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Impact of CD34 Cell Dose and Conditioning Regimen on Outcomes after Haploidentical Donor Hematopoietic Stem Cell Transplantation with Post-Transplantation Cyclophosphamide for Relapsed/Refractory Severe Aplastic Anemia

Leonardo Javier Arcuri[1,*], Samir Kanaan Nabhan[2], Renato Cunha[3], Samantha Nichele[2], Andreza Alice Feitosa Ribeiro[1], Juliana Folloni Fernandes[1,4], Liane Esteves Daudt[5], Ana Luiza Melo Rodrigues[6], Celso Arrais-Rodrigues[7], Adriana Seber[8], Elias Hallack Atta[9], Jose Salvador Rodrigues de Oliveira[7], Vaneuza Araujo Moreira Funke[10], Gisele Loth[2], Luiz Guilherme Darrigo Junior[3], Alessandra Paz[5], Rodolfo Froes Calixto[11], Alessandra Araujo Gomes[12], Carlos Eduardo Sa Araujo[13], Vergilio Colturato[14], Belinda Pinto Simoes[3], Nelson Hamerschlak[1], Mary Evelyn Flowers[15], Ricardo Pasquini[2], Vanderson Rocha[4,16], Carmem Bonfim[2]

[1] *Hospital Israelita Albert Einstein, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*
[2] *Universidade Federal do Parana, Bone Marrow Transplantation Unit, Curitiba, Brazil*
[3] *Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Bone Marrow Transplantation Unit, Ribeirao Preto, Brazil*
[4] *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*
[5] *Hospital das Clinicas de Porto Alegre, Bone Marrow Transplantation Unit, Porto Alegre, Brazil*
[6] *Hospital Pequeno Principe, Bone Marrow Transplantation Unit, Curitiba, Brazil*
[7] *Universidade Federal de São Paulo, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*
[8] *Hospital Samaritano, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*
[9] *Instituto Nacional de Cancer, Bone Marrow Transplantation Unit, Rio de Janeiro, Brazil*
[10] *Hospital Nossa Senhora das Graças, Bone Marrow Transplantation Unit, Curitiba, Brazil*
[11] *Real Hospital Portugues de Beneficencia em Pernambuco, Bone Marrow Transplantation Unit, Recife, Brazil*
[12] *Hospital Sirio-Libanes, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*
[13] *Instituto de Cardiologia do Distrito Federal, Bone Marrow Transplantation Unit, Brasilia, Brazil*
[14] *Fundação Amaral Carvalho, Bone Marrow Transplantation Unit, Jau, Brazil*
[15] *Fred Hutchinson Cancer Research Center, Seattle, Washington*
[16] *Rede D'or, Bone Marrow Transplantation Unit, Sao Paulo, Brazil*

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A B S T R A C T

Severe aplastic anemia (SAA) is a life-threatening disease that can be cured with allogeneic cell transplantation (HCT). Haploidentical donor transplantation with post-transplantation cyclophosphamide (haplo-PTCy) is an option for patients lacking an HLA-matched donor. We analyzed 87 patients who underwent haplo-PTCy between 2010 and 2019. The median patient age was 14 years (range, 1 to 69 years), most were heavily transfused, and all received previous immunosuppression (25% without antithymocyte globulin). Almost two-thirds (63%) received standard fludarabine (Flu)/cyclophosphamide (Cy) 29/total body irradiation (TBI) 200 cGy conditioning, and the remaining patients received an augmented conditioning: Flu/Cy29/TBI 300-400 (16%), Flu/Cy50/TBI 200 (10%), or Flu/Cy50/TBI 400 (10%). All patients received PTCy-based graft-versus-host disease (GVHD) prophylaxis. Most grafts (93%) were bone marrow (BM). The median duration of follow-up was 2 years and 2 months. The median time to neutrophil recovery was 17 days. Primary graft failure occurred in 15% of the patients, and secondary or poor graft function occurred in 5%. The incidences of grade II-IV acute GVHD was 14%, and that of chronic GVHD was 9%. Two-year overall survival and event-free survival (EFS) were 79% and 70%, respectively. EFS was higher for patients who received augmented Flu/Cy/TBI (hazard ratio [HR], .28; P = .02), and those who received higher BM CD34 cell doses (>3.2 × 10E6/kg) (HR, .29; P = .004). The presence of donor-specific antibodies before HSCT

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*Correspondence and reprint requests: Hospital Israelita Albert Einstein, Av. Albert Einstein, 627 - Jardim Leonor, São Paulo – SP, Brazil, CEP 05652-900 Tel.: +55 (21)981334715
E-mail address: leonardojavier@gmail.com (L.J. Arcuri).

