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ORIGINAL ARTICLE

Comparison between horse and rabbit antithymocyte globulin as first-line treatment for patients with severe aplastic anemia: a single-center retrospective study

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Abstract The best antithymocyte globulin preparation for first-line immune suppression in patients with severe aplastic anemia is still not clear. The aim of this study was to compare hematological response and overall survival in patients submitted to horse or rabbit antithymocyte globulin as first-line treatment for severe aplastic anemia. We retrospectively compared 71 consecutive patients with severe aplastic anemia, classified according to the antithymocyte globulin preparation. Analyses included variables related to patients and to immune suppression. Forty two patients (59.1%) received horse and 29 (40.9%) rabbit antithymocyte globulin. Response rates were higher at 6 months in patients submitted to horse in comparison to rabbit antithymocyte globulin (59.5% versus 34.5% respectively, $p=0.05$). Median time to response was similar between the two groups (99 versus 88.5 days, respectively, for horse and rabbit antithymocyte globulin; $p=0.98$). Overall survival at 2 years was significantly higher in patients submitted to horse in comparison to rabbit antithymocyte globulin (78.4% versus 55.4%, $p=0.03$). Post-treatment response was strongly associated with survival at 2 years (97% in responders versus 41.2% in non-responders, $p<0.001$). Use of rabbit antithymocyte globulin was an independent predictor of death (odds ratio 2.5; 95% confidence interval 1.03–6.04; $p=0.04$). Rabbit antithymocyte globulin was associated with a significant and prolonged lymphopenia in comparison with horse antithymocyte globulin. Our data suggest the superiority of horse over rabbit antithymocyte globulin as first-line treatment for severe aplastic anemia, both regarding hematological response and survival.

Keywords Severe acquired aplastic anemia · Immune suppression · Antithymocyte globulin · Hematologic response · Overall survival

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Introduction

Severe aplastic anemia (SAA) is a life-threatening bone marrow failure syndrome associated with a high mortality rate without proper treatment [1]. Primary treatment for patients with SAA depends on the age of the patient, the availability of an HLA-identical sibling donor, and the ability to tolerate bone marrow transplantation (BMT) [2]. Antithymocyte globulin (ATG) is the drug of choice for immune suppression in patients with SAA unsuitable for BMT [3]. The association of cyclosporine (CSA) with ATG improves the overall response rate and is the current protocol for immune suppression in SAA [4–6].

The exact mechanism of action of ATG in aplastic anemia (AA) is not fully elucidated, although it appears to depend upon a non-selective depletion of T lymphocytes [7]. ATG is obtained through the immunization of animals, generally horses or rabbits, with thymocytes or T-cell lines, followed by the purification of the IgG fraction of the sera from these animals. The standard ATG preparation for first-line immune suppression in AA is horse ATG because of the larger experience and the results already reported with this preparation [3–6]. Rabbit ATG is used mainly as

second-line treatment for patients with refractory or relapsed AA after horse ATG [8, 9]. Despite sharing some properties, rabbit and horse ATG are strictly different drugs, and probably should not be used interchangeably [7].

The aim of this study was to retrospectively analyze patients submitted to horse or rabbit ATG as first-line treatment for SAA to compare hematological response and survival rates.

Design and methods

Eligibility

For this retrospective analysis, all patients with AA who received ATG in the period between January 2000 and December 2008 at Hemorio, Rio de Janeiro, Brazil, were identified. From January 2000 until the end of 2005, horse ATG (Lymphoglobuline®, Genzyme, Cambridge, MA, USA) was used as first line for immune suppression in patients with SAA unsuitable for allogeneic hematopoietic stem cell transplantation. Due to the unavailability of the former product in Brazil, rabbit ATG (Thymoglobuline®, Genzyme, Cambridge, MA, USA) became the standard ATG preparation for immune suppression in AA since 2006. The manufacturing of Lymphoglobuline® and Thymoglobuline® relies on the immunization of animals with fresh human thymocytes, differing in the animal species utilized [7].

Patients were included in this analysis if the criteria for SAA were met and if ATG was used as first-line treatment. Patients younger than 40 years only received immune suppression with ATG as first-line treatment if an HLA-identical sibling donor was not available or in cases unsuitable for BMT [2]. SAA was defined by a bone marrow cellularity of less than 30% with at least two of the three following criteria in the peripheral blood: (1) absolute neutrophil count less than 0.5×10^9/L, (2) absolute reticulocyte count less than 60×10^9/L, and (3) platelet count less than 20×10^9/L [6]. The higher number of absolute reticulocyte count adopted reflects the more accurate automated reticulocyte counting. All the patients included in this study fulfilled the criteria for SAA at the moment of diagnosis. Exclusion criteria for this study were: (1) abnormal cytogenetics, (2) morphology consistent with myelodysplasia, (3) pregnancy-associated aplastic anemia, and (4) hepatitis-associated aplastic anemia.

