

# Long-term outcome of 25 children and adolescents with severe aplastic anemia treated with antithymocyte globulin

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## Abstract

Severe aplastic anemia (SAA) is probably an immune-mediated disorder, and immunosuppressive therapy is recommended for patients with no available donor for bone marrow transplant. Between October 1984 and November 1987, 25 consecutive children and adolescents with SAA with no HLA-compatible marrow donor received equine antithymocyte globulin (ATG) ( $15 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) for 10 days. The patients were evaluated 6 weeks, 6 months, and 12 months after starting ATG treatment. Thereafter, patients were evaluated yearly until July 1998. Median age was 10 years (range, 1.5-20 years), granulocyte counts on referral ranged from  $0.032$  to  $1.4 \times 10^9/\text{l}$  (median  $0.256 \times 10^9/\text{l}$ ), and 12 patients had granulocyte counts  $<0.2 \times 10^9/\text{l}$ . At a median follow-up of 9.6 years (range, 8.6-11.8 years), 10 patients (40%) remained alive with good marrow function. No morphologic evidence of hematological clonal disorders has been observed, although two patients probably have acquired clonal chromosomal abnormalities (trisomy 8 and  $\text{del}(6)\text{q}21$ , respectively). Responses to ATG were observed between 6 weeks and 6 months from the start of treatment in 60% of evaluable patients. The response rate was not different in patients whose granulocyte count at diagnosis was  $<0.2 \times 10^9/\text{l}$ , or in those who were  $<10$  years of age. This study supports the view that, when compared with supportive measures, ATG is an effective treatment for children or adolescents with SAA. Although these results are inferior to those reported for marrow transplantation or more intensive immunosuppressive regimens, these patients who responded to ATG are long-term survivors with stable peripheral blood counts and a low rate of relapse.

## Key words

- Aplastic anemia
- Children
- Adolescents
- Antithymocyte globulin

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## Introduction

Severe aplastic anemia (SAA) is a heterogeneous group of disorders characterized by pancytopenia and bone marrow failure. SAA has several causes, but an immune-mediated

process is suspected to underlie most cases (1-5). Immunosuppressive treatment of aplastic anemia began after it was observed that autologous hematopoiesis was recovered in patients who rejected partially matched bone marrow after an antithymocyte globulin

(ATG) or antilymphocyte globulin preparative regimen. The cytotoxicity of these agents to T lymphocytes, and the consequent reduced production of bone marrow inhibitory lymphokines are probably the chief mechanisms of action (6). In large cohorts of patients, approximately 50% of those with SAA have responded to ATG; in rare cases, there was complete normalization of blood counts (7-9). But evolution to other clonal hematologic disorders, such as paroxysmal nocturnal hemoglobinuria, myelodysplasia, and leukemia was observed, and patients with severe neutropenia ( $<0.2 \times 10^9/l$ ) who were  $<20$  years of age had the poorest prognosis (10-14). Although responses to ATG have been consistently documented in children and adolescents with SAA, the quality of response, the predictors of response, and the long-term consequences of treatment are not as well established.

This report is the first to describe a Brazilian sizable cohort of children treated with ATG at one institution in which we investigated the quality of long-term marrow function ( $>10$  years post-diagnosis) and examined factors associated with the hematologic response.

Table 1 - Clinical features of children at diagnosis of severe aplastic anemia.

No. patients	25
Age	1.5 to 20 years (median, 10 years)
$\leq 10$ years	16 patients
$> 11$ years	9 patients
Sex M:F	17:8
Etiology	
Unknown	18
Drugs (non-hormonal antiinflammatory agents)	2
Insecticides	3
Viral hepatitis	2
Interval between diagnosis and treatment	1 to 36 months (median, 4 months)
Granulocyte count	0.03 to $1.4 \times 10^9/l$ (median, $0.25 \times 10^9/l$ )
$\leq 0.2 \times 10^9/l$	12 patients
$> 0.2 \times 10^9/l$	13 patients
Platelet count	1.0 to $15.0 \times 10^9/l$ (median, $8 \times 10^9/l$ )

## Material and Methods

### Patients

Twenty-five patients with SAA, less than 20 years of age, were treated with ATG at the University Hospital, Federal University of Parana, Brazil (Table 1). Patients, or their legal representatives, signed an informed consent to participate in the study, which was approved by the Ethics Commission of the hospital. All patients had severe aplastic anemia as defined by established criteria. Cases of constitutional aplastic anemia were not included. None of the patients had HLA-compatible bone marrow donors, nor had any patient received prior immunosuppressive treatment.

