RESEARCH PAPER

Survival After Immunosuppressive Therapy in Children with Aplastic Anemia

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Received: April 6, 2011; Initial review: May 2, 2011; Accepted: June 21, 2011.

Objective: To determine the survival of children ≤18y, treated with immunosuppresive therapy (IST) using equine antithymocyte globulin (e-ATG) and cyclosporine (CsA).

Design: Prospective data entry as per a specified format.

Setting: Tertiary care hospital.

Patients: From January 1998 to December 2009, 40 children were diagnosed with acquired aplastic anemia; 33 patients, who received IST, were analyzed. 31 children (94%) received one course of e-ATG and CsA. 2 patients (6%) received two courses of ATG.

Intervention: Immunosuppressive therapy using equine ATG and cyclosporine.

Main Outcome Measures: Overall response and overall survival.

Results: The overall response (complete response + partial response) to IST at 6 months was 87.9%. 8 (24.2%) patients achieved CR, 21 (63.6%) patients had PR and 4 (12.1%) patients did not respond to IST. Median follow-up was 24 (6-102) months. Overall survival at 24 months was 90%, with an acturial survival of 85.4% at 5 years. Seventeen patients (51.5%) received G-CSF for a median duration of 32 (23-64) days. The patients who received G-CSF had fewer infectious complications (*P*=0.002), but G-CSF administration did not influence survival/ outcome. No patient developed myelodysplastic syndrome or acute leukemia.

Conclusions: The survival of patients who respond to IST is excellent. Also, G-CSF reduces the infectious complications without conferring any survival advantage.

Key words: *Antithymocyte globulin, Aplastic anemia, Ciclosporine, Granulocyte-Colony Stimulating Factor (G-CSF), Immunosuppressive therapy, India, Treatment.*

Published online: 2011 October 30. P II: S09747559110000298 – 1

Aplastic anemia is a bone-marrow failure disorder characterized by immune mediated bone marrow destruction, and immunosuppressive therapy forms an essential aspect of therapy [1]. In general, the outcome after hematopoietic-stem-cell-transplantation has been found to be better than immuno-suppressive therapy, but since most children lack a histocompatible donor, it is often administered as the initial therapy. The standard regimen includes anti-thymocyte globulin (ATG) plus cyclosporine (CsA) [2-4].

Several independent studies have predicted survival ranging from 67.5% to 80% [5-8]. Furthermore, though the role of Granulocyte-Colony Stimulating Factor (G-CSF) addition to immunosuppressive therapy is debatable, many centers use additional G-CSF, particularly in pediatric patients [8-10]. To gain insights into the survival of children treated with immune suppressive therapy, we conducted a single center analysis of overall response and overall survival in children with aplastic anemia treated with ATG plus CsA.

METHODS

All patients ≤18y of age, diagnosed as aplastic anemia at a tertiary care center in India, from January 1998 to December 2009 were included in the study. Patients were excluded if they were diagnosed with an inherited marrow failure syndrome before treatment or if they underwent stem cell transplant. The details regarding medical history, physical examination, complete blood count, bone marrow aspirate and biopsy were retrieved. The inherited bone marrow failure syndromes were excluded based on medical history, family history, physical examination, bone marrow cytogenetics, and chromosomal fragility studies with diepoxybutane, echocardiogram and ultrasound of the abdomen.

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Paroxysmal Nocturnal Hemoglobinuria (PNH) was excluded by Hams's test and urine for hemosiderin (till 2004), and by flow cytometry for determination of CD55 and CD59 from January 2005 onwards. Additional tests including liver function tests, renal function tests, and serology for hepatitis A,B,C, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and parvovirus B-19 were performed depending upon clinical setting.

In addition, the data regarding the therapeutic profile of the patients' therapy with ATG and CsA, supportive therapy with antibiotics, transfusions and G-CSF was obtained. All the courses of ATG and CsA were documented and response to therapy at 6 month, 12 month, 18 month and last follow-up was recorded. The data was entered as per a pre-specified proforma.

