Predicting response to immunosuppressive therapy in childhood aplastic anemia

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ABSTRACT

In aplastic anemia, predictive markers of response to immunosuppressive therapy have not been well defined. We retrospectively evaluated whether clinical and laboratory findings before treatment could predict response in a pediatric cohort from the multicenter AA-97 study in Japan. Between 1997 and 2006, 312 newly diagnosed children were enrolled and treated with a combination of antithymocyte globulin and cyclosporine. In multivariate analyses, lower white blood cell count was the most significant predictive marker of better response; patients with white blood cell count less than 2.0×10^9/L showed a higher response rate than those with white blood cell count of 2.0×10^9/L or more (P=0.0003), followed by shorter interval between diagnosis and therapy (P=0.01), and male sex (P=0.03). In conclusion, pre-treatment clinical and laboratory findings influence response to therapy. The finding that response rate worsens with increasing interval between diagnosis and treatment highlights the importance of prompt immunosuppressive therapy for patients with aplastic anemia.

Key words: aplastic anemia, children, immunosuppressive therapy, predictive marker.

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Introduction

Aplastic anemia (AA) is defined as peripheral blood pancytopenia caused by bone marrow failure, and the pathogenesis is thought to involve autoimmune processes.[1-3] Several studies have confirmed immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine (CyA) as a promising therapeutic option for patients lacking HLA-identical related donors.[4-8] Although several potential markers of IST response that appear to reflect the immune pathophysiology of aplastic anemia have been suggested, mainly from adult studies,[9-11] none have been widely accepted. We have already investigated the clinical relevance of HLA, a minor population of paroxysmal nocturnal hemoglobinuria-type cells, and a specific autoantibody associated with aplastic anemia in pediatric patients, finding no correlation between these markers and response to therapy.[12]

Some groups have recently shown that pre-treatment laboratory variables are associated with good response to immunosuppressive therapy, but those results remain controversial, as the numbers of children included in the study was relatively small and the drugs used for immunosuppressive therapy have not been consistent.[13-15] The present study, therefore, evaluated whether clinical and laboratory findings before treatment could predict immunosuppressive therapy response in a large population of children with aplastic anemia enrolled in a multi-center study.

Design and Methods

Patients

Between October 1997 and September 2006, a total of 312 Japanese children with aplastic anemia (AA) from 118 hospitals were enrolled in the AA-97 multicenter study conducted by the Japan Childhood Aplastic Anemia Study Group. Patients with acquired AA were eligible if the following criteria were met: age under 18 years; newly diagnosed disease (≤180 days) without specific prior treatment; and moderate to very severe AA. The disease was considered severe if at least 2 of the following were noted: neutrophil count less than 0.5×10^9/L; platelet count less than 20×10^9/L; or reticulocyte count less than 20×10^9/L with hypocellular bone marrow.[16] AA was considered very severe if the criteria for severe disease were fulfilled and neutrophil count was less than 0.2×10^9/L. Moderate disease was defined by at least 2 of the following: neutrophil count less than 1.0×10^9/L; platelet count less than 50×10^9/L; or reticulocyte count less than 60×10^9/L.[6] Patients with congenital AA or paroxysmal nocturnal hemoglobinuria were excluded. Allogeneic stem cell transplantation was recommended for patients with severe or very severe disease who had an HLA-matched sibling, so these patients were not included in the AA-97 study. Written informed consent was obtained from all parents and all patients over the age of ten years. All study protocols were approved by the ethics committee of each participating hospital. The study also conforms to the recently revised Declaration of Helsinki.