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was associated with lower EFS (HR, 3.92; $P = .01$). Graft failure (HR, 7.20; $P < .0001$) was associated with an elevated risk of death. Cytomegalovirus reactivation was frequent (62%). Haploidentical HCT for SAA is a feasible procedure; outcomes are improved with augmented conditioning regimens and BM grafts with higher CD34 cell doses.

INTRODUCTION

Severe aplastic anemia (SAA) is a life-threatening disease characterized by immune destruction of the hematopoietic stem cells. Current reviews recommend treatment with hematopoietic stem cell transplantation (HSCT) from an HLA-matched sibling donor or immunosuppression with or without eltrombopag [1,2].

Immunosuppressive therapy with rabbit antithymocyte globulin (r-ATG) yields poorer results compared with horse ATG (h-ATG) [3], despite having a better immunosuppressive profile. h-ATG offers a superior 6-month remission rate with lower early mortality and possibly lower relapse rates [4]; however, h-ATG is not available in Brazil.

HSCT is currently the sole curative approach for SAA. When immunosuppression fails and an HLA-matched donor is not available, few options remain. The use of HLA-matched unrelated donors (MUDs) for SAA has been associated with inferior survival and a higher incidence of graft-versus-host disease (GVHD). A recent study reported significantly higher cumulative incidences of acute GVHD (25% versus 15%) and chronic GVHD (26% versus 14%) with MUD HSCT compared with HLA-matched sibling donor HSCT [5]. For patients with SAA who fail to respond to treatment with immunosuppression, any delays in performing HSCT is a major setback, given the high risk of infection and death in this population. In contrast, for patients without a matched sibling donor, identification and availability of a haploidentical family member suitable to serve as an HCT donor can be accomplished as early as in 2 weeks.

Haploidentical transplantation has been reported in 3 small series of patients with SAA [6-8]. The incidence of graft failure was 11% in a prospective trial (n = 37) and 33% in a European retrospective study (n = 33). Owing to the small number of patients, risk factors have not been analyzed. Two-year overall survival (OS) ranged from 78% to 94%.

Here we report risk factors for outcomes after related haploidentical donor HSCT in 87 Brazilian patients with SAA.

METHODS

This retrospective multicenter study was conducted on behalf of the Brazilian Society of Bone Marrow Transplantation and the Brazil-Seattle Transplant-Related Complications Consortium. The study cohort comprised 87 consecutive patients who underwent a related haploidentical HSCT for relapsed/refractory SAA and who received a fludarabine (Flu)/cyclophosphamide (Cy)/total body irradiation (TBI)-based conditioning regimen and PTCy-based GVHD prophylaxis (Cy 50 mg/kg on days +3 and +4) in 14 Brazilian centers between 2010 and June 2019. All patients lacked an MUD. The conditioning regimen was standard Flu/Cy/TBI [9] (Flu 30 mg/mg^2 for 5 days from day -6 to day -2 or 40 mg/m^2 for 4 days from day -6 to day -3, Cy 14.5 mg/kg on days -6 and -5, and TBI 200 cGy on day -1) in 55 patients (63%). Thirty-two patients (37%) received an augmented conditioning regimen, either with an increased TBI dose to 300 cGy (single dose; 3 patients) or 400 cGy (in two 200 cGy doses given 12 hours apart; 11 patients), an increased Cy dose to 50 mg/kg (25 mg/kg on days -6 and -5; 9 patients), or both Cy 50 mg/kg and TBI 200 cGy given twice (9 patients). PTCy was administered at a dose of 50 mg/kg on days +3 and +4. Tacrolimus or cyclosporine (to maintain trough levels of 5 to 15 ng/mL and 200 to 400 ng/mL, respectively) and mycophenolate mofetil 30 to 45 mg/kg (in 3 divided daily doses) were started on day +5 and continued for 1 year and day +35 post-HSCT, respectively. Sixteen of the patients have been reported in a previous study [10]. All patients met the criteria for SAA. Patients with Fanconi anemia were excluded. Viral surveillance and antibiotic prophylaxis were provided according to the protocols of the local centers. All patients underwent pretransplantation testing for donor-specific anti-HLA antibodies (DSAs). The study was conducted in accordance with the ethical principles specified in the Declaration of Helsinki and Good Clinical Practices.

