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Cryopreserved CD34⁺ Cell Dose, but Not Total Nucleated Cell Dose, Influences Hematopoietic Recovery and Extensive Chronic Graft-versus-Host Disease after Single-Unit Cord Blood Transplantation in Adult Patients

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A B S T R A C T

Low cryopreserved total nucleated cell (TNC) dose in a cord blood (CB) unit has been shown to be associated with engraftment failure and mortality after single-unit cord blood transplantation (CBT) in adults. Although CB banks offer specific characteristics of cryopreserved cell dose, such as TNC, CD34⁺ cells, and colony-forming unit for granulocyte/macrophage (CFU-GM), the impact of each cell dose on engraftment and outcomes after single-unit CBT in adults remains unclear. We retrospectively analyzed the results of 306 CBTs for 261 adult patients in our institution between 1998 and 2016. The median age was 43 years (range, 16 to 68), the median actual body weight (ABW) was 56.2 kg (range, 36.2 to 104.0), the median ideal body weight (IBW) was 62.3 kg (range, 39.7 to 81.3), the median TNC dose was 2.46×10^7/ABW kg (range, 1.07 to 5.69), the median CD34⁺ cell dose was $.91 \times 10^5$/ABW kg (range, .15 to 7.75), and the median CFU-GM dose was 24.46×10^3/ABW kg (range, .04 to 121.81). Among patients who achieved engraftment, the speed of neutrophil, platelet, and red blood cell engraftment significantly correlated with CD34⁺ cell dose, but not with TNC and CFU-GM dose, based on both ABW and IBW. In multivariate analysis, the incidence of extensive chronic graft-versus-host disease (GVHD) was significantly higher in patients receiving the highest CD34⁺ cell dose, based on both ABW and IBW. Nevertheless, no cell dose was associated with survival, transplantation-related mortality, and relapse. In conclusion, cryopreserved CD34⁺ cell dose was the best predictor for hematopoietic recovery and extensive chronic GVHD after CBT. The cryopreserved CD34⁺ cell dose should be used for unit selection criteria in single-unit CBT for adults.

INTRODUCTION

Cord blood transplantation (CBT) is an acceptable alternative approach to allogeneic hematopoietic stem cell transplantation (HSCT) for adult patients without HLA-matched related or unrelated donors [1-4]. The limited cell dose in a single cord blood (CB) unit remains 1 of the major concerns of CBT because it might contribute to a higher risk of engraftment failure and delayed hematopoietic recovery. This may result in higher mortality in the early phase of CBT, particularly for adult patients.

Historically, most of the early studies involving both children and adults showed that cryopreserved [5-7] or post-thaw [8] total nucleated cell (TNC) dose based on actual body weight (ABW) was 1 of the most significant indicators of hematopoietic engraftment and survival after CBT. Some registry data restricted to adult patients showed that cryopreserved TNC dose was also associated with engraftment but not with survival [9,10], whereas Cohen et al. showed that the cryopreserved TNC dose was significantly associated with mortality in single-unit CBT [11]. Conversely, an early prospective study of single-unit CBT for adults could not show the association between cryopreserved TNC dose and hematopoietic recovery [12]. In addition, several registry-based and single-center studies have shown that cryopreserved CD34⁺ cell dose [13,14], or colony-forming unit (CFU) dose [15,16] had greater influence on engraftment than the TNC dose in CBT. Several studies among both children and adults have demonstrated that post-thaw CD34⁺ cell dose [9,13,14,17-21], CD8⁺ cell dose [18,19], CFU dose [16,21], or CFU for granulocyte/macrophage (CFU-GM) dose [20,22] had greater

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influence on engraftment and transplantation outcomes than the TNC dose in CBT. However, the impact of cryopreserved cell dose, such as TNC, $CD34^+$ cell, and CFU-GM dose, on engraftment and outcomes has been largely unclear for adult patients in single-unit CBT. Moreover, there are no data indicating if the use of ABW or ideal body weight (IBW) for calculation of the cell dose is better for predicting outcomes and should be used for CB unit selection. In Japan, single-unit CBT is a common procedure because double-unit CBT is not approved for use outside of clinical trials. Therefore, we retrospectively investigated the characteristics of cryopreserved cell dose based on ABW or IBW associated with engraftment and outcomes after single-unit CBT for adult patients.

