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Transplantation of Ex Vivo Expanded Umbilical Cord Blood (NiCord) Decreases Early Infection and Hospitalization

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A B S T R A C T

Delayed hematopoietic recovery contributes to increased infection risk following umbilical cord blood (UCB) transplantation. In a Phase 1 study, adult recipients of UCB stem cells cultured ex vivo for 3 weeks with nicotinamide (NiCord) had earlier median neutrophil recovery compared with historical controls. To evaluate the impact of faster neutrophil recovery on clinically relevant early outcomes, we reviewed infection episodes and hospitalization during the first 100 days in an enlarged cohort of 18 NiCord recipients compared with 86 standard UCB recipients at our institution. The median time to neutrophil engraftment was shorter in NiCord recipients compared with standard UCB recipients (12.5 days versus 26 days; $P < .001$). Compared with standard UCB recipients, NiCord recipients had a significantly reduced risk for total infection (RR, 0.69; $P = .01$), grade 2-3 (moderate to severe) infection (RR, 0.36; $P < .001$), bacterial infection (RR, 0.39; $P = .003$), and grade 2-3 bacterial infection (RR, 0.21; $P = .003$) by Poisson regression analysis; this effect persisted after adjustment for age, disease stage, and grade II-IV acute GVHD. NiCord recipients also had significantly more time out of the hospital in the first 100 days post-transplantation after adjustment for age and Karnofsky Performance Status (69.9 days versus 49.7 days; $P = .005$). Overall, transplantation of NiCord was associated with faster neutrophil engraftment, fewer total and bacterial infections, and shorter hospitalization in the first 100 days compared with standard UCB transplantation. In conclusion, rapid hematopoietic recovery from an ex vivo expanded UCB transplantation approach is associated with early clinical benefit.

INTRODUCTION

Umbilical cord blood (UCB) extends the curative potential of stem cell transplantation to adult patients without an HLA-compatible donor [1]. After UCB transplantation, overall survival is comparable to that of matched related or unrelated donor transplantation, but treatment-related mortality is significantly higher [2,3]. UCB grafts are limited by low total and stem cell doses, which are associated with delayed hematopoietic and immunologic recovery. Delayed neutrophil engraftment likely contributes to the increased risk of life-threatening infection and longer hospitalization in the early post-UCB transplantation period [4,5].

To overcome the limitation of low UCB cell dose, several techniques have been developed to expand cord blood–derived hematopoietic stem and progenitor cells ex vivo before transplantation [6,7]. Although each of these techniques uses a different mechanism for ex vivo expansion, all have shown promise in reducing the time to neutrophil and platelet engraftment [8-11]. NiCord is an UCB-derived cell product that uses a small molecule, nicotinamide, to inhibit the differentiation and enhance the functionality of hematopoietic stem and progenitor cells (HSPCs) expanded in ex vivo culture [12]. The NiCord graft consists of 2 fractions from the UCB unit. The CD133-positive fraction containing HSPCs is expanded for 21 days in the presence of hematopoietic stem cell active cytokines plus nicotinamide. The CD133-negative fraction containing lymphoid cells is retained, cryopreserved, and ultimately coinfused with the expanded CD133-positive cell fraction on the day of transplantation. Results from a Phase I trial of transplantation with NiCord along with a second unmanipulated UCB unit showed earlier median neutrophil recovery compared with historical controls, and long term engraftment with the NiCord unit was also observed in the

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majority of patients [10]. Rapid neutrophil recovery has also been observed in a subsequent ongoing Phase II trial exploring the use of NiCord as a single unit graft [13].

Because use of the NiCord ex vivo expanded UCB grafts resulted in rapid hematopoietic recovery, we hypothesized that NiCord transplantation would improve clinically relevant early outcomes by decreasing the risk of infection and length of hospitalization. Therefore, we analyzed infection episodes and hospitalization during the first 100 days after transplantation in an enlarged cohort of patients undergoing NiCord transplantation compared with a historical control cohort of consecutive adult patients undergoing standard UCB transplantation at our institution.

METHODS

Patients and Transplantation Approach

Two cohorts of adult patients age ≥18 years with hematologic malignancies who underwent UCB transplantation at Duke University were compared in this study. Cohort 1 included 18 consecutive adults who underwent transplantation with an expanded NiCord graft as part of 2 Phase I and II clinical trials conducted from January 2010 to March 2015 [10,13]. Cohort 2 included 86 consecutive adults transplanted with unmanipulated standard single or double UCB grafts from January 2005 to March 2015. All patients received a myeloablative total body irradiation (TBI; 1350 cGy)- and fludarabine (160 mg/m^2)-based conditioning regimen. No patient underwent in vivo T cell depletion. Cord blood units were matched to the recipient at 4 or more HLA loci (intermediate resolution for HLA-A and -B, high resolution for -DRB1). In cohort 1, 11 of 18 patients underwent double UCB transplantation with 1 NiCord expanded cord blood unit of minimum 1.5×10^7 total nucleated cells (TNCs)/kg and 1 unmanipulated cord blood unit of minimum 2.5×10^7 TNCs/kg as described previously [10]. The other 7 patients in cohort 1 underwent transplantation at Duke Medical Center with a single NiCord expanded cord blood unit of minimum 1.8×10^7 TNCs/kg before expansion as part of a multicenter Phase II trial [13].

In cohort 2, patients received either a single cord blood unit with a minimum cryopreserved cell dose of 3×10^7 TNCs/kg or 2 cord blood units each containing a minimum cryopreserved cell dose of 1.5×10^7 TNCs/kg. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus (target level, 10 to 15 ng/mL) for at least 6 months and mycophenolate mofetil for at least 60 days after transplantation.

