Relapse of aplastic anemia in children after immunosuppressive therapy: a report from the Japan Childhood Aplastic Anemia Study Group

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ABSTRACT

Background
Although the therapeutic outcome of acquired aplastic anemia has improved markedly with the introduction of immunosuppressive therapy using antithymocyte globulin and cyclosporine, a significant proportion of patients subsequently relapse and require second-line therapy. However, detailed analyses of relapses in aplastic anemia children are limited.

Design and Methods
We previously conducted two prospective multicenter trials of immunosuppressive therapy for children with aplastic anemia: AA-92 and AA-97, which began in 1992 and 1997, respectively. In this study, we assessed the relapse rate, risk factors for relapse, and the response to second-line treatment in children with aplastic anemia treated with antithymocyte globulin and cyclosporine.

Results
From 1992 to 2007, we treated 441 children with aplastic anemia with standard immunosuppressive therapy. Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) relapsed. The cumulative incidence of relapse was 11.9% at 10 years. Multivariate analysis revealed that relapse risk was significantly associated with an immunosuppressive therapy regimen using danazol (relative risk, 3.15; P=0.001) and non-severe aplastic anemia (relative risk, 2.51; P=0.02). Seventeen relapsed patients received additional immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight patients responded within 6 months. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation and five are alive. Eleven patients underwent hematopoietic stem cell transplantation directly and seven are alive.

Conclusions
In the present study, the cumulative incidence of relapse at 10 years was relatively low compared to that in other studies mainly involving adult patients. A multicenter prospective study is warranted to establish optimal therapy for children with aplastic anemia.

Key words: children, aplastic anemia, relapse, risk factors, immunosuppressive therapy.

Citation: Kamio T, Ito E, Ohara A, Kosaka Y, Tsuchida M, Yagasaki H, Mugishima H, Yabe H, Morimoto A, Ohga S, Muramatsu H, Hama A, Kaneko T, Nagasawa M, Kikuta A, Osugi Y, Bessho F, Nakahata T, Tsukimoto I, and Kojima S, on behalf of the Japan Childhood Aplastic Anemia Study Group. Relapse of aplastic anemia in children after immunosuppressive therapy: a report from the Japan Childhood Aplastic Anemia Study Group. Haematologica 2011;96(6):814-819. doi:10.3324/haematol.2010.035600

Manuscript received on October 20, 2010. Revised version arrived on February 13, 2011. Manuscript accepted on March 14, 2011.

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Introduction

Aplastic anemia (AA) is thought to be an immune-mediated bone marrow disease, characterized by bone marrow aplasia and peripheral blood pancytopenia. Currently, two effective treatments are available for this disorder: allogeneic bone marrow transplantation and immunosuppressive therapy. Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling donor can cure the majority of transplanted patients with severe AA.[1] The outcome after bone marrow transplantation has been markedly better in children than in adults, with less frequent and severe graft-*versus*-host disease and better overall survival.[2,3] However, most children with severe AA have no matched sibling donor and rely on immunosuppressive therapy as first-line treatment.

The combination of antithymocyte globulin and cyclosporine is now considered the standard immunosuppressive regimen for children with severe AA who lack a matched sibling donor.[4] Recent large trials of combined immunosuppressive therapy for severe AA in children demonstrated that the response rate is greater than 60% and the 3- to 5-year survival rate is approximately 90%.[5-7] However, relapse and clonal evolution with transformation to myelodysplasia or acute myeloid leukemia remain significant problems after immunosuppressive therapy, and long-term, event-free survival is less impressive than after bone marrow transplantation.[4,8] We previously reported the results of a multicenter trial of immunosuppressive therapy for children with AA (AA-92 study).[5] In the AA-92 study, the response rate at 6 months was 71%, with the probability of survival at 4 years being greater than 90%. However, a significant proportion of patients subsequently relapsed and required second-line therapy. To select the optimal therapy for such patients, a detailed analysis concerning relapse after response to immunosuppressive therapy is very important; however, analyses of relapse of AA in children after the standard combined immunosuppressive regimen are very limited.[9-11] Although the European Group for Blood and Marrow Transplantation (EMBT) reported an analysis of relapse of AA after immunosuppressive therapy in a large number of patients, the study populations were primarily adults treated in the 1970s and 1980s with antithymocyte globulin monotherapy.[9] A report from the Italian Association of Pediatric Hematology and Oncology focused mainly on the response to cyclosporine and dependence after immunosuppressive therapy.[10] A single-center retrospective analysis from the National Institutes of Health showed excellent long-term survival with a 33% cumulative incidence of relapse at 10 years in children with severe AA who responded to the standard immunosuppressive therapy; however, a detailed analysis of relapse that included risk factors was not provided.[11]

We previously conducted two prospective multicenter studies: the AA-92 and AA-97, which began in November 1992 and October 1997, respectively.[5,12] From 1992 to 2007, 473 children with AA were treated with immunosuppressive therapy in these studies, and 441 of the children were treated with antithymocyte globulin plus cyclosporine. In the present study, we assessed the relapse rate, risk factors for relapse, response to second-line treatment, and prognosis after relapse in AA children treated with an antithymocyte globulin/ cyclosporine-based regimen.

Design and Methods

Patients

Two consecutive prospective studies were designed by the Japan Childhood Aplastic Anemia Study Group and involved 79 hospitals in Japan. The eligibility criteria have been described previously.[5] The severity of disease was determined according to currently used criteria.[13,14] Disease was considered severe if at least two of the following were present: (i) neutrophil count less than 0.5×10^9/L; (ii) platelet count less than 20×10^9/L; and (iii) reticulocyte count less than 20×10^9/L with a hypocellular bone marrow. AA was considered very severe if the above criteria for severe disease were fulfilled and the neutrophil count was less than 20×10^9/L. Non-severe disease was defined by at least two of the following: (i) neutrophil count less than 1.0×10^9/L, (ii) platelet count less than 50×10^9/L; and (iii) reticulocyte count less than 60×10^9/L with a hypocellular bone marrow. Allogeneic bone marrow transplantation was recommended for those patients with severe or very severe disease who had a matched sibling donor. This study was approved by the Ethic Committee of Hyogo Children Hospital.

Treatment

The details of the immunosuppressive therapy administered were described in previous reports.[5,12] Immunosuppressive therapy consisted of horse antithymocyte globulin (Lymphoglobulin; Genzyme Corp., Cambridge, MA, USA) (15 mg/kg per day, days 1 to 5), cyclosporine (6 mg/kg per day, days 1 to 180, with subsequent adjustments to maintain the whole blood cyclosporine concentration between 100 to 200 ng/mL), and methylprednisolone for prophylaxis against allergic reactions (2 mg/kg per day for 5 days, with subsequent halving of the dose every week until discontinuation on day 28). Patients with very severe AA were treated with immunosuppressive therapy plus granulocyte-colony stimulating factor (G-CSF) (Filgrastim; Kirin, Tokyo, Japan) [400 $\mu g/m^2$ on day 1, with responding patients (neutrophil count > 1.0×10^9/mL) receiving the same dose three times a week for 3 months in the AA-92 study and for 60 days in the AA-97 study]. In the AA-92 study, the addition of G-CSF to immunosuppressive therapy for patients with severe AA and non-severe AA was randomized, while in the AA-97 study, G-CSF was not given to patients with severe AA or non-severe AA except to those with documented severe infection. All patients in the AA-92 study received danazol at a dose of 5 mg/kg/day for 6 months, and danazol was discontinued without tapering.

