Antithymocyte globulin and cyclosporine for treatment of 44 children with hepatitis associated aplastic anemia

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ABSTRACT

We analyzed the outcomes of 44 children with hepatitis associated aplastic anemia (HAA) who received immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine (CsA). Fourteen (31.8%) patients achieved complete response and 17 (38.6%) achieved partial response, for an overall response rate of 70.4% after 6 months. Seven non-responders received bone marrow transplantation from an HLA-matched unrelated donor and 6 out of 7 are alive. The probability of overall survival at 10 years was 88.3±4.9%, which supports the role of IST with ATG and CsA as treatment of choice for children with HAA without an HLA identical sibling donor.

Key words: hepatitis associated aplastic anemia, antithymocyte globulin, cyclosporine, children

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Hepatitis associated aplastic anemia (HAA) is a variant of acquired aplastic anemia (AA) in which an episode of hepatitis precedes AA by a period of weeks or months.[1,2] Immunologic mechanisms are thought to be involved in the pathogenesis of HAA,[3,4] and the result of immunosuppressive therapy (IST) has been reported in a small study.[5] We have been conducting a prospective multicenter trial of IST for children with acquired AA since 1992. To evaluate the efficacy of IST for HAA, we analyzed 44 patients with HAA who were registered for the protocols of the Japan Childhood Aplastic Anemia Study Group between 1992 and 2001.

Design and Methods

The patients with HAA were enrolled in the prospective studies conducted by the Japan Childhood AA Study Group. HAA was defined as AA developed within 12 months after a documented episode of hepatitis which required an increase in serum aminotransferase level more than three times the upper limit of normal range. Patients with HAA were eligible if they met the following criteria: age <18 years, recently diagnosed disease (within 180 days from diagnosis) without specific prior treatments, and moderate to very severe AA. Severity of disease was classified according to currently used criteria.[6] Chromosome-breakage study and Ham/sugar water test were performed to rule out Fanconi's anemia and paroxymal nocturnal hemoglobinuria (PNH) in all patients. From November 1992 to September 1997, 21 patients were treated according to the Childhood AA 92 protocol. After stratification for severity of disease, patients were randomly assigned to treatments with horse ATG (Lymphoglobuline; IMTIX-SANGSTAT, Lyon, France), cyclosporine, and danazol with or without recombinant human granulocyte colony-stimulating factor (rhG-CSF).[7] During the next study, 23 patients were treated by Childhood AA 97 protocol which slightly modified the previous treatment.[8] Danazol was omitted, and administration of rhG-CSF was restricted to patients with very severe AA. Hematologic response was evaluated at 3 and 6 months after the start of the therapy. The definition of complete response (CR), partial response (PR), and relapse was as previously described.[7] Patients who failed to respond to first IST underwent bone marrow transplantation if a suitable donor was avail-

able. Toxicity of the treatment was evaluated according to World Health Organization criteria.[9] All participating hospitals were required to complete case report forms. Survival was analyzed using the Kaplan-Meier method. Informed written consent was obtained from all patients or their parents and the protocol study was approved by the ethics committee of each center.

Results

From November 1992 to October 2001, a total of 318 children with newly diagnosed AA were enrolled into the two consecutive protocol studies. AA was associated with hepatitis in 44 patients (14%). The analysis of data was performed in April 2006. Patient characteristics are summarized in Table 1.

The onset of hepatitis was documented by jaundice and markedly elevated liver enzyme levels. The median level of peak alanine aminotransferase (ALT) was 1,181, ranging from 521 to 2,570 IU/L. The median interval between onset of hepatitis and development of AA was 30 days (range 0-240 days). All patients except 1 developed pancytopenia within 6 months of diagnosis of hepatitis. AA and hepatitis developed simultaneously in 3 patients. Data of hepatitis virus were available for 38 patients. Serologic tests for hepatitis A, hepatitis B and hepatitis C were all negative. The response rate is shown in Table 2. At 3 months after start of therapy, CR was observed in 2 (4.5%) and PR in 15 patients (34.1%), for an overall response rate of 38.6%. At 6 months, CR was observed in 14 (31.8%) and PR in 17 (38.6%) patients. The overall response rate increased to 70.4%. Patients with lower neutrophil counts took longer to respond to therapy. After 3 months, only 24.0% of patients with a neutrophil counts of $<0.2\times10^9$/L responded compared with 57.9% of patients with a count of $>0.2\times10^9$/L ($p<0.05$).