This study was approved by the local medical ethics committee and was in accordance with Brazilian legislation and the Declaration of Helsinki. All patients gave written informed consent allowing the use of their medical records for clinical research. Data were obtained from written and computerized medical records.

Patient and immune suppression data

Baseline patient characteristics analyzed were gender, age at the time of diagnosis, and hematologic counts at diagnosis: absolute neutrophil count (ANC), absolute reticulocyte count, absolute lymphocyte count (ALC), and platelet count. Patients with criteria for SAA with an ANC at diagnosis less than 0.2×10^9/L were categorized as very severe aplastic anemia (VSAA) [10]. The hematologic parameters were obtained from an automated Cell-Dyn 3700 blood cell counter (Abbott Diagnostics, Chicago, IL, USA). Screening of paroxysmal nocturnal hemoglobinuria (PNH) was done by the Ham and sucrose tests or by flow cytometric analysis of CD55 and CD59 expression on red blood cells and neutrophils; the latter test only became available in 2002. In this way, immunophenotypic screening for PNH was done in 60.1% of patients submitted to horse ATG, in comparison to 100% of those submitted to rabbit ATG. Fanconi anemia was ruled out by the diepoxybutane chromosomal breakage test in young adults whenever possible. Immune suppression treatment data included: any treatment prior to ATG, time elapsed between diagnosis of SAA and ATG, the ATG preparation, the dose administered, and the hematologic parameters immediately before ATG. All patients received ATG infusion during five consecutive days. Administration of the following drugs until 6 months after ATG was also analyzed: CSA, corticosteroids, granulocyte colony-stimulating factor (G-CSF), recombinant erythropoietin (R-EPO), and androgens. CSA dose was adjusted to maintain blood levels between 200 and 400 ng/mL both in the group treated with horse or rabbit ATG. Mean corpuscular volume (MCV) and ALC were also analyzed 30, 60, and 90 days after ATG.

Response and relapse definitions

Response to immune suppression was evaluated until 6 months after ATG and categorized into two groups: complete remission (hemoglobin >10 g/dL, ANC $>1.5\times10^9$/L, and platelet count $>100\times10^9$/L) and partial remission (no longer meeting criteria for SAA and transfusion independent) [11]. Response was confirmed by two or more complete blood counts at least 4 weeks apart.

Relapse was defined as the loss of one or more criteria for response, regardless of the need for transfusion of red blood cells and/or platelets.

End points

The end points of this study were overall response (complete or partial response) in the first 6 months after ATG and overall survival (OS) in SAA patients treated with horse or rabbit ATG as first-line therapy.

Supportive care

Corticosteroids, usually methylprednisolone, were given for at least 2 weeks after ATG to prevent serum sickness. G-CSF was administered if clinically indicated, usually in patients with severe neutropenia and evidence of infection. Trimethoprim–sulfamethoxazole was administered for primary prophylaxis of *Pneumocystis jiroveci* pneumonia for at least 3 months after ATG treatment. Other prophylactic antibiotics and antifungal drugs were not routinely administered because of the concern about the emergence of resistant pathogens [12]. Red blood cells were transfused in patients with symptomatic anemia or to maintain the hemoglobin level higher than 9 g/dL in patients with cardiopulmonary disease. Platelets were transfused prophylactically in all patients with a platelet count lower than 10×10^9/L. Platelets were also transfused prophylactically in patients with fever and a platelet count below 20×10^9/L or in patients with higher counts with clinical bleeding. All cellular blood products administered were leukocyte-depleted through the use of blood filters. Cellular blood products were not routinely irradiated in view of the lack of evidence recommending this practice after ATG administration [2].

Statistical analysis

To compare the differences between the groups according to type of ATG used, χ^2 or Fisher's exact test were used for categorical variables and the Mann–Whitney U non-parametric test was used for continuous variables. Times to response, relapse, and death were determined from the time of ATG administration until the event under evaluation. For OS analysis, patients were censored at the time of last visit, even in the cases where a second course of ATG was administered, with or without switching to a different animal source. Time-to-event analyses were done by the Kaplan–Meier method and compared by the log-rank test. The prognostic significance of the type of ATG preparation was evaluated by the Cox proportional hazards regression method, adjusting for other potential prognostic factors. All p values represented were two-sided, with $p<0.05$ indicating statistical significance. Registration and analysis of data were carried out using SPSS version 15 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Comparison of patients treated with horse and rabbit ATG