Disease severity: Patients were classified according to published severity criteria [11,12]. Aplastic anemia was considered severe if the marrow cellularity was <25%, and at least 2 of the following criteria were met: neutrophil count $<0.5\times10^9$/L, platelet count $<20\times10^9$/L, or reticulocyte count $<20\times10^9$/L. It was considered very severe if the above criteria were fulfilled, and the neutrophil count was $<0.2\times10^9$/L. Moderate aplastic anemia was defined as hypocellular bone marrow with at least two of the following hematological values: neutrophil count $<1\times10^9$/L, platelet count $<50\times10^9$/L, or reticulocyte count $<60\times10^9$/L, but not sufficient for severe category. Hepatitis-associated aplastic anemia was defined when it occurred either concurrent or within 6 months after presentation with an increase in serum alanine amino- transferase level by at least five times the upper reference limit.

Treatment protocol: The treatment was initiated only after obtaining consent from the parents of the child. Equine ATG (e-ATG, Atgam, Pfizer Inc, New York, NY) was administered intravenously at a dose of 40 mg/kg/day for 4 days as continuous infusion over 12-18h. On day 1, it was administered at a very slow rate intravenously initially to evaluate for immediate hypersensitivity reaction, and if there was no reaction to infusion, it was infused at the regular rate for next 12-18h. CsA was administered orally from day 21 of ATG at a dose of 8-10 mg/kg/day in 2 divided doses and adjusted to maintain serum levels between 150-200 µg/L or for renal/hepatic toxicity.

Oral prednisolone at a dose of 2 mg/kg/day was administered for 7 days followed by a 1 week taper for prevention of serum sickness. Platelets were transfused prophylactically for levels $<10\times10^3$/L and at higher levels in setting of symptomatic bleeding. Red blood cell transfusions were given for hemoglobin <70g/L or symptomatic anemia. All blood products were irradiated [12]. Most patients received single donor platelet units. However, random donor platelets were also used. Febrile neutropenia was managed with intravenous antibiotics with addition of antifungals after 3-4 days of unresponsive fever in accordance with the institutional antimicrobial policy. No prophylactic oral antifungals or antibiotics were administered. G-CSF was administered at a dose of 5µg/kg/day subcutaneously. The point of initiating G-CSF was not predefined and was variable in different patients. G-CSF was used in all patients of very severe aplastic anemia, and was not used in patients in moderate category. In patients with, G-CSF was administered if the patient had febrile neutropenia or sepsis, based on the treating physicians discretion. A second course of e-ATG or rabbit ATG (r-ATG, Thymoglobulin, Genzyme Corporation, Cambridge, MA) was administered, if the patient had not responded after 6 months of initial treatment or relapsed after initial response. CsA was administered for 12 months and was, thereafter, tapered gradually over next 3-6 months, so that each patient received CsA for 15-18 months.

Response criteria: A complete response was defined as neutrophils $>1.5\times10^9$/L, platelets $>100\times10^9$/L and hemoglobin value normal for age and sex. A partial response was defined when the counts were not sufficient for a complete response and the absolute neutrophil count (ANC) was $>0.5\times10^9$/L, platelets $>20\times10^9$/L and hemoglobin >80 g/L in patients with severe, and ANC $>1.0\times10^9$/L and platelets $>30\times10^9$/L and hemoglobin >80 g/L in patients with moderate aplastic anemia. The response was assessed 6 months after sATG administration. Relapse was indicated by a decline in peripheral blood cell counts to levels meeting the definition of severe or moderate aplastic anemia.

For detecting clonal disorders, the patients were followed up using peripheral blood counts and biochemistry. The bone marrow and cytogenetic studies were attempted only if the blood counts or biochemical profile showed any abnormality.

