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Platelet Transfusion Refractoriness in Single-Unit Cord Blood Transplantation for Adults: Risk Factors and Clinical Outcomes

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A B S T R A C T

Platelet transfusion refractoriness (PTR) is frequently observed after allogeneic hematopoietic cell transplantation (HCT). However, the incidence of and risk factors for PTR, and impact of PTR on transplant outcomes after cord blood transplantation (CBT) have not been fully investigated. We retrospectively analyzed 185 adult patients who received single-unit CBT in our institute. The mean 16-hour corrected count increment (CCI) for the 5840 platelet transfusions was 3.68×10^9/L. Among them, 3196 transfusions (54.7%) were associated with a PTR with 16-hour-CCI $<4.5 \times 10^9$/L. Results of multivariate analysis indicated that the following factors were significantly associated with decreased platelet transfusion responses: female sex with pregnancy history, male sex, the presence of HLA class I antibody, lower cord blood total nucleated cell dose, lower cord blood $CD34^+$ cell dose, 3 locus HLA disparities, body temperature ≥38°C, C-reactive protein ≥10 mg/dL, cytomegalovirus reactivation, use of foscarnet, and use of liposomal amphotericin B. By contrast, graft-versus-host disease prophylaxis including methotrexate, ABO minor mismatch, use of ganciclovir, and use of linezolid were significantly associated with better platelet transfusion responses. PTR had a significant effect on poor neutrophil and platelet recovery, and overall mortality after CBT. These data suggest that early phase PTR may be predictive of engraftment and mortality after single-unit CBT for adults.

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INTRODUCTION

It is necessary to perform prophylactic and therapeutic platelet transfusions to prevent hemorrhagic complications in patients undergoing allogeneic hematopoietic cell transplantation (HCT) [1,2]. However, some patients do not achieve the expected platelet count increment after platelet transfusion. Several studies have demonstrated that platelet transfusion refractoriness (PTR) is common after allogeneic HCT [3-13].

For adult patients without HLA-matched related or unrelated donors, cord blood transplantation (CBT) is an acceptable alternative to the allogeneic HCT [14-17]. The limited cell dose in a single cord blood (CB) unit may contribute to delayed hematopoietic recovery, which may increase platelet transfusion requirements in the early phase of CBT. Therefore, PTR tends to be problematic, especially in adult patients undergoing CBT. Although risk factors and survival impact of PTR in pediatric [3,4] and adult [5-10] patients undergoing allogeneic HCT from other graft sources have been extensively analyzed, the effects of PTR on the outcomes after CBT are limited [10]. In the present study, we retrospectively evaluated (1) risk factors for PTR and (2) the impact of PTR during early and mid-phase CBT on engraftment and survival after single-unit CBT.

PATIENTS AND METHODS

Patients and Transplant Procedures

This retrospective study included data from 185 adult patients at the Institute of Medical Science, the University of Tokyo who underwent their first allogeneic HCT from single-unit unrelated CB, between March 2004 and November 2016. CB units were provided by the Japan cord blood bank. To avoid graft failure, the existence of any anti-HLA antibodies resistant to donor-specific antigens is evaluated before we start conditioning regimens. This has been done for patients in our institution since 2004. Different methods for detecting anti-HLA antibodies were used during different time periods. Since 2004, patients have been screened for anti-HLA antibodies by WAKFlow HLA antibody Class I using the Luminex method (Wakunaga Pharmaceutical Co, Ltd, Osaka, Japan), and patients have also been screened for anti-human platelet antigen antibodies using the anti-human platelet antigen mixed passive hemagglutination panel (Olympus, Tokyo, Japan) at the Japanese Red Cross Blood Center. Since 2012, class I and class II anti-HLA antibodies have also been tested using LAB Screen PRA and Single Antigen (One Lambda, Canoga Park, CA) [18]. Conditioning regimen and graft-versus-host-disease (GVHD) prophylaxis were determined by the physicians. Almost all patients received same supportive care, such as antibacterial, antifungal,

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and antiviral agents [16]. The Institutional Review Board of the Institute of Medical Science, the University of Tokyo approved this retrospective study.

Platelet Transfusion and Platelet Transfusion Refractoriness

All platelet components (PCs) were produced by the Japanese Red Cross Blood Center using the apheresis from a single donor. All packed platelets that were transfused underwent leukoreduction before or after storage. Platelets were irradiated to 15 to 50 Gy. Most of the blood used in this study contained at least 2.0×10^{11} platelets, known as Units 10 in Japan.

Prophylactic platelet transfusions were given to patients with platelet counts $<20 \times 10^9$/L. Platelet transfusions were also given to patients with platelet counts $>20 \times 10^9$/L if active bleeding was present. After starting of conditioning regimen, the ABO and Rh (D) platelets transfused were determined by the blood groups of donors and recipients. Patients with anti-HLA antibodies (identified by WAKFlow HLA antibody Class I) usually received only HLA-compatible platelet transfusions.

