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Cord Blood

Development of Pre-Engraftment Syndrome, but Not Acute Graft-versus-Host Disease, Reduces Relapse Rate of Acute Myelogenous Leukemia after Single Cord Blood Transplantation

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A B S T R A C T

The different effects of pre-engraftment syndrome (PES) and acute graft-versus-host disease (aGVHD) on outcomes after cord blood transplantation (CBT) are unclear. We retrospectively evaluated the impact of PES and aGVHD on relapse and survival after single-unit CBT in 138 adult patients with hematologic malignancies at our institution between 2004 and 2016. Multivariate analysis demonstrated that development of grade III-IV aGVHD, particularly with gut or liver involvement, significantly contributed to higher nonrelapse mortality ($P < .001$), but PES and grade II-IV aGVHD did not. In subgroup analyses of underlying disease type, the development of PES had a significant effect on decreased relapse ($P = .032$) and better disease-free survival (DFS) ($P = .046$) in patients with acute myelogenous leukemia (AML). These data suggest that PES is associated with a reduced relapse rate and better DFS in AML, indicating that the early immune reaction before neutrophil engraftment may provide a unique graft-versus-leukemia effect after single-unit CBT.

INTRODUCTION

Pre-engraftment syndrome (PES) is a unique clinical manifestation involving noninfectious fever and erythematous skin rash occurring before neutrophil engraftment after cord blood transplantation (CBT) [1-11]. Although the incidence and severity of graft-versus-host disease (GVHD) after single-unit CBT is usually lower than that after conventional adult donor hematopoietic cell transplantation (HCT) [12-15], the incidence of PES after CBT has been reported to be high, ranging from 20% to 78% [1-7].

The development of GVHD is associated with a lower incidence of relapse of leukemia in conventional adult donor HCT, indicating that GVHD may be an indicator of a graft-versus-leukemia (GVL) effect [16-22]. Several studies have demonstrated that the GVL effect is usually accompanied by the development of GVHD in single- and double-unit CBT in adult patients [15,23-25]. Although our previous study suggested that a similar mechanism might be involved in the development of PES and acute GVHD (aGVHD) [11], the impact of PES on relapse after CBT is unclear, and no previous study has evaluated the different clinical impacts of PES and aGVHD after CBT. Therefore, in this study, we retrospectively evaluated the impact of PES and aGVHD on relapse and survival after single-unit CBT in adult patients with hematologic malignancies at our institution.

METHODS

Patient Selection

This retrospective study initially included 185 consecutive adult patients who underwent single-unit CBT for hematologic malignancies at our institution between March 2004 and November 2016. Eleven patients who did not achieve neutrophil engraftment and 36 patients with documented infection (bacteremia, n = 14; *Clostridium difficile* colitis, n = 1; invasive aspergillus infection, n = 1; cytomegalovirus [CMV] reactivation, n = 14; human herpes virus-6 reactivation, n = 14; and adenovirus cystitis, n = 1, with some cases overlapping) before neutrophil engraftment were excluded. Thus, a total of 138 patients were included in the analysis. All procedures were approved by the Institutional Review Board of the Institute of Medical Science, University of Tokyo.

Endpoints and Definition

The primary study endpoint was the impact of PES and aGVHD on relapse after CBT. The secondary endpoints were the impact of PES and aGVHD on nonrelapse mortality (NRM), overall survival (OS), and disease-free survival (DFS) after CBT. Relapse was defined as morphological evidence of disease. NRM was defined as death during remission. OS (the inverse of overall mortality) was defined as the time between CBT and death or last contact. DFS (the inverse of treatment failure) was defined as the time between CBT and relapse, death, or last contact. PES was defined as noninfectious fever >38.3° C with an unexplained erythematous skin rash occurring before neutrophil engraftment, as described previously [1]. Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of $>.5 \times 10^9$/L. aGVHD was graded after neutrophil engraftment by the treating

