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Preengraftment Syndrome after Unrelated Cord Blood Transplant Is a Strong Predictor of Acute and Chronic Graft-versus-Host Disease

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Preengraftment syndrome (PES) is a known complication following unrelated cord blood transplant (CBT) that has not been well characterized. We sought to determine the incidence and clinical outcome of PES among 326 patients <18 years of age who were prospectively enrolled on a multicenter CBT trial. All patients received a myeloablative (MA) transplant and a single cord blood unit (CBU). PES developed in 20% of the patients at a median of 10 days (range: 5-24). Patients receiving a CBU with a total nucleated cell (TNC) count of $>5 \times 10^7$/kg had significantly higher risk of developing PES ($P = .02$). There were significantly higher rates of grade II-V ($P < .001$), grade III-IV ($P < .001$) acute and chronic ($P = .002$) graft-versus-host disease (aGVHD, cGVHD) in those who developed PES. In a multivariate analysis, PES did not significantly affect overall survival (OS) ($P = .38$). We conclude that PES is common following CB transplant (CBT) and additional more intensive immune suppression might be considered to decrease the risk of developing aGVHD and cGVHD.

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KEY WORDS: Preengraftment syndrome, Cord blood transplant, Graft-versus-host disease

INTRODUCTION

Preengraftment syndrome (PES) is a poorly defined clinical entity following hematopoietic stem cell transplant (HSCT). Early immune reactions, cytokine storm, or PES have been described following unrelated cord blood transplant (CBT) in adult patients with an incidence of 23% to 78% [1-4]. The classic clinical manifestations of this syndrome are high fever in the absence of infection and diffuse erythematous skin rash resembling acute graft-versus-host disease (aGVHD) with or without fluid retention. Patients generally develop this syndrome as early as 5 days posttransplant, and often respond to a short course of corticosteroid therapy. This syndrome is distinct from aGVHD in its timing as well as its manifestations. Prior studies failed to show any correlation between PES and subsequent risk of GVHD. Limitations of prior studies include retrospective analysis, small numbers of patients, and inclusion of patients who received various GVHD prophylaxis regimens and supportive care. To our knowledge, this is the first comprehensive analysis of the incidence, risk factors for developing PES, and clinical outcome of 326 pediatric patients following CBT enrolled on a multicenter clinical trial.

PATIENTS AND METHODS

The cord blood transplant (COBLT) study is a multi institutional trial of CBT sponsored by the National Heart, Lung and Blood Institute (NHLBI) branch of the National Institutes of Health. A total of 364 adult and children were prospectively enrolled on the study between 1999 to 2003. The protocol was approved by the institutional review board (IRB) of all participating centers. Data from the study were made available by the NHLBI for analysis via a limited data agreement with Vanderbilt University. The analysis was approved by the IRB of Vanderbilt University Medical Center. Three hundred twenty-six patients ≤18 years of age who lacked HLA matched related donors with malignant and nonmalignant disease were enrolled. Only subjects receiving 1 cord blood unit (CBU) were eligible. Searches for CBU were conducted by using low/intermediate-resolution molecular typing for HLA class I (A and B) and high-resolution molecular typing for HLA DRB1. Selection of the unit was based on providing the highest number of total nucleated cell (TNC) count and matching at a minimum of 4/6 HLA loci.

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Transplant Procedure

The conditioning regimen consisted of busulfan (Bu), cyclophosphamide (Cy), and antithymocyte globulin (ATG) (equine) for patients with nonmalignant diseases. For patients with malignant diseases the conditioning regimen consisted of total body irradiation (TBI), Cy, and ATG. One stratum evaluated the safety of Bu, melphalan (Mel), and ATG in young patients and those who were not able to tolerate TBI. The details of the preparative regimens has been previously reported [5-7]. GVHD prophylaxis consisted of methylprednisolone 0.5 mg/kg twice daily on day +1 through +4 and then 1 mg/kg twice daily from day +5 to day +19 or until the first day the absolute neutrophil count (ANC) reached 500/μL, at which time the dose was tapered at the rate of 0.2 mg/kg/week. Cyclosporine was started on day −3 and continued until at least day 180. Then the dose was tapered at the rate of 5% of the initial dose per week if the recipients had no evidence of GVHD.