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IST

All patients were treated with a combination of intravenous ATG (Lymphoglobulin; Genzyme, Cambridge, USA) at 15 mg/kg/day for five days and oral CyA at 6 mg/kg/day. The dose of CyA was adjusted to maintain trough levels between 100 and 200 ng/mL, and the appropriate dose was administered for at least six months. Granulocyte colony-stimulating factor (Filgrastim; Kirin, Tokyo, Japan) was administered intravenously or subcutaneously at 400 μg/m^2 for three months only to patients with very severe disease.[17] Response to IST was evaluated at six months after initiation of therapy. Complete response (CR) was defined as a neutrophil count more than 1.5×10^9/L, a platelet count more than 100×10^9/L, and a hemoglobin level more than 11.0 g/dL.[17] Partial response (PR) was defined as a neutrophil count more than 0.5×10^9/L, a platelet count more than 20×10^9/L, and a hemoglobin level more than 8.0 g/dL in patients with severe or very severe AA, and as a neutrophil count more than 1.0×10^9/L, a platelet count more than 30 ×10^9/L, and a hemoglobin level more than 8.0 g/dL in patients with moderate AA.[17] Overall response was defined as CR or PR at six months after IST.

Statistical analyses

Parameters for univariate analyses to determine predictors of response to IST included age at diagnosis, sex, interval between diagnosis and treatment, etiology, severity of disease, white blood cell (WBC) count, neutrophil count, lymphocyte count, hemoglobin level, reticulocyte count, and platelet count. Pre-treatment laboratory values were defined as the lowest value without transfusions during the four weeks preceding IST. Continuous variables were divided into quartile categories, and these cut offs were used for categorical analysis. To evaluate correlations between these parameters and response, differences in continuous variables were analyzed using the Mann-Whitney U-test and differences in frequencies were tested using the χ^2 or Fisher's exact test. For multivariate analyses, logistic regression modeling was performed. Important covariates in the multivariate models were chosen using stepwise variable selection procedures. Values of $P<0.05$ were considered statistically significant.

Results and Discussion

Patients' characteristics are shown in Table 1. A total of 312 patients fulfilled the eligibility criteria. Median age at diagnosis was eight years. Severity of AA was considered very severe in 156 patients, severe in 107 patients, and moderate in 49 patients. The median interval between diagnosis and treatment was 15 days. A total of 176 of the 312 (56.4%) patients improved with IST and achieved PR (n=131) or CR (n=45) at six months. All of them achieved transfusion independence.

To determine predictors of IST response, we compared differences in potential pre-treatment variables between IST responders and non-responders. The following were analyzed both for prevalence in categorical variables and differences in continuous variables: age at diagnosis, interval between diagnosis and treatment, WBC count, neutrophil count, lymphocyte count, hemoglobin level, reticulocyte count, and platelet count. In univariate analyses, WBC count, lymphocyte count, interval between diagnosis and therapy, and gender showed associations with IST response (Table 2). We also performed multivariate logistic regression analysis to assess the simultaneous contributions of each of the variables in predicting response. In these analyses, lower WBC count (P=0.0003), shorter interval between diagnosis and therapy (P=0.012), and male sex (P=0.036) represented significant predictors of better response (Table 2).

Boys displayed better response than girls (Figure 1A). This relationship was also observed in a retrospective European study in which a young female cohort experienced delayed recovery of bone marrow function following IST.[18] Median WBC count before treatment was significantly lower in patients who achieved response (1.9×10^9/L) than in those who did not (2.3×10^9/L; P= 0.007). In addition to the analysis with continuous variable, lower WBC count according to categorical analysis also associated with favorable response, with 93 of 144 patients (65%) with WBC less than 2.0×10^9/L and 83 of 168 patients (49%) with WBC of 2.0×10^9/L or more showing improvement with IST (P=0.009; Figure 1B). When lymphocyte count was applied to the analysis instead of WBC count, a correlation between lower lymphocyte count and response to IST was also observed (Table 2); 82 of 123 patients (67%) with lymphocyte count less than 1.5×10^9/L improved with IST, a significantly higher frequency than the 94 of 189 patients (50%) with lymphocyte count of 1.5×10^9/L or more who improved with IST (P=0.004). Neither neutrophil count nor severity of disease was predictive of response.

Regarding the association between pre-treatment neutrophil count and response, conflicting results have been reported. A European study reported superior response rates in children with very severe AA compared to severe AA[5] but, in contrast, some studies including a recent report of a Korean cohort of adult patients have produced the opposite results.[13,19] The present findings differ from those published studies, with favorable responses correlating well with lower WBC count rather than neutrophil count or disease severity. Indeed, WBC count was the strongest predictor of response to IST in multivariate analysis. In patients with AA, pre-treatment WBC count may mainly reflect the size of lymphocyte populations, due to the severe neutropenia in this condition. These results suggest that poor response to IST might possibly be ascribed to higher WBC

Table 1. Patients' characteristics.