### Treatment

All patients received horse antihuman thymocyte globulin (ATGAM, Upjohn Co., Kalamazoo, MI, USA),  $15 \text{ mg kg}^{-1} \text{ day}^{-1}$  by 6-h intravenous infusion, daily for 10 days. ATG was diluted in 500 ml of 0.9% NaCl solution and administered via a central venous access device after premedication with oral acetaminophen (500 mg), intravenous diphenhydramine hydrochloride (50 mg) and intravenous hydrocortisone (50 mg). Before the first ATG dose, 0.1 mg of ATG was administered intradermally to detect sensitization. All treated patients tested negatively. Several lots of ATG were utilized during the study. Serum sickness was treated with prednisone ( $1 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) until clinical manifestations improved. By protocol design, a second course of ATG was not administered within 12 months after the start of the first course, regardless of patients' hematologic responses.

### Evaluations and response criteria

Clinical and laboratory evaluations were conducted 6 weeks, 6 months, and 12 months

after the end of the 10-day ATG course. The last follow-up date was July 1998. The first three evaluations included physical examination, complete blood count, platelet count, and bone marrow aspiration and biopsy. The Ham test and cytogenetic studies of the bone marrow cells were included in the subsequent yearly evaluations.

Responses to treatment were defined prospectively as type I (patients became independent of red cell or platelet transfusions and were not hospitalized for fever), type II (patients required red cell or platelet support and/or were admitted due to fever and neutropenia, despite increased hemoglobin, platelet counts, or granulocyte counts), and type III (no increase in hemoglobin, platelet count, or granulocyte count within 12 months after the completion of ATG).

### Supportive care

All patients were treated in double-occupancy nonsterile rooms. Patients who had an absolute neutrophil count less than  $0.5 \times 10^9/l$  and an axillary temperature exceeding  $38.0^\circ\text{C}$  were treated empirically with broad-spectrum antibiotics. All blood products were irradiated. Red cells and platelets were transfused when the hemoglobin level fell below  $7.0 \text{ g/dl}$  and the platelet count below  $20 \times 10^9/l$ , respectively. All patients received care at the University Hospital for the first 12 months and were then discharged in the care of the referring physician. Return visits to the University Hospital for follow-up evaluation were scheduled on a yearly basis.

### Statistical analysis

Survival time was measured from the first day of ATG infusion. Product limit estimates of the survival time distribution were calculated using the method of Kaplan and Meier. The log-rank test was used to compare survival distribution.

## Results

### Response

The clinical responses to ATG are listed in Table 2. At the 6-week evaluation, four patients (16%) had responded (type II responses) and three had died from hemorrhagic or infectious complications. The remainder had type III responses. At 6 months, six patients achieved a type I response and one patient had a type II response. Seven patients died during this period from complications of bone marrow failure. At one year, two more patients had achieved type I responses and three patients continued to show no response. Of these three patients, two later achieved a type I response (one after further immunosuppression with cyclosporine plus prednisone), and two died.

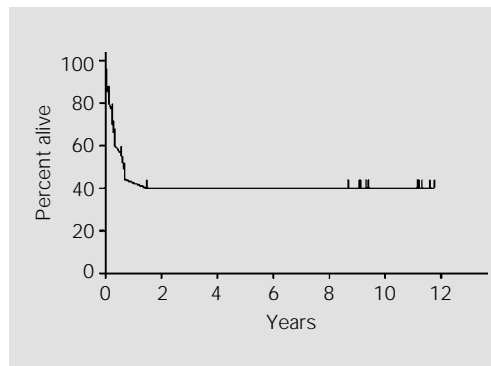
Of the eight patients who responded within one year of ATG treatment, one developed severe thrombocytopenia 6 years later that resolved after cyclosporine and prednisone therapy. Currently, ten patients (40%) are alive with good bone marrow function (Figure 1). Response to ATG ap-

Table 2 - Outcome of children treated with anti-thymocyte globulin for severe aplastic anemia.

Type I: Patients became independent of red cell or platelet transfusions and were not hospitalized for fever; type II: patients required red cell or platelet support and/or were admitted due to fever and neutropenia; type III: no increase in hemoglobin, platelet count, or granulocyte count by 12-month follow-up. \*Includes two patients who received additional immunosuppressive therapy.

Evaluation	Response type (No. patients)			Status (No. patients)	
	I	II	III	Alive	Dead
6 weeks	-	4	18	22	3
6 months	6	1	8	15	7
12 months	8	1	3	12	3
Last follow-up	10*	-	-	10	2

Figure 1 - Kaplan-Meier estimates of overall survival of 25 children and adolescents with severe aplastic anemia treated with antithymocyte globulin.



pears to be independent of age or severity of neutropenia. Seven of the 10 responders were less than 10 years of age, and five had absolute neutrophil counts less than  $0.2 \times 10^9/l$  at diagnosis. However, the number of patients is too small for meaningful statistical analysis.

