

Artigo / Article

Allogeneic bone marrow transplantation for severe aplastic anemia patients with risk factors for poor prognosis: is fludarabine a requirement?

Transplante alogênico de medula óssea em portadores de anemia aplásica severa com fatores de mau prognóstico: é necessário fludarabina?

Carlos R. de Medeiros
Elenaide Coutinho
Eliane R. Carmes
Marco A. Bitencourt
Jefferson Ruiz
Carmen S. Bonfim
Vaneusa M. Funke
Daniela C. Setubal
José Zanis-Neto
Ricardo Pasquini

Hematopoietic progenitor cell transplantation from HLA-identical sibling donors cures 70-90% of Severe Aplastic Anemia (sAA) patients. Older age, heavy exposure to transfusions, immunosuppression treatment (IST) with a long interval from diagnosis to transplant and infection at procedure are associated with poor outcomes. We transplanted 18 patients with sAA and at least one risk factor (RF) for poor prognosis (age >35 years, >50 transfusions prior to transplant, unresponsiveness to previous IST and bacterial or fungal infection at transplant) from 2001 to 2005, using cyclophosphamide (CY - 5 patients) or busulfan plus CY (13 patients). Sixteen patients engrafted, two died with no engraftment, three patients had evidence of graft failure at days +67, +524 and +638 (two died and one was rescued with IST). Grade III/IV mucositis occurred in 39% but neither aGVHD nor cGVHD were observed. The Kaplan-Meier probability of survival was 75% at 2.14 years, with a trend favoring survival by number of RF (1 versus =2 RF) (P = 0.06). These results are comparable to recent data reported with fludarabine-based conditioning in patients with poor prognosis sAA. Due to the small sample size, prospective clinical trials with larger cohorts of patients are needed to confirm the real benefits of fludarabine-based conditioning, and also to define the best agent(s) to be associated with Fludarabine as preparative regimen for sAA patients with poor prognosis. Rev. bras. hematol. hemoter. 2008; 30(4):292-296.

Key words: Severe aplastic anemia; fludarabine-based conditioning; poor prognosis sAA; graft failure.

Introduction

Hematopoietic progenitor cell transplantation (HPCT) from an HLA-identical sibling donor is the curative treatment for 70 to 90% of Severe Aplastic Anemia patients (sAA).¹ However, older age, long duration of disease with consequent heavy exposures to transfusion and active infection at time of HPCT have a negative influence on outcome, favoring graft failure (GF) and quite probably graft versus host disease (GVHD).² Several patients with sAA, even those with an HLA-identical available donor, have been considered unsuitable for this procedure, due to the presence of these risk factors

for poor survival. Immunosuppression (IST), in spite of being a non-curative treatment for sAA patients, may promote partial recovery of hematopoiesis, and has been used as an alternative for patients with an available donor but presenting poor survival risk factors at the time of HPCT.³ Unfortunately, ~35% of IST treated patients do not respond and another therapeutic approach is then needed.

Nonmyeloablative HPCT (NMHPCT) has been successfully used in the treatment of hematological malignancies. Older age, pretransplantation comorbidities and extensive previous treatments are the main factors leading to the use of less aggressive conditioning regimens.⁴

Bone Marrow Transplantation Service, Hospital de Clínicas, Universidade Federal do Paraná (UFPR) – Curitiba-PR.

Correspondence: Carlos R. de Medeiros
Bone Marrow Transplantation Service, Hospital de Clínicas, UFPR
Rua General Carneiro, 181 – 15º andar
Zip code 80060-900 – Curitiba-PR – Brazil
Phone: 55 41-33361110; Fax: 55 41-33361110
E-mail: crdemedeiros@terra.com.br

Fludarabine (FLU), a nonmyeloablative immunosuppressant with mild systemic activity, has been used as part of conditioning regimens in allogeneic NMHPCT, allowing engraftment of patients with hematological malignancies, even when the donor is unrelated. When compared to conventional conditioning regimens, at least in patients with hematological malignancies, NMHPCT has promoted engraftment with less toxicity and a lower incidence of acute (a) GVHD.⁵ Early in 2000, several case reports demonstrated the feasibility of FLU-based regimens used as conditioning for HPCT, allowing long-lasting engraftment with low regimen-related toxicity for non-malignant hematological conditions.⁶⁻¹⁰