Assessments

A complete response was defined for all patients as a neutrophil count greater than 1.5×10^9/L, a platelet count greater than 100×10^9/L, and a hemoglobin level greater than 11.0 g/dL. For patients with severe AA and very severe AA, a partial response was defined as a neutrophil count greater than 0.5×10^9/L, a platelet count greater than 20×10^9/L, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions. For patients with non-severe AA, a partial response was defined as a neutrophil count greater than 1.0×10^9/L, a platelet count greater than 30×10^9/L, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions.[5] In patients with a complete response on day 180, the cyclosporine dose was tapered down slowly (10% of adjusted dose per month). In those with a partial response, cyclosporine was continued for another 6 months to allow further improvement of blood counts. Tapering of cyclosporine was started on day 360 (10% every 2 weeks) regardless of response.

The hematologic response was evaluated 6 months after the

initiation of therapy. Relapse was defined by conversion to no response from a partial or complete response and/or the requirement for blood transfusions.[5]

Statistical analysis

Failure-free survival curves were calculated by the Kaplan-Meier method, and evaluated by the log-rank test. The Cox proportional hazards model was used to assess the risk factors for relapse after immunosuppressive therapy using both univariate and multivariate analyses. The estimated magnitude of the relative risk (RR) is shown along with the 97.5% confidence interval (CI). Cumulative incidence using the competing risk method, as described by Fine and Gray,[15] was used for the assessment of factors predicting relapse. The competing events of relapse were death and transplantation.

Results

Patients' characteristics

In the AA-92 and AA-97 studies, 441 AA children were treated with antithymocyte globulin plus cyclosporine between 1992 and 2007. The characteristics of all the patients studied are summarized in Table 1. There were 112 and 329 patients in the AA-92 and AA-97 studies, respectively. The median age of all these patients was 8.3 years (range, 0 to 17 years). Patients with very severe (n=210), severe (n=149) and non-severe disease (n=82) received initial immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 264 patients (59.9%) had achieved a complete response (n = 91) or partial response (n=173). Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) subsequently relapsed. The cumulative incidence of relapse was 11.9% at 10 years and the median time from diagnosis to relapse was 21 months (range, 6 to 138 months). The median time from response to antithymocyte globulin therapy to relapse was 22 months (range, 2 to 135 months).

Risk factors for relapse

Two hundred and sixty-four patients with a total of 42 events were eligible for analyses of risk factors for relapse. In univariate analysis, two parameters, non-severe disease (RR=2.98, 97.5% CI 1.40 - 6.34, P=0.0047) and use of danazol (RR=3.44, 97.5% CI 1.78 - 6.65, P=0.00023), were statistically significant risk factors (Table 2). In contrast, the relative risk of relapse for patients with post-hepatitis AA was significantly lower than the relative risk for patients with idiopathic AA (RR=0.234, P=0.043). Gender, age, duration of AA prior to initial treatment, early response (within 90 days after immunosuppressive therapy), use of G-CSF, and HLA-DR2 could not be identified as risk factors. In multivariate analysis, two factors, non-severe AA (RR=2.51, 97.5% CI 1.15 - 5.46, P=0.02) and use of danazol (RR=3.15, 97.5% CI 1.62 - 6.12, P=0.001) remained statistically significant. Figure 1A shows the cumulative incidence of relapse of patients with non-severe AA (35.3%), severe AA (12.9%), and very severe AA (12.0%) 10 years after the first immunosuppressive therapy. The cumulative relapse rate of patients with non-severe AA was significantly higher than that of patients with severe AA (P=0.025) or very severe AA (P=0.005). Figure 1B shows the actuarial risk of relapse at 10 years among patients treated with danazol (29.0%) and in the group not treated with danazol (9.8%) (P<0.001).

Repeated immunosuppressive therapy versus hematopoietic stem cell transplantation as second-line therapy

Among 42 relapsed patients, 17 received a second course of immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight of these 17 patients responded within 6 months and are alive. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation (HSCT) as salvage therapy. The hematopoietic stem cell donors were HLA-matched unrelated bone marrow donors (n=4), unrelated cord blood donors (n=2) and one matched sibling donor. Five of seven patients are alive following HSCT. Eleven patients underwent HSCT directly from an alternative donor (unrelated bone marrow donor, n=7; unrelated cord blood donor, n=1, HLA-mismatched family donor, n=3) and seven are alive. The estimated failure-free survival from the beginning of second-line therapy was 63.6% in the HSCT group compared with 47.1% in the groups treatment with repeated immunosuppressive therapy (P=0.96).

Table 1. Patients' pretreatment characteristics.

	Very severe AA	Severe AA	Non-severe AA
Registered	210	149	82
Sex (male/female)	115/95	83/66	47/35
Median age, years (range)	8.1 (0-17)	8.3 (1-17)	8.5 (2-16)
Etiology of AA			
Ideopathic	168	125	74
Hepatitis	37	21	7
Viral infection	2	1	0
Drug	3	2	1
Median days from diagnosis to treatment (range)	20.4 (1-146)	30.6 (1-180)	44.8 (3-180)
Study (AA-92/AA-97)	46/164	38/111	28/54
Response (complete/partial) (%)	128 (40/88) (61.0%)	91 (38/53) (61.1%)	45 (13/32) (54.9%)
Relapse (AA-92/AA-97)	6/8	9/5	11/3

Table 2. Risk factors for relapse in patients with aplastic anemia by univariate analysis.

Variable	Relative risk (97.5% CI)	P
Sex, male	0.977 (0.514-1.86)	0.94
Age	1.01 (0.947-1.08)	0.78
Etiology of AA		
Ideopathic	4.97 (1.22-20.2)	0.025
Hepatitis	0.234 (0.0577-0.952)	0.043
Duration of AA prior to initial treatment	1.01 (0.998-1.02)	0.11
Response at 90 days	1.07 (0.517-2.21)	0.86
Severity of disease		
Non-severe	2.98(1.40-6.34)	0.0047
Severe	1.21(0.561-2.63)	0.62
Very severe	1	
Study, AA-92 (Danazol+)	3.44 (1.78-6.65)	0.00023
G-CSF (+)	0.915 (0.363-2.31)	0.85
HLA-DR2	0.905 (0.307-2.67)	0.86

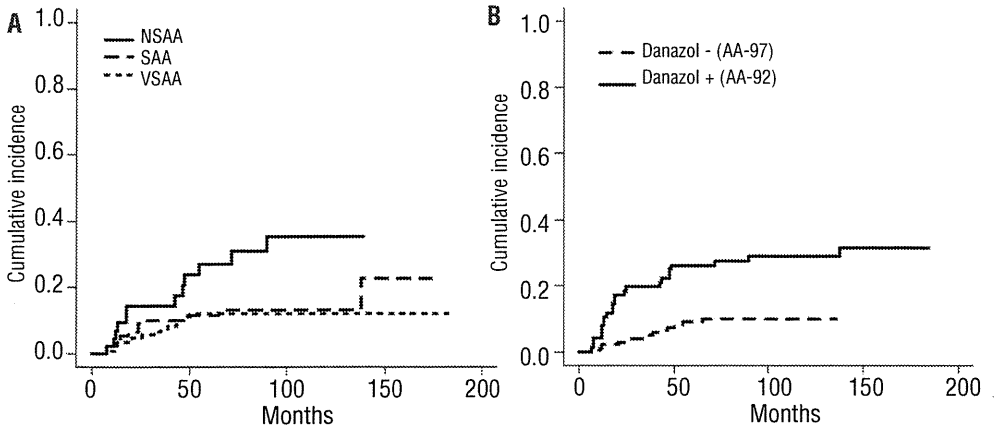

Figure 1. Cumulative incidence of relapse after immunosuppressive therapy in children with aplastic anemia. (A) The cumulative relapse rate of patients with non-severe aplastic anemia (NSAA) was significantly higher than that of patients with severe aplastic anemia (SAA) (P=0.025) and very severe aplastic anemia (VSAA) (P=0.005) 10 years after the first immunosuppressive therapy. (B) The actuarial risk of relapse at 10 years was significantly higher in the group treated with danazol (29.0%) than in the group not treated with danazol (9.8%) (P<0.001).