Statistical analysis: Overall response was calculated as the sum of partial and complete response. Overall survival (OS) was measured from the time of onset of treatment to the time of last follow-up or death. Summary statistics, including means, medians, and proportions were used to describe patients' baseline characteristics. The multi-variate Cox regression model was used to analyze the risk factors for death. Variables with *P* values <0.1 in univariate analysis were entered in stepwise selection models and hazard ratios (HR) with 95% confidence intervals (CI) were calculated. Survival

analysis was done using the Kaplan-Meier curves. Statistical analysis was done using GraphPad Prism version 5.00 for MacOsX (GraphPad Software, San Diego California USA) and SPSS version 16.0 (SPSS Inc, Ill, USA).

RESULTS

From January 1998 to December 2009, 40 children were diagnosed as acquired aplastic anemia; 7 were excluded (one died on day 2 of ATG administration and six did not consent for receiving IST). In total, 33 patients who received IST were included for final analysis (***Table* I**). No PNH positive cases were detected during the study.

Of the 33 patients, one had hepatitis-associated-aplastic anemia; the serological tests for hepatitis A, B, C, CMV, and EBV were negative. No other causes for secondary aplastic anemia were found in any other patient. Twenty-eight patients had idiopathic severe aplastic anemia, 4 had very severe (3 idiopathic, one hepatitis associated), and one child had idiopathic moderate aplastic anemia.

Response to immunosuppressive therapy

Thirty-one children (94%) received one course of e-ATG. Two patients (6%) received two courses of ATG. One child who failed to respond to first course of e-ATG was administered a second course of e-ATG, but he continued to be a non-responder. The other patient received r-ATG as the second course for relapse, after first course of e-ATG and he responded to the therapy. r-ATG was not consistently available and its availability was the factor determining whether the patient received e-ATG or r-ATG.

The overall response to therapy was seen in 29/33 (87.9%) patients. Eight (24.2%) patients achieved complete response, 21 (63.6%) patients had partial response and 4 (12.1%) patients did not respond (***Table* II**). The median time to achieve complete response was 9 months.

Overall survival: Median follow-up in our study was 24 (6-102) months. OS at 24 months was 90% (30 patients). One patient who was a non-responder was alive at 24 months and was receiving supportive care. The actual survival at 5 years was 85.4% (***Fig.*** 1***a***). ***Figure*** 1***b*** highlights the survival comparison between the CR, PR and NR group of patients. Three patients died; two at 6 months and one within 12 months of receiving IST. Sepsis, acute pancreatitis, and pulmonary aspergillosis accounted for one fatality each. All the 3 patients who died were non-responders to IST.

Complications: During the first 90 days after ATG administration, there were 39 episodes of infection noted in 19 patients. One patient with partial response relapsed 360 days after ATG administration. However, no clonal disorders were detected in any patient during the follow-up.

TABLE I PRETREATMENT CHARACTERISTICS OF STUDY POPULATION *(N*=33*)*

Variable	
Age (yr)*	14 (7y-18y)
M:F	1.54:1
Duration of symptoms*	2.5 (1-15) mo
Fever, *n* (%)	16/33 (48%)
Bleeding diathesis, *n* (%)	22/33 (66.7%)
Pallor, *n* (%)	23/33 (70%)
Hemoglobin (g/dL)*	6.9 (3.5-9.3)
Absolute reticulocyte count ($X10^9$/L)*	19 (14-22)
WBC count ($X10^9$/L)*	1.6 (0.85-3.35)
ANC count ($X10^9$/L)*	0.35 (0.145-0.820)
Platelets ($X10^9$/L)*	14 (4-28)

**Values in median (range).*

G-CSF: Seventeen patients (51.5%) received G-CSF for a median duration of 32 days (23-64 days) In total, 94.5% (16/17) patients who received G-CSF and 81.3% (13/16) patients who did not receive G-CSF responded to immune suppressive therapy (Relative Risk=1.16, 95% CI=0.89-1.51, P=0.26). In patients who received G-CSF, one patient died (5.9%), compared to two deaths (12.5%) in those who did not receive G-CSF (RR=1.08, 95%CI=0.86-1.34, P=0.51).

