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Late chimerical status after bone marrow transplantation in severe aplastic anemia according to two different preparatory regimens

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ABSTRACT

Background: This study investigated the influence of two conditioning regimens on the chimerical status of 104 patients with acquired severe aplastic anemia.
Methods: Patients were monitored for at least 18 months after related bone marrow transplantation and reaching partial or complete hematologic recovery. Group I patients ($n = 55$) received 200 mg/kg cyclophosphamide alone and Group II ($n = 49$) received 120 mg/kg cyclophosphamide associated with 12 mg/kg busulfan. Patients were classified in three chimerism levels according to the percentage of donor cells in the peripheral blood.
Results: Chimerism ≤50% occurred in 36.4% of Group I and none of Group II; chimerism 51–90% was found in 20.0% of Group I and 10.2% of Group II; and chimerism >90% was found in 43.6% of Group I versus 89.8% of Group II. A significant association (p-value < 0.001) was found between conditioning type and chimerism levels. A higher number of infused cells was associated with higher levels of chimerism only in Group I (p-value = 0.013). Multivariate analysis showed that chimerism >90% is associated with the cyclophosphamide plus busulfan conditioning (p-value < 0.001) and higher number of infused cells (p-value = 0.009), suggesting that these factors are predictive of graft outcome. Regarding hematological recovery, higher chimerism levels were associated with higher neutrophil (p-value = 0.003) and platelet counts (p-value < 0.001) in Group I only. These results show that myeloablative conditioning favors full donor chimerism and non-myeloablative conditioning predisposes to mixed chimerism or autologous recovery of hematopoiesis.
Conclusion: These data show that autologous recovery depends on the intensity of immunosuppression and that the immunosuppressive function of cyclophosphamide alone can induce this type of hematopoietic recovery.

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Introduction

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for young patients with severe aplastic anemia (SAA).[1] An intensity of conditioning regimen that achieves immunosuppressive effects to avoid graft rejection is a crucial factor in the allogeneic bone marrow transplantation (BMT) setting for SAA patients. The standard conditioning for patients who have an human leukocyte antigen (HLA)-matched sibling donor comprises high doses of cyclophosphamide (CY – 200 mg/kg) with or without antithymocyte globulin (ATG).[2]

Until the early 90s, SAA patients transplanted at Hospital de Clínicas (HC) of the Universidade Federal do Paraná (UFPR) received non-myeloablative conditioning such as 200 mg/kg of body weight of CY. Subsequently, it was observed that patients who received more than 15 transfusions prior to transplant had higher rejection rates and lower survival compared to those receiving fewer transfusions (unpublished data). Piccin et al.[3] also found a significant difference in the survival rates of patients with SAA or Fanconi Anemia who received more than 20 units of red blood cells and/or platelets. For this reason and because of the irregular supply of ATG, from 1993 all polytransfused SAA patients received CY (120 mg/kg) associated with busulfan (BUS – 12 mg/kg of body weight). The CY + BUS combination showed a marked reduction in rejection rates.[4]

Transplant outcome is assessed by hematologic recovery and chimerism analysis that provide information on the hematopoietic reconstitution of patients, which can be autologous, allogeneic or chimerical. The determination of chimerism status has been based on the amplification of markers with a variable number of tandem repeats (VNTRs) or short tandem repeats (STRs) scattered throughout the genome.[5–7]

This retrospective study analyzed the levels of chimerism in two groups of patients with acquired SAA who received different conditioning regimens before undergoing BMT with HLA-identical sibling donors. The goal was to investigate the association between chimerism levels with different conditioning as well as with other variables in order to identify factors that could predict the outcome of the allogeneic hematopoietic stem cell graft. Among the study variables were pre-transplant characteristics such as patient's age and gender, time between diagnosis and BMT, and transplant characteristics such as donor's age and gender and number of infused cells. The chimerism status of patients with normal or near normal hematological levels was also analyzed. Peripheral blood hematimetric levels, such as the neutrophil and platelet counts, and hemoglobin concentration were evaluated in the period equal to or greater than 18 months after transplantation. Patients with time equal to or greater than 18 months post-transplant were included because they had already reached stable chimerism and because hematological changes rarely occur after this time.

Methods

Patients

The Bone Marrow Transplantation Service at HC-UFPR started its activities in 1979, and from that time up to October 2011, this center has performed 516 transplants in SAA patients. This study involved 104 SAA patients who underwent the first transplant at HC-UFPR between August 1987 and July 2009. Data on chimerism levels were collected up to April 2011. The inclusion criteria for the patients analyzed in this cohort were a diagnosis of SAA, first BMT with a related donor, alive at the time of this study, chimerism analysis performed at a time ≥18 months after the transplant, partial or complete hematologic recovery, and conditioned with CY alone or with CY + BUS (Figure 1). Pre-transplant characteristics of these patients are shown in Table 1. The characteristics of patients who passed away (not included in this study) are described in Table 2.

Transplant characteristics

All patients who received bone marrow from HLA-identical siblings were divided into two groups according to the conditioning regimen. The choice of the conditioning protocol was based on the number of previous transfusions with this information being obtained from medical records as well as from reports from the patient or family members. Forty-four patients with less than 16 transfusions were treated with CY alone at 200 mg/kg body weight in four doses and those with ≥16 transfusions ($n = 49$), in general, received CY at 120 mg/kg body weight divided in two doses associated with BUS at 12 mg/kg of body weight in 16 oral doses.[4] However, eleven patients who received ≥16 transfusions and were included in the CY alone group ($n = 44 + 11$) because they were transplanted before the implementation of the CY + BUS protocol. The decision to combine CY + BUS was based on the experience of Lucarelli et al.[8] who used this regimen to treat thalassemia patients, but the doses utilized in our center were modified. The transplant characteristics of this cohort are shown in Table 3.

Late hematological status

The evaluation of late chimerism status was based on the last test performed for each patient at a time ≥18 months with the date of post-BMT sample collection for testing varying from 18.8 to 187.8 months (median: 55.5 months) according to the clinical follow-up. The neutrophil and platelet counts as well as hemoglobin level were obtained from the last available blood count with the date also varying according to the clinical follow-up of each patient, but it was always ≥18 months after BMT (range: 21.4–285.1; median: 113 months).