The overall survival rate did not differ between the immunosuppressive therapy group (84.7%) and the HSCT group (63.6%) after second-line treatment (P=0.07). Other patients were treated with cyclosporine alone (n=6) or bone marrow transplantation from a matched sibling donor (n=6). Two patients did not receive second-line treatments. One patient developed clonal evolution to myelodysplastic syndrome after 65 months, and the second developed acute myeloid leukemia after 37 months. Two patients showed clonal evolution to paroxysmal nocturnal hemoglobinuria after 138 months and 55 months. There were seven deaths among the 42 patients who initially relapsed. The causes of death were HSCT-related complications (n=5), acute myeloid leukemia (n=1) and bacteremia (n=1). The overall 10-year survival rates for patients with very severe AA, severe AA, and non-severe AA were 82.2±3.3%, 82.1±4.7% and 98.2±1.8%, respectively.

Discussion

Analysis of relapse in children with AA responding to immunosuppressive therapy will provide valuable information for the management of childhood AA. Here, we present the results of a comprehensive analysis of the largest consecutive series of AA children treated with standard immunosuppressive therapy. Relapse of AA after immunosuppressive therapy is relatively common, with actuarial risks of 30 - 40% having been reported.[16-18] In the present study, the cumulative incidence of relapse at 10 years was 11.9%, which is relatively low compared with that found in other studies that primarily involved adult patients.[16-18] Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies. A recent Italian study of childhood AA showed a 16% cumulative incidence of relapse, which is comparable with that found in our study.[10]

Multivariate analysis of the data from this retrospective multicenter study shows that the use of danazol was the most statistically significant risk factor for relapse. From 1992 to 2007, 441 children with newly diagnosed AA were treated with immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine with (the AA-92 study) or without danazol (the AA-97 study). There are several reports of the efficacy of anabolic steroids in the treatment of AA. A randomized trial from the EBMT SAA working party demonstrated that the addition of an anabolic steroid (oxymetholone) to antithymocyte globulin treatment improved the response rate of patients with treated AA.[14] In our study, consistent with that report, the response rate at 6 months was higher in the patients who received immunosuppressive therapy with danazol (67.9%) than in the group of patients who received immunosuppressive therapy without danazol (57.1%). Furthermore, our results also showed that the cumulative relapse rate was significantly higher in the patients treated with immunosuppressive therapy plus danazol (Figure 1B). The reason danazol has an impact on relapse is unknown. However, it is possible that a number of cases with an androgen-responsive congenital bone marrow failure syndrome such as dyskeratosis congenita were hidden in our series of AA patients, and discontinuation of danazol was responsible for relapse. Recent reports have shown that a bone marrow failure syndrome of variable severity due to dyskeratosis congenita may be present in otherwise phenotypically normal individuals, and can masquerade as acquired AA.[19-22] We found mutations in the telomerase reverse transcriptase (*TERT*) gene, which is one of the genes causing dyskeratosis congenita, in two of 96 Japanese children with acquired AA.[23] Recently, more dyskeratosis congenita genes have been discovered. It is possible that more cases with an androgen-responsive dyskeratosis congenita were hidden in our series of AA patients. Alternatively, danazol may inhibit complete eradication of pathological T-cell clones by antithymocyte globulin through an unknown mechanism. Understanding the effects of androgens and developing androgen-mimetic drugs could be of significant benefit.

In our cohort of patients with non-severe AA, most patients were transfusion-dependent. In the AA-92 and AA-97 studies, 82 patients with non-severe AA were treated with the standard immunosuppressive regimen consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 13 patients had achieved a complete response and 32 patients achieved a partial response. Among the 32 patients who achieved a partial response, 14 patients later relapsed. However, 18 patients with non-severe AA patients who achieved a partial response maintained their hematologic response, and 12 of them subsequently achieved a complete response. When childhood non-severe AA is treated with supportive care, 67% of patients progress to develop severe AA, suggesting that it is important to consider early immunosuppressive therapy.[24] Our data indicate that

immunosuppressive therapy is beneficial for some patients with non-severe AA.

A previous Japanese study showed that the addition of G-CSF to immunosuppressive therapy increased the hematologic response rate after 6 months and reduced the relapse rate in adult patients with severe AA.[25] Recently, Gurion *et al.* conducted a systematic review and meta-analysis of randomized controlled trials comparing treatments with immunosuppressive therapy with or without hematopoietic growth factors in patients with AA. The addition of hematopoietic growth factors did not affect mortality, response rate, or occurrence of infections, but did significantly decrease the risk of relapse.[26] The data from our AA-92 trial were included in this meta-analysis. In contrast to the other five studies in the meta-analysis, only our study included patients with non-severe AA, who had a significantly higher relapse rate than that of patients with either severe AA or very severe AA. Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies in the meta-analysis. To compare our results with the other studies, we excluded patients with non-severe AA from the statistical analysis, and compared the risk of relapse between patients who did or did not receive G-CSF. The results again showed no significant differences in the relative risk between them (RR=2.71, 97.5% CI 0.614 - 12.0, *P*=0.19).

The majority of patients who experienced relapse responded to reintroduction of immunosuppressive agents.[27] Our present study also demonstrates that a second course of immunosuppressive therapy was a safe and effective treatment for the patients who relapsed after the first immunosuppressive therapy. However, an optimal second immunosuppressive therapy regimen has not yet been established. Furthermore, about half of the relapsing patients eventually received HSCT in our study. The treatment choice was based on center-related preferences or on anecdotal evidence. A multicenter prospective study is warranted to establish optimal therapy for these patients.