#### Complications of therapy

Fever was the most common clinical sign, occurring in 19 patients (76%). A maculopapular skin rash occurred in 17 patients (68%), and 6 (24%) developed cutaneous vasculitis. Five patients (20%) developed hypertension, which was managed with sublingual nifedipine and diuretics. Serum sickness, characterized by fever, skin rash, arthralgia, and serpiginous lesions on palms and soles, was observed in two patients (8%) and resolved with administration of prednisone. Treatment was not discontinued in any patient due to ATG-associated complications.

Although no hematologic disease has been noted in the long-term survivors (except for the patient with thrombocytopenia), one patient has abnormal mean corpuscular volume ( $>100$  fl), and two patients have developed clonal karyotypic abnormalities (trisomy 8 and del(6)q21, respectively). Central nervous system bleeding was the cause of death for 10 patients, bacterial infection for four, and fulminant viral hepatitis for one.

#### Discussion

We set out to characterize the quality of bone marrow function in long-term survivors of SAA who were initially managed with ATG. The bone marrow function of our ten long-term survivors has been normal. Interestingly, two of these patients developed clonal cytogenetic abnormalities (trisomy 8 and del(6)q21), and a third patient has an elevated red cell mean corpuscular volume. Although these abnormalities can be found in patients with myelodysplastic syndromes (MDS) and lymphoid malignancies, our patients' bone marrow studies have not been revealing evidence of them. Recently, Geary et al. (15) reported the follow-up of 13 SAA patients with an abnormal cytogenetic clone (including trisomy 8), detected at or sometime after diagnosis. They received immunosuppressive therapy and transformation to acute leukemia or MDS was not observed after 4.1 years (range 1.2 to 11.2 years). Surprisingly, in four patients the abnormal clone disappeared after treatment (15). So, the clinical significance of these findings is presently unknown.

Adults with SAA who are treated with ATG alone have a 9.6% and 6.6% cumulative risk at 10 years for myelodysplasia and leukemia, respectively (10). Those treated mainly with ATG have the same probability (9% at 4 years) (16). Approximately 15% of these patients develop evidence of paroxysmal nocturnal hemoglobinuria (17). The incidence of MDS and acute leukemia as a late complication of immunosuppressive treatment in children may vary with the type of drugs used, being more frequent when cyclosporine and G-CSF are used together (18). Children with SAA may be less prone to developing clonal hematologic disorders after treatment with ATG. Paroxysmal nocturnal hemoglobinuria has not been detected in any of our patients, although we have used only the acidified Ham test for screening.

Early mortality, defined as death within

100 days of the beginning of therapy, had an important negative impact on the overall survival of our patients. Seven patients (47%) died during this period, most from CNS hemorrhage or infectious complications, in contrast to recent reports of mortality below 10% (19). However, most patients in our series were admitted with serious medical complications, such as active infection and bleeding.

The intensity of the initial immunosuppressive regimen can also influence early mortality. ATG is only mildly immunosuppressive, prolonging the time for bone marrow recovery. The mortality rates reported for patients treated with more intense regimens, such as combinations of cyclosporine and ATG with or without G-CSF, are 8 to 12% (20). Taken together, the data support the opinion that early intensive immunosuppression is associated with decreased rates of early death and increased rates of long-term survival.

Relapse can be a significant problem for patients whose SAA initially responded to immunosuppression. Rates of relapse reach 35% at 10 years, as reported by Schrezenmeier et al. (21). In our series, only one patient had a relapse after achieving a type I response. Rescue with cyclosporine A plus prednisone was effective, and after 4 years the patient maintains normal hematologic parameters, demonstrating that this immunosuppressive combination should be considered for patients who relapse or initially fail to respond to ATG (22).

The number of patients in our study was too small to allow meaningful analysis of prognostic factors. In general, patients with presenting granulocyte counts less than  $0.2 \times 10^9/l$  tend to have a worse prognosis than others, mainly with respect to early mortality (23). In our series, 4 of the 12 patients who had granulocyte counts  $<0.2 \times 10^9/l$  responded to ATG, and another subsequently responded to cyclosporine plus prednisone. This rate of response did not differ from that of patients who presented with higher granulocyte counts, suggesting that initial granulocyte count is probably not associated with outcome if patients survive the first few months after immunosuppressive therapy.

Young age at diagnosis has been associated with poor outcome in some studies, but not in others (24-26). In our series, 6 of 16 patients  $<10$  years of age (37.5%) are long-term disease free survivors, as compared with 4 of 9 patients (44.4%) older than age 10 years. Thus, age  $<10$  years does not appear to be associated with poorer outcome.

The overall response and survival rates achieved in our study are inferior to those reported for sibling HLA-matched stem cell transplantation (24,25) and for more intensive immunosuppressive regimens (20,26). However, our survivors have enjoyed long-term stable peripheral blood counts, Karnofsky scores of or near 100, and a low rate of relapse, demonstrating that normal long-term survival is possible for children treated with ATG.

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