Within the first 3 months of therapy, 3 patients died from infections. Eight out of 10 patients without response at 6 months received second IST after which only 2 patients achieved a sustained response. One died of bacteremia 17 months after registration. The remaining 7 patients subsequently received BMT from an HLA-matched unrelated donor. Six out of 7 patients restored bone marrow function and are alive with a median follow-up period of 60 months following BMT (range 46-102 months).

None of the 33 patients who responded subsequently relapsed. At the time of diagnosis, cytogenetic studies were available for 38 out of 44 patients. No patients showed cytogenetic abnormality. During the follow-up period, new clonal abnormalities appeared in 2 patients. One showed trisomy 8 at 12 months after the start of therapy and the other showed del (13) (q14q21) at 19 months. Neither patient showed distinctive morphologic findings of myelodysplastic syndrome (MDS). They are alive 118 and 115 months after the cytogenetic abnormality was identified without transformation to malignant disease. Figure 1 shows the probability of survival at 10 years (88.3±4.9%). There were 4 deaths in the very severe AA group and 1 in the severe AA group. Causes of death included interstitial pneumonitis (n=2), bacterial or fungal infections (n=2), and transplant related toxicity (n=1). Antigenemia assay identified CMV was detected in the peripheral blood cells of two patients who died of pneumonitis. Overall, treatment was well tolerated. At the start of IST, 7 patients showed an increase in serum ALT levels to at least two times the upper limit of the normal range. Serum ALT levels rapidly decreased within one

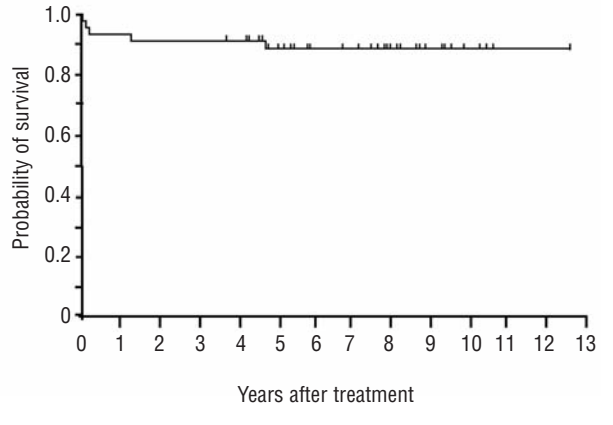

Figure 1. Survival of 44 patients who received IST. Probability of overall survival at 10 years was 88.3±4.9%.

Table 1. Patients' characteristics.

Characteristics	*N. of patients*
Patient number	44
Sex; male/female	28/16
Median age; year (range)	9 (1-18)
Median days from diagnosis of AA to treatment	12 (1-146)
Severity of disease	
very severe	25
severe	15
moderate	4
Median neutrophil count; $\times10^9$/L (range)	0.15 (0-1.05)
Median platelet count; $\times10^9$/L (range)	12 (1-58)
Median reticulocyte count; $\times10^9$/L (range)	13 (0-59)
Peak blood chemistry	
median T-Bil; mg/dL (range)	8.8 (1.1-23.9)
median AST; IU/L (range)	1012 (200-2893)
median ALT ; IU/L (range)	1181 (521-2570)
Median days between hepatitis and AA (range)	30 (0-240)

AA: aplastic anemia; T-Bil: total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

week after treatment in all patients except one. Serum ALT levels returned to normal in 50% of patients within 4 weeks after treatment.