The patients who received G-CSF had fewer infectious complications (7/17 [41.2%] patients; 9/39 [23.1%] infection episodes) as compared to those who did not receive any G-CSF therapy (12/16 [75%] patients; 30/39 [76.9%] episodes (RR=0.33, 95% CI=0.16 to 0.70; *P*=0.002).

The number of days of hospitalization among patients who received G-CSF *versus* those who did not receive G-CSF could not be determined as this data was not available.

Factors predicting outcome: In a stepwise multivariate regression analysis, none of the factors were predictive of response or outcome. We compared the covariates between 29 patients (responders) and 4 patients (non-responders).

The variables that might influence response to IST and OS, including age, sex, duration of disease before onset of therapy, clinical presentation, blood counts at

TABLE II OUTCOME OF CHILDREN TREATED WITH IMMUNOSUPPRESSIVE THERAPY

Evaluation	*Complete response*	*Partial response*	*No response*	*Alive*	*Cumulative Mortality*
6 months	3	26	2	31	2
12 months	7	21*	2*	30	3
18 months	8	21#	1^	30	3
Last follow-up	8	21	1	30	3

**One patient with partial response relapsed;#One patient who relapsed responded to second course of rabbit ATG; ^One non-responder continued to be non-response even after second course of equine ATG.*

presentation, and use of G-CSF therapy, were tested in a univariate analysis. The following factors had a significant influence on OS (P<0.1): age<12years, absolute neutrophil count (ANC) <300/μL, absolute lymphocyte count (ALC) >1000/μL. The use of G-CSF and other variables did not influence outcome. Including all these variables in a multivariate Cox regression analysis, age<12 years ANC<300/μL and ALC>1000 were not predictive of response/ outcome.

DISCUSSION

HSCT often is the initial treatment in children who have an HLA-matched sibling donor [1]. The primary treatment of these children when a matched related donor is unavailable is immune suppressive therapy [13]. Our study shows an overall response of 87.9% to immune suppressive therapy with ATG and CsA. The results from other studies also demonstrate response rates from 74% to 81%, with OS varying from 67.5% to 88% over 3 to 5 years. Our results confirm an excellent response to IST among children with aplastic anemia. Unlike most of the published series, in our study only one patient (3%) relapsed, possibly due to prolonged duration of CsA therapy and slow tapering.

Our results are better than most of the published Indian series. In a recently published trial where the response to IST was compared to HSCT, authors reported only 43.5% response to IST and an overall survival of 70% post HSCT [14]. Similarly, an earlier series demonstrated 40% response to IST at 6 months and 45% response to IST at one year [15]. Our results are definitely superior to previously published Indian data and are comparable to those from the developed countries. This is probably due to similar care, both the primary treatment and the supportive care, being delivered to all children irrespective of the socio-economic background as the complete expense of treatment was borne by the armed forces.