To evaluate responses to each PC transfusion, we used the corrected count increment (CCI) [19,20]. The 16-hour CCI was chosen as the parameter for evaluation because platelet transfusions (usually completed around 5 p.m.) and the post-transfusion platelet counts (obtained at approximately 9 a.m. the following morning) occur 16 hours apart in our institution. The 16-hour CCI was calculated using the following formula; the 16-hour CCI (/μL) = (16-hour post-transfusion platelet count [/μL] – pretransfusion platelet count [/μL]) ×body surface area / number of platelets transfused [$\times 10^{11}$]). PTR was defined as the 16-hour CCI $<4.5 \times 10^9$/L [6]. A total of 5880 platelet transfusions were performed for 185 patients between the start of the conditioning regimen and 100 days after CBT, or the data of graft failure or relapse before 100 days after CBT. To evaluate PTR, 40 platelet transfusions were excluded from this study, including transfusions that were discontinued due to severe transfusion reaction (n = 7). Platelet transfusions with unevaluated platelet counts before (n = 7), after (n = 24), or both before and after transfusion (n = 2) were also excluded. The remaining 5840 platelet transfusions were retrospectively analyzed in this study.

To evaluate the impact of PTR on hematopoietic recovery and on transplant outcomes after CBT, the PTR patient group was defined as having a median 16-hour CCI of $<4.5 \times 10^9$/L at each interval, whereas the non-PTR patient group was defined as having a median 16-hour CCI of more than 4.5×10^9/L at each interval. The median 16-hour CCI was evaluated according to the 5 intervals (before CBT, 0 to 15 days after CBT, 16 to 30 days after CBT, 31 to 45 days after CBT, 46 to 100 days after CBT).

Definitions

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. For hematopoietic engraftment, death before going 28 days without hematopoietic engraftment was defined as a competing event. The overall survival (OS) (inverse of overall mortality) was defined as the time between CBT and death or last contact. Transplant-related mortality (TRM) was defined as death during remission. Relapse was defined by hematological evidence of disease. For TRM, relapse was defined as a competing event. By contrast, TRM was defined as a competing event for relapse. The number of HLA disparities was defined by low-resolution for HLA-A, -B, and -DR. Myeloablative conditioning (MAC) regimens were defined according to criteria from the Center for International Blood and Marrow Transplant Research, and others were classified as reduced-intensity conditioning (RIC) [21]. Disease status at CBT was assessed using the refined disease risk index, which categorizes disease type, disease status, and cytogenetic risk [22]. Cytomegalovirus (CMV) reactivation, which was evaluated using an antigenemia assay with the monoclonal antibodies C10/C11, was defined as being achieved when there was more than 1 positive cell within a week before the day of platelet transfusion. Administrating more than .5 mg/kg of prednisolone or more than .4 mg/kg of methylprednisolone was defined as systemic treatment with glucocorticoids.

Statistical Analysis

Continuous variables were compared using the Mann-Whitney *U* test for 2 groups. For 3 groups, Kruskal-Wallis and Steel-Dwass tests were used for multiple comparisons. Categorical variables were compared using the chi-square test or the Fisher's exact test. The Spearman rank correlation coefficient was calculated to assess the correlation between 16-hour CCI and serum C-reactive protein (CRP) levels.

The risk factors for PTR were evaluated using a logistic regression model for univariate and multivariate analysis. The following factors were taken into consideration: age (<45 versus ≥45 years); sex and pregnancy history (female without pregnancy history versus female with pregnancy history versus male); HLA class I antibody (negative versus positive); recipient CMV serostatus (negative versus positive); disease risk index (low to intermediate versus high to very high); conditioning regimen (MAC versus RIC); GVHD prophylaxis (cyclosporine with methotrexate versus without methotrexate); cryopreserved CB total nucleated cell (TNC) dose ($<2.5 \times 10^7$/kg versus $\geq 2.5 \times 10^7$/kg); cryopreserved CB CD34$^+$ cell dose ($<1 \times 10^5$/kg versus $\geq 1 \times 10^5$/kg); HLA disparities (0 versus 1 versus 2 versus 3); ABO incompatibility between donor and recipient (match versus minor mismatch versus major mismatch versus bidirectional mismatch); sex incompatibility (female donor to male recipient versus others); body temperature (<38°C versus ≥38°C), CRP (<10 mg/dL versus ≥10 mg/dL); CMV reactivation (yes versus no); and concomitant use of foscarnet (yes versus no), ganciclovir (yes versus no), linezolid (yes versus no), vancomycin (yes versus no), liposomal amphotericin B (yes versus no), and glucocorticoids (yes versus no). Multivariate analysis was performed with the variables identified as significant using univariate analysis.