PATIENTS AND METHODS

Patients and Transplantation Procedures

Between August 1998 and June 2016, we performed 316 CBTs for 271 patients at the Institute of Medical Science, University of Tokyo. To evaluate the effect of cryopreserved cell numbers on engraftment and outcomes after single-unit CBT in adult patients, 7 patients younger than 15 years, 2 patients who received double-unit CBT in a clinical trial, and 1 patient who was the victim of a nuclear accident were excluded. Finally, the remaining 306 CBTs for 261 patients were retrospectively analyzed in this study. CBT units were provided by the Japan cord blood bank. The cell numbers of TNC, $CD34^+$ cells, and CFU-GM were measured before cryopreservation at each CB bank. The method of $CD34^+$ cell measurement according to the International Society of Hematotherapy and Graft Engineering guidelines [23,24] was standardized among all CB banks after 2008. To avoid graft failure, the existence of anti-HLA antibodies against donor-specific antigens was evaluated for all patients in our institution since 2004. According to our institutional policy, CB is an alternative first-line graft source option for patients without a matched sibling donor, unless the patient has anti-HLA antibodies against donor-specific antigens. The institutional review board of the Institute of Medical Science, University of Tokyo approved this retrospective study.

Endpoint and Definitions

The primary endpoint was neutrophil engraftment. Secondary endpoints were platelet and RBC engraftment, graft-versus-host disease (GVHD), overall survival (OS), event-free survival (EFS), transplantation-related mortality (TRM), and relapse. *Neutrophil engraftment* was defined as being achieved on the first of 3 consecutive days when the absolute neutrophil count was higher than $.5 \times 10^9$/L. *Platelet engraftment* was defined as being achieved on the first of 7 consecutive days when the platelet count was higher than 50×10^9/L from the last platelet transfusion. *RBC engraftment* was defined as being achieved on the first of 3 consecutive days when the reticulocyte count was higher than 1%. For hematopoietic engraftment, death before 28 days without hematopoietic engraftment was a competing event. Acute GVHD and chronic GVHD were graded according to the standard criteria [25,26]. The incidences of acute and chronic GVHD were evaluated in all engrafted patients. For GVHD, death without GVHD was a competing event. The OS (inverse of overall mortality) was defined as the time from the date of CBT to the date of death or last contact. *EFS* (inverse of treatment failure for EFS) was defined as the time from the date of CBT to the date of graft failure, relapse, death or last contact. *TRM* was defined as death during remission. Relapse was defined by hematological evidence of disease. For TRM, relapse was a competing event. In contrast, TRM was a competing event for relapse. IBW was calculated by a standard formula [27] using sex and body height: $50 + (2.3 \times [\text{height, inches-60}])$ for male and $45.5 + (2.3 \times [\text{height, inches-60}])$ for female. The number of HLA disparities was defined as a low-resolution for HLA-A, -B, and -DR, because HLA-DR mismatches were previously evaluated at the low-resolution level at CB unit selection in Japan [28]. Myeloablative conditioning (MAC) regimens were defined according to the criteria of the Center for International Blood and Marrow Transplant Research, and others were classified as reduced-intensity conditioning [29]. Disease status at HSCT was assessed according to the refined Disease Risk Index, which is determined by disease type, disease status, and cytogenetic risk [30].