Appendix

The following centers and persons participated in the Japan Childhood Aplastic Anemia Study Group: Japanese Red Cross Nagoya First Hospital (K. Kato); Kyoto Prefectural University of Medicine (S. Morimoto); Kobe University School of Medicine (Y. Takeshima); Hyogo College of Medicine (Y. Ohtsuka); Tokai University (H. Yabe); Shizuoka Children's Hospital (J. Mimaya); Fukushima Medical University (A. Kikuta); Tokyo Metropolitan Children's Medical Center, Tokyo (T. Kaneko); Osaka City General Hospital (J. Hara); Nagoya University (S. Kojima) ; Jichi Medical School (T. Yamauchi); Kagoshima University (Y. Kawano); Okayama University (M. Oda); Hokkaido University (R. Kobayashi); Hiroshima University (S. Nishimura); Kanazawa University (S. Koizumi); Keio University (T. Mori); Hiroshima Red Cross Atomic Bomb Hospital (K. Hamamoto); Chiba University (T. Sato); Hirosaki University (E. Ito); Teikyo University School of Medicine (F. Ohta); Tottori University (T. Kawakami); Dokkyo University School of Medicine (K. Sugita); Kumamoto National Hospital (K. Takagi); Seirei Hamamatsu Hospital (T. Matsubayashi); Hyogo Children's Hospital (Y. Kosaka); Yokohama City University (K. Ikuta); Yamaguchi University (H. Ayukawa); Kanagawa Children's Medical Center (T. Kigasawa); Hirakata City Hospital (C. Kawakami); Nakadohri General Hospital (A. Watanabe); Gumma Children's Hospital (T. Shitara); National Defence Medical College (I. Sekine); Gifu University School of Medicine (K. Isogai); Kumamoto University School of Medicine (S. Morinaga); University of Ryukyu (N. Hyakuna); Narita Red Cross Hospital (K. Sunami); Asahikawa Medical College (M. Yoshida); Nagoya City University (Y. Ito).

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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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.

Citation: Yoshida N, Yagasaki H, Hama A, Takahashi Y, Kosaka Y, Kobayashi R, Yabe H, Kaneko T, Tsuchida M, Ohara A, Nakahata T, Kojima S. Predicting response to immunosuppressive therapy in childhood aplastic anemia. Haematologica 2011;96(05):771-774. doi:10.3324/haematol.2010.032805

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.

Manuscript received on September 27, 2010. Revised version arrived on November 27, 2010. Manuscript accepted on January 13, 2011.
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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, $\times 10^9$/L	1.900	2.255	0.007
$\geq 2.0\times10^9$/L, n. of patients (%)	87(47)	85(63)	0.009
$< 2.0\times10^9$/L, n. of patients (%)	93(53)	51(37)	
Median lymphocyte count, $\times 10^9$/L	1.600	2.016	0.006
Median neutrophil count, $\times 10^9$/L	0.218	0.200	NS
Median Hb level, g/dl	6.8	6.8	NS
Median reticulocyte count, $\times 10^9$/L	15.730	17.600	NS
Median platelet count, $\times 10^9$/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, $< 2.0\times10^9$/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, $>25\times10^9$/L	1.589	0.843-2.997	NS
Platelet count, $>20\times10^9$/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

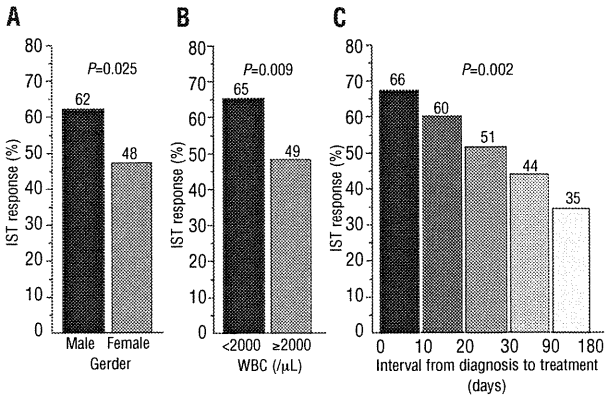

Figure 1. Response to IST in 312 patients according to WBC count, gender, and interval from diagnosis to treatment. (A) **Response rate according to gender. Boys showed better response than girls (62% *vs.* 48%, respectively; P=0.025).** (B) **Response rate according to WBC counts. Patients with WBC count $<2.0\times10^9$/L displayed a significantly higher response rate than patients with WBC $\geq2.0\times10^9$/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

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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Int J Hematol (2011) 93:832–837
DOI 10.1007/s12185-011-0873-0

MEETING REPORT

The Third Consensus Conference on the treatment of aplastic anemia

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Received: 19 January 2011 / Revised: 28 April 2011 / Accepted: 6 May 2011 / Published online: 27 May 2011

1 Introduction

Acquired aplastic anemia (AA) is characterized by bone marrow hypoplasia and peripheral blood pancytopenia. Although the pathogenesis of AA is not well understood, it is thought to be an immune-mediated disease in most patients [1, 2]. The main treatment options for patients with AA include allogeneic bone marrow transplantation (BMT) and immunosuppressive therapy (IST). Recent studies of BMT from an HLA-matched family donor (MFD) showed excellent survival for AA patients. The long-term survival rate of children and young adults with severe aplastic anemia (SAA) after BMT from an MFD ranges from 70 to 90% [3, 4], and BMT currently represents first-line therapy if an MFD is available. The combination of antithymocyte globulin (ATG) and cyclosporine (CsA) results in a response rate of 60–70% in AA patients [5–7] and is indicated as first-line therapy in children and young adults if MFD is unavailable, as well as in all patients older than 40–50 years.

BMT from an HLA-matched unrelated donor (MUD BMT) is indicated for patients who have failed at least one course of ATG and CSA. Better HLA-typing and less-toxic preparative regimens have resulted in substantial increases in survival among patients undergoing MUD BMT [8–10].

Bearing these issues in mind, experts from Europe, America, and Asia presented recent advances in understanding of the pathophysiology and current clinical trials for the treatment of AA (Table 1) at the Third Consensus Conference on the treatment of aplastic anemia on February 21, 2010, in Hamamatsu, Japan. After all speakers had presented, a general consensus was held to establish guidelines for the diagnosis and treatment of AA. Participants included clinicians and scientists from 13 countries, including seven countries in Asia.

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2 Pathogenesis of AA

In Session 1, four scientists presented the latest data regarding the pathogenesis of AA. Dr. Hirano identified two AA-associated antigens, kinectin and anti-postmeiotic segregation increased 1 (PMS1), by screening antibodies in a patient's sera against a peptide library of fetal liver cells [11]. The putative T cell epitope derived from kinectin triggered a cytotoxic T cell response in vitro, and inhibited granulocyte–macrophage colony forming unit formation. However, kinectin-specific T cells were not seen in AA patients. These auto-antibodies are present only in sera of AA patients, and become undetectable in the patients who achieve clinical remission, suggesting that these auto-antibodies may serve as a biomarker for AA, and may correlate with or predict disease activity in AA patients. However, a prospective study conducted by the Japan Childhood Aplastic Anemia Study Group failed to demonstrate a correlation between the presence of anti-PMS1 and response to IST [12].

Dr. Nakao discussed the clinical implication of detecting small paroxysmal nocturnal hemoglobinuria (PNH) clones by sensitive flow cytometric analysis. The presence of an increased number of PNH-type cells was predictive of a response to IST and a favorable prognosis among patients with AA. Ninety percent of patients with increased PNH-type cells responded to ATG + CSA, whereas only 50% of

Table 1 Program

Session 1: S. Nakao, N. Young
Pathogenesis of aplastic anemia (AA)
(1) Autoimmunity in AA
N. Hirano, Dana-Farber Cancer Institute, MA, USA
(2) Application of SNP-array in bone marrow failure syndromes
J.P. Maciejewski, Cleveland Clinic Foundation, OH, USA
(3) PNH clones as a marker of autoimmunity
S. Nakao, Kanazawa University Graduate School of Medicine, Japan
(4) Genetic risk factors for AA
N. Young, National Heart, Lung, and Blood Institute, MD, USA
Session 2: S. Kojima, A. Bacigalupo
Stem cell transplantation
(1) Optimal conditioning regimen
S. Kojima, Nagoya University Graduate School of Medicine, Japan
(2) Role of antithymocyte globulin
A. Bacigalupo, Ospedale San Martino, Italy
(3) Long- term outcome after stem cell transplantation
H.J. Deeg, Fred Hutchinson Cancer Research Center, WA, USA
Session 3: K. Ozawa, G. Socie
Immunosuppressive Therapy
(1) Optimal dose of rabbit-antithymocyte globulin
J.C.W. Marsh, Kings College London, UK
(2) ATG + Cyclosporine vs High-dose Cyclophosphamide for treatment of aplastic anemia
F.K. Zhang, Institute of Hematology & Blood Disease Hospital, China
(3) Role of G-CSF
G. Socie, Hospital Saint Louis, France
(4) Role of iron chelator
J.W. Lee, The Catholic University of Korea, Korea
Session 4: S. Nakao, S. Kojima, A. Bacigalupo
Discussion for General Consensus

patients without PNH-type cells responded. Failure-free survival rates were significantly higher among patients with minor PNH clones than among those without these cells [13].