Discussion

HAA is documented in 5-10% of patients with acquired AA.[10,11] Because of the rarity of the disease, few patients enter the large prospective studies of IST for patients with acquired AA. Only a retrospective data of 10 patients are available for the evaluation of IST with ATG and CsA in patients with HAA.[5] In this report, 7 out of 10 patients responded to intensive IST. Recently, Savage *et al*. reported that 4 out of 5 patients with HAA responded to high dose cyclophosphamide and are in remission without further immune suppression.[12] In the current study, we analyzed the data of 44 patients with HAA who were registered for our prospective studies. Overall, 70.4% of patients responded within 6 months and the probability of 10 year survival was 88.3±4.9%. The response rate of ATG and CsA therapy ranged from 60-80% in several large prospective studies for idiopathic AA.[13-15] In our study, the response rate was 72% for 90 children with idiopathic AA.[7] Given that the same response rate was observed for both HAA and idiopathic AA, it has been suggested that both diseases are mediated by a common immunopathologic mechanism. At the beginning of the AA 92 study, 3 patients developed typical interstitial pneumonitis at 38, 60, and 68 days after the start of IST, 2 of whom died. CMV-positive antigenemia was detected in peripheral blood cells from 2 of them. CMV pneumonitis is a common cause of death in severely immunocompromised hosts. A marked decrease in $CD4^+$ lymphocytes was reported in patients with HAA.[3-5] Therefore, it seems that ATG and CsA cause more intensive immunosuppression to reactivate CMV. We gave prophylactic therapy for interstitial pneumonitis as for patients who received allogeneic stem cell transplantation after which no patients suffered from interstitial pneumonitis. Therefore, prophylactic therapy and virologic surveillance for CMV are recommended after IST for patients with HAA.

Liver toxicity is one of the most common adverse effects in patients receiving ATG and CsA. In 70% of patients, liver dysfunction was observed within 3 weeks after treatment.[13] It is, therefore, an important question whether IST exacerbates the preceding hepatitis. However, we did not observe any grade II to IV liver toxicity. Liver enzyme levels rapidly decreased after treatment even in patients with liver dysfunction. Cytotoxic T lymphocytes are thought to cause liver damage in viral hepatitis.[16,17] A recent study showed that the Fas/Fas-ligand pathway was involved in this process.[18] ATG and CsA may eliminate the cytotoxic T lymphocytes and improve liver dysfunction. IST may therefore be useful even in patients with active hepatitis and it is recommended that therapy should start early.

In conclusion, these observations support the view that, for HAA patients without an HLA matched related donor, treatment with ATG and CsA should be started immediately after diagnosis, and BMT from an alternative donor is indicated as a salvage therapy for non-responders to the first IST.

Table 2. Response to immunosuppressive treatment.

	Very severe AA (n=25)	*Severe AA (n=15)*	*Moderate AA (n=4)*	*Total (n=44)*
3 months				
CR	0 [24%]	1 [53%]	1 [75%]	2 [39%]
PR	6	7	2	15
NR	19	7	1	27
Alive	18	6	1	25
Dead	1	1	0	2
6 months				
CR	4 [60%]	7 [80%]	3 [100%]	14 [70%]
PR	11	5	1	17
NR	10	3	0	13
Alive	8	2	10	
Dead	2	1	3	
12 months				
CR	10 [68%]	9 [80%]	3 [100%]	22 [75%]
PR	7	3	1	11
NR	6	3	0	9
Alive	4	2	6	
Dead	2	1	3	
Non-evaluable*	2	0	2	

*CR: complete response; PR: partial response; NR: no response; AA : aplastic anemia. *Two patients received bone marrow transplantation from unrelated donors at 7 months from diagnosis.*

Authors' Contributions

SK is primarily responsible for the paper: SK, MY,AO and IT promoted and designed the study. HY collected and analyzed the data; YO, MS, YK, TT, TI, TS and JM took care of the patients. YO and HY wrote the report with a contribution from SK.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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