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

Characteristic		Total	No PES	PES	*P* Value
Number of patients		138	21	117	
Age at CBT, median (range), yr		44 (17-68)	44 (19-68)	44 (17-68)	.842
Body weight, median (range), kg		55.9 (38.4-83.6)	53.6 (40.8-75.2)	56.2 (38.4-83.6)	.380
Sex, n (%)					
	Male	79 (57.2)	13 (61.9)	66 (56.4)	.811
	Female	59 (42.8)	8 (38.1)	51 (43.6)	
CMV-seropositive recipient, n (%)		120 (87.0)	18 (85.7)	102 (87.2)	.738
Disease type, n (%)					
	AML	73 (52.9)	11 (52.4)	62 (53.0)	.089
	ALL	30 (21.7)	3 (14.3)	27 (23.1)	
	MDS	16 (11.6)	6 (28.6)	10 (8.5)	
	MPN	1 (.7)	1 (4.8)	0 (.0)	
	CML	8 (5.8)	0 (.0)	8 (6.8)	
	CMML	2 (1.4)	0 (.0)	2 (1.7)	
	NHL	5 (3.6)	0 (.0)	5 (4.3)	
	ATL	3 (2.2)	0 (.0)	3 (2.6)	
Disease Risk Index, n (%)					
	Low, intermediate	80 (58.9)	6 (30.0)	74 (63.8)	**.006**
	High, very high	56 (41.1)	14 (70.0)	42 (36.2)	
Conditioning regimen, n (%)					
	MAC	135 (97.8)	20 (95.2)	115 (98.3)	.393
	RIC	3 (2.1)	1 (4.8)	2 (1.7)	
GVHD prophylaxis, n (%)					
	CSP with MTX	122 (88.4)	16 (76.2)	106 (90.6)	.071
	CSP without MTX	16 (11.6)	5 (23.8)	11 (9.4%)	
TNC, $\times 10^7$/kg, median (range),		2.52 (1.32-5.50)	2.52 (1.51-4.64)	2.52 (1.32-5.50)	.497
$CD34^+$ cells, $\times 10^5$/kg, median (range)		.97 (.28-2.84)	.97 (.38-1.55)	.97 (.28-2.84)	.797
HLA disparities, n (%)					
	0 or 1	29 (21.0)	7 (33.3)	22 (18.8%)	.150
	2 or 3	109 (79.0)	14 (66.7)	95 (81.2%)	
ABO incompatibility, n (%)					
	Match/minor mismatch	75 (54.3)	11 (52.4)	64 (54.7%)	1.000
	Major mismatch/bidirectional mismatch	63 (45.7)	10 (47.6)	53 (45.3%)	
Sex incompatibility, n (%)					
	Female donor to male recipient	40 (29.0)	7 (33.3)	33 (28.2%)	.612
	Others	98 (71.0)	14 (66.7)	84 (71.8%)	
Neutrophil engraftment, d, median (range)		21 (15-34)	21 (16-34)	21 (15-32)	.422
ALC in PB at 1 mo, cells/μL, median (range)		403 (29-1453)	316 (67-956)	413 (29-1453)	.060
ALC in PB at 3 mo, cells/μL, median (range)		1400 (125-7784)	943 (168-5676)	1526 (125-7784)	**.040**

P values in bold type are statistically significant (<.05).
ALL indicates acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; RIC, reduced-intensity conditioning; CSP, cyclosporine; TNC, total nucleated cell; PB, peripheral blood.

physician according to standard criteria [26]. The onset of aGVHD after preceding PES was defined as the day of neutrophil engraftment. Skin biopsy specimens were not obtained for any patient for diagnosis of PES or skin GVHD. The intensity of conditioning regimen was classified as myeloablative conditioning (MAC) or reduced-intensity conditioning based on the criteria of the Center for International Blood and Marrow Transplant Research [27]. Disease status at the time of CBT was assessed according to the refined Disease Risk Index [28].

