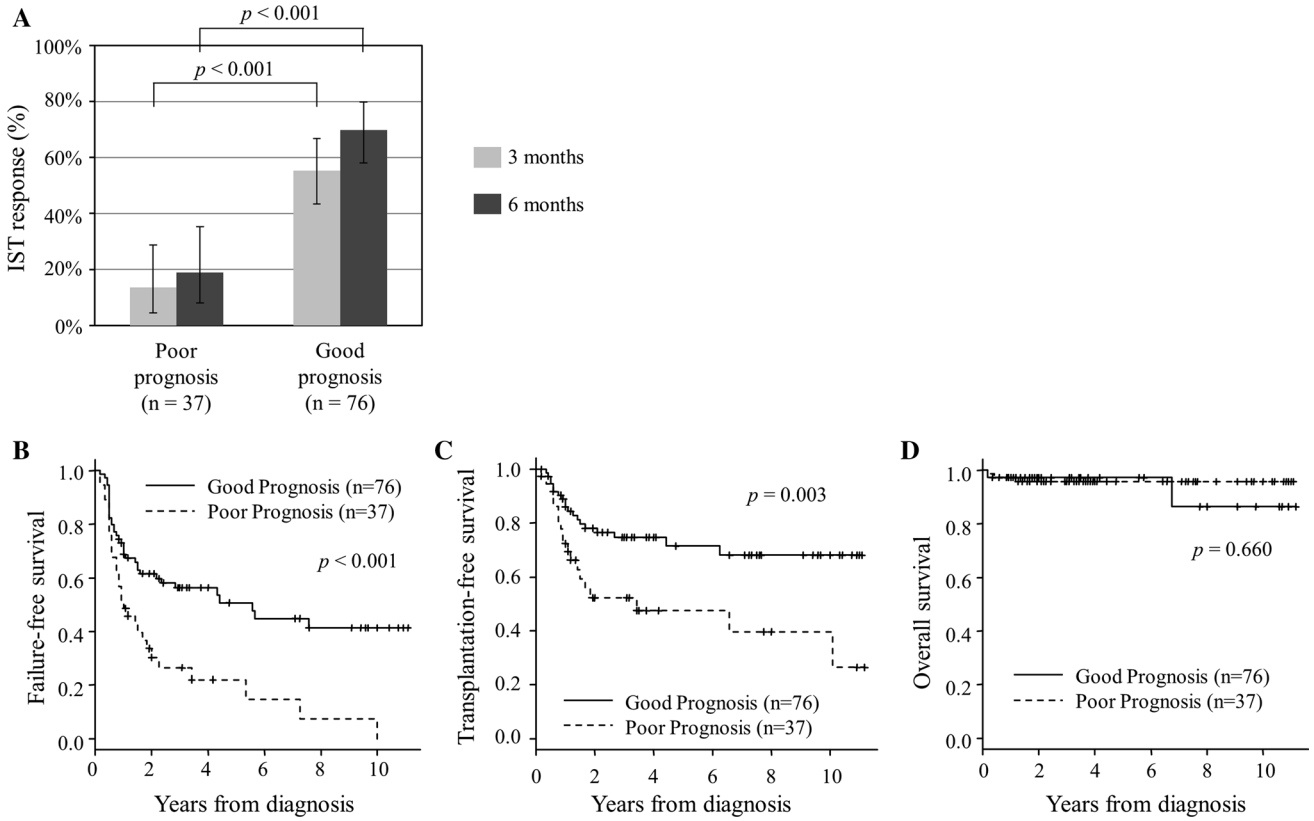

Fig. 1 **a** Response rates to immunosuppressive therapy (IST) at 3 and 6 months according to predictive stratification. Risk stratification based on minor paroxysmal nocturnal hemoglobinuria (PNH) populations and telomere length (TL) clearly demonstrated worse responses in the unfavorable group than in the favorable group. Prognosis after IST in the good prognosis and poor prognosis groups. **b** Failure-free survival. **c** Transplantation-free survival. **d** Overall survival