Among the 71 patients with SAA who were eligible for this study, 42 (59.1%) received horse ATG and 29 (40.9%) rabbit ATG as first-line therapy (Table 1). The distribution of the following baseline variables was similar between the groups (horse versus rabbit): median age (19 versus 21 years, $p=0.21$), male gender (54.8% versus 62.1%, $p=0.62$), median ANC at diagnosis (0.195 versus 0.216×10^9/L, $p=0.70$), median reticulocyte count at diagnosis (34.450 versus 37.250×10^9/L, $p=0.37$), median platelet count at diagnosis (9.950 versus 8×10^9/L, $p=0.19$), median ALC at diagnosis (1.510 versus 1.570×10^9/L, $p=0.77$), diagnosis of VSAA (52.4% versus 48.3%, $p=0.81$), presence of PNH clone without clinical manifestations (2.4% versus 10.3%, $p=0.30$), any treatment prior to ATG (66.7% versus 65.5%, $p=1$), and the interval between diagnosis of SAA and ATG greater than 30 days (45.2% versus 65.5%, $p=0.15$). The median duration between diagnosis of SAA and ATG was higher in the group treated with rabbit ATG, although this difference was not statistically significant (54 versus 27 days, $p=0.12$). Median ANC and ALC immediately before ATG were similar between patients submitted to horse and rabbit ATG (0.306 versus 0.337×10^9/L, $p=0.93$ and 1.800 versus 1.550×10^9/L, $p=0.64$, respectively). Median horse and rabbit ATG doses were respectively 15 mg/kg (range 10–21.4 mg/kg) and 2.5 mg/kg (range 1.5–3 mg/kg), administered for five consecutive days. Patients also received the following drugs in the 6 months after ATG (horse versus rabbit): CSA (71.4% versus 89.7%, $p=0.08$), corticosteroids for more than 4 weeks after ATG (64.3% versus 31%, $p=0.008$), G-CSF (59.5% versus 69%, $p=0.46$), R-EPO (9.5% versus 6.9%, $p=1$), and androgens (45.2% versus 41.4%, $p=0.81$). The group treated with horse ATG received more corticosteroids because of the protocol adopted in our institution until 2002, which was later changed. In patients receiving CSA after ATG, the administration of this drug for more than 6 months was similar between patients submitted to horse and rabbit ATG (53.3% versus 42.3%, $p=0.43$, respectively).

Comparison of response and relapse between patients treated with horse and rabbit ATG

Response rates were higher at 6 months in the group of patients submitted to horse in comparison to rabbit ATG, 59.5% versus 34.5%, respectively ($p=0.05$) (Table 2). In spite of this, the median time to response was similar between the two groups (99 versus 88.5 days, respectively, for horse and rabbit ATG; $p=0.98$) (Fig. 1). Both partial and complete remissions in the first 6 months after ATG were more frequent in the group treated with horse ATG than with rabbit ATG, although these differences were not statistically significant when analyzed by each category. The median ATG dose administered daily was identical between responders and non-responders, both in the group submitted to horse (15 mg/kg, $p=0.43$) and rabbit ATG (2.5 mg/kg, $p=0.54$).

Table 1 Comparison of patients treated with horse and rabbit ATG

	Horse ATG (*N*=42)	Rabbit ATG (*N*=29)	*p* value
Age (years) (median, range)	19 (1–66)	21 (4–63)	0.21
Male gender	23 (54.8%)	18 (62.1%)	0.62
Baseline hematologic parameters			
ANC ($\times 10^9$/L) (median, range)	0.195 (0.011–0.672)	0.216 (0.026–0.478)	0.70
Reticulocyte ($\times 10^9$/L) (median, range)	34.450 (1.193–118.600)	37.250 (1.200–223.400)	0.37
Platelet ($\times 10^9$/L) (median, range)	9.950 (1.000–20.000)	8.000 (0.400–18.900)	0.19
ALC (median)	1.510 (0.099–5.120)	1.570 (0.419–4.350)	0.77
Diagnosis of VSAA	22 (52.4%)	14 (48.3%)	0.81
Presence of PNH clone without clinical manifestations	1 (2.4%)	3 (10.3%)	0.30
Prior treatment to ATG	28 (66.7%)	19 (65.5%)	1
Interval between diagnosis and ATG >30 days	19 (45.2%)	19 (65.5%)	0.15
Pre-ATG hematologic parameters			
ANC ($\times 10^9$/L) (median, range)	0.306 (0.004–2.460)	0.337 (0.007–2.150)	0.93
ALC ($\times 10^9$/L) (median, range)	1.800 (0.039–10.400)	1.550 (0.030–7.800)	0.64
Drugs in the 6 months after ATG			
CSA	30 (71.4%)	26 (89.7%)	0.08
Corticosteroids >4 weeks	27 (64.3%)	9 (31%)	0.008
G-CSF	25 (59.5%)	20 (69%)	0.46
R-EPO	4 (9.5%)	2 (6.9%)	1
Androgens	19 (45.2%)	12 (41.4%)	0.81