A single nucleotide polymorphism array (SNP-array) has recently been applied widely as a powerful karyotyping tool that detects deletions, amplifications, and loss of heterozygosity (LOH) at high resolution [14]. Dr. Maciejewski used this new tool in a series of 102 AA patients. Using conventional metaphase cytogenetics, 13% of patients showed cytogenetic abnormalities, which increased to 26% when a SNP-array was used. Early detection of clonal lesions was also possible when using a SNP-array. Interestingly, loss of the short arm of chromosome 6, which encompasses the HLA locus, was detected in three patients before IST. This finding suggests that escape from immune attack may work through the loss of the HLA haplotype in AA patients.

Telomeres are repeated nucleotide sequences that cap the ends of chromosomes and protect them from damage. Telomeres are short in one-third of AA patients [15]. Children with congenital bone marrow failure syndrome, and in particular, dyskeratosis congenita (DC), have extremely short telomeres [16]. Dr. Young demonstrated the presence of mutations in telomerase-complex genes such as TERT and TERC in a small percentage of AA patients without phenotypic characteristics of DC [17, 18]. A family study showed that healthy relatives of patients carrying these mutations also had short telomeres and mild hematologic abnormalities. Although telomere length does not predict response to IST, patients with short telomeres are at high risk of relapse and clonal evolution to myelodysplasia and acute myeloid leukemia after IST [19]. Dr. Young's group recently reported the significant correlation between absolute reticulocyte count (ARC) and absolute lymphocyte count at initial diagnosis and response to IST [20]. A further addition of telomere length increased the predictive capacity. Patients with both high ARC and long telomeres showed excellent survival, whereas those with low ARC and short telomeres had poor outcomes; patients with one of the two variables had intermediate outcomes.

3 Stem cell transplantation

In Session 2, three experts from Asia, Europe, and America discussed the optimization of stem cell transplantation for AA. Until the late 1990s, fewer than 40% of AA patients who underwent MUD BMT survived long-term, and there was a high incidence of graft failure and graft versus host disease (GVHD) [21]. Recent data have shown improved results through better selection of HLA-matched donors and changes in conditioning regimens [22, 23].

Dr. Kojima analyzed a Japanese cohort of 301 AA patients who received MUD BMT through the Japan Marrow Donor Program. Using matched-pair analysis, he showed the superiority of a fludarabine (Flu) + cyclophosphamide (CY) + ATG and radiation regimen compared with a CY + ATG + total body irradiation (TBI) regimen. The current recommended regimen in Japan includes Flu (100 mg/m^2) + CY (3,000 mg/m^2) + rabbit ATG (5 or 10 mg/kg) + 3 Gy TBI. He also used matched-pair analysis to compare tacrolimus (FK)/methotrexate (HTX) with CsA/MTX for the prophylaxis of GVHD in AA patients who received a MUD BMT. Results showed the superiority in overall survival of FK/MTX over CsA/MTX [24].

Dr. Bacigalupo proposed optimized protocols for BMT from an MFD for AA patients. For children and young adults, the recommended regimen is CY (200 mg/kg) + rabbit ATG (7.5 mg/kg). The stem cell source should be bone marrow rather than peripheral blood [25]. GVHD prophylaxis consists of CsA + MTX [26]. There is controversy concerning the upper age limit for BMT in AA patients. A large amount of data from the Europe Group for Blood and Marrow Transplantation (EBMT) showed an inferior outcome in AA patients older than 50 years, although outcomes for patients aged 30–40 years were similar to this aged 40–50 years. To improve outcome, Dr. Bacigalupo proposed a conditioning regimen with Flu (120 mg/kg), CY (1,200 mg/m^2), and rabbit ATG (7.5 mg/kg) for older patients. Dr. Bacigalupo also referred to previously published conditioning regimens for MUD BMT [22]. The current EBMT regimen recommended for children is Flu (120 mg/kg), CY (1,200 mg/m^2), and rabbit ATG (15 mg/kg). For adult patients, the addition of TBI (2 Gy) with a reduced dose of ATG (7.5 mg/kg) is recommended. However, a recent analysis of 100 patients treated according to these protocols revealed that graft failure and Epstein Barr virus (EBV)-lymphoproliferative disease (LPD) still remain significant causes of death [27]. Consequently, Dr. Bacigalupo modified the current EBMT protocol with an increased dose of CY (from 1,200 mg/m^2 to 120 mg/kg), a reduction of rabbit ATG (from 15 to 7.5 mg/kg), and prophylactic administration of rituximab for EBV-LPD.

In the United States, Dr. Deeg previously demonstrated an improved outcome in patients receiving CY + ATG + 2 Gy TBI for MUD BMT, compared with a higher dose of TBI [23]. The ongoing CTNN study in the United States is designed to find the best dose of CY (0, 50, 100, or 150 mg/kg) combined with a regimen of Flu, ATG, and 2 Gy TBI. The 0- and 150-mg trials stopped due to rejection and toxicities, respectively. Both regimens currently undergoing testing in Europe and the United States are similar to the regimen recommended by the Japanese group. Dr. Deeg discussed the late effects of stem cell transplantation and its major adverse effect, i.e., chronic GVHD. There are no benefits associated with chronic GVHD in patients with non-malignant diseases and it increases the risk of secondary malignancy [28]. The most significant risk factor for developing chronic GVHD is the use of peripheral blood stem cells [25]. Dr. Deeg recommended bone marrow, not peripheral blood, as the source of stem cells for AA patients. He analyzed risk factors for chronic GVHD in AA patients who received a matched-related BMT. Patients who received a nucleated marrow cell dose greater than 3.4 × 108/kg developed chronic GVHD 7.7 times more often than those who received a marrow cell dose less than 2.3 × 108/kg ($P = 0.004$). This finding was further reflected in overall survival, which was significantly worse in patients who a received higher dose of bone marrow cells than in those who received a lower dose, although CD34 cell dose was not analyzed in this study.

4 Immunosuppressive therapy

Dr. Marsh summarized the clinical trials of IST with rabbit ATG and CsA for AA as the initial course of treatment [29, 30]. The dose of rabbit ATG varied between 10 and 18.75 mg/kg among studies. Response rates ranged from 50 to 70%, which was equivalent with rates seen with of horse ATG, although patient numbers reported from some of these studies were small. However, immunosuppression of rabbit ATG is more potent than horse ATG, resulting in an increased incidence of infectious complications [31]. Dr. Marsh concluded that it is warranted to conduct a prospective study to find the optimal dose of rabbit ATG, and that larger prospective studies comparing rabbit ATG with horse ATG are needed.