Definitions

Neutrophil engraftment was defined as achieving an ANC of at least 500/μL for 3 consecutive measurements on different days and demonstrated donor chimerism of >90%. The grading of aGVHD followed the GVHD consensus grading scheme [8]. An algorithm calculated the maximum GVHD clinical grade based on the weekly organ staging in skin, upper and lower gastrointestinal tract, and liver. This calculated organ stage was decreased by 1 stage if a listed specific differential diagnosis was reported for either gastrointestinal tract or liver. An independent panel reviewed all weekly records and assigned each patient a final maximum grade, similar to the methods described by Weisdorf et al. [8]. PES, or "cytokine storm," as it was referred to in the protocol and data forms, was defined as unexplained fever in the absence of documented infection and diffuse erythematous skin rash prior to neutrophil engraftment. The protocol recommended therapy for treatment of PES was methylprednisolone at a dose of 500 mg/m^2 given in 2 divided doses.

Statistical Analyses

Time to chronic GVHD (cGVHD) and preengraftment syndrome were analyzed using proportional subdistribution hazards regression model considering death as the competing event. Time to neutrophil engraftment and aGVHD were analyzed using extended proportional hazards regression model treating death as the competing event and preengraftment syndrome as the time-dependent covariate. All statistical analyses were done with R software (version 2.8.1).

RESULTS

There were 326 patients with a median age of 4.65 years (range: 0.04-17.9) who were included in this analysis. The majority of patients (70%) had malignant diseases and the majority of those received a TBI-based preparative regimen. The median TNC count was 6.94 $\times$ 10^7/kg (range: 0.08-80.9) and 45% of the patients received a HLA matched or single antigen mismatch cord blood. The median follow-up of surviving patients is 21 months (range: 3-64). Patient's characteristics are listed in Table 1. Sixty-four patients (20%) developed PES at a median of 10 days (range: 5-24). Patients who developed PES were younger (median age of 2.47 versus 5.57 years) and were more likely to have nonmalignant disease (48% versus 25%). The median precryopreserved TNC infused was 8.58 $\times$ 10^7/kg (range: 1.54-27.49) in those who developed PES compared to 6.68 $\times$ 10^7/kg (range: 0.08-80.90) for those who did not. After adjusting for patients age, sex, performance status, HLA match (6/6 or 5/6 versus 4/6 or 3/6 match), and cytomegalovirus (CMV) status, patients with nonmalignant disease and those who received a nucleated cell (NC) count of >5 $\times$ 10^7/kg had significantly higher risk of PES, $P = .05$ and $= .02$, respectively. The cumulative incidence of neutrophil engraftment at day 100 in those with PES was 0.84 (95% confidence interval [CI] 0.80-0.89) compared to 0.77 (95% CI 0.75-0.80) in

Table 1. Patient and Transplant Characteristics

Number of patients	326
Median age in years (range)	4.65 (range: 0.04-17.9)
Sex, male/female	197/129
Recipient ethnicity	
Caucasian	240
African American	45
Other	41
Performance status	
>80	263
≤80	63
Median TNC (×10^7/kg) (range)	6.94 (0.08-80.9)
Diagnosis	
Malignant disease	229
Lymphoid	128
Myeloid	65
Other	36
Nonmalignant disease	97
Immune deficiency	24
Storage diseases	69
Other	4
Conditioning regimen	
TBI/Cy/ATG	195
Bu/Cy/ATG	97
Bu/Mel/ATG	34
HLA match	
6 of 6 or 5 of 6	148
4 of 6 or 3 of 6	178
Recipient CMV	
Positive	139
Negative	180
Inconclusive or not tested	7

TNC indicates total nucleated cell count; TBI, total body irradiation; Cy, cyclophosphamide; ATG, antithymocyte globulin; Bu, busulfan; Mel, melphalan; CMV, cytomegalovirus.

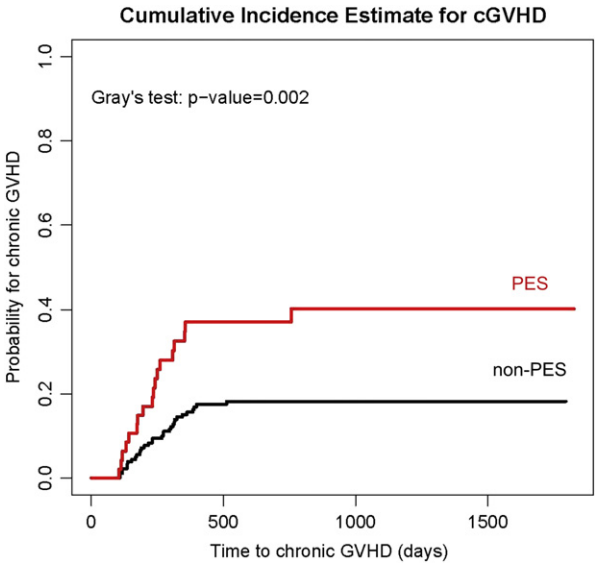

Figure 1. Cumulative incidence of cGVHD at 1 year for patients with PES is 37% (95% CI, 23-51) compared to those without 16% (95% CI 10-21).