ALC absolute lymphocyte count, *ANC* absolute neutrophil count, *ATG* antithymocyte globulin, *CSA* cyclosporine, *G-CSF* granulocyte colony-stimulating factor, *PNH* paroxysmal nocturnal hemoglobinuria, *R-EPO* recombinant erythropoietin, *VSAA* very severe aplastic anemia

Relapse rates were similar between patients receiving horse or rabbit ATG (36% versus 30%, $p=1$, respectively). Interestingly, patients submitted to horse ATG relapsed later than those submitted to rabbit ATG (median time of 371 versus 66 days, $p=0.02$, respectively). A second course of ATG was administered in 11 out of the 42 patients originally submitted to horse ATG (26.1%). Nine of them received horse ATG again, whereas two of them switched to rabbit ATG. Hematological response was observed in six patients (54.5%). In patients treated originally with rabbit ATG, seven out of 29 patients (24.1%) received a second course of rabbit ATG, and none of them developed a hematological response.

Analysis of survival after ATG treatment

Survival rates were significantly higher in the group of patients submitted to horse in comparison to rabbit ATG (Fig. 2). OS at 2 years was 78.4% in recipients of horse ATG and 55.4% in those treated with rabbit ATG ($p=0.03$). Median OS was not reached in either group after a median follow-up of 3.9 years for all surviving patients (median

Table 2 Analysis of response after ATG

	Horse ATG (*N*=42)	Rabbit ATG (*N*=29)	*p* value
Partial remission	20 (47.6%)	8 (27.6%)	0.14
Complete remission	5 (11.9%)	2 (6.9%)	0.69
Total response (PR+CR)	25 (59.5%)	10 (34.5%)	0.05
Interval between ATG and response (days) (median, range)	99 (39–166)	88.5 (39–161)	0.98
Relapse	9 (36%)	3 (30%)	1
Interval between ATG and relapse (days) (median, range)	371 (60–1,707)	66 (34–98)	0.02

ATG antithymocyte globulin, *CR* complete response, *PR* partial response

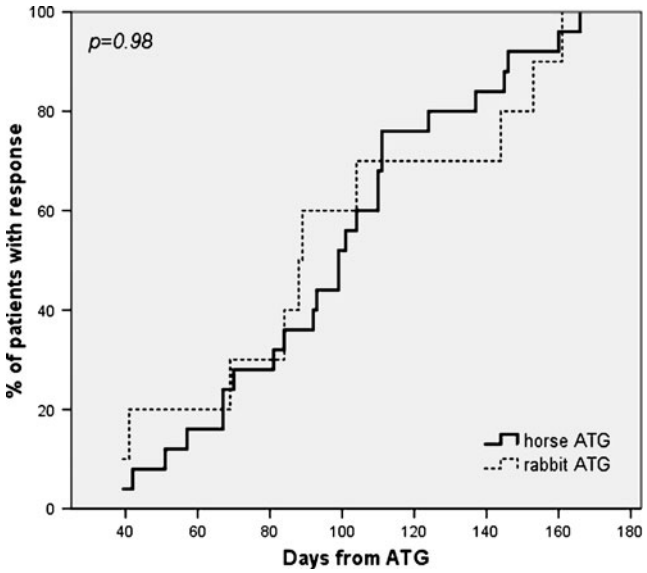

Fig. 1 Comparison of the time between horse and rabbit antithymocyte globulin (ATG) and hematologic response. Median times to response were 99 and 88.5 days in patients submitted to horse and rabbit ATG, respectively

follow-up of 5.1 and 1.1 years for horse and rabbit ATG groups, respectively). Post-treatment response was strongly associated with survival (Fig. 3). OS at 2 years was 97% in patients who responded to ATG and 41.2% in those who did not ($p<0.001$). Median OS was 8.1 months for non-responders and not reached in responders.

The source of ATG was independently associated with survival (Table 3). Patients submitted to rabbit ATG were 2.5 times more likely to die than those who received horse ATG