The probabilities of neutrophil and platelet engraftment, 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 probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. To evaluate the impact of PTR on hematopoietic recovery and transplant outcomes, univariate and multivariate analyses were performed using a Cox proportional hazards model for overall mortality, and a Fine and Gray proportional hazards model was used to evaluate the other endpoints using these factors: PTR before CBT, 0 to 15 days after CBT, 16 to 30 days after CBT, or 31 to 45 days after CBT (yes versus no); increased platelet transfusion requirement during same periods (yes versus no), which was defined as requirement of platelet transfusion more than every second day; age at CBT (16 to 44 years versus ≥45 years); sex (male versus female); disease risk index (low/intermediate versus high/very high); conditioning regimen (MAC versus RIC); CB TNC dose ($<2.5 \times 10^7$/kg versus $\geq 2.5 \times 10^7$/kg); CB CD34$^+$ cell dose ($<1 \times 10^5$/kg versus $\geq 1 \times 10^5$/kg); HLA disparities (0 or 1 versus 2 or 3); and ABO compatibility between donor and recipient (match, minor mismatch versus major, bidirectional mismatch). Final model variables were confirmed using a backward selection procedure where the level of significance was $P = .05$. All *P* values were 2 sided and all statistical analyses were performed using either GraphPad Prism 6 for Mac OS X (GraphPad Software, La Jolla, CA) or EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [23], a graphical user interface for the R 3.0.2 software program (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of Patients and Transplantations

The characteristics of patients, CB units, and transplantations are shown in Table 1. The median age of participants was 44 years (range, 16 to 68 years). Forty-seven (25%) were female who had pregnancy history. Twenty (11%) patients had antibodies against HLA class I. The most common disease type was acute myeloid leukemia (51%). The majority of conditioning regimens were MAC (97%), and the most common GVHD prophylaxis was cyclosporine A and methotrexate (88%). Among MAC regimens, the most common conditioning regimen was total body irradiation 12 Gy, cyclophosphamide, and cytosine arabinoside with or without granulocyte colony-stimulating factor in patients with myeloid or lymphoid malignancies, respectively [24,25]. The median TNC dose was 2.52×10^7/kg (range, 1.32 to 5.69×10^7/kg). The median CD34$^+$ cell dose was $.94 \times 10^5$/kg (range, .28 to 2.84×10^5/kg). The number of HLA mismatches between CB unit and recipient was 0 in 5 (3%), 1 in 32 (17%), 2 in 140 (76%), and 3 in 8 (4%) patients.

Characteristics and Risk Factors for Platelet Transfusion Refractoriness

The median 16-hour CCI for the 5840 platelet transfusions received by 185 patients was 3.68×10^9/L (range, –41.72 to 41.67×10^9/L) (Figure 1). The percentage of transfusions with a 16-hour CCI $<4.5 \times 10^9$/L was 54.7%, indicating frequent poor response to platelet transfusion in adult patients undergoing CBT. From the start of conditioning regimen to 100 days after CBT, 1 patient died from a severe bleeding complication (diffuse alveolar hemorrhage on day 8 after CBT).

Table 1
Characteristics of the Patients, Cord Blood Units, and Transplantations

Characteristic	Value
CBTs	185
Age at CBT, yr	44 (16–68)
Body surface area, m^2	1.63 (1.27–2.20)
Sex and pregnancy status	
Female without pregnancy history	28 (15)
Female with pregnancy history	47 (25)
Male	110 (59)
HLA class I antibody	
Negative	165 (89)
Positive	20 (11)
Recipient CMV serostatus	
Positive	160 (86)
Negative	25 (14)
Disease	
AML	94 (51)
ALL	41 (22)
MDS	24 (13)
CML/MPN	13 (7)
NHL	11 (6)
Benign (CAEBV, SAA)	2 (1)
Disease risk index*	
Low	12 (6)
Intermediate	94 (51)
High	70 (38)
Very high	5 (3)
Conditioning regimen	
MAC	179 (97)
RIC	6 (3)
GVHD prophylaxis	
CSP + MTX	163 (88)
CSP + MMF	21 (11)
CSP	1 (<1)
Cryopreserved TNC, $\times 10^7$/kg	2.52 (1.32–5.69)
Cryopreserved $CD34^+$ cells, $\times 10^5$/kg	.94 (.28–2.84)
HLA disparities	
0	5 (3)
1	32 (17)
2	140 (76)
3	8 (4)
ABO incompatibility	
Match	51 (28)
Minor mismatch	52 (28)
Major mismatch	57 (31)
Bidirectional mismatch	25 (13)
Sex incompatibility	
Female donor to male recipient	57 (31)
Others	128 (69)

Data are presented as median (range) or n (%).
AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin lymphoma; CAEBV, chronic active Epstein-Barr virus infection; SAA, severe aplastic anemia; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; CSP, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil.
* Data of 4 patients were not available in the refined disease risk index.