The baseline data of the patients and transplantations are presented as percentage or median. Survival and the cumulative incidence of events were estimated using the Kaplan-Meier or Gray methods. Cumulative incidence curves were compared with using the Gray test, and survival curves were compared with the log-rank test. OS was defined as the time from HSCT to death or last follow-up.

Graft failure was defined as failure of hematologic recovery by day +28 after HSCT or as the absence of chimerism in unfractionated bone marrow (BM) and/or blood cells, and poor graft function was defined as persistent cytopenia despite evidence of chimerism. Diagnosis and grading of acute GVHD were done according to the Glucksberg criteria [11], and diagnosis and grading of chronic GVHD were done according to the National Institutes of Health consensus criteria [12]. Event-free survival (EFS) was defined as time from HSCT to first event (graft failure or death). Patients were considered at risk for cytomegalovirus (CMV) reactivation if the donor or recipient was CMV IgG$^+$. CMV reactivation was defined as any positive antigenemia or PCR that prompted preemptive therapy.

The univariate analyses for survival were performed with Cox models for survival and EFS and with the Fine and Gray model for engraftment, graft failure, and CMV reactivation, with death as a competing event. The variables listed in Table 1 were included in the univariable analyses. Data on CD34$^+$ cell dose were missing in 5 patients; all of these patients received a BM graft, and their missing cell doses were imputed using a linear function of total nucleated cells in patients who received BM grafts using linear regression.

Graft failure was included in the multivariate model for OS as a time-dependent variable. Multivariate models were built only for EFS, OS, and engraftment, owing to the relatively small number of events in the other outcomes (eg, GVHD). A 2-sided α error of $<.05$ was considered statistically significant.

RESULTS

The characteristics of all 87 patients are presented in Table 1. The median patient age was 14 years (range, 1 to 69 years), and 51 (60%) were male. All patients had refractory/relapsed SAA. Most had been heavily transfused, and 68 had received more than 20 transfusions. Twenty-five percent had not received previous ATG therapy, because h-ATG (the most efficacious form), is not available in Brazil, and the accessibility of r-ATG is low. The median time from diagnosis to haploidentical HSCT was 16 months (range, 2 to 108 months). The graft source was BM in 81 patients (93%), peripheral blood stem cells in 3 patients, and a combination of the two in 3 patients. r-ATG (Thymoglobulin) was added for 8 patients (9%) in the standard Flu/Cy/TBI group and for 4 patients (5%) in the augmented Flu/Cy/TBI group. The median infused CD34 cell dose was 3.6 × 10E6/kg (range, 1.0 to 11.3× 10E6/kg; available for 82 patients), and the median total nucleated cell count was 5.3 × 10E8/kg (range, 1.0 to 19.4 × 10E8/kg; available for 84 patients). The main outcomes are summarized in Table 2.

Engraftment and Graft Failure

The median time to neutrophil recovery was 17 days (range, 8 to 29 days), and the median time to platelet engraftment was 21 days (range, 11 to 200 days). The cumulative incidence of neutrophil recovery at day +30 was 85%; it was higher in patients who received an augmented conditioning regimen (94% versus 80%; $P = .003$) and in patients who received CD34 doses $>3.2 \times 10E6/kg$ (94% versus 74%; P = .0009). Children age ≤ 10 years received higher CD34 cell doses ($P = .001$) and thus has a higher rate of engraftment (91% versus 82%; $P = .03$). The results of univariable and multivariable analyses for neutrophil recovery