those without PES. The median time to neutrophil engraftment was 25.5 days (15-45 days) for patients with PES and 23 days (11-90 days) for those without PES. The median time to developing grade II-IV aGVHD among those with and without PES was 15 and 23 days, respectively. After controlling for patient's age, gender, disease (malignant versus nonmalignant), HLA match (6/6 and 5/ 6 versus other), performance status, CMV status, and total cell count, there was significantly increased risk of grade II-IV (P <0.01) and more severe grade III-IV (P <0.001) aGVHD among those with PES. Among patients who survived >100 days, the probability of developing cGVHD is significantly higher in patients with history of PES (P = .002) (Figure 1). In a multivariate analysis, patients with history of PES had a significantly higher risk of developing cGVHD (P = .0012) after adjusting for patient's age, sex, disease (malignant versus nonmalignant), HLA match (6/6 and 5/ 6 versus other), performance status, CMV status, and total cell count. Using Cox regression analysis, overall survival (OS) was significantly worse among patients with malignant disease (hazard ratio [HR] 1.73, 95% CI 1.07-2.80, P = .025), female patients (HR 1.78, 95% CI 1.27-2.49 , P < .001), and those who were CMV positive (HR 1.46, 95% CI 1.04-2.06, P = .029). In this multivariable model, PES did not significantly affect OS (HR 0.81, 95% CI 0.50-1.30, P = .38).

DISCUSSION

The etiology of PES is not well known. Some reports suggested that this clinical entity is mediated by a cytokine storm that is thought to contribute to this clinical syndrome [1,9]. PES seems to be a distinct clinical syndrome from engraftment syndrome, which has been described immediately prior to engraftment in autologous and allogeneic HSCT patients [10,11]. Prior studies have failed to identify a specific risk factor for developing PES, although 1 study suggested that using methotrexate containing GVHD prophylaxis might reduce its incidence [3]. Patel et al. [4] recently reported on their centers experience with 52 patients who received unrelated cord blood after MA (n = 36) and NMA (n = 16) conditioning regimens. The incidence of PES in that study was 31%, and the authors did not find an association between PES, teatment-related mortality (TRM), and aGVHD or cGVHD. Although all patients in that study received identical GVHD prophylaxis, the numbers were too small to be able to detect any differences. In our analysis, patients receiving a TNC of $>5 \times 10^7$/kg have a significantly higher risk of developing PES. The trend toward increased risk in patients with nonmalignant disease might be because of using chemotherapy-based preparative regimen compared to TBI-based regimen in the majority of patients with malignant diseases. Additional studies should be performed to better understand the effect of the preparative regimen on developing PES. Our study found a strong association between PES and the development of severe aGVHD and cGVHD. This is a novel finding that has not been reported in prior retrospective analysis that included patients receiving various GVHD prophylaxis, supportive care regimens, and smaller number of patients. The strong association between PES and GVHD raises the question if the preengraftment syndrome represents an early form of GVHD or hyperacute GVHD that has been described in patients following related and unrelated donor transplant [12,13]. Hyper-aGVHD has been associated with increased risk of transplant-related mortality in recipients of unrelated donor stem cell transplant [12]. In our study we do not have a biopsy to confirm that the rash observed in patients with PES was not early or hyper-aGVHD. One limitation of our study is the lack of response data to increased dose of steroids, but in a multivariable analysis PES did not adversely affect OS.

We conclude that PES is a common complication following unrelated CBT and is associated with significantly higher risk of aGVHD and cGVHD. Future studies should be aimed at better understanding the etiology of PES and weather using additional immune suppression in patients who develop PES could decrease the risk of developing aGVHD and cGVHD.

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AUTHOR'S CONTRIBUTIONS

H. Frangoul did the study design, analysis, and writing the paper; L. Wang did the analysis of the data and writing the paper; F.E. Harrell did the analysis of the data and writing the paper; R. Ho did the study design and writing of the paper; J. Domm did the study design, analysis, and writing of the paper.

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