Statistical Analysis

The baseline characteristics of patients who developed PES and those did not develop PES after CBT were compared using the chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. The probabilities of relapse, NRM, PES, and aGVHD were estimated as cumulative incidence, taking into account competing risks, and the groups were compared using Gray's test. The competing risk for relapse was NRM, whereas the competing risk for NRM was relapse. For PES and aGVHD, relapse or death without PES or aGVHD, respectively, were competing risks. The probabilities of OS and DFS were estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The Fine and Gray proportional hazards model was used to estimate hazard ratios (HRs) for aGVHD. The Cox proportional hazards regression model was used to estimate HRs for relapse, NRM, overall mortality, and treatment failure in univariate and multivariate analyses, treating the development of PES or aGVHD as time-dependent covariates. To evaluate HRs for relapse and NRM, patients who experienced NRM or relapse were censored. The following variables other than PES or aGVHD were considered in the multivariate analysis: age (<45 versus ≥45 years), CMV serostatus (positive versus negative), refined Disease Risk Index (low/intermediate versus high/very high), cryopreserved cord blood total nucleated cell count (<2.5×10^7/kg versus ≥2.5×10^7/kg), HLA disparities (≤1 versus ≥2), sex incompatibility (female donor to male recipient versus others), and ABO blood group incompatibility (match/minor mismatch versus major/bidirectional mismatch). All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [29], a graphical user interface for the R 3.3.2 software program (R Foundation for Statistical Computing, Vienna, Austria). All *P* values were 2-sided, and *P* values <.05 were considered statistically significant.

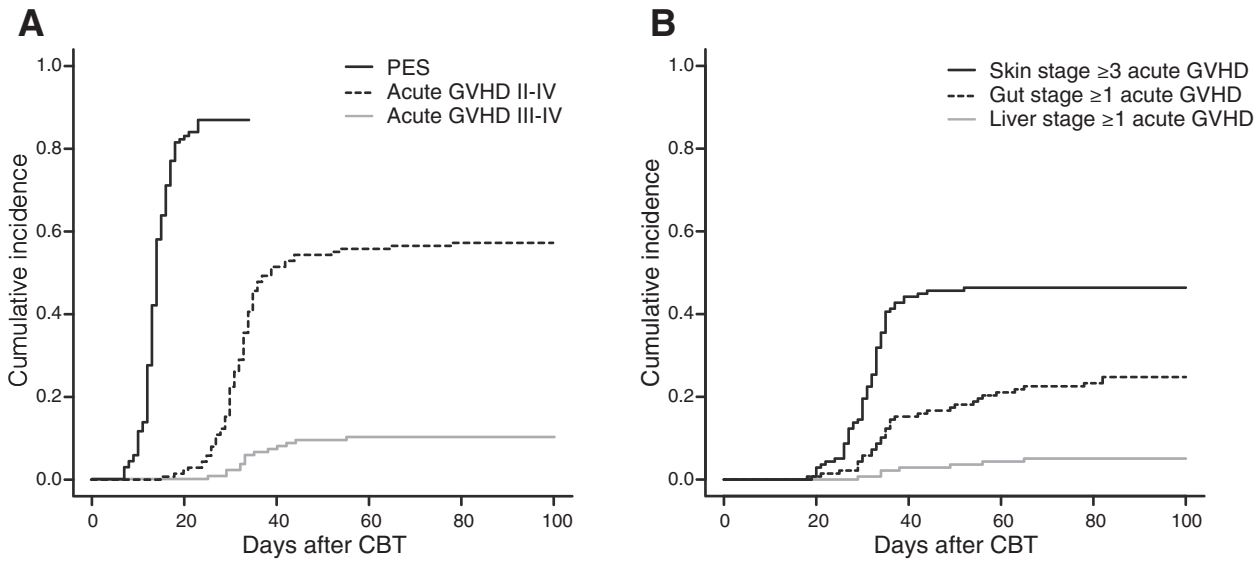

Figure 1. Cumulative incidences of PES and aGVHD (A) and aGVHD according to target organ (B).