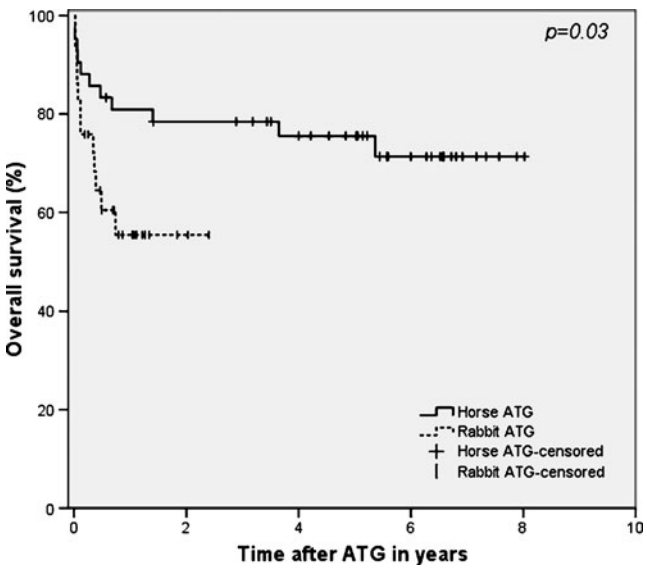

Fig. 2 Overall survival (OS) of 71 patients with acquired severe aplastic anemia according to antithymocyte globulin (ATG) source: OS at 2 years was 78.4% in recipients of horse ATG and 55.4% in those treated with rabbit ATG ($p=0.03$). Median OS was not reached in both groups

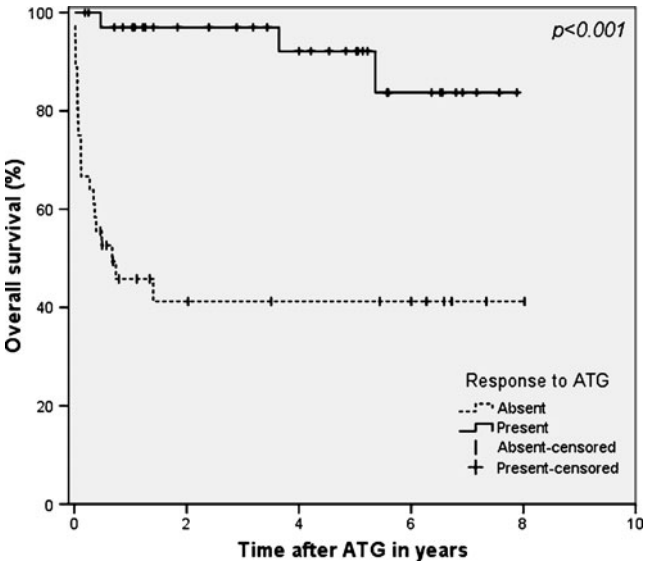

Fig. 3 Overall survival (OS) of 71 patients with acquired severe aplastic anemia according to response after antithymocyte globulin (ATG): OS at 2 years was 97% in patients with hematologic response after ATG and 41.2% in non-responders ($p<0.001$). Median overall survival was 8.1 months in patients without response after ATG and not reached in the other group

(odds ratio 2.5; 95% confidence interval 1.03–6.04; $p=0.04$). Age above 40 years, diagnosis of VSAA, presence of PNH clone without clinical manifestations, interval between diagnosis and ATG greater than 30 days, pre-ATG hematologic parameters (ANC and ALC), and administration of other drugs in the 6 months following ATG (CSA, corticosteroids for more than 4 weeks, G-CSF, R-EPO, and androgens) were not associated with survival in the univariate analyses.

Analysis of causes of death after ATG treatment

Mortality was higher among patients submitted to rabbit ATG in comparison to horse ATG, and this difference was more profound in the early period after ATG (Table 4). The relationship between the number of deaths and the initial number of patients in each category was used for comparison between the early and late period after ATG. The proportion of deaths in the first 60 days after ATG was 11.9% in the group submitted to horse ATG and 24.1% in the group receiving rabbit ATG. This proportion was more balanced after 60 days from ATG: 14.2% and 17.2% in patients submitted to horse and rabbit ATG, respectively. Early mortality in patients submitted to rabbit ATG was either related to infectious complications (five out of seven patients) or to severe bleeding (two out of seven patients). Infection-related mortality in patients treated with rabbit ATG was mainly due to bacterial and fungal infections, in spite of adequate antibiotic and antifungal therapy.

Table 3 Analysis of factors associated with overall survival after ATG

	Univariate analysis		Multivariate analysis	
	p value	Odds ratio (95% CI)	*p* value	Odds ratio (95% CI)
Age ≥40 years	0.87	0.90 (0.26–3.04)		
Diagnosis of VSAA	0.34	1.49 (0.65–3.40)	–	–
Presence of PNH clone without clinical manifestations	0.97	1.03 (0.13–7.74)		
Interval between diagnosis and ATG >30 days	0.30	1.54 (0.66–3.58)	–	–
Pre-ATG hematologic				
ANC	0.42	NA	–	–
ALC	0.66	NA	–	–
Rabbit vs. horse ATG	0.04	2.50 (1.03–6.04)	0.04	2.50 (1.03–6.04)
Drugs in the 6 months after ATG				
CSA	0.60	1.33 (0.45–3.93)	–	–
Corticosteroids >4 weeks	0.47	1.53 (0.47–4.92)	–	–
G-CSF	0.07	2.47 (0.91–6.68)	–	–
R-EPO	0.35	1.77 (0.52–6.01)	–	–
Androgens	0.31	0.64 (0.27–1.51)	–	–

