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Significance of Increased Blastic-Appearing Cells in Bone Marrow Following Myeloablative Unrelated Cord Blood Transplantation in Adult Patients

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An abnormal increase of nonleukemic blastic-appearing lymphocytes in bone marrow (BM) specimens has been reported after unrelated cord blood transplantation (UCBT). This study analyzed the incidence, chronology, biological features, and clinical significance of elevated numbers of these cells in a series of 165 consecutive adult patients demonstrating myeloid engraftment after myeloablative UCBT in a single institution. The patients' BM samples were routinely evaluated by cytomorphology at different time points after UCBT. When ≥5% of blastic-appearing cells were detected by cytomorphology in the BM, samples were also evaluated by multiparametric flow cytometry to characterize these cells. Systematic chimerism analyses of BM samples using PCR amplification of short tandem repeat markers were performed. Forty-three patients (cumulative incidence, 26.1%) demonstrated ≥5% of nonmalignant blastic-appearing cells in BM after a median of 101 days after UCBT (range, 28-377 days). All of these patients had full-donor chimerism and a clinical course without leukemic relapse. Multiparametric flow cytometry analyses performed in 36 of the 43 patients showed a polyclonal expansion of B lymphocytes with a broad spectrum of maturation stages. An increased number of nonmalignant blastic-appearing cells was significantly associated with a high number of lymphocytes infused at the time of UCBT and with low rates of acute and chronic extensive graft-versus-host disease, suggesting a potential immunoregulatory role of these cells. The observation of ≥5% nonmalignant blastic-appearing cells in BM samples after myeloablative UCBT is frequent, and these should be distinguished from malignant blasts.

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INTRODUCTION

Abnormal expansion of naïve B lymphocytes has been reported after unrelated cord blood transplantation (UCBT) [1,2]. In some cases, the posttransplantation bone marrow (BM) specimens may contain immature blastic-appearing lymphocytes that might be difficult to distinguish from malignant blasts and raise a concern about possible relapsed acute leukemia [1-3]. Although this phenomenon is apparently associated with allogeneic hematopoietic stem cell transplantation (HSCT) from cord blood progenitors [3], data regarding the abnormal increase of blastic-appearing lymphocytes in the setting of UCBT remain limited. Thus, an examination of the incidence, chronology, biological features, and clinical significance of an increased number of lymphoblast-like cells in BM after UCBT is warranted.

The present study aimed to evaluate the incidence of an increased percentage of nonleukemic blasts in the BM after myeloid engraftment in 165 consecutive adult patients undergoing myeloablative UCBT at a single center. BM specimens were also examined by multiparametric flow cytometry (MPFC) to characterize the immature blastic-appearing cells. The factors associated with the occurrence of increased nonleukemic blasts, as well as the possible impact of

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this increase on posttransplantation outcomes, were analyzed.

MATERIALS AND METHODS

Patients and Transplantation Characteristics

Between May 1997 and May 2010, XXX adults with hematologic malignancies underwent UCBT at Hospital Universitari i Politècnic La Fe, Valencia, Spain. All patients included in the study had myeloid engraftment, defined as more than 3 days with an absolute neutrophil count $>0.5 \times 10^9$/L with full-donor chimerism. All patients provided informed consent in accordance with institutional guidelines. The transplantation protocols were approved by the hospital's Research Ethics Board and conformed to the Declaration of Helsinki. Patients undergoing UCBT with a reduced-intensity conditioning regimen were excluded from the study. All UCBTs were performed on a single unit in the hospital. No graft manipulation was performed. Donor–recipient matching was based on low-resolution HLA typing for HLA-A and HLA-B and on high-resolution typing for HLA-DRB1. Early disease stage at UCBT was defined as chronic myelogenous leukemia in the chronic phase, acute leukemia in first or second complete remission, myelodysplastic syndrome untreated or in complete remission, and lymphoma in complete remission.

Preparative Regimens and GVHD Prophylaxis

In 58 patients (35%), the conditioning regimen comprised thiotepa, oral or i.v. busulfan, cyclophosphamide, and antithymocyte globulin (ATG) [4]. Of these 58 patients, 27 received horse ATG (Lymphoglobulin; bio Mérieux, Lyon, France), and 31 received rabbit ATG (Thymoglobulin; Genzyme, Framingham, MA). The other 107 patients (65%) received a conditioning regimen composed of i.v. busulfan, fludarabine, and ATG (Thymoglobulin) [5].

Acute graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine plus prednisone in 111 patients (67%) and cyclosporine plus mycophenolate in the remaining 54 patients (33%). Acute and chronic GVHD were graded according to published criteria [6,7]. Cytomegalovirus (CMV) monitoring and prophylaxis were performed according to previously reported protocols [8].

BM Analyses

BM samples of patients were routinely evaluated by cytomorphology at different time points after UCBT (days +28, +56, +100, +180, and +365). BM samples were stained with May-Grünwald-Giemsa stain and viewed under a light microscope by a local pathologist. A minimum 300-cell differential count was performed in each case. When an excess of blastic-appearing cells (ie, high nuclear-to-cytoplasmic ratio and uncondensed chromatin, with or without granulation or nucleolus) was detected, samples were evaluated by MPFC to characterize the blastic-appearing cells. Blastic cells were gated on the basis of their $CD34^+$ and $CD45^w$ content and low side light scatter. Immature blastic-appearing B lymphocytes were discriminated by MPFC on the basis of the normal B cell maturation pattern into several stages using a combination of CD10, CD19, CD20, CD22, CD34, CD38, TdT, and CD45 antigen surface markers [9,10]. In patients with antecedents of leukemia, malignant blastic cells were characterized according to the leukemic blast phenotype at baseline diagnosis. Chimerism analyses of BM samples using multiplex PCR amplification of short tandem repeat markers were performed systematically at each time point.

Study Definitions and Endpoints

The study's primary endpoint was the incidence of increased blastic-appearing cells in BM after UCBT, defined as ≥5% of nonmalignant blastic cells detected on cytomorphology. Relapse of leukemia or myelodysplastic syndrome, development of secondary myeloid neoplasms, and posttransplantation lymphoproliferative syndrome were excluded at the time of detection of increased blastic-appearing cells. The differential diagnosis between nonmalignant blastic-appearing cells and malignant blasts was done by chimerism analyses (with full-donor chimerism required for diagnosis), MPFC, and cytogenetics or molecular biology when the baseline hematologic malignancy had a characteristic genetic abnormality. The secondary study endpoint was time to increased blastic-appearing cells.

Overall survival (OS), nonrelapse mortality (NRM), risk of relapse (RR), disease-free survival (DFS), and development of chronic GVHD after UCBT were assessed as well. All patients were followed until death or last follow-up.

Data Collection and Prognostic Factors

Data were collected prospectively and registered. Twenty-two patient and transplantation characteristics were examined to establish their relationship with the occurrence of increased blastic-appearing cells in BM. Demographic data and transplantation characteristics included age; sex; weight; previous autologous or allogeneic HSCT; underlying disease; disease stage; patient CMV serostatus; degree of HLA mismatch; ABO incompatibility; donor sex, number of total nucleated and $CD34^+$ cells at cryopreservation; viability and number of total lymphocytes, $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+/56^+$, and $CD19^+$ cells infused; conditioning regimen; type of ATG used; and acute GVHD prophylaxis. The rate of increased blastic-appearing cells in BM was assessed in relation to the development of acute GVHD.