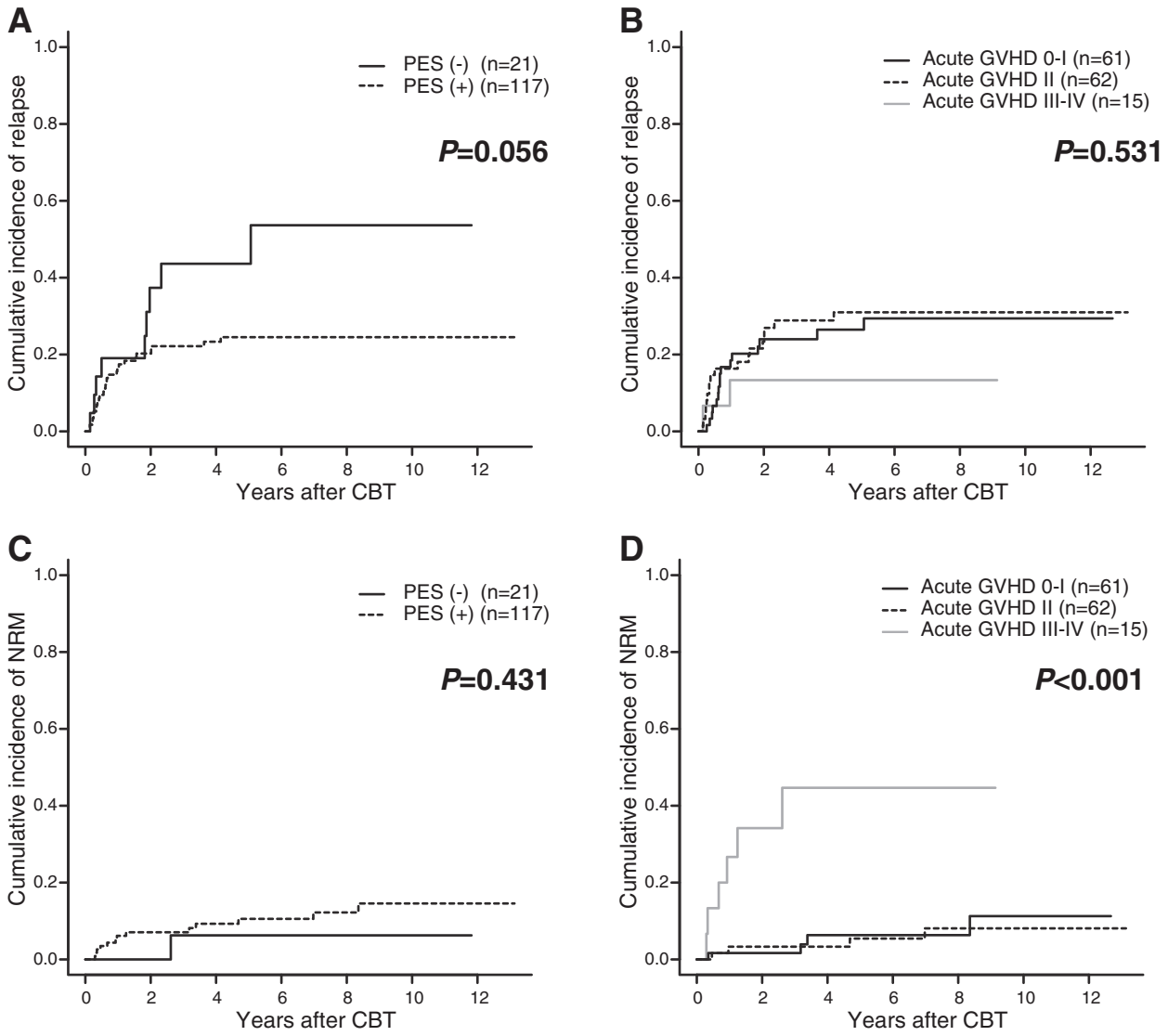

Figure 2. Cumulative incidences of relapse (A and B) and NRM (C and D) according to the development of PES and aGVHD.

Table 2
Univariate and Multivariate Analyses of Transplantation Outcomes According to the Development of PES and aGVHD