95% CI 95% confidence interval, *ALC* absolute lymphocyte count, *ANC* absolute neutrophil count, *ATG* antithymocyte globulin, *CSA* cyclosporine, *G-CSF* granulocyte colony-stimulating factor, *NA* not applicable, *PNH* paroxysmal nocturnal hemoglobinuria, *R-EPO* recombinant erythropoietin, *VSAA* very severe aplastic anemia

Comparison of the mean corpuscular volume and the absolute lymphocyte count after horse and rabbit ATG

Although the difference was not statistically significant, MCV was slightly higher 3 months after horse ATG in comparison to rabbit ATG (93.5 versus 88.7 fl, $p=0.14$, respectively). This difference became statistically significant when only patients with hematologic response after ATG were analyzed: MCV was 100 fl (range=85–114 fl) in comparison with 89.4 fl (range=82–103 fl) 3 months after horse and rabbit ATG, respectively ($p=0.04$).

No difference was found between median ALC before treatment with horse or rabbit ATG (1.550 versus 1.800×10^9/L, $p=0.64$) (Fig. 4). Differently, median ALC was significantly higher in patients submitted to horse ATG in comparison to rabbit ATG on days 30 (2.220 versus 0.683×10^9/L, $p<0.001$, respectively), 60 (1.945 versus 0.554×10^9/L, $p<0.001$, respectively), and 90 after ATG (2.065 versus 0.675×10^9/L, $p<0.001$, respectively). Median ALC before ATG was similar between patients with and without hematologic response both in the group submitted to horse (1.770 versus 1.830×10^9/L, $p=0.98$, respectively) and to rabbit ATG (1.550 versus 1.425×10^9/L, $p=0.51$, respectively). Median ALC 30 days after ATG was slightly higher in patients with hematologic response in comparison to those without response, both in the group submitted to horse (2.460 versus 1.580×10^9/L, $p=0.12$) and to rabbit ATG (0.820 versus 0.636×10^9/L, $p=0.31$).

Discussion

First-line treatment in patients with SAA who are ineligible for bone marrow transplantation consists of immune suppression based on the combination of ATG and CSA [4–6]. Traditionally, horse ATG is the preferred ATG preparation for patients with AA [3–6]. The use of rabbit ATG was evaluated in patients with relapse or lack of response after horse ATG with conflictive results, probably reflecting differences regarding the time from horse to rabbit ATG and the criteria adopted for hematological

Table 4 Analysis of early and late deaths after ATG

	Horse ATG (*N*=42)	Rabbit ATG (*N*=29)
All deaths	11 (26.1%)	12 (41.3%)
Deaths in the first 60 days after ATG	5 (11.9%)	7 (24.1%)
Deaths after 60 days from ATG	6 (14.2%)	5 (17.2%)
Deaths related to infection in the first 60 days after ATG	5 (11.9%)	5 (17.2%)
Deaths related to bleeding in the first 60 days after ATG	0	2 (6.9%)

ATG antithymocyte globulin

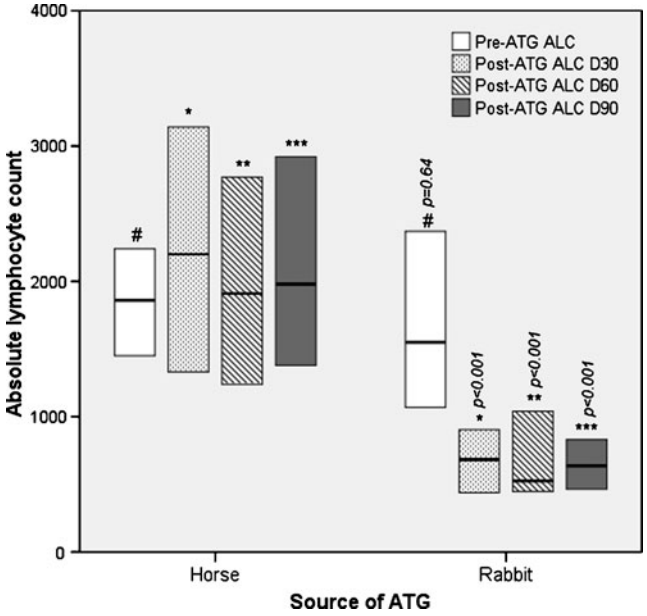

Fig. 4 Evolution of the absolute lymphocyte count (ALC) before and after antithymocyte globulin (ATG): median ALC pre-ATG was similar between patients submitted to horse and rabbit ATG (1.800 versus 1.550×10^9/L, $p=0.64$, respectively) and significantly superior in patients submitted to horse ATG in comparison to rabbit ATG on days 30 (2.220 versus 0.683×10^9/L, $p<0.001$, respectively), 60 (1.945 versus 0.554×10^9/L, $p<0.001$, respectively), and 90 after ATG (2.065 versus 0.675×10^9/L, $p<0.001$, respectively)