Unless contraindicated, patients from both cohorts received antimicrobial prophylaxis with acyclovir 800 mg twice daily to day +365, ciprofloxacin 500 mg twice daily to day +180, voriconazole 200 mg twice daily to at least day +100, and trimethoprim-sulfamethoxazole 400/80 mg once daily to at least day +180 following transplantation. Supportive care measures, including evaluation and management of febrile neutropenia, weekly PCR surveillance for cytomegalovirus (CMV; for the entire study period) and human herpesvirus 6 (HHV-6) viremia (starting in 2010), and infection control practices were maintained in accordance with institutional protocol in both cohorts. G-CSF (5 μg/kg) was administered daily starting on day 1 after transplantation until the absolute neutrophil count (ANC) exceeded 1000 cells per μL of blood. Patients were eligible for discharge from the hospital when the ANC exceeded 500 cells/μL.

Definitions

The time to neutrophil engraftment was defined as the first of 3 consecutive days with an ANC of $\geq 0.5 \times 10^9$/L. Disease status at the time of transplantation was classified as early for patients with acute leukemia in first complete remission (CR), myelodysplastic syndrome (MDS) untreated or in first CR, chronic myelogenous leukemia in first chronic phase, or non-Hodgkin lymphoma (NHL) or multiple myeloma in first CR; all other patients were considered nonearly [14]. Acute GVHD was defined and graded according to standard criteria [15].

Infection Data

Early infection episodes through day +100 after transplantation were retrospectively identified and categorized by organism type and severity in accordance with BMT CTN Technical MOP Version 3.0, Appendix 4-A (Supplemental Table S1). Recurrence interval definitions in Appendix 4-A were also used to determine whether a given infection was part of a prior episode or a new episode. Each new infection episode was first classified by type as bacterial, fungal, viral, parasitic, or nonmicrobiologically defined, then further characterized by severity as grade 1 (mild), grade 2 (moderate), or grade 3 (severe/life-threatening). Patients were considered at risk of early infection through day +100 after transplantation, day of relapse, day of second transplantation, or day of death, whichever occurred first.

Hospitalization Data

Hospitalization was defined as days alive and out of the hospital in the first 100 days, to account for the incongruous association of earlier mortality with shorter hospitalization, as reported by Ballen et al. [5]. For patients who survived to day +100, days alive and out of the hospital in the first 100 days was calculated by subtracting the total number of days in the hospital during the initial admission and any readmissions from 100. For patients who died before day +100, days alive and out of the hospital in the first 100 days was calculated by subtracting the total number of days in the hospital during the initial admission and any readmissions from the day of transplantation to the day of death. In this way, if a patient's death occurred during the initial hospitalization, then there were no days alive and out of the hospital.

Statistical Analysis

Patient baseline and transplantation characteristics were compared using Fisher's exact test for categorical variables and the *t* test for continuous variables. The cumulative incidence of neutrophil recovery was compared between study groups with death, relapse, or subsequent transplant as competing risks, and the hazard ratio (HR) and 95% confidence interval (CI) for study group was estimated from a proportional hazards model that also accounted for competing risks. Infection rates were calculated as the number of patients who experienced each infection at least once during the observation period, and Fisher's exact test was used to test for differences between groups. To account for multiple infections in an individual patient as well as differing periods of risk, infection density was calculated as the total number of infections per patient per days at risk. Individual patient infection densities were then averaged over all patients in a group to calculate the mean number of infections experienced per 100 patient-days, and Wilcoxon's rank-sum test was used to test for differences between groups. Poisson regression was used to estimate the effect of NiCord versus standard UCB transplantation on the rates of total infection, grade 2-3 infection, bacterial infection, grade 2-3 bacterial infection, and grade 2-3 nonviral infection, both univariately and after adjustment for covariates known to affect the risk of infection, including age, disease status, and acute GVHD [16]. An offset was included in the model to account for the observation time for each patient. Risk ratio (RR) and 95% CI were estimated from the Poisson model. Analysis of variance was used to univariately examine the association of time alive and out of the hospital during the first 100 days post-transplantation with study group, and analysis of covariance was used to examine the association after adjustment for known covariates, including age and Karnofsky Performance Status (KPS). To determine differences between the group of standard UCB recipients who underwent transplantation between 2010 and 2015 (n = 50) and the complete group of control patients, a sensitivity analysis comparing these 2 groups was performed. A second sensitivity analysis was performed by comparing the NiCord cohort with the contemporaneous cohort of standard UCB recipients who underwent transplantation between 2010 and 2015. All statistical analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC). This retrospective analysis was approved by the Duke University Medical Center's Institutional Review Board.

RESULTS

Patients

A total of 104 patients, 18 who underwent NiCord transplantation and 86 who underwent standard UCB transplantation, were included in this study. Patient baseline and transplantation characteristics are summarized in Table 1. NiCord recipients were older than standard UCB recipients (median age, 45.5 years; interquartile range [IQR], 42 to 57 years versus 37.5 years; IQR, 28 to 51 years; $P = .007$). Patient sex, pretransplantation weight, CMV serostatus, and KPS were similar in the 2 groups. The underlying malignant disease (acute leukemia/myelodysplastic syndrome, 89%; lymphoid, 11%) and disease status at transplantation were also similar in the 2 groups. All patients received a myeloablative TBI- and fludarabine-based conditioning regimen, and no patients underwent in vivo T cell depletion. In the NiCord group, 11 patients received NiCord with a second unmanipulated unit, and 7 patients (39%) received NiCord as a single UCB graft, whereas only 4 patients (5%; $P < .001$) in the standard UCB group underwent single UCB transplantation. There was a range of recipient-to-UCB unit HLA matching in both groups, and cryopreserved total nucleated cell dose was similar in the 2 groups. No significant differences between the groups