Before embarking on a new protocol involving FLU as part of conditioning to treat sAA patients with poor survival risk factors, we decided to analyze our transplant results with conventional (CONV) conditioning. Herein we describe the outcomes of sAA patients, who had at least one risk factor for poor survival at the time of procedure, transplanted from 2001 to 2005 using an alkylating agent alone (cyclophosphamide - CY) or in combination (busulfan and CY).

Patients and Method

Patients with sAA were included in our HPCT protocol from January 2001 to June 2005 with all receiving unmanipulated bone marrow from an HLA-identical sibling donor. Patients were eligible for analysis if they presented at least one of the following risk factors: (1) age older than 35 years; (2) >50 transfusions prior to transplant; (3) defined or probable invasive fungal infection and/or confirmed bacterial infection at transplant; (4) unresponsiveness to IST prior to transplant. Median age of participants was 18.2 years (range 6 to 46 years) with ten patients being male.

Conditioning regimen was 50 mg/kg CY once daily i.v. on days 1 to 4 (total dose 200 mg/kg – 5 patients) or 3 mg/kg BU p.o. in divided doses daily for 4 days (total dose 12 mg/kg) plus CY 60 mg/kg once daily i.v. on days 1 and 2 (total dose 120 mg/kg – 13 patients). Patients were isolated in single or double rooms with high efficiency particulate air filters. In case of ABO incompatibility, red blood cells or plasma were removed by starch sedimentation. Patients seronegative for cytomegalovirus (CMV) were transfused with CMV-negative blood or leukodepleted blood. Acyclovir prophylaxis were used for the herpes virus and trimethopim-sulfamethoxazole for *Pneumocystis carinii* for all patients as was fluconazole for fungus prevention. Weekly antigenemia, to monitor CMV viremia, and preemptive treatment with ganciclovir were routine. Grade II to IV acute (a) GVHD was defined using published criteria¹¹ and patients surviving >28 days post-transplant with engraftment were considered to be at risk. Patients engrafted and surviving >90 days were at risk for chronic (c) GVHD.¹² Neutrophil engraftment was defined as

an absolute neutrophil count >0.5 x 10⁹/L on at least two consecutive days without treatment using a growth factor. Time to platelet engraftment was defined as three consecutive days on which the platelet count was higher than 50 x 10⁹/L without transfusion for seven days. GF was classified as primary graft failure when no hematological recovery in patients surviving >28 days post-transplant occurred; and as transient engraftment (TE) when complete or partial recovery of hematopoiesis of donor origin was detected, followed by recurrent pancytopenia with markedly hypocellular marrow in the absence of GVHD. Documented marrow donor engraftment was confirmed by ABO typing in patients with major red blood cell group differences, by chromosomal analysis for sex-mismatched patient/donor pairs and by PCR targeting a variable number of tandem repeats loci. Prophylaxis for graft versus host disease was identical for all patients using Methotrexate and Cyclosporine. The protocol was approved by the Ethics Committee of the Hospital de Clinicas and all patients or parents gave written informed consent. Typing of both donor and recipient pairs involved low resolution for class I HLA by reverse sequence specific oligonucleotides using Luminex technology (One Lambda Cat. RSSO1A/RSSO1B, Canoga Park, USA), and high resolution for class II HLA using sequence specific primers (One Lambda Cat. SSP2H, Canoga Park, USA).