response [8, 9]. Results with rabbit ATG as first-line treatment in SAA were inferior in comparison to horse ATG in two prospective clinical trials [13, 14]. In spite of this, the use of rabbit ATG in association with CSA as initial therapy for SAA is now frequent in the USA and the only option in many countries where horse ATG is no longer available. In this study, we sought to retrospectively analyze patients with SAA submitted to horse or rabbit ATG as first-line treatment, aiming to compare hematologic response and survival.

Our results suggest that horse ATG is superior to rabbit ATG in terms of hematological response in the first 6 months after immune suppression. The hematologic response observed with horse ATG was 59.5%, very close to the results described in a cohort of 122 patients treated with horse ATG and CSA in a single center [6]. The response rate observed in our patients treated with rabbit ATG was only of 34.5%, which is a very poor result. Nevertheless, it should be highlighted that previous studies which compared horse with rabbit ATG as first-line treatment in SAA also reported inferior response rates with the rabbit preparation. In a small prospective double-blind randomized trial conducted in a pediatric population, the hematological response reported after horse ATG and CSA was 93% in comparison with 47% in the group treated with rabbit ATG and CSA [13]. In a similar way, Zheng et al. also observed in a prospective randomized open-label trial a superior response after horse ATG and CSA in comparison with rabbit ATG and CSA (79% versus 53%, respectively) [14]. Finally, Vallejo et al. reported a higher complete response rate after horse ATG in comparison with rabbit ATG (38% versus 22%, respectively). Moreover, the patients originally treated with rabbit ATG were more prone to receive a second course of immune suppression or an alternative approach because of the greater rate of partial remission reported in this group [15]. The time to hematological response in our study was similar with both ATG preparations. The higher relapse rate observed in our study is probably a consequence of the broader relapse definition adopted. Relapse rates were similar between horse and rabbit ATG groups. The time between response and relapse was significantly shorter in patients responding to rabbit ATG in comparison to those responding after horse ATG, although this was not necessarily translated into the reinstitution of immune suppression and/or transfusion requirement.

In the present study, the odds for survival were considerably higher in patients submitted to horse ATG, probably reflecting the better hematological response achieved in this group. Also, our results demonstrated that the source of ATG was independently associated with OS. Only one previous study which compared horse with rabbit ATG as first-line treatment in SAA evaluated survival, reporting a 5-year OS higher for horse ATG in comparison to rabbit ATG (81% versus 66%, respectively) [14]. Diagnosis of VSAA was not associated with a lower survival in our study, in accordance with the results of a recent report by the European Group for Blood and Marrow Transplantation (EBMT) which also found no difference in survival between SAA and VSAA patients submitted to immune suppression [16]. We found that hematological response was strongly associated with survival after ATG, as patients who did not respond to ATG demonstrated a dismal prognosis with a median OS of only 8.1 months. The positive impact of achieving a hematological response after immune suppression on long-term survival was also reported in previous studies [6, 17]. Rosenfeld et al. found a 5-year OS of 86% for patients classified as responders compared to 40% for non-responders [6]. Not surprisingly, the excess of deaths observed in our patients receiving rabbit ATG occurred mainly in the 60 days after immune suppression, probably reflecting the inferior hematological response observed in this group. These deaths were related to infectious complications and severe bleeding. The proportion of deaths between horse and rabbit ATG was more balanced after 60 days from immune suppression, pointing to a similar prognosis once a hematological response is achieved, independently of the ATG source. In spite of the availability of new antibiotics and antifungal drugs when rabbit ATG was used in our patients, the infectious mortality observed was higher.

Interestingly, we observed a higher MCV in patients with hematologic response after horse ATG in comparison with rabbit ATG. The observation that responders to immune suppression with horse ATG develop higher MCV was reported in previous studies, and it merely reflects the generation of new red blood cells [18]. The reason why patients with a response to rabbit ATG did not develop an increase in MCV comparable to the one observed in responders to horse ATG remains unclear. Unfortunately, the absolute reticulocyte count was not available in all patients in the moment when MCV was analyzed, hampering possible explanations for this observation.