Table 1
Characteristics of the Study Population

Characteristic	Value
No. of patients	87
No. of centers	14
Patient age, yr, median(range)	14 (1-69)
Donor age, yr, median (range)	36 (10-59)
Female donor to male recipient, n (%)	19 (22)
Donor, n (%)	
Parent	61 (70)
Offspring	1 (1)
Sibling	22 (25)
Other	3 (4)
ABO match, n (%)	
Matched	60 (69)
Minor mismatch	14 (16)
Major mismatch	10 (12)
Bidirectional	3 (3)
CMV Donor or recipient positive CMV serostatus, n (%)	86 (99)
Previous exposure to r-ATG or alemtuzumab, n (%)	65 (75)
Other pre-HSCT immunosuppression without r-ATG or alemtuzumab, n (%)	22 (25)
Time from diagnosis to HSCT, mo, median (range)	16 (2-108)
BM graft, n (%)	81 (93)
Conditioning regimen, n (%)	
Flu/Cy29/TBI200	55 (63)
Flu/Cy29/TBI300	3 (3)
Flu/Cy29/TBI400	11 (13)
Flu/Cy50/TBI200	9 (10)
Flu/Cy50/TBI400	9 (10)
r-ATG in the conditioning regimen	12 (14)
GVHD prophylaxis, n (%)	
PTCy/CSA/MMF	76 (87)
PTCy/tacrolimus/MMF	11 (13)

CSA indicates cyclosporine A; MMF, mycophenolate mofetil.

Table 2
Major Outcomes after Haploidentical HSCT for SAA (N= 87)

Outcome	100 Days, % (n)	1 Year, % (n)	2 Years, % (n)
OS	90 (9)	80 (17)	79 (18)
EFS	75 (22)	71 (25)	70 (26)
Graft failure or poor graft function	18 (16)	20 (17)	20 (17)
Acute GVHD grade II-IV	13 (11)	14 (12)	NA
Acute GVHD grade III-IV	3 (3)	3 (3)	NA
Chronic GVHD (any)	1 (1)	9 (7)	10 (8)
CMV reactivation	61 (53)	62 (54)	62 (54)

are presented in Tables 3 and 4; the data show that only augmented conditioning and CD34 dose remained significant.

Seventeen patients had primary (13 patients; 15%) or secondary (3 patients; 4%) graft failure or poor graft function (1 patient; 1%), and the 1-year cumulative incidence of graft failure/poor graft function was 20% (95% confidence interval [CI], 12% to 32%). The rate of graft failure was significantly lower in patients who received an augmented conditioning regimen (7% versus 27%: P = .02), and in those who received higher CD34 doses (>3.2 × 10E6/kg; 11% versus 31%; P = .02). In multivariable analysis, previous use of r-ATG was also protective (HR, .28; P = .01 (Table 4)).

Six patients had a positive DSA, including 3 patients with 2 different DSAs, the higher titers with a mean fluorescent intensity [MFI] of 2683, 2660, and 1256; 1 patient with an MFI of 7696; and 2 patients with an MFI >10,000). Two of these patients had primary graft failure, and 1 had secondary graft failure.

Fourteen of the 17 patients with graft failure or poor graft function underwent a second HCT, from a different haploidentical donor (n = 13) or with double umbilical cord blood units (n = 1). The 1-year OS was 47% after a diagnosis of graft failure/poor graft function and 57% after a second HCT. Two patients needed a third HCT (1 haploidentical, 1 umbilical cord blood), but neither survived.

Chimerism analyses were available for 62 patients who had not experienced graft failure; 59 patients had complete chimerism, and 3 had mixed chimerism at last follow-up. Two of the latter patients were alive at the time of this report (92% and 70% chimerism), transfusion-independent, and hemoglobin concentration >12 g/dL, but the other patient died of thrombotic microangiopathy.

GVHD

Acute GVHD grade II-IV was observed in 12 patients (9 with grade II and 3 with grade III). The cumulative incidence of acute GVHD grade II-IV at 100 days was 13% (95% CI, 8% to 25%) and that grades III-IV was 4% (95% CI, 1% to 12%). Chronic GVHD was observed in 8 patients, including 5 with mild, 1 with moderate, and 2 with severe chronic GVHD by National Institutes of Health classification. At 2 years, the cumulative incidence of chronic GVHD was 10% to 11% (95% CI, 5% to 20%).