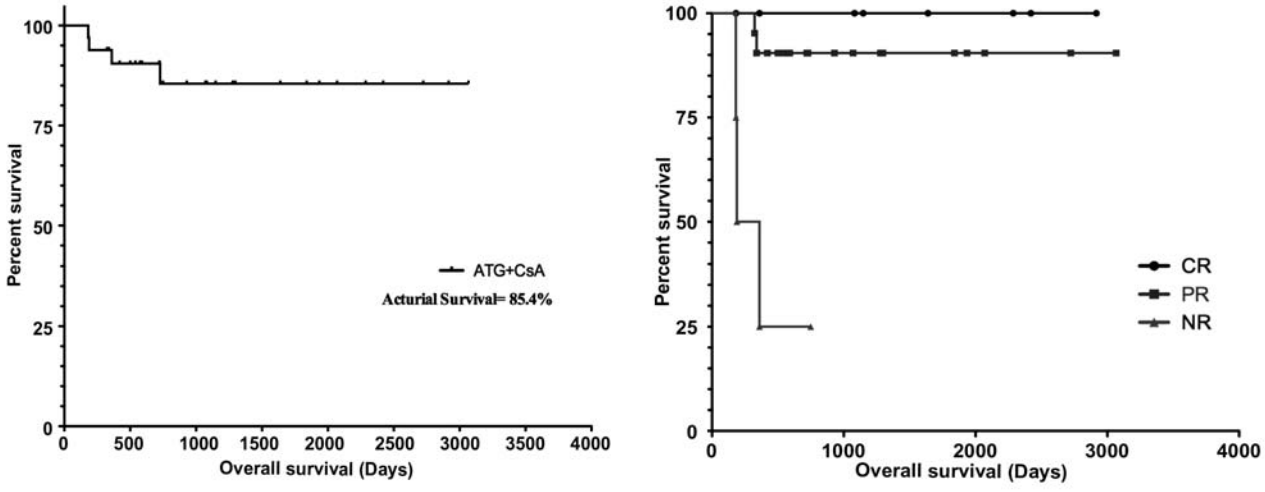

FIG.1 (*a) Kaplan-Meier estimates of overall survival of 33 children and adolescents with aplastic anemia treated with antithymocyte globulin and ciclosporine; (b) The comparison of survival among the complete responders, partial responders and non-responders. Log rank (Mantle-Cox Test) to compare survival between CR and PR failed to show any statistical significance (Hazard Ratio=0.26, 95% confidence interval-0.003 to 24.36, P=0.56.*

WHAT IS ALREADY KNOWN?

- Immunosuppressive therapy using anti-thymocyte globulin and cyclosporine is effective as first line therapy in children with aplastic anemia.

WHAT THIS STUDY ADDS?

- With Immunosuppressive therapy using e-ATG and CsA, response rates and overall survival of >85% can be achieved.
- G-CSF may reduce the episodes of infection in these children but fails to offer any survival advantage or influence outcome.

The use of G-CSF in the immediate neutropenic phase after ATG administration is controversial. We demonstrate a reduction in the number of infectious episodes in the first 90 days after ATG administration in patients who received G-CSF. Though G-CSF did not confer any advantage in terms of OS or improved response rates, the reduction of infectious episodes can translate into shorter hospitalization and lesser morbidity. These results corroborate those of Tichelli, *et al*. [16], where they failed to demonstrate impact of G-CSF on OS, event free survival or on remission.

None of our patients developed clonal disorders with use of G-CSF. The results from other researchers have been conflicting with some studies suggesting a higher risk of clonal disorders with the use of G-CSF [17,18], while others, including a meta-analysis, failing to substantiate it [8,19-21]. However, the follow-up time in our study is too short for a definitive statement and we cannot draw any inference in this respect from our study.

Although some methods to predict response to IST have been suggested, but none has been standardized. In an analysis of 300 patients of all ages, younger age, higher pretreatment absolute reticulocyte count (ARC>25000/μL), and higher pretreatment absolute lymphocyte count (ALC>1000/μL) were predictive of a favorable response to IST [22]. In the same study, on subset analysis authors found that only ARC (and not ALC) correlated with response in pediatric age group (<18y) [6]. Similarly, in a large European study, a low pretreatment absolute neutrophil count (ANC<200/μL) was found to be predictive of response to IST in children [10]. In multi-variate regression analysis, we failed to demonstrate any predictors of response/survival. However, due to a small sample size and only four non-responders, the analysis for predicting variables for survival/ response may be skewed and not definitive.

The limitations of our study include a median follow-up period of 24 month and a small sample size. Aplastic anemia is a rare disorder and hence, a single center accrual is scarce. To summarize, we demonstrate that the outcome with equivalent ATG/CsA as first line therapy in children is excellent and this corroborates with a similar response demonstrated from other countries.

Acknowledgements: Commandant, Army Hospital (Research and Referral Centre) for providing treatment cost. We are also grateful to the office of DGMS (Army) and the office of DGAFMS for supporting our endeavor.
Contributors: VN was the principal investigator and will act as guarantor of the study. VN, AS, SD, and SS recruited the patients. VS participated in the statistical analysis. VN, AS, and SD coordinated the research. VN and VS wrote the paper. The final manuscript was approved by all authors.
Funding: Nil; *Competing interests: None stated.*

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