Our results demonstrated a significant and prolonged lymphopenia in patients submitted to rabbit in comparison to horse ATG. Scheinberg et al. also observed a more severe lymphopenia after rabbit ATG when compared to horse ATG, which translated in more severe immune suppression and, consequently, in a higher rate of subclinical Epstein–Barr virus reactivation [19]. The enhanced lymphocytotoxicity of rabbit ATG is probably related to its higher affinity to human lymphocytes and an extended half-life [20]. We observed a slightly higher ALC 1 month after either horse or rabbit ATG in patients who later developed a hematological response, although this difference was not statistically significant. Marsh et al. also found that responders to immune suppression tended to have higher ALC than non-responders [18]. A recent study also demonstrated that baseline ALC $\geq 1 \times 10^9$/L was independently associated with better response and survival in patients with SAA submitted to immune suppression [21].

One of the main limitations of our study is its retrospective design, which compromises treatment homogeneity. Although some patients received some sort of treatment prior to ATG, hematologic parameters immediately before horse or rabbit ATG were not different. Also, the extended course of corticosteroids, the lack of administration of CSA after ATG, and the longer interval between diagnosis and ATG in some patients were not associated with survival in Cox regression analyses. Finally, another limitation of our study was that the dose of rabbit ATG used was equivalent to the lowest dose recommended by the manufacturer for treatment of SAA (i.e., 2.5 mg/kg/day). The dose currently recommended for treating SAA is 3.75 mg/kg/day, although no study compared the effectiveness of different doses of rabbit ATG in SAA [2]. Nevertheless, we were able to observe a severe and long-lasting lymphopenia in our patients even with the lower dose of rabbit ATG used, which is an important surrogate marker of its activity.

The exact mechanism behind the supposed superiority of horse ATG for first-line treatment in SAA is not fully clear. There are two possible explanations for the superiority of horse ATG. Firstly, patients submitted to rabbit ATG develop a more profound immune suppression, and this could be translated into a higher infection rate and mortality. A second explanation is that rabbit ATG promotes depletion of regulatory T cells (Tregs) in vivo. As previously demonstrated, Tregs are decreased at presentation in almost all patients with AA, suggesting a pivotal role for these cells in the pathogenesis of AA [22]. Moreover, the infusion of functional Tregs was able to abrogate the expansion of auto-reactive T lymphocytes and to protect against immune-mediated bone marrow failure in an experimental model [23]. The severe lymphopenia observed after rabbit ATG in our study may point to a non-selective and robust depletion of T lymphocytes. This possibility is strengthened by the observation that the administration of rabbit ATG as induction therapy in kidney transplantation resulted in the disappearance of Tregs, which were undetectable 1 week after ATG in these patients [24]. Tregs slowly reappeared only after 4 weeks and recovered to approximately 30% of baseline at 26 weeks after rabbit ATG. Depletion of Tregs may be related to the higher lymphocytotoxicity of rabbit in comparison to horse ATG, implying that the intensification of the non-selective depletion of T lymphocytes is probably undesirable [20]. Different results were obtained when peripheral blood mononuclear cells were cultured with rabbit ATG, promoting the expansion of Tregs; but these results were obtained in vitro [25–27]. The exact mechanism behind the lower response to rabbit ATG demands more studies.

In summary, our data suggest the superiority of horse ATG over rabbit ATG, both regarding hematological response and survival, as first-line treatment for SAA. Also, we observed a profound lymphopenia after rabbit ATG, which may be associated with the poor response observed. As far as we know, there are two open clinical trials comparing horse and rabbit ATG as first-line treatment of SAA. The first is a phase II clinical trial conducted by the EBMT (NCT00471848) aiming to compare the results of rabbit ATG and CSA as first-line treatment in approximately 35 patients with newly diagnosed SAA with a matched historical control treated with horse ATG and CSA. The second is also a phase II clinical trial sponsored by the National Heart, Lung, and Blood Institute (NCT00260689) which aims to compare the effectiveness of three immunosuppressive regimens in patients with SAA: horse ATG and CSA, rabbit ATG and CSA, and alemtuzumab alone. An interim analysis of this trial suggested that the hematological response may not be comparable between horse and rabbit ATG, which prompted the opening of a third trial designed to evaluate horse ATG plus CSA in patients unsuccessfully treated with rabbit ATG plus CSA (NCT00944749). The results of these

prospective studies are urgently needed to provide insights about the best ATG preparation for first-line immune suppression in SAA.

Conflict of interest The authors reported no potential conflicts of interest.

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Author contributions

EHA: conception and design of the study, analysis and interpretation of data, drafting the manuscript; DSDP: conception and design of the study, analysis and interpretation of data; VLNM: analysis and interpretation of data, drafting the manuscript; AMA: analysis and interpretation of data, drafting the manuscript. All authors approved the final version submitted for publication.