Outcome		Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Relapse*					
	PES	.50 (.23-1.06)	.072	.58 (.27-1.28)	.177
	Grade II-IV aGVHD	.98 (.51-1.89)	.945	.82 (.41-1.64)	.574
	Grade III-IV aGVHD	.64 (.15-2.68)	.543	.48 (.11-2.06)	.321
	Stage ≥3 skin aGVHD	1.06 (.55-2.03)	.873	.93 (.47-1.83)	.834
	Stage ≥1 gut aGVHD	.68 (.28-1.64)	.392	.55 (.22-1.38)	.205
	Stage ≥1 liver aGVHD	.86 (.12-6.33)	.883	.42 (.05-3.26)	.407
NRM†					
	PES	1.87 (.24-14.4)	.549	1.59 (.20-12.5)	.661
	Grade II-IV aGVHD	1.85 (.58-5.91)	.300	1.53 (.45-5.15)	.493
	Grade III-IV aGVHD	9.46 (3.24-27.6)	**<.001**	46.52 (7.32-295.6)	**<.001**
	Stage ≥3 skin aGVHD	2.15 (.72-6.42)	.171	2.78 (.88-8.76)	.081
	Stage ≥1 gut aGVHD	4.21 (1.46-12.2)	**.007**	3.15 (1.01-9.78)	**.046**
	Stage ≥1 liver aGVHD	17.2 (4.87-60.9)	**<.001**	8.26 (1.74-39.2)	**.008**
Overall mortality‡					
	PES	1.10 (.43-2.81)	.844	1.16 (.44-3.04)	.764
	Grade II-IV aGVHD	.84 (.45-1.56)	.570	.67 (.35-1.32)	.249
	Grade III-IV aGVHD	2.57 (1.13-5.82)	**.024**	2.26 (.90-5.69)	.084
	Stage ≥3 skin aGVHD	.86 (.46-1.62)	.647	.82 (.43-1.56)	.540
	Stage ≥1 gut aGVHD	1.44 (.73-2.83)	.293	1.15 (.57-2.35)	.694
	Stage ≥1 liver aGVHD	3.79 (1.34-10.7)	**.012**	1.56 (.49-4.99)	.449
Treatment failure§					
	PES	.65 (.32-1.30)	.221	.68 (.33-1.38)	.286
	Grade II-IV aGVHD	1.10 (.63-1.91)	.749	.94 (.53-1.69)	.844
	Grade III-IV aGVHD	2.11 (.99-4.50)	.053	1.79 (.79-4.06)	.167
	Stage ≥3 skin aGVHD	1.23 (.71-2.13)	.466	1.18 (.67-2.07)	.569
	Stage ≥1 gut aGVHD	1.26 (.68-2.33)	.461	1.02 (.53-1.95)	.949
	Stage ≥1 liver aGVHD	3.50 (1.37-8.98)	**.009**	1.77 (.63-4.93)	.279

P values in bold type are statistically significant (<.05).

* A high or very high risk by the Refined Disease Risk Index was the sole significant variable for relapse in the multivariate analysis according to each analysis of PES, grade II-IV aGVHD, grade III-IV aGVHD, stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, or stage ≥1 liver aGVHD.

† ABO major/bidirectional mismatch was a significant variable for NRM in the multivariate analysis according to each analysis of PES, grade II-IV aGVHD, grade III-IV aGVHD, stage ≥3 skin aGVHD, or stage ≥1 gut aGVHD. Age ≥45 years at the time of CBT was also a significant variable for NRM in the multivariate analysis according to each analysis of grade III-IV aGVHD or stage ≥3 skin aGVHD.

‡ A high or very high risk by the refined Disease Risk Index was the sole significant variable for overall mortality in the multivariable analysis according to each analysis of PES, grade II-IV aGVHD, stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, or stage ≥1 liver aGVHD. HLA disparity ≥2 was also a significant variable for overall mortality in the multivariate analysis according to analysis of grade III-IV aGVHD. ABO major/bidirectional mismatch was also a significant variable for overall mortality in the multivariate analysis according to analysis of PES.

§ High or very high risk by the Refined Disease Risk Index was the sole significant variable for relapse in the multivariate analysis according to each analysis of PES, grade II-IV aGVHD, grade III-IV aGVHD, stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, or stage ≥1 liver aGVHD.