Hemorrhagic Cystitis and CMV Reactivation

Twelve patients experienced hemorrhagic cystitis (cumulative incidence of 12%) at a median of 35 days after HCT (range, 24 to 180 days). All but 1 patient were at risk for CMV reactivation (78 donor [D]+/recipient [R]+, 2 D+/R-, 5 D-/R+, 1 D-/R-, and 1 D+/R unknown), which occurred in 54 individuals, with a cumulative incidence of 62% and at a median of 36 days (range, 12 to 109 days). In multivariate analysis, risk factors for CMV reactivation were inclusion of ATG in the conditioning regimen (HR, 2.40; P = .03), grade II-IV acute GVHD as a time-dependent covariate (HR, 5.07; P = .0001), and donor age (HR, .96 for each year; P = .008).

OS and EFS

With a median follow-up of 2 years and 2 months (range, 5 months to 8.6 years), the 2-year OS was 79% (95% CI, 71% to 88%; Figure 1). The sole risk factor for death was graft failure (HR, 7.20; P < .0001, time-dependent covariate). OS was 50% in patients with positive DSA and 81% in patients without DSA (P= .07).

Eighteen patients died, including 9 out of 17 patients with graft failure (sepsis, n = 2; fungal, n = 5; CMV disease, n = 1; other, n = 1) and 9 out of 70 who engrafted (sepsis, n = 2; fungal infection, n = 3; disseminated toxoplasmosis, n = 1; CMV disease, n = 1; post-transplantation lymphoproliferative disease-Epstein-Barr virus, n = 1; and GVHD, n = 1).

The estimated 2-year EFS was 70% (95% CI, 61% to 80%). It was higher in patients who received the augmented conditioning regimen (88% versus 60%, P = .01; Figure 2A), higher CD34 doses (>3.2 × 10E6/kg; 82% versus 54%, P = .002; Figure 2B), and in those without DSA (72% versus 33%, P = .03). These 3 variables remained significant in multivariable analysis. The results of univariate analysis of risk factors for EFS survival are

Table 3
Univariate Analyses of Risk Factors for Neutrophil Recovery and EFS for Patients with SAA Undergoing Haploidentical HSCT with PTCy

Risk Factor	HR (95% CI)	*P* Value
Neutrophil recovery		
Age (>10 years versus ≤10 y/o)	.62 (.39-.99)	.05*
Sex (female versus male)	1.10 (.69-1.74)	.70
Time to transplantation >24 mo	.87 (.53-1.42)	.58
Donor-specific antibodies (presence or absence)	.76 (.28-2.08)	.59
Previous immunosuppressive treatment (with or without anti-T serotherapy)	.80 (.46-1.40)	.44
Donor relationship, others versus parent	.83 (.50-1.38)	.47
ABO, mismatched vs matched	.97 (.59-1.59)	.91
Total nucleated cells >4 × 10E8/kg	1.40 (.83-2.37)	.21
CD34 dose infused (each 1 × 10E6/kg increase)	1.09 (1.01-1.18)	.03*
No. CD34 infused > 3.2 × 10E6/kg	2.27 (1.42-3.65)	.0007*
Cy 50 mg/kg and/or TBI > 200 cGy	1.91 (1.19-3.05)	.007*
r-ATG in the conditioning	1.01 (.51-1.96)	.99
EFS		
Age (> 10 yr versus ≤10 yr)	2.62 (.99-6.94)	.05
Sex (female vs male)	1.03 (.47-2.25)	.94
Time to transplantation >24 mo	.80 (.34-1.91)	.62
Donor-specific antibodies (presence or absence)	3.06 (1.05-8.92)	.04*
Previous immunosuppressive treatment (with or without anti-T serotherapy)	.67 (.29-1.53)	.34
Donor relationship, others versus parent	.92 (.39-2.21)	.86
ABO mismatched versus matched	.64 (.25-1.58)	.33
TNC >4 × 10E8/kg	.51 (.23-1.13)	.10
CD34 dose infused (each 1 × 10E6/kg increase)	.79 (.64-.98)	.03*
CD34 dose infused >3.2 × 10E6/kg	.29 (.12-.66)	.003*
Cy 50 mg/kg and/or TBI >200 cGy	.27 (.09-.79)	.02*
r-ATG in the conditioning regimen	1.49 (.56-3.97)	.41

* Statistically significant.