High-dose CY (HD-CY) without stem cell rescue has been developed as a promising therapy for AA by the John's Hopkin's group [32]. However, a randomized trial conducted by the National Institutes of Health showed unacceptable toxicities, leading to early closure [33]. Dr. Zhang compared HD-CY + CsA with ATG + CsA for treatment of AA. The dose of CY was decreased to 120 mg/kg from the original report of 200 mg/kg. The costs of drugs were much cheaper in the CY group than the ATG group in China. The response rate at 6 months was comparable between both groups at 70%. The overall survival at 3 years was also comparable between the two groups, at 85%. It is noteworthy that the rate of early death was less than 5% in the CY group. Dr. Zhang's data justify conducting a randomized study to compare a modified dose of CY therapy with standard ATG therapy for newly diagnosed.

5 AA patients

To date, three prospective randomized studies have addressed the role of granulocyte-colony stimulating factor (G-CSF) in combination with IST [6, 34, 35]. Dr. Socie summarized the results of these studies, which showed faster recovery of neutrophils in the G-CSF group but failed to show significant differences in study endpoints including response rate, incidence of infections, and overall survival between the G-CSF group and the non-G-CSF group. He also presented the latest EBMT study, which enrolled more than 200 newly diagnosed patients with AA [36]. The study also confirmed the results of previous

studies; there was no difference in overall survival or event-free survival between the two arms.

Dr. Lee discussed the role of iron chelation therapy in patients with AA. Regular transfusions lead to the development of iron overload, which is increasingly recognized as a risk factor following HSCT [37]. He presented the results of the EPIC trial, which evaluated the efficacy and safety of deferasirox, an oral iron chelator, in a large cohort of AA patients [38]. After 1 year of treatment, median serum ferritin levels decreased significantly with concomitant improvement of liver dysfunction. The therapy was generally well tolerated, but one quarter of patients suffered from an increase in serum creatinine levels. The concomitant use of CsA had a significant impact on serum creatinine levels.

6 Consensus panel

After all speakers had presented, a general consensus session was held. This session was chaired by S. Nakao, S. Kojima, and A. Bacigalupo. A number of questions were raised by the chairperson, and the following consensus was reached.

6.1 New diagnostic tests

The panelists discussed the relevance of incorporating new diagnostic tests into the management of AA patients. The new diagnostic tests include AA-associated autoantibodies, SNP-array, sensitive flow cytometric assay for PNH clones, and measurement of telomere length. All panelists felt that these new tests may be useful in the investigation of the pathophysiology of AA, but that it is too early to incorporate them into general practice for AA. The findings presented by each speaker must be confirmed by other investigators.

6.2 Stem cell transplantation

All panelists agreed that bone marrow should be used as the source of stem cells. The use of peripheral blood is indicated when a voluntary donor donates peripheral blood. A consensus was reached regarding the upper age limit both for BMT from an HLA-identical sibling and from an unrelated donor. The limit should be 50 years.

The chairperson proposed (1) CY + ATG for young patients and (2) CY + Flu + ATG for older patients as conditioning regimens in the case of HLA-matched sibling transplants. For adult patients transplanted from an unrelated donor, CY + Flu + ATG + low-dose TBI regimen was proposed. Although the panelists did not recommend other conditioning regimens, no general consensus was reached on conditioning regimens. Results of ongoing CTNN study in the United States are expected to reveal the optimal conditioning regimen for unrelated BMT. A higher dose of stem cell infusion has been recommended to facilitate engraftment. According to the presentation by Dr. Deeg, however, a higher dose of stem cell infusion was harmful because of the associated increase with chronic GVHD. The panelists discussed the optimal dose of stem cells, but no agreement was reached. Dr. Deeg emphasized that all of the patients who receive HSCT for a rare disease such as AA should be enrolled into prospective studies to address unsolved questions. All panelists agreed that the donor should be matched at 10/10 or 9/10 levels by HLA high-resolution typing. In the case of patients in whom an appropriate donor is unavailable, unrelated cord blood transplantation or haploidentical transplantation may be indicated.

6.3 Immunosuppressive therapy

The combination of ATG and CsA remains the gold standard for immunosuppressive therapy. Because the supply of horse ATG was stopped in Europe and Asia, rabbit ATG replaced horse ATG in these areas. Because the optimal dose of rabbit ATG has not been clarified, a prospective study to compare two doses of rabbit ATG is proposed. In addition, the panelists discussed the rationale of performing a randomized study to compare a modified dose of high-dose CY + CsA with ATG + CsA as first-line therapy.

Although several panelists agreed with the need for such a study, the majority of the panelists did not place a high priority on this type of study. Most panelists thought that G-CSF is indicated only in limited cases, for example, patients with severe bacterial or fungal infections. Although all published randomized studies revealed that G-CSF has no proven effect on clonal evolution in AA, several panelists felt that a longer follow-up period is necessary to reach a definitive conclusion on this issue.

Appendix

The following persons participated in the conference

Hoon Kook (Gwangiu, Korea), Dae Chul Jeong (Seoul, Korea), Jong Wook Lee (Seoul, Korea), Surapol Issaragagrisil (Bankok,Thailand), Xiao-Fan Zhu (Tianjin, China), Feng-Kui Zhang (Tianjin, China), Jing-Yan Tang (Shanghai, China), Jianping Shen (Hangzhou, China), Minghui Duan (Beijing, China), Jun Ma (Harbin, China), Honorata Baylon (Manila, Philippine), See Voon Seow (Singapore), Michelle Poon (Singapore), Lily Wong Lee (Sabah, Malaysia), Naoto Hirano (Boston, USA), Jaroslaw Maciejewski (Cleveland, USA), Neal Young (Bethesda, USA),

Joachim Deeg (Seattle, USA), Andrea Bacigalupo (Genova, Italy), Judith Marsh (London, UK), Gerard Socie (Paris, France), Keiya Ozawa (Tochigi, Japan), Masao Tomonaga (Nagasaki, Japan), Shinji Nakao (Kanazawa, Japan), Hiroto Yamazaki (Kanazawa, Japan), Akio Urabe (Tokyo, Japan), Seishi Ogawa (Tokyo, Japan), Hiroki Yamaguchi (Tokyo, Japan), Masanao Teramura (Tokyo, Japan), Kensuke Usuki (Tokyo, Japan), Chitose Ogawa (Tokyo, Japan), Ohara Akira (Tokyo, Japan), Tatsutoshi Nakahata (Kyoto, Japan), Hiromasa Yabe (Isehara, Japan), Etsuro Ito (Hirosaki, Japan), Kazuko Kudo (Shizuoka, Japan), Seiji Kojima (Nagoya, Japan), Yoshiyuki Takahashi (Nagoya, Japan), Haruhiko Ohashi (Nagoya, Japan), Koichi Miyamura (Nagoya, Japan).