Median 16-hour CCI 16 to 30 days after CBT was significantly lower than other time periods (Figure 2). A body temperature ≥38°C (the febrile condition) was significantly associated with the lower 16-hour CCI (Figure 3A). The correlation between serum CRP level and 16-hour CCI was found to be significant ($r = -.256$, $P < .001$) (Figure 3B).

Results of univariate analysis indicated that the following were significantly associated with decreased platelet transfusion responses: age ≥45 years, female sex with pregnancy history, male sex, the presence of HLA class I antibody, lower CB TNC dose, lower CB $CD34^+$ cell dose, 3 locus HLA disparities, body temperature ≥38°C, CRP ≥10 mg/dL, CMV reactivation, use of foscarnet, use of vancomycin, and use of liposomal amphotericin B (Table 2). Results of multivariate analysis indicated that the following were also significantly associated with decreased platelet transfusion responses: female sex with pregnancy history, male sex, the presence of HLA class I antibody, lower CB TNC dose, lower CB $CD34^+$ cell dose, 3 locus HLA disparities, body temperature ≥38°C, CRP ≥10 mg/dL, CMV reactivation, use of foscarnet, and use of liposomal amphotericin B (Table 2).

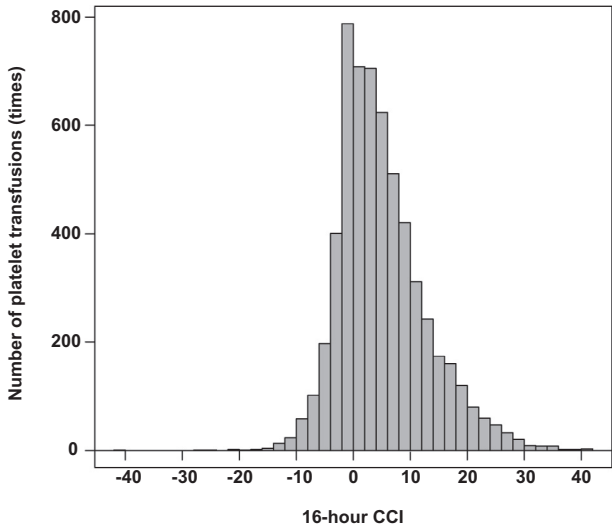

Figure 1. Distribution of 16-hour CCI after 5840 platelet transfusion given to 185 patients undergoing CBT.

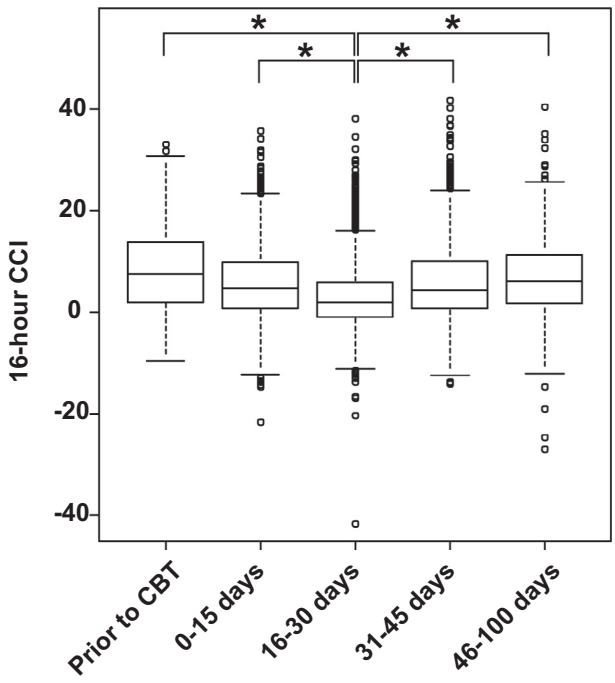

Figure 2. Sixteen-hour CCI according to the different time points during CBT. The line inside the box, the lower and upper box ends, and the lower and upper whiskers represent the median value, the 25th and 75th percentiles, and the 10th and 90th percentiles of 16-hour CCI, respectively. The statistical differences between each of the 2 groups among 16-hour CCI of before CBT, 0 to 15 days, 16 to 30 days, 31 to 45 days, and 46 to 100 days after CBT were analyzed by the Mann-Whitney test. *$P < .05$.