Results

Eighteen patients with sAA and, at least, one poor prognostic risk factor were included in our HPCT protocol from January 2001 to June 2005. The baseline characteristics of patients are shown in Table 1. Sixteen patients engrafted and two died on days +10 (septic shock) and +22 (central

Table 1. Baseline characteristics of 18 patients

Number of patients [n (%)]	18 (100)
Sex	
male [n(%)]	10 (56)
Age in years [median (range)]	18.2 ± 9.4 (6-46)
>35 years [n (%)]	1 (6)
Infection at HPCT [n (%)]	4 (22)
Previous IST [n (%)]	16 (89)
Prior transfusion [median (range)]	27 (6 - 51)
>50 (%)	2 (11)
Interval DX ? HPCT months [median (range)]	5.5 (1 - 47)
Risk factors [n (%)]	
1	15 (83)
≥ 2	3 (17)
Cell dose x 10 ⁸ /kg	1.9 - 6.6 (M: 3.4)

HPCT: hematopoietic progenitor cell transplantation; IST: immunosuppressive therapy; DX: diagnosis

nervous system fungal infection) with no engraftment. Three patients had only TE with subsequent GF on days +67, +524 and +638. The former two died secondary to fungal infection and central nervous system bleeding, respectively, and the latter was rescued with maintenance IST. Grade 0/II mucositis was observed in 11 patients (61%), and grade III/IV in 7 patients (39%). Neither aGVHD nor cGVHD were presented by our patients. Fourteen patients survived the

procedure, hence the probability of survival calculated by the Kaplan-Meier method was 75% at 2.14 years (Figure 1). When we compared survival by number of risk factors (1 versus ≥ 2 risk factors), we observed a trend for better survival of patients with only one risk factor at transplant ($P=0.06$). (Figure 2). Survival of patients with only one risk factor determined by Kaplan-Meier was 85% at 2.14 years.

Statistical analysis

Demographic, clinical and laboratory data are summarized in Table 1 using percentages, means and standard deviation or median and range values. The Shapiro-Wilk W test is used to test for normality. If the W statistic is significant, then the hypothesis that the respective distribution is normal should be rejected. GF was analyzed in patients surviving at least 28 days post-transplantation. Patients surviving 28 days with engraftment were considered at risk for aGVHD and those surviving 90 days, for cGVHD. Survival curves were calculated using the Kaplan-Meier product limit method. The log-rank test was used to test differences in survival. All P-values presented are 2-sided. A probability below 0.05 was considered significant. Statistical analyses were carried out using the Statistica 7.0 (StatSoft, Inc, Tulsa, OK, USA) software.

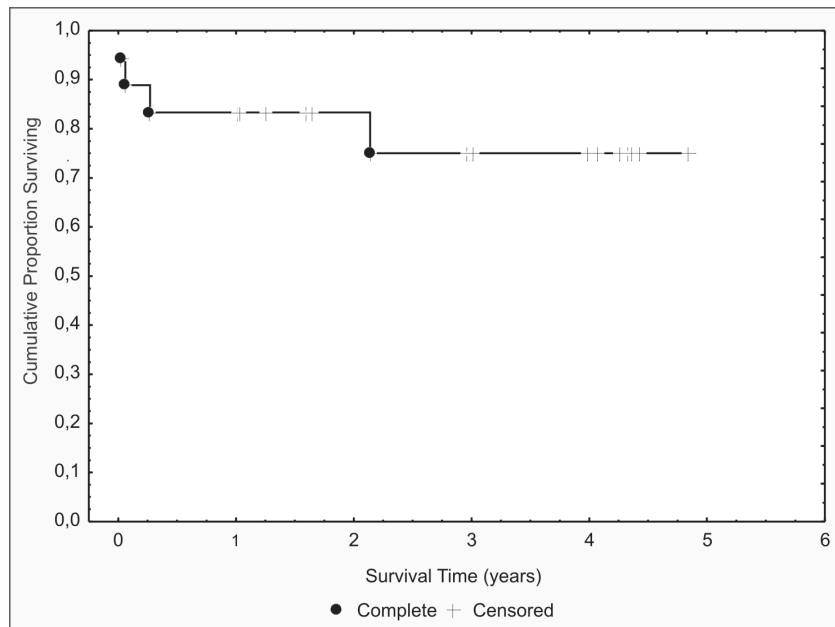


Figure 1 – Cumulative probability of survival (n=18)