Table 4
Multivariate Analysis for Neutrophil Recovery and EFS for Patients with SAA Undergoing Haploidentical HSCT with PTCy

Variable	HR (95% CI)	*P* Value
Neutrophil recovery		
CD34 dose infused >3.2 × 10E6/kg	2.13 (1.32-3.44)	.002*
Cy 50 mg/kg and/or TBI > 200 cGy	1.72 (1.07-2.77)	.03*
EFS		
Donor-specific antibodies	3.92 (1.32-11.7)	.01*
CD34 dose infused >3.2 × 10E6/kg	.29 (.13-.68)	.004*
Cy 50 mg/kg and/or TBI >200 cGy	.28 (.09-.81)	.02*
Graft failure		
CD34 dose infused >3.2 × 10E6/kg	.27 (.09-.78)	.02*
Cy 50 mg/kg and/or TBI >200 cGy	.19 (.04-.84)	.03*
Previous ATG exposure	.28 (.11-.73)	.01*

* Statistically significant.

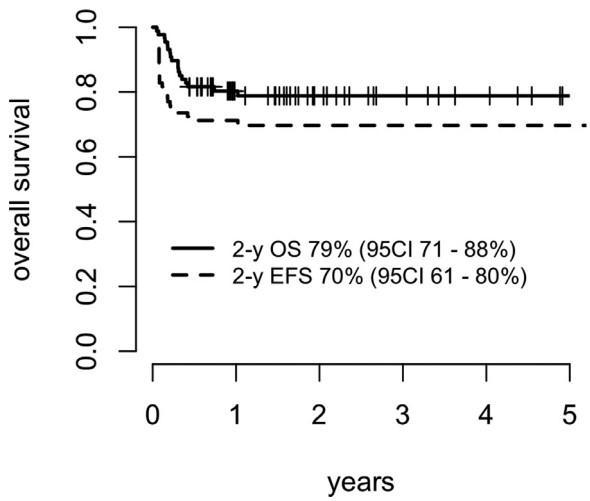

Figure 1. OS and EFS after haploidentical HSCT for SAA.

shown in Table 3, and results of multivariable analysis are presented in Table 4.

When we included only patients who received BM grafts, the impacts of CD34 dose >3.2 × 10E6/kg on engraftment (HR, 2.33, *P* = .0007), graft failure (HR, .33; *P* = .03), and EFS (HR, .33, *P* = .009) remained significant.

DISCUSSION

Our results show that haploidentical HCT with PTCy for patients with aplastic anemia is a feasible procedure for patients without an HLA-matched donor, with a 2-year OS of 79%. Impressively, early transplantation-related mortality was 10%, and the incidence of acute GVHD was 14%. Despite encouraging outcomes, rates of primary and secondary graft failure were as high as 20%, as have been observed in other series (<38 patients), varying from 11% to 33% [6-8]. This is the largest cohort reported so far, which allowed us to perform a risk factor analysis for engraftment and EFS. Among patient-, disease-, donor-, and transplantation-related factors, we found that the presence of 2 modifiable factors, such as higher BM $CD34^+$ cell dose and a more intensive conditioning regimen, can increase engraftment and EFS rates.