$p = 0.022$), absence of a PNH population (OR, 6.50; 95 % CI 2.44–17.30; $p < 0.001$), and shorter TL (OR, 3.61; 95 % CI 1.43–9.11; $p = 0.007$) were identified as independent unfavorable predictors of response to IST at 6 months. When the cohort was stratified according to poor prognosis (PNH^- and a shorter TL, < -1.21 SD, $n = 37$) or good prognosis (PNH^+ and/or a longer TL, $n = 76$), the response rate at 6 months in the poor prognosis group was significantly lower (19 %) than that in the good prognosis group (70 %; $p < 0.001$; Fig. 1a). No significant differences in the probability of 5-year cumulative incidence of relapse (0 %; 95 % CI 0–0 % vs 16 %; 95 % CI 4–27 %; $p = 0.392$) or clonal evolution (5 %; 95 % CI, 0–13 % vs 3 %; 95 % CI 0–8 %; $p = 0.847$) were observed between the poor prognosis and good prognosis groups. Five-year transplantation free survival (TFS) and failure free survival (FFS) were significantly lower in the poor prognosis group than in the good prognosis group (TFS, 48 %; 95 % CI 29–64 % vs 72 %; 95 % CI 59–82 %; $p = 0.003$; FFS, 22 %; 95 % CI 9–38 % vs 52 %; 95 % CI 39–64 %; $p < 0.001$; Fig. 1b, c). However, no difference in 5-year OS was observed between groups (poor prognosis, 97 %; 95 % CI 82–100 % vs good prognosis, 96 %; 96 % CI 88–99 %; $p = 0.660$), possible due to effective salvage HSCT (Fig. 1d).

Future directions

Biomarkers of hematologic response, relapse, and clonal transformation have the potential to greatly enhance risk-stratification and allow for better treatment allocation in AA. Recently, the outcomes of unrelated and mismatched-related donor transplantation in AA patients have improved dramatically, with an OS in the range of 70–80 % expected in selected patient populations [38, 39]. In particular, young patients with disease duration of <1 year have achieved better results than adults did [40]. The EBMT group demonstrated that upfront matched or mismatched unrelated donor HSCT has similar outcomes to matched sibling donor HSCT in idiopathic severe AA of childhood and adolescence [41]. Further, a Japanese study found no differences in OS and FFS between matched sibling donor and unrelated donor HSCT [42]. Therefore, despite the risk of complications, younger patients with a low probability

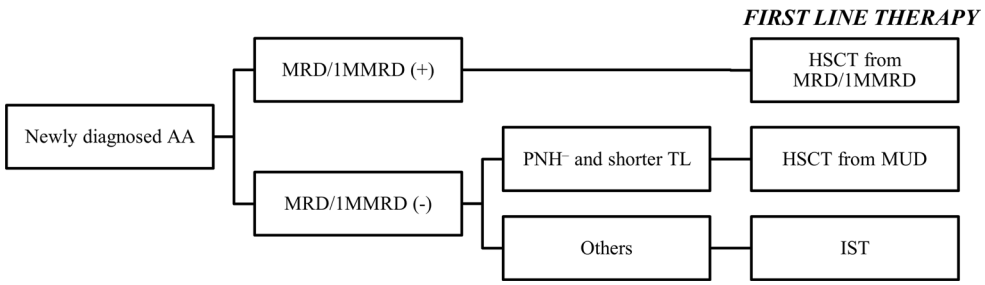

Fig. 2 Treatment algorithm for children with severe aplastic anemia. Hematopoietic stem cell transplantation (HSCT) is considered the most appropriate initial treatment in patients with a matched related donor (MRD) or a HLA 1 locus mismatched related donor (1MMRD). For patients without a MRD or 1MMRD, upfront HSCT from a matched unrelated donor (MURD) could be considered as a potential option in children with the combination of a paroxysmal nocturnal hemoglobinuria (PNH) clone and short telomere length (TL). Immunosuppressive therapy (IST) is considered the most appropriate initial therapy in other cases

of response and high probability of late events may benefit from unrelated donor HSCT as a first therapy (Fig. 2). On the other hand, previous studies of the association between biomarkers and clinical response to IST have been limited by retrospective and heterogeneous cohorts. Large and prospective studies are warranted to determine the utility of biomarkers in the risk stratification of patients with AA.

Conclusion

The present review provides a summary of previous studies of predictive biomarkers of clinical prognosis in AA. Several potential markers of IST response that appear to reflect the immune pathophysiology of AA have been proposed, with a proportion identified as candidate markers reflecting clinical adaptations. In particular, combination of minor PNH clones and TL may represent a promising tool for future personalized IST in AA. Further prospective studies in large study populations may facilitate the development of novel therapeutic algorithms for AA patients.

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