N. of patients		312
Age at diagnosis, years, median (range)		8 (1-17)
Gender	male / female	186 /126
Etiology	n. of patients (%)	
Idiopathic		261 (83.7)
Hepatitis		44 (14.1)
Others		7 (2.2)
Severity of AA	n. of patients (%)	
VSAA		156 (50.0)
SAA		107 (34.3)
MAA		49 (15.7)
Peripheral blood data at diagnosis		
Median WBC count, ×10^9/L (range)		2.02 (0.20-8.70)
Median neutrophil count, ×10^9/L (range)		0.22 (0.00-3.13)
Median lymphocyte count, ×10^9/L (range)		1.82 (0.10-8.50)
Median Hb level, g/dl (range)		6.9 (2.1-13.2)
Median reticulocyte count, ×10^9/L (range)		16.0 (0.0-98.0)
Median platelet count, ×10^9/L (range)		11.0 (1.0-109.0)
Interval from diagnosis to treatment, days, median (range)		15 (1-180)

VSAA: very severe aplastic anemia; SAA: severe aplastic anemia; MAA: moderate aplastic anemia; WBC: white blood cell; Hb: hemoglobin.

Table 2. Univariate and multivariate analysis for IST response in 312 patients with AA.

Univariate variables	Responder	Non-responder	*P*
N. of patients (%)	176 (56.4)	136 (43.6)	
Median age at diagnosis, years	8	8	NS
Gender, male / female	115/61	71/65	0.025
Etiology, n. of patients (%)			
Idiopathic	141(80)	120(88)	NS
Hepatitis	29(17)	15(11)	
Others	6(3)	1(1)	
Severity of AA, n. of patients (%)			
VSAA	90(51)	66(49)	NS
SAA	62(35)	45(33)	
MAA	24(14)	25(18)	
Median WBC count, $\times10^9$/L	1.900	2.255	0.007
≥ 2.0×10^9/L, n. of patients (%)	87(47)	85(63)	0.009
< 2.0×10^9/L, n. of patients (%)	93(53)	51(37)	
Median lymphocyte count, $\times10^9$/L	1.600	2.016	0.006
Median neutrophil count, $\times10^9$/L	0.218	0.200	NS
Median Hb level, g/dl	6.8	6.8	NS
Median reticulocyte count, $\times10^9$/L	15.730	17.600	NS
Median platelet count, $\times10^9$/L	10.000	11.000	NS
Interval from diagnosis to treatment, days	13	19	0.002

Multivariate variables	Odds ratio	95% CI	*P*
WBC count, < 2.0×10^9/L	3.219	1.707-6.070	0.0003
Interval from diagnosis to treatment, < 30 days	2.571	1.225-5.396	0.012
Gender, male	1.873	1.042-3.366	0.036
Reticulocyte count, >25×10^9/L	1.589	0.843-2.997	NS
Platelet count, >20×10^9/L	1.362	0.657-2.826	NS
Etiology, hepatitis/others	1.223	0.504-2.966	NS

VSAA: very severe aplastic anemia; SAA, severe aplastic anemia; MAA, moderate aplastic anemia; WBC, white blood cell; Hb, hemoglobin.

count, that is, a relative increase in lymphocytes. Given the dramatic effects of T-cell suppressants including ATG and CyA on *in vivo* hematopoiesis, autoreactive T-cell responses against hematopoietic stem cells have been suggested to play a major role in the pathogenesis of AA, and *in vitro* studies have also supplied supportive evidence for this idea. Early experiments demonstrated inhibitory effects of autologous lymphocytes on hematopoietic progenitor cell growth through overproduction of cytokines such as interferon-γ and tumor necrosis factor-α by activated cytotoxic T cells in AA patients.[20-22] More recently, oligoclonal T-cell expansions have been described in AA patients, disappearing with clinical improvement following IST.[23] Taking our results and previous findings together, a higher WBC count before treatment may indicate the presence of numerous autoreactive T cells that need to be eliminated and thus a high potential to destroy marrow function through lymphocytes, rather than better residual marrow function. In this scenario, patients with a lower WBC count could be seen to have a better probability of hematopoietic recovery following IST.