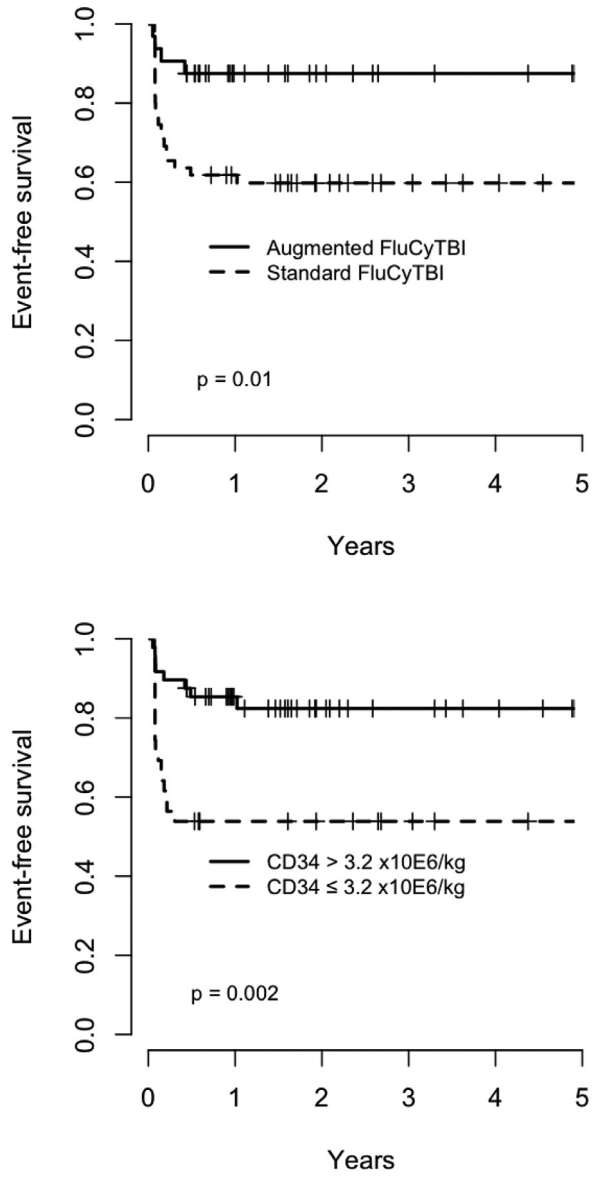

Figure 2. EFS after haploidentical HSCT for SAA according to conditioning regimen (A) and CD34 cell dose (B).

In our study, augmented conditioning regimen, with an increased TBI dose (300 or 400 cGy) or Cy dose (50 mg/kg), effectively overcame graft failure (6% versus 27%) and increased EFS (88% versus 60%). In hemoglobinopathies at least, increasing the TBI dose to 400 cGy (in a population that also received ATG and sirolimus) has also successfully addressed graft failure after haploidentical HSCT [13]. The potential concern that 400 cGy TBI will substantially increase long-term morbidity or subsequent neoplasms might not be justified [14]. However, high rates of graft failure (33% primary or secondary) after haploidentical HSCT with PTCy for SAA were reported by Prata et al [6] in series with 33 patients on behalf of the European Group for Blood and Marrow Transplantation (EBMT). One-half of their patients received the original Baltimore conditioning regimen (Flu 150 mg/m^2, Cy 29 mg/kg, and TBI 200 cGy). In these patients, the cumulative incidence of neutrophil engraftment was only 69%. DeZern et al [7] published a series of 20 relapsed/refractory patients using the standard Flu/Cy/TBI or with TBI 400 cGy, combined with ATG. Only 1 patient (5%) experienced graft failure. In our cohort, 4 of the 12 patients who received ATG in the conditioning regimen had graft failure. Moreover, ATG in the conditioning regimen was a risk factor for CMV reactivation. Based on our results, the role of ATG in the conditioning regimen for patients with aplastic anemia undergoing HCT remains controversial.

Improved EFS was also associated with higher CD34 cell dose. This effect was present even when we analyzed only patients who received BM grafts. The importance of high-CD34 grafts in BM has been reported in several HCT clinical settings, including HLA-matched sibling donor [15], unrelated donor [16], and haploidentical HSCT for malignant diseases [17], but the beneficial effect of high- CD34 grafts in haploidentical BM transplantation for aplastic anemia has not been reported previously. High-CD34 BM grafts can effectively overcome graft failure and thereby improve EFS. The BM harvesting technique has deteriorated in recent years [18]; nonetheless, for nonmalignant disorders, BM is still the superior stem cell source owing to an undesirable higher incidence of chronic GVHD associated with peripheral blood stem cells (PBSCs). One could argue that for heavier patients for whom a BM CD34$^+$ cell dose of 3.2 $\times$ 10^6/kg cannot be achieved, PBSCs could be an option; however, in our study, we cannot even speculate on the role of PBSCs in this setting, owing to the very small number of patients receiving PBSCs.

In the BMT-CTN 0301 trial that defined the ideal Cy dose in combination with Flu and TBI for unrelated donor transplantations, the graft failure rate was 11% [19]. Latour et al [20] also reported an 11% rate of graft failure after umbilical cord blood transplantation for SAA using reduced-intensity ATG/Flu/Cy/TBI-based conditioning. Taken together, these studies highlight the favorability of our results for haploidentical HSCT with the augmented Flu/Cy/TBI regimen. In addition, a haploidentical donor can be cleared for donation in as little as 2 weeks, whereas unrelated donor transplantations are seldom performed in less than 2 months. One the other hand, the availability of eltrombopag may reduce the need for HSCT from alternative donors, given that the overall response rate tops 80% when eltrombopag is added to the standard immunosuppressive regimen [21].