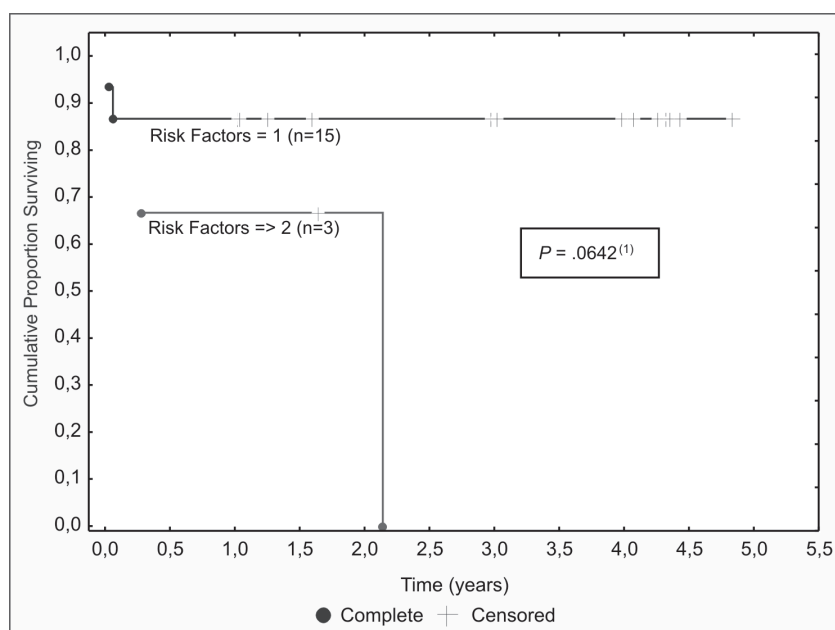


Figure 2 - cumulative probability of survival stratified by risk factors

Discussion

Several risk factors negatively influence survival of sAA patients treated with allogeneic HPCT. Older age for reasons not completely understood, exposure to a large number of transfusions due to sensitization of the recipient to histocompatibility antigens, and long duration of disease are recognized as risk factors for GF, a major procedure problem. In addition, low neutrophil and monocyte counts put sAA patients at risk for bacterial and fungal infections¹³ and worse HPCT results are expected when performed in the presence of an infectious process. Also, patients undergoing HPCT after failing IST have usually been heavily transfused and are inclined to acquire bacterial and fungal infections, thereby affecting survival.¹⁴

Increasing of conditioning regimens has been a strategy to reduce GF.¹⁵ Incorporation of total body irradiation (TBI)

to conditioning has overcome the risk of graft rejection, but leads to several complications. High doses or the combination of alkylating agents may reduce GF but generally with no better outcome in heavily transfused patients.¹⁶ In addition, more toxicity at conditioning is undesirable for patients with past or present bacterial and/or fungal infections at transplant. Fludarabine, as has been demonstrated in NMHPCT to treat malignancies, as a useful drug capable of immunoablation. This medication when associated to low doses of an alkylating agent with or without antithymocyte globulin (ATG) and low-dose TBI, should create the desired conditioning regimen: high immunosuppression and low toxicity.