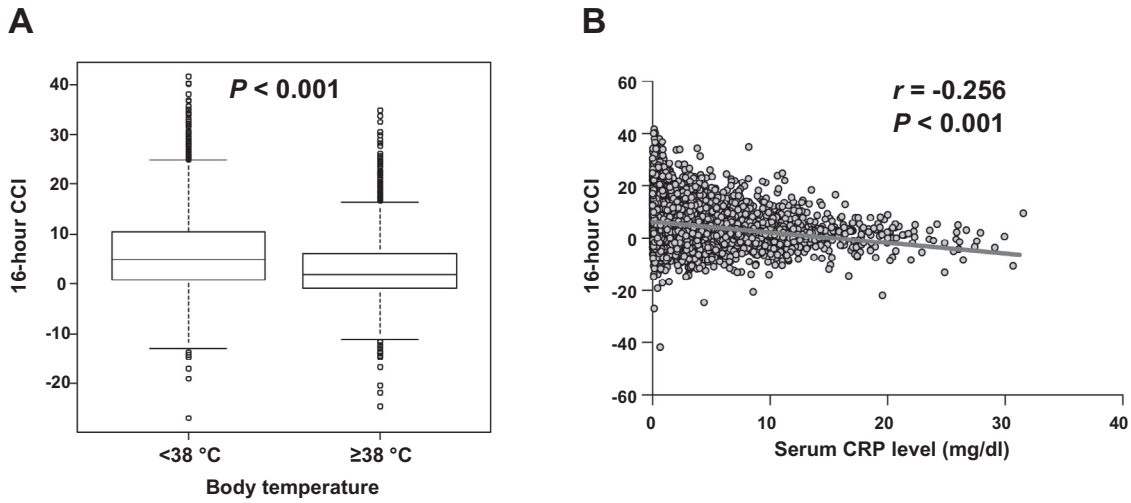

Figure 3. (A) The relationship between 16-hour CCI and body temperature and (B) the correlation between 16-hour CCI and serum CRP level. The line inside the box, the lower and upper box ends, and the lower and upper whiskers represent the median value, the 25th and 75th percentiles, and the 10th and 90th percentiles of 16-hour CCI, respectively.

By contrast, GVHD prophylaxis including methotrexate, ABO minor mismatch, use of ganciclovir, and use of linezolid were significantly associated with better platelet transfusion responses in both univariate and multivariate analyses (Table 2).

Among 185 patients, 175 patients experienced at least one 16-hour CCI $<4.5 \times 10^9$/L. The mean number of platelet transfusion received before development of PTR, which was defined as a 16-hour CCI $<4.5 \times 10^9$/L, per each patient was 4.31 (range, 1 to 16) between the start of the conditioning regimen and 100 days after CBT, or the data of graft failure or relapse before 100 days after CBT. The median days of platelet transfusion received before development of PTR per each patient was 2 days (range, –9 to 23 days) after CBT.

Impact of Platelet Transfusion Refractoriness on Hematopoietic Recovery

The cumulative incidence of neutrophil and platelet engraftment was 96.4% (95% confidence interval [CI], 91.2% to 98.6%) at day 42 and 71.6% (95% CI, 64.3 to 77.7%) at day 60, respectively. Among patients who achieved neutrophil and platelet engraftment, median time to neutrophil and platelet engraftment was 22 days (range, 15 to 46 days) and 46 days (range, 27 to 204 days), respectively.

Compared with the non-PTR patient group before CBT ($P = .03$) or 0 to 15 days after CBT ($P < .001$), the cumulative incidence of neutrophil engraftment was significantly lower in the PTR patient group during same periods (Figure 4A, 4B). The cumulative incidence of platelet engraftment was significantly lower in the PTR patient group 0 to 15 days after CBT ($P < .001$), 16 to 30 days after CBT ($P < .001$), and 31 to 45 days after CBT ($P < .001$), compared with the non-PTR patient group during each time period (Figure 4C-E). Results of multivariate analysis indicated that the PTR patient group before CBT and 0 to 15 days after CBT was an independent factor in achieving neutrophil recovery (Table S1). The PTR patient group before CBT, 0 to 15 days after CBT, and 16 to 30 days after CBT was also an independent factor in achieving platelet recovery (Table S1).

Impact of Platelet Transfusion Refractoriness on Survival, Relapse, and TRM

At a median follow-up of survivors 78 months (range, 3 to 158 months) after CBT, the probabilities of OS at 5 years was 67.6% (95% CI, 59.7% to 74.3%). The cumulative incidences of relapse and TRM at 5 years were 26.2% (95% CI, 19.8% to 33.1%) and 12.4% (95% CI, 7.9% to 18.0%), respectively.

Results of the univariate analysis indicated that the probability of overall survival was significantly lower in the PTR patient group 31 to 45 days after CBT compared with the non-PTR patient group during the same period ($P = .03$) (Figure 5). The PTR patient group during any period did not affect relapse and TRM (Table S2). Results of multivariate analysis indicated that the PTR patient group 31 to 45 days after CBT was an independent factor of overall mortality (Table S2).