Statistical Analysis

The Spearman rank correlation coefficient was calculated to assess the correlation between number of TNC, $CD34^+$ cells, and CFU-GM or between each cell number based on ABW or IBW and hematopoietic recovery. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, TRM, and relapse were estimated based on a cumulative incidence method to accommodate competing risks, and the groups were compared using Gray's test. The probabilities of OS and EFS were estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. Univariate and multivariate analyses were performed with a Cox proportional hazard model for overall mortality and treatment failure for EFS, and a Fine and Gray proportional hazards model for the other endpoints. Separate multivariate analysis was performed for each cell dose based on ABW or IBW using all of the following factors: age at CBT (16 to 44 versus ≥45 years), cytomegalovirus serostatus (positive versus negative), Disease Risk Index (low/intermediate versus high/very high), number of allogeneic transplantations (1 versus ≥2), conditioning regimen (MAC versus reduced intensity), TNC dose based on ABW or IBW (<2 versus 2 to 2.49 versus 2.5 to 2.99 versus $\geq 3 \times 10^7$/kg), $CD34^+$ cell dose based on ABW or IBW (<.5 versus .5 to .99 versus 1 to 1.49 versus $\geq 1.5 \times 10^5$/kg), CFU-GM dose based on ABW or IBW (<15 versus 15 to 24.9 versus 25 to 34.9 versus $\geq 35 \times 10^3$/kg), HLA disparities (0 and 1 versus 2 and 3), ABO compatibility between donor and recipient (major, bidirectional mismatch versus match, minor mismatch), sex compatibility between donor and recipient (female donor to male recipient versus others), and year of CBT (1998 to 2006 versus 2007 to 2016). In these analyses, TNC dose 2 to 2.49×10^7/kg, $CD34^+$ dose .5 to $.99 \times 10^5$/kg, and CFU-GM dose 15 to 24.9×10^3/kg were considered the reference groups, because these ranges included the median cell dose, and the main purpose of this study was to evaluate the effect of a lower or higher cell doses on engraftment and outcomes compared with cell doses that are usually used. Final model variables were confirmed with a backward selection procedure at a level of significance of $P = .05$. All P values were 2-sided and all statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [31], a graphical user interface for the R 3.0.2 software program (R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism 6 for Mac OS X (GraphPad Software, San Diego, CA).

RESULTS

Characteristics of CBTs and CB Units

The characteristics of CBTs, and CB units are shown in Table 1. The median age was 43 years (range, 16 to 68 years), median ABW was 56.2 kg (36.2 to 104.0 kg), median IBW was 62.3 kg (39.7 to 81.3 kg), median height was 167 cm (range, 146 to 187 cm), and median body mass index was 20.5 kg/m^2 (range, 14.2 to 33.5 kg/m^2). The ABW was more than 25% over IBW in 7 CBTs (2%). The most common disease type was acute myeloid leukemia (54%). The majority of conditioning regimens were MAC (89%), and the most common GVHD prophylaxis was cyclosporine A and methotrexate (75%). Among MAC regimens, the most common conditioning regimen was total body irradiation + cyclophosphamide + cytosine arabinoside with or without granulocyte colony-stimulating factor in patients with myeloid or lymphoid malignancies for first CBT, respectively [32,33]. The median TNC dose was 2.46×10^7/kg (range, 1.07 to 5.69×10^7/kg) based on ABW and 2.36×10^7/kg (range, .90 to 5.39×10^7/kg) based on IBW. The median $CD34^+$ cell dose was $.91 \times 10^5$/kg (range, .15 to 7.75×10^5/kg) based on ABW and $.83 \times 10^5$/kg (range, .12 to 7.12×10^5/kg) based on IBW. The median CFU-GM dose was 24.46×10^3/kg (range, .04 to 121.81×10^3/kg) based on ABW and 23.06×10^3/kg (range, .05 to 111.87×10^3/kg) based on IBW. Although the TNC dose, $CD34^+$ cell dose, and CFU-GM dose per 1 CB unit significantly correlated with each other, the correlation was the strongest between $CD34^+$ cell dose and CFU-GM dose (Supplementary Figure S1).

Hematopoietic Recovery

Among patients who achieved neutrophil, platelet, and RBC engraftment, the speed of each recovery significantly correlated with $CD34^+$ cell dose, but not with TNC and CFU-GM dose, based on both ABW and IBW (Figure 1).

In the entire cohort, the cumulative incidence of neutrophil engraftment was 92.5% (95% confidence interval [CI], 88.2% to 95.3%) at day 42. In univariate analysis, the $CD34^+$ cell dose and CFU-GM dose were significantly associated with achievement of neutrophil engraftment, but TNC dose was