Recently, four groups published their experience with HPCT in patients with marrow failure syndromes and increased graft failure risk using a FLU-based conditioning regimen.¹⁷⁻²⁰ Kumar *et al.*¹⁷ transplanted five sAA patients with PBSC from HLA-identical sibling donors, conditioned with FLU, CY and ATG. GVHD prophylaxis was by cyclosporine (CSA). At a median follow-up of 171 days, all patients had engrafted with no rejection; aGVHD was present in one and cGVHD in two patients. Resnick *et al.*¹⁸ published the results of 13 sAA patients receiving PBSC cells from a family matched donor. Conditioning was by FLU, CY and ATG and prevention of GVHD used CSA and methotrexate (MTX). Twelve patients achieved sustained engraftment, cumulative aGVHD was 8.3%, only one patient evolved with limited cGVHD, and with a median follow-up of 45 months, the 5-year overall probability of survival was 84%. Ramaprasad *et al.*¹⁹ treated 26 patients with marrow failure syndromes (sAA, pure red cell aplasia and paroxysmal nocturnal hemoglobinuria) with HPCT from a HLA-compatible relative. Conditioning was by FLU and CY with or without ATG, and CSA alone or with mycophenolate mofetil or MTX were used as GVHD prophylaxis. All patients achieved durable engraftment and transfusion-independence, but cumulative aGVHD and cGVHD were 65% and 56% respectively; 24 patients are alive at a median of 21 months following transplantation (Kaplan-Meier estimate of overall survival was 77%). Finally, Gomez-Almaguer *et al.*²⁰ transplanted 23 sAA patients, previously unresponsive to IST, with PBSC from a sibling donor. Conditioning used FLU, CY and BU and GVHD prophylaxis was CSA and MTX. Twenty-one patients engrafted and 19 remain in complete remission (two after a second infusion of PBSC). aGVHD occurred in four and limited cGVHD in six patients.

In contrast, over 17 months we transplanted 18 sAA patients with at least one risk factor for poor prognosis, utilizing CY alone or BU+CY as preparative regimen, both recognized as nonmyeloablative conditioning, with 14 patients remaining alive (Kaplan-Meier survival is 75% at 2.14 years). TE occurred in three patients, one was rescued with maintenance of IST and the other two died secondary to fungal and bacterial infections, respectively. We also noticed

a possible relationship between number of risk factors and survival. Our patients presenting with one risk factor had a trend for better survival compared to patients with ≥ 2 risk factors ($P = 0.07$). Kaplan-Meier survival of patients with only one risk factor was 85% at 2.14 years. We neither observed aGVHD nor cGVHD, contrasting with the data of Ramaprasad *et al.*,¹⁹ who described 65% of grade II-IV aGVHD and 65% of cGVHD among their patients. So, with a CONV conditioning our results are very similar to those recently published utilizing FLU, at least in patients with one risk factor for poor survival.

Prospective clinical trials with larger cohorts of patients are needed, to establish the real benefits of FLU-based conditioning regimens and also to define the best agent(s) to be associated with FLU, when transplanting sAA patients with risk factors for poor prognosis.

Resumo

Transplante de medula óssea de doador irmão HLA-idêntico pode curar 70%-90% dos portadores de anemia aplásica severa (AAs). Pacientes mais idosos, muito transfundidos, longamente tratados com imunossupressão (IS) e com infecções ao tempo do transplante têm pior evolução. Nós transplantamos 18 pacientes com AAs e pelo menos um dos fatores associados a pior prognóstico (idade >35 anos, >50 transfusões antes do transplante, falta de resposta à imunossupressão prévia e infecção bacteriana ou fúngica ao transplante de 2001 a 2005, com ciclofosfamida (Cy - 5 pacientes) ou bussulfan mais Cy (13 pacientes). Dezesesseis pacientes apresentaram pega do enxerto, dois morreram sem pega, três tiveram rejeição aos dias +67, +524 e +638 (dois morreram e um foi resgatado com IS). Mucosite grau III/IV ocorreu em 39% e não observamos DECH aguda ou crônica. A probabilidade de sobrevida pelo método de Kaplan-Meier foi de 75% aos 2,14 anos, e uma tendência a melhor sobrevida foi encontrada entre os portadores de apenas um fator de risco ao transplante ($P: .06$). Estes resultados são comparáveis a recentes relatos de literatura envolvendo condicionamentos baseados em fludarabina para tratar pacientes com alto risco. Devido à pequena amostra analisada, estudos clínicos prospectivos com maior número de pacientes são necessários, visando comprovar o real benefício dos condicionamentos baseados em fludarabina, definir o melhor agente a ser a ela associado e assim obter o melhor condicionamento para portadores de AAs com fatores de mau prognóstico para o transplante. Rev. bras. hematol. hemoter. 2008; 30(4):292-296.

Palavras-chave: Anemia aplásica severa; condicionamento baseado em fludarabina; AAs de mau prognóstico; falha de pega.

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