DISCUSSION

The purpose of this single-center retrospective study was to evaluate the incidence of and risk factors for PTR in adult patients from the start of a conditioning regimen to 100 days after CBT, and to identify the impact of PTR on transplant outcomes after CBT. Of the total 5840 platelet transfusions given to 185 adults, 3196 transfusions (54.7%) with 16-hour CCI $<4.5 \times 10^9$/L were associated with a poor transfusion response, indicating that PTR was a relatively common problem in adult patients undergoing CBT. This is consistent with a previous report [10]. Risk factors for PTR included the following: presence of HLA class I antibody, lower CB TNC dose, lower CB $CD34^+$ cell dose, 3 locus HLA disparities, body temperature ≥38°C, CRP ≥10 mg/dL, CMV reactivation, use of foscarnet, and use of liposomal amphotericin B. By contrast, better platelet transfusion responses were significantly associated with the following: GVHD prophylaxis including methotrexate, ABO minor mismatch, use of ganciclovir, and use of linezolid. Finally, the PTR patient group significantly affected poor neutrophil and platelet recovery, and overall mortality after CBT. This result suggested that early phase PTR after single-unit CBT may be predictive of engraftment and mortality in adults.

Table 2
Risk Factors for PTR in Univariate and Multivariate Analysis

	Univariate Analysis	*P* Value	Multivariate Analysis	*P* Value
	HR (95% CI)		HR (95% CI)	
Age at CBT				
<45 yr	Reference			
≥45 yr	1.14 (1.03–1.27)	.01*		
Sex and pregnant status				
Female without pregnancy history	Reference		Reference	
Female with pregnancy history	1.33 (1.11–1.59)	.002*	1.38 (1.14–1.69)	.001*
Male	1.70 (1.45–2.00)	<.001*	1.82 (1.51–2.20)	<.001*
HLA class I antibody				
Negative	Reference		Reference	
Positive	1.51 (1.29–1.77)	<.001*	1.75 (1.45–2.12)	<.001*
Recipient CMV serostatus				
Negative	Reference			
Positive	1.03 (.88–1.21)	.72		
Disease risk index				
Low/intermediate	Reference			
High/very high	1.06 (.95–1.17)	.31		
Conditioning regimen				
RIC	Reference			
MAC	.93 (.68–1.25)	.62		
GVHD prophylaxis				
CSP without MTX	Reference		Reference	
CSP with MTX	.67 (.57–.78)	<.001*	.65 (.55–.77)	<.001*
Number of TNC				
$\geq 2.5 \times 10^7$/kg	Reference		Reference	
$<2.5 \times 10^7$/kg	1.31 (1.18–1.45)	<.001*	1.13 (1.00–1.28)	.04*
Number of $CD34^+$ cells				
$\geq 1.0 \times 10^5$/kg	Reference		Reference	
$<1.0 \times 10^5$/kg	1.42 (1.28–1.58)	<.001*	1.33 (1.18–1.50)	<.001*
HLA disparities				
0	Reference		Reference	
1	.90 (.63–1.29)	.58	1.11 (.74–1.66)	.62
2	1.05 (.75–1.47)	.78	1.31 (.88–1.96)	.18
3	1.69 (1.12–2.54)	.01*	1.82 (1.15–2.90)	.01*
ABO incompatibility				
Match	Reference		Reference	
Minor mismatch	.77 (.67–.89)	<.001*	.78 (.67–.91)	.001*
Major mismatch	.97 (.85–1.11)	.65	.98 (.85–1.13)	.80
Bidirectional mismatch	1.00 (.84–1.20)	.98	.99 (.82–1.20)	.91
Sex incompatibility				
Other	Reference			
Female donor to male recipient	.91 (.82–1.02)	.09		
Body temperature				
<38°C	Reference		Reference	
≥38°C	2.26 (2.01–2.53)	<.001*	2.23 (1.97–2.52)	<.001*
CRP				
<10 mg/dL	Reference		Reference	
≥10 mg/dL	2.60 (2.04–3.33)	<.001*	1.55 (1.18–2.02)	.001*
CMV reactivation				
No	Reference		Reference	
Yes	1.27 (1.08–1.50)	.004*	1.57 (1.26–1.96)	<.001*
Use of foscarnet				
No	Reference		Reference	
Yes	2.09 (1.68–2.59)	<.001*	1.36 (1.07–1.73)	.01*
Use of ganciclovir				
No	Reference		Reference	
Yes	.65 (.56–.75)	<.001*	.46 (.37–.56)	<.001*
Use of linezolid				
No	Reference		Reference	
Yes	.65 (.51–.83)	.001*	.61 (.47–.80)	<.001*
Use of vancomycin				
No	Reference			
Yes	1.23 (1.11–1.37)	<.001*		
Use of liposomal amphotericin B				
No	Reference		Reference	
Yes	1.77 (1.48–2.12)	<.001*	2.18 (1.78–2.67)	<.001*
Use of glucocorticoids				
No	Reference			
Yes	1.02 (.86–1.21)	.79		

HR indicates hazard ratio.
* $P < .05$.