RESULTS

Patient and Transplantation Characteristics

The characteristics of patients, cord blood units, and transplantations are summarized in Table 1. The median patient age was 44 years (range, 17 to 68 years). Seventy-nine patients (57.2%) were male, and 59 (42.8%) were female. Disease types included acute myelogenous leukemia (AML; n = 73), acute lymphoblastic leukemia (n = 30), myelodysplastic syndrome (n = 16), chronic myelogenous leukemia (n = 8), myeloproliferative neoplasm (n = 1), chronic myelomonocytic leukemia (n = 2), non-Hodgkin lymphoma (n = 5), and adult T cell leukemia (n = 3). The majority of conditioning regimens were MAC (97.8%), and the most common GVHD prophylaxis was cyclosporine A and methotrexate (88.4%). Among the intensified MAC regimens for patients age ≤59 years and those without comorbidities, the most common regimen was 12 Gy total body irradiation, cyclophosphamide 120 mg/kg, and cytosine arabinoside 12 g/m^2 with or without granulocyte colony-stimulating factor in patients with myeloid or lymphoid malignancies, respectively [30-32]. The reduced-intensity MAC regimen included 4 Gy total body irradiation, i.v. busulfan 9.6 mg/kg, fludarabine 180 mg/m^2, and cytosine arabinoside 12 g/m^2 with granulocyte colony-stimulating factor for patients age ≥60 years and those with comorbidities [33]. No patients received antithymocyte globulin or alemtuzumab as a conditioning regimen or GVHD prophylaxis. The median total nucleated cell dose was 2.52×10^7/kg (range, 1.32 to 5.50×10^7/kg), and the median CD34$^+$ cell dose was $.97 \times 10^5$/kg (range, .28 to 2.84×10^5/kg). The median duration of follow-up for survivors after CBT was 75 months (range, 3 to 159 months). There were no significant differences in baseline characteristics between patients who developed PES and those who did not develop PES after CBT, except that a low or intermediate risk by the refined Disease Risk Index was significantly more common in the patients who developed PES (*P*=.006).