We identified a significantly inverse correlation between response and interval between diagnosis and treatment; median intervals among responders and non-responders were 13 and 19 days, respectively (*P*=0.002). In categorical analysis, response rates of patients with intervals less than 30 and of 30 days or more were 60% and 43%, respectively (*P*=0.013). Figure 1C clearly indicates the inverse relationship. Notably, response rates to IST were considerably low among AA patients with long-standing disease; only 35% of patients treated 90 days or more after diagnosis responded, suggesting that patients with this condition may receive irreversible damage to hematopoietic progenitor cells or stromal elements that progresses over time, possibly due to

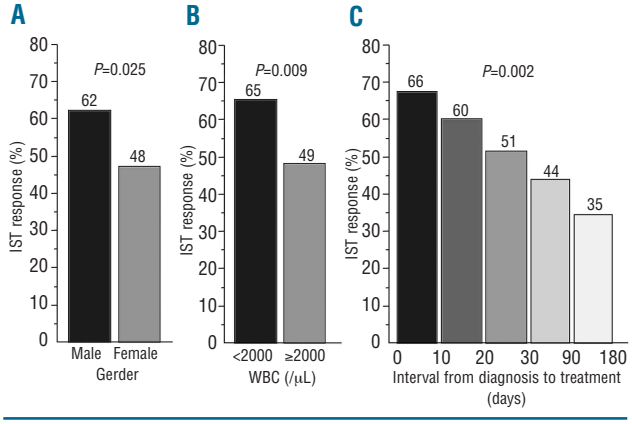

Figure 1. Response to IST in 312 patients according to WBC count, gender, and interval from diagnosis to treatment. (A) Response rate according to gender. Boys showed better response than girls (62% *vs.* 48%, respectively; *P*=0.025). (B) Response rate according to WBC counts. Patients with WBC count <2.0×10^9/L displayed a significantly higher response rate than patients with WBC ≥2.0×10^9/L (65% *vs.* 49%, respectively; *P*=0.009). (C) Response rate according to the interval between diagnosis and treatment. Response rate was inversely associated with the interval between diagnosis and treatment (*P*=0.002).

immune attack through autoreactivated lymphocytes. The present study indicates the importance of prompt IST therapy for patients with AA. We, therefore, recommend offering IST as soon as possible in all children with AA who lack a matched sibling donor.

Other variables did not differ significantly between responders and non-responders (Table 2). Particularly with regard to reticulocyte count, 122 patients showed reticulocyte count more than 25×10^9/L, of whom 67 (55%) responded to IST, and 186 patients had reticulocyte count of 25×10^9/L or less, of whom 107 (58%) responded to IST. Correlations of higher reticulocyte count and higher lymphocyte count at initial diagnosis with better response to IST in patients of all ages have recently been described by the National Institutes of Health (NIH) group.[15] However, when the same analysis was applied to their 77 pediatric patients, lymphocyte count was not predictive.[14] More recently, another relatively small study in adults with AA found no such association.[13] These studies were limited by inconsistency of regimens used for IST. The current study investigated a large cohort of children with AA treated using a unified regimen, but failed to confirm any correlation between reticulocyte count and response to IST, suggesting a limited contribution of this clinical parameter to the prediction of hematopoietic recovery, at least in children.

In conclusion, pre-treatment clinical and laboratory findings influence response to IST. Favorable response correlates better with lower WBC count than with neutrophil count or disease severity, and this blood count parameter might help in clinically assessing bone marrow function. Unlike the situation in adult AA, reticulocyte count is not predictive of response to IST in pediatric patients. IST should be started as soon as possible after diagnosis of AA, given that the response rate worsens as the interval between diagnosis and treatment increases.

Authorship and Disclosures

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