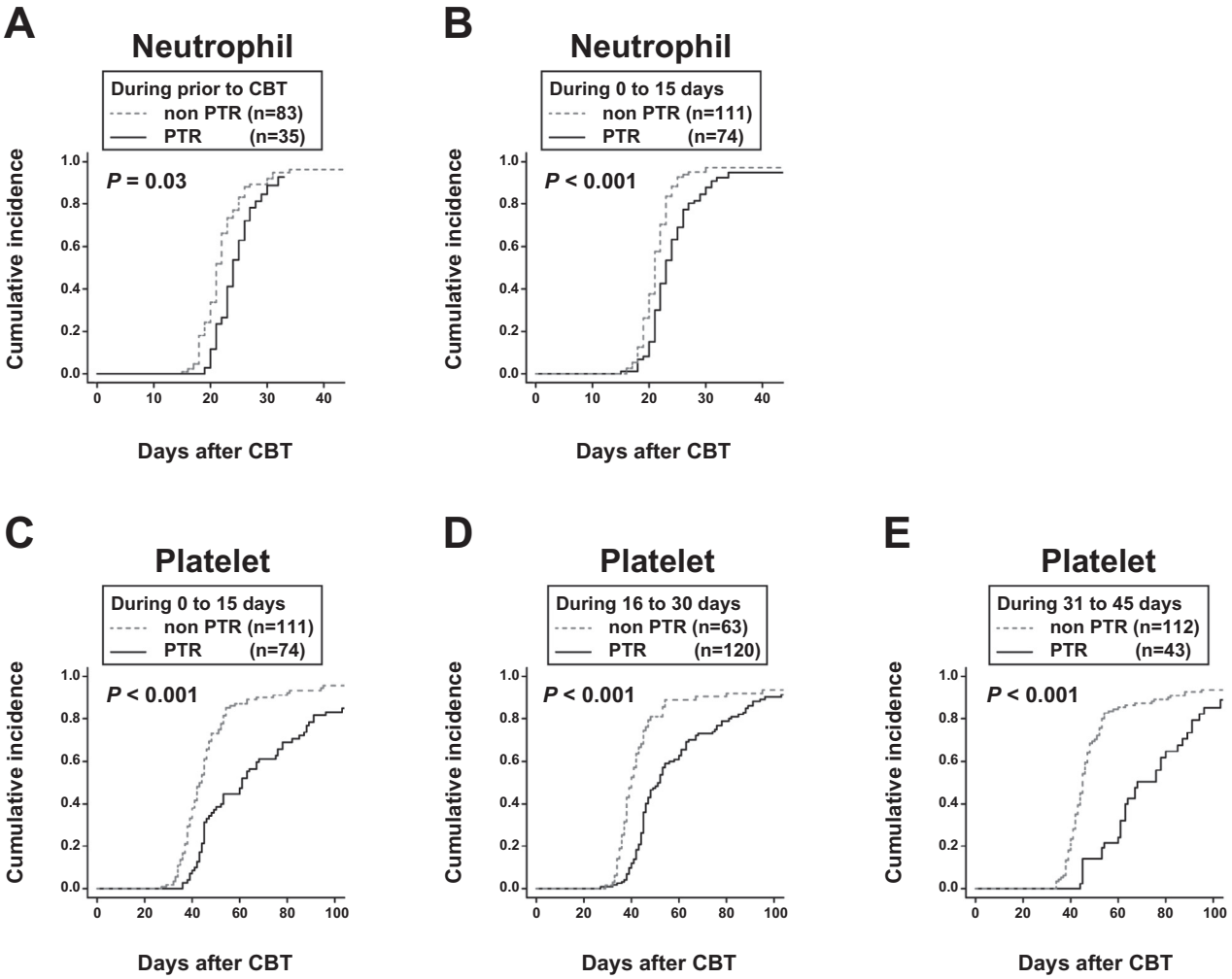

Figure 4. The cumulative incidences of neutrophil and platelet engraftment after CBT according to PTR. The cumulative incidences of neutrophil engraftment according to PTR (A) before CBT and (B) 0 to 15 days after CBT, and the cumulative incidences of platelet engraftment according to PTR (C) 0 to 15 days after CBT, (D) 16 to 30 days after CBT, and (E) 31 to 45 days after CBT.

Our data showed that a median 16-hour CCI was significantly lower 16 to 30 days after CBT, which is around the potential onset period of pre-engraftment syndrome (PES). PES is a unique clinical manifestation of fever, skin rash, and CRP elevation that occurs before neutrophil engraftment [26,27]. PES has frequently been observed during this period after CBT. In our study, fever is the factor most strongly associated with PTR. The significant association between increased CRP levels and decreased 16-hour CCI may be explained by the frequent occurrence of PES after CBT. In addition, GVHD prophylaxis using methotrexate may contribute to better platelet transfusion responses found in our study, probably because using methotrexate for GVHD prophylaxis has been reported to reduce the incidence of PES and acute GVHD after CBT [28-30]. These findings suggested that development of PES may be strongly associated with PTR after CBT.