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Bone Marrow Transplantation (2011) 46, 1148–1150

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LETTER TO THE EDITOR

Alternative donor marrow transplantation in children with aplastic anemia using low-dose irradiation and fludarabine-based conditioning

Bone Marrow Transplantation (2011) **46,** 1148–1150; doi:10.1038/bmt.2010.241; published online 18 October 2010

Allogeneic BMT is a curative treatment for patients with severe aplastic anemia (SAA). In particular, BMT from a HLA-identical sibling is an established treatment for children with acquired SAA, but the results of alternative donor (AD) transplantation have been less favorable because of the high rates of graft failure and severe acute GVHD.[1] However, recent data have shown improvements in the survival of patients receiving unrelated donor (UD) transplantation possibly due to better donor matching attributable to the use of high-resolution HLA typing data.[2] Two major studies have been performed in the setting of UD transplantation for SAA. The first is a Japanese study that reported 154 SAA patients undergoing UD transplantation using CY/TBI/anti-thymocyte globulin (ATG) and also CY/limited field irradiation/ATG: 11% showed rejection whereas 56% survived.[3] The second involves a group in the USA, which reported a study that tested the deescalating doses of TBI (from 6 to 2 Gy) in combination with CY and ATG in UD transplantation for SAA, and showed the best outcomes in patients receiving 2 Gy, with 8 of 13 patients surviving.[4] The current series presents the results of AD transplantation in 13 SAA patients undergoing BMT with the addition of low-dose irradiation to fludarabine (Flu)-based conditioning.

The characteristics of the 13 SAA patients who underwent AD BMT at Tokai University Hospital between September 2001 and May 2008 are shown in Table 1. Of these 13 patients, 12 received prior immunosuppressive therapy, and all were transfusion dependent at the time of AD transplantation. Specifically, the donors were a UD for 11 patients and an HLA mismatched family member for 2 patients. The HLA-incompatible loci are described in Table 1 and detailed data of HLA typing were received from the Japan Marrow Donor Program. Follow-up data were collected on 30 June 2010. Patients were conditioned with 3 Gy thoracoabdominal irradiation (TAI) on day −6, Flu 25 mg/m^2 once daily (i.v.) on days −5, −4, −3 and −2 (total dose 100 mg/m^2), CY 750 mg/m^2 once daily (i.v.) on days −5, −4, −3 and −2 (total dose 3000 mg/m^2) and ATG (thymoglobulin; Genzyme, Cambridge, MA, USA) 1.25 mg/kg once daily (i.v.) on days −5, −4, −3 and −2 (total dose 5 mg/kg) Unmanipulated BM was infused on day 0. Ovary and testis shielding during TAI was performed for all patients, and thyroid gland shielding was provided for five successive patients from patient 9 onwards to prevent secondary thyroid cancer. GVHD prophylaxis was carried out with short-term MTX (15 mg/m^2 on day 1; 10 mg/m^2 on days 3, 6 and 11) and continuous i.v. infusion of tacrolimus 0.02–0.03 mg/kg. Mycophenolate mofetil 15 mg/kg per day (days 14–42) was added for patients who received antigen mismatched donor marrow. All patients were administered G-CSF from day 5 until their neutrophil counts exceeded 1.5×10^9/L.

The clinical outcomes of BMT are summarized in Table 2. All patients achieved neutrophil engraftment ($>0.5 \times 10^9$/L) at a median of day +20 (range, 12–25 days), but one patient showed rejection 31 days after transplantation caused by hemophagocytic syndrome following sepsis. This patient received a second successful BMT from his haploidentical mother with Flu, melphalan and TAI conditioning. The day 30 chimerism by STR analysis of BM mononuclear cells was 100% in all but one patient (96%), and that of PBMCs was 100% in all patients more than 12 months after BMT. None of the patients showed grade III/IV regimen-related toxicity using the Bearman's criteria at any evaluation point. All patients are alive and well; their hemograms are normal with complete donor cell engraftment, and with a Lansky/Karnofsky score of 100% at a median of 64 months following BMT. There were no malignancies observed during the follow-up period.

Both graft rejection and severe acute GVHD were the major causes of failure in the AD BMT for SAA, particularly in mismatched UD.[5] The risk of rejection can be reduced with the addition of 8–10 Gy irradiation,[3] but this is associated with delayed effects on growth, pulmonary toxicity and secondary malignancies.[6] One approach to improve conditioning by the European Group for Blood and Marrow Transplantation SAA working party has been the substitution of TBI with Flu to reduce the risk of secondary tumors.[7] The results are also less favorable in older patients aged more than 15 years, with 61% survival and 32% rejection. Although radiation increases the risk of secondary tumors, very low dose irradiation (2–3 Gy) may reduce such risk, and this procedure is used in many regimens for UD transplantations such as in our study to achieve a stable engraftment.[8] We previously reported that a Flu-based conditioning regimen (low-dose TAI/Flu/CY/ATG) that was used in 27 Fanconi anemia patients for AD transplantations led to successful engraftment in 25 of 26 evaluable patients without severe toxicity.[9] The advantage of Flu may be its strong cytotoxic activity against lymphocytes, which consistently prolongs immunosuppression, thus facilitating the engraftment of hematopoietic stem cells. The combination of Flu and low-dose TAI might establish a definitive engraftment in SAA patients transplanted from an AD.

Table 1 Patient and donor characteristics

Patient no.	*Age (years)*	*Sex*	*Time from diagnosis to BMT (months)*	*Prior treatment*	*Number of pretransplant transfusions*		*Donor type*	*Donor matching*	
					RBC	*Platelet*		*Identity of HLA allele*	*Mismatched HLA allele*
1	13	F	116	ATG, HDMP, CSA, G-CSF, androgen	10	7	UD	6/10	A, C, DRB1, DQB1
2	16	M	47	ATG, HDMP, CSA, androgen	20	5	UD	8/10	C, C
3	10	M	79	ATG, HDMP, CSA, androgen	17	27	UD	7/10	A, C, DQB1
4	15	F	18	ATG, HDMP, CSA	22	10	UD	7/10	A, DRB1, DQB1
5	11	M	21	ATG × 2, HDMP, CSA	22	29	UD	6/10	C, DRB1, DQB1, DQB1
6	4	F	14	ATG × 2, HDMP, CSA	29	66	Mother	4/8	A, B, C, DRB1
7	5	M	32	ATG × 2, HDMP, CSA	10	2	UD	7/10	C, DRB1, DQB1
8	16	M	64	ATG × 2, HDMP, CSA	29	67	UD	7/10	A, C, DQB1
9	15	M	101	ATG × 2, HDMP, CSA	75	46	UD	6/6	No
10	17	M	115	CSA, androgen	9	2	UD	8/10	A, C
11	5	F	17	ATG, HDMP, CSA, G-CSF	20	73	UD	6/6	No
12	14	F	93	None[a]	4	3	Brother	5/6	A
13-1	15	M	35	ATG, HDMP, CSA, androgen	48	7	UD	6/8	B, C
13-2	—	—	—	—	—	—	Mother	4/8	A, B, C, DRB1

Abbreviations: ATG = anti-thymocyte globulin; F = female; HDMP = high-dose methylprednisolone; M = male; UD = unrelated donor.
[a]The status at diagnosis of patient 12 was mild aplastic anemia and she developed very severe aplastic anemia 91 months after diagnosis with recurrent infection.