Graft failure—and consequent infection—was the primary cause of death, even though most patients underwent a subsequent HSCT, and OS following graft failure was substantially higher than that reported by the EBMT [22] (47% versus against 16%). The rapid availability of a haploidentical donor might have contributed to our results. Unless there is an already identified backup donor [23], the time between the preliminary search to stem cell infusion averages around 100 days for unrelated donors [24], hampering successful rescue transplantation, whereas rescue haploidentical transplantation can be done within a shorter interval and achieve excellent outcomes [25].

The 2-year OS of 79% achieved in this study is comparable to that a recent study of haploidentical HSCT for refractory aplastic anemia performed in Europe (78% at 2 years) [6]. In this study, 96% of the patients had been previously treated with ATG-based therapies, and one-half of the patients received the classical Baltimore conditioning regimen with TBI 200 cGy and Cy 29 mg/kg. DeZern et al [7] reported 100% survival in 20 refractory patients using the classical Baltimore conditioning regimen plus ATG; TBI 400 cGy was given only to treatment-naïve patients. Clay et al [8] reported 6 of 8 successful engraftments in patients with refractory SAA or SAA who failed to engraft after unrelated donor or umbilical cord blood

transplantation. All patients received PBSCs and the classic Baltimore conditioning regimen. The survival achieved in our study is even higher than that reported in a cohort of pediatric patients who underwent unrelated donor transplantation in Brazil [26]. Our mortality rate was not higher in patients with a >2 year interval between diagnosis and transplantation. This is in contrast with reports by Bacigalupo et al [27] and Locasciulli et al [28], who identified early transplantation for AA as a significant factor contributing to superior outcomes. Potential sources of bias in our study include high-risk patients who may have died before being considered for HCT. The long interval between diagnosis and transplantation in our population (>12 months in more than one-half of the patients and >24 months in one-third of the patients) suggests a milder disease course in our population, but a high number of blood products transfused and potentially more infections by the time of HSCT, and thus the direction of the bias unpredictable. Patients might not have been referred early to a transplantation center for several reasons.

Rates of both chronic and acute GVHD were low, similar to other reports of haploidentical transplantation with BM grafts and PTCy [29]. The incidence of CMV was quite high (62%) but not unexpected in a population with only 1% of the donor-host pairs CMV-negative. ATG in the conditioning regimen may increase the risk of CMV reactivation.

In our study, there were 32 children age 10 years, which makes our series the largest pediatric series reported to date as well. The OS in this population was 91%. These children received grafts with higher CD34 counts and thus had a higher engraftment rate and better EFS (84% versus 62%).

This study has several limitations inherent to a retrospective analysis. For instance, some information was not available, such as doses and dates of r-ATG administration in the immunosuppressive treatment. In Brazil, most patients are first treated with immunosuppression for SAA; thus, most of our haploidentical transplantation recipients had received previous immunosuppression with r-ATG. Another potential source of bias in our results include delayed referral to a transplantation center, leading to early death from lack of access to the best supportive therapy and blood transfusions. Predominantly, patients surviving immunosuppressive therapy without definite improvement were the ones referred to transplantation centers. On the other hand, to our knowledge, this is the largest series of recipients of haploidentical transplantations with PTCy in relapsed/refractory SAA reported to date.

In conclusion, we have shown that haploidentical related donor HSCT for SAA is feasible and can benefit many patients but is associated with an ~20% rate of graft failure/poor graft function. Increasing the conditioning regimen intensity and BM $CD34^+$ cell dose may benefit patients. Taking into account the nearly universal availability of haploidentical donors, stem cell transplantation with the use of PTCy for SAA can be a valuable option. Whether Haplo-PTCy is a better option or at least comparable to HLA-unrelated or -matched sibling donor HSCT merits further investigation.

CONCLUSIONS

Haploidentical HSCT may be the sole option for patients with relapsed/refractory SAA. CD34 cell dose and conditioning regimen are factors that can be modified and improve outcomes.

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