Our study also confirmed that CMV reactivation was significantly associated with PTR, which is consistent with previous report of allogeneic HCT [4]. Although CMV reactivation is usually attended by either foscarnet or ganciclovir use, ganciclovir use and CMV reactivation itself mediate myelosuppression. Several studies have demonstrated that

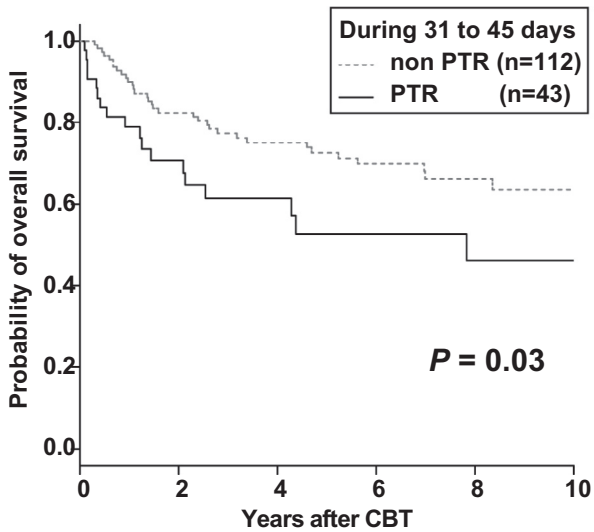

Figure 5. The probability of overall survival after CBT according to PTR. The probabilities of OS at 5 years were 52.7% (95% CI, 34.1% to 68.3%) for patients with PTR 31 to 45 days after CBT and 72.7% (95% CI, 62.8% to 80.3%) for patients without PTR during the same period ($P = .03$ by log-rank test).

higher number of antibiotics and several types of drugs, such as vancomycin, amphotericin B, and glucocorticoids, may decrease platelet transfusion responses [4,5,10,11,19,20,31]. The finding that ganciclovir and linezolid use were significantly associated with better platelet transfusion responses was unexpected because ganciclovir and linezolid use can lead to thrombocytopenia. By contrast, foscarnet use was significantly associated with decreased platelet transfusion responses. These results should be interpreted with caution because the patients in our study who had myelosuppression received foscarnet, but not ganciclovir and linezolid. If our patients had also used ganciclovir and linezolid, they might have been included with those patients who had better platelet transfusion responses. Therefore, further studies are required to clarify the impact of concomitant use of drugs on the PTR after CBT.

PTR is considered to be associated with multifactorial mechanisms. Although PTR can be due to immune and nonimmune causes, immune factors are less common cause of PTR than nonimmune factors in patients undergoing induction chemotherapy for acute myeloid leukemia or those undergoing autologous or allogeneic HCT [6,10,32,33]. Our data also confirmed that the presence of HLA class I antibody and pregnancy history among women were significantly associated with PTR after CBT, which is consistent with previous reports of allogeneic HCT [3-7,10,11]. Three locus HLA disparities were also found to cause PTR after CBT. In the CBT-specific setting of our study, lower CB TNC dose and CB $CD34^+$ cell dose were significant predictors of PTR after CBT. Gluckman et al. [34] reported that engraftment was log linearly related to the number of HLA disparities. We previously demonstrated that after single-unit CBT, $CD34^+$ cell dose was the best predictor for hematopoietic recovery [35]. All these findings suggested that common factors, such as lower CB TNC dose and CB $CD34^+$ cell dose, and higher HLA disparities, could influence PTR and graft failure after CBT.

Previous studies have shown that delayed platelet recovery was associated with inferior outcomes following HCT [36-39]. However, investigation of the impact of PTR on transplant outcomes has been limited [7,8,10,12,13]. Scott et al. reported that the occurrence of PTR before HCT was not associated with failure of platelet engraftment after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation from related and unrelated donors [12], but our study clearly demonstrated that the PTR patient group significantly influenced neutrophil and platelet engraftment following CBT. Several studies have shown an association between PTR and inferior survival in patients undergoing allogeneic HCT [7,8,10,12]. Interestingly, although previous studies reported that there was an association between PTR and hemorrhagic complication [7,8] and that CBT was a significant risk factor for hemorrhagic complication [40], the higher mortality rate of the PTR patient group in our cohort was not caused by the higher incidence of hemorrhagic complication, which is consistent with previous studies [8,10]. These data suggest that PTR may be surrogate marker for toxicity and mortality following allogeneic HCT.

In summary, this retrospective single-institute analysis confirmed that PTR is frequently observed after CBT in adults. Nonimmune factors were the main determinants of PTR after CBT. PTR was the significant indicator for poor neutrophil and platelet engraftment, and overall mortality after CBT. Although the exact mechanism of PTR is unclear, further studies are warranted to establish the optimal management strategies for PTR after CBT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.05.006.

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