Table 2 Outcomes of BMT

Patient no.	*Status at BMT*	*Infused TNC ($\times 10^8$/kg)*	*ANC after BMT $>0.5 \times 10^9$/L*	*GVHD*		*Chimerism status (% donor cells)*		*Complication*	*Survival (months)*
				Acute	*Chronic*	*BM At the day 30 chimerism test*	*PB At the last chimerism test (months after BMT)*		
1	SAA	2.07	20	0	No	100	100 (58)	No	>94
2	SAA/+8	3.05	14	0	No	100	100 (36)	No	>75
3	SAA	5.05	20	0	Yes	100	100 (35)	No	>73
4	SAA	3.92	21	0	No	100	100 (53)	No	>69
5	SAA	3.93	15	0	No	100	100 (29)	No	>69
6	SAA	3.95	18	I	Yes	100	100 (27)	No	>68
7	SAA	3.58	21	0	No	100	100 (24)	No	>64
8	SAA	1.12	25	II	No	100	100 (18)	Sepsis	>59
9	SAA	2.67	15	0	No	96	100 (20)	No	>48
10	SAA	1.60	21	I	No	100	100 (27)	EB duodenitis	>47
11	SAA	4.29	21	0	No	100	100 (33)	Sepsis	>43
12	VSAA	3.02	12	0	No	100	100 (16)	No	>34
13-1	VSAA	2.94	16	NA	0	0	0 (0.7)	Sepsis, HPS	>27
13-2	—	5.13	17	0	Yes	100	100 (14)	No	—

Abbreviations: EB = Epstein–Barr virus; HPS = hemophagocytic syndrome; NA = not applicable; SAA = severe aplastic anemia; TNC = total nucleated cells; VSAA = very severe aplastic anemia; +8 = trisomy 8 chromosome positive.

In addition, the total dose of CY (3000 mg/m^2) was reduced by about half from the standard dose at 200 mg/kg for SAA patients to reduce cardiac complications,[10] as many SAA patients have cardiac dysfunction due to persistent anemia and/or iron accumulation.

The second goal of this series was to prevent severe acute GVHD after AD transplantation including mismatched transplants. None of the patients in our study developed grade III/IV acute GVHD. The low-dose ATG administered prior to transplantation might exert a preventive effect on GVHD in conjunction with tacrolimus and short-term MTX.[9]

Taken together, this series demonstrated that a conditioning regimen containing low-dose irradiation and a minimum dose of Flu, CY and ATG could enable the successful engraftment of AD marrow in children with SAA. Long-term follow-up and larger studies are warranted to confirm the high engraftment rates and absence of severe GVHD and secondary malignancies.

Conflict of interest

The authors declare no conflict of interest.

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Bone Marrow Transplantation (2012) 47, 159–160

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LETTER TO THE EDITOR

Successful allo-HSCT with a minimal myeloablative conditioning regimen in an adult patient with Fanconi's anemia

Bone Marrow Transplantation (2012) **47,** 159–160; doi:10.1038/bmt.2011.34; published online 7 March 2011

Fanconi's anemia (FA) is an autosomal recessive (and rarely X-linked) disorder, usually characterized by several congenital malformations, progressive BM failure and an increased incidence of malignancies. Cells from FA patients are characterized by chromosomal instability and marked sensitivity to DNA crosslinking alkylating agents such as mitomycin C[1], which is pathognomonic. The median age at diagnosis of FA is under the age of 7 years; the reported age range is up to 56 years.[2] A report from the International Fanconi Anemia Registry revealed that nearly 40% of patients had no reported physical findings.[3] The heterogeneity of symptoms and the variety of the clinical course may lead to difficulties in diagnosing FA, especially in adult patients.

Here, we describe a case of adult FA without any reported physical findings receiving allo-HSCT from an unrelated donor after a minimal myeloablative conditioning regimen.

The patient was admitted to a reference hospital at the age of 25 years with a chief complaint of general fatigue. The patient's general appearance was normal with no skeletal anomaly, except for slight gross skin pigmentation. Laboratory findings revealed severe anemia (hemoglobin 2.2 g/dL) with an elevated mean corpuscular volume (104 fL), and decreased number of both WBC (2.2×10^6/L) and platelet (1.1×10^{10}/L). Biopsy of BM revealed a severe hypocellularity, and consequently a diagnosis of aplastic anemia was made. Notably, the abnormal chromosomal breakage test with mitomycin C with patient's PBMCs strongly suggested the chromosomal fragility of somatic cells (21 breaks/gap out of 45 cells in the patient, 22 breaks/gap out of 100 cells in the normal control). Because an HLA-identical sibling donor was available, immunosuppressive therapy using antithymocyte globulin, CYA and G-CSF was initiated 2 months after admission. Androgen therapy that was initiated after failure of the immunosuppressive therapy also failed. Eight months later, the patient was referred to our hospital for allo-HSCT with an unrelated donor.

The abnormal chromosomal breakage test is indicative of FA among patients with BM failure syndrome,[4] and a subsequent diepoxybutane test for peripheral T-lymphocytes also revealed the increase sensitivity to the reagent as well, and he lacked FANCD2 monoubiquitination, which has been developed for a rapid diagnosis and subtyping screen for FA based on the FA signaling pathway.[5] These findings lead to a definitive diagnosis of FA.[4] Although awaiting definitive proof of FA, he had no alternative but to receive frequent blood transfusions (10 RBC units/month, 80 platelet concentrates/month).

The first-line treatment for patients under the age of 40 years with severe AA refractory to immunosuppressive therapy is an unrelated allo-HSCT. However, accumulated evidence has demonstrated that a survival rate for FA patients having received unrelated allo-HSCT was only 33%.[6] A recent pilot study of unrelated allo-HSCT using a minimal myeloablative conditioning regimen for FA patients reported that the 1-year OS was 96.3%;[7] thus, we proceeded with unrelated allo-HSCT at the age of 26 years. The number of transplanted nucleated cells was 1.78×10^8/kg. The patient was conditioned with a minimal myeloablative conditioning regimen consisting of 3-Gy thoraco–abdominal irradiation on day −8, 25 mg/m^2 of i.v. fludarabine from day −7 to day −2, 10 mg/kg of i.v. cyclophosphamide from day −5 to day −2, and 1.25 mg/kg of antithymocyte globulin from day −5 to day −2.[7] As prophylaxis for acute GVHD, administration of 0.02 mg/kg FK506 was started on day −1 and a short course of methotrexate was given on days +1, +3, +6 and +11. The patient also received 5 μg/kg of G-CSF from day +5. Neutrophil and platelet engraftment was achieved on days +23 and +46, respectively. Complete donor chimerism in BM cells was achieved on day +64. Despite several other regimen-related toxicities (grade 3 sepsis, grade 3 mucositis, grade 3 pulmonary invasive aspergillosis, grade 2 convulsion during BM cells infusion), the patient was discharged on day +73. He is healthy without any immunosuppressant drug, blood transfusion or secondary malignancy 4 years after HSCT.

Even though G-CSF alone or combined with EPO and/or androgens may be helpful, allo-HSCT represents the only alternative, which is capable of providing corrected long-term hematopoiesis in FA. Results of allo-HSCT from alternative donors have still been insufficient, with a survival rate of 29–38% because of an increased rate of graft rejection, severe GVHD or opportunistic infections.[6,8] However, an increased OS of 72.2–96.3% has been achieved by unrelated allo-HSCT using fludarabine-based conditioning regimen.[7,9,10]

Making a prompt and definite diagnosis of FA in patients with BM failure is pivotal, especially in patients who take advantage of unrelated allo-HSCT using minimal myeloablative conditioning for the following reasons:

(1) Immunosuppressive therapy is revealed to be ineffective, rather time consuming and harmful, considering the requirement for frequent blood transfusions and immunosuppressive therapy-related mortality because of life-threatening infection.