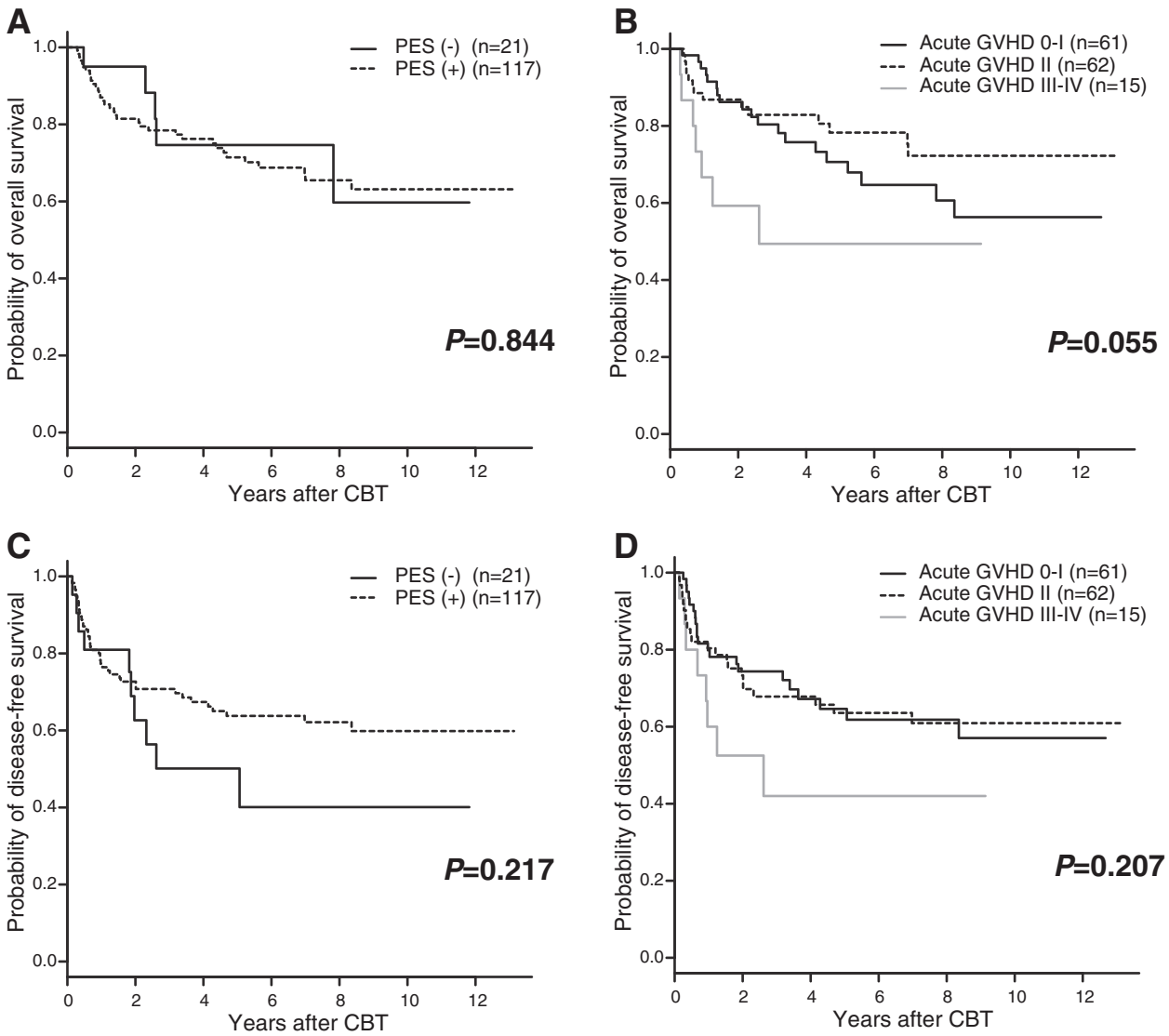

Figure 3. Probabilities of OS (A and B) and DFS (C and D) according to the development of PES and aGVHD.

We also analyzed whether the development of PES could affect the time to neutrophil engraftment and the recovery of absolute lymphocyte count (ALC) in peripheral blood at 1 month and 3 months after CBT. There was no significant difference in the time to neutrophil engraftment between patients who developed PES and those who did not develop PES after CBT, but the ALC at 3 months was significantly higher in the patients who developed PES ($P = .040$) (Table 1).

Incidence of and Risk Factors for PES and aGVHD after CBT

All patients achieved neutrophil recovery, with a median time to neutrophil engraftment of 21 days (range, 15 to 34 days). The cumulative incidences of PES, grade II-IV aGVHD, and grade III-IV aGVHD at 100 days after CBT were 86.8% (95% confidence interval [CI], 78.8% to 91.8%), 57.2% (95% CI, 48.1% to 64.8%), and 10.2% (95% CI, 4.97% to 15.0%), respectively (Figure 1A). The median times to the development of PES, grade II-IV aGVHD, and grade III-IV aGVHD were 14 days (range, 7 to 23 days), 32 days (range, 15 to 78 days), and 33 days (range, 25 to 55 days), respectively. According to the target organs, the cumulative incidences of stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, and stage ≥1 liver aGVHD were 47.8% (95% CI, 38.8% to 55.5%), 24.8% (95% CI, 17.2% to 31.6%), and 5.11% (95% CI, 1.35% to 8.72%), respectively (Figure 1B).

All 117 patients who developed PES had episodes of spiking fever, and the median maximum temperature during the course of PES was 39.5 °C (range, 38.4 °C to 41.8 °C). The percent body surface area of skin rash was 25% to 50% in 25 patients (21.3%) and >50% in 76 patients (64.9%). Sixty-four patients (54.7%) had a weight gain of 3% of baseline body weight. Seven patients (5.9%) developed transaminase elevation without total bilirubin elevation. No patient developed pulmonary edema or central nervous system symptoms.

On evaluation of risk factors for the development of PES, in univariate analysis, high or very high risk by the refined Disease Risk Index was significantly associated with a lower incidence of PES (HR, .66; 95% CI, .45 to .98; $P = .040$). Multivariate analysis identified no significant risk factors for the development of PES, but a trend toward the development of PES was observed for CMV-negative status (HR, 1.80; 95% CI, .99 to 3.25; $P = .050$), high or very high risk by the refined Disease Risk Index (HR, .67; 95% CI, .44 to 1.00; $P = .050$), and ≥2 HLA disparities (HR, 1.54; 95% CI, .93 to 2.55; $P = .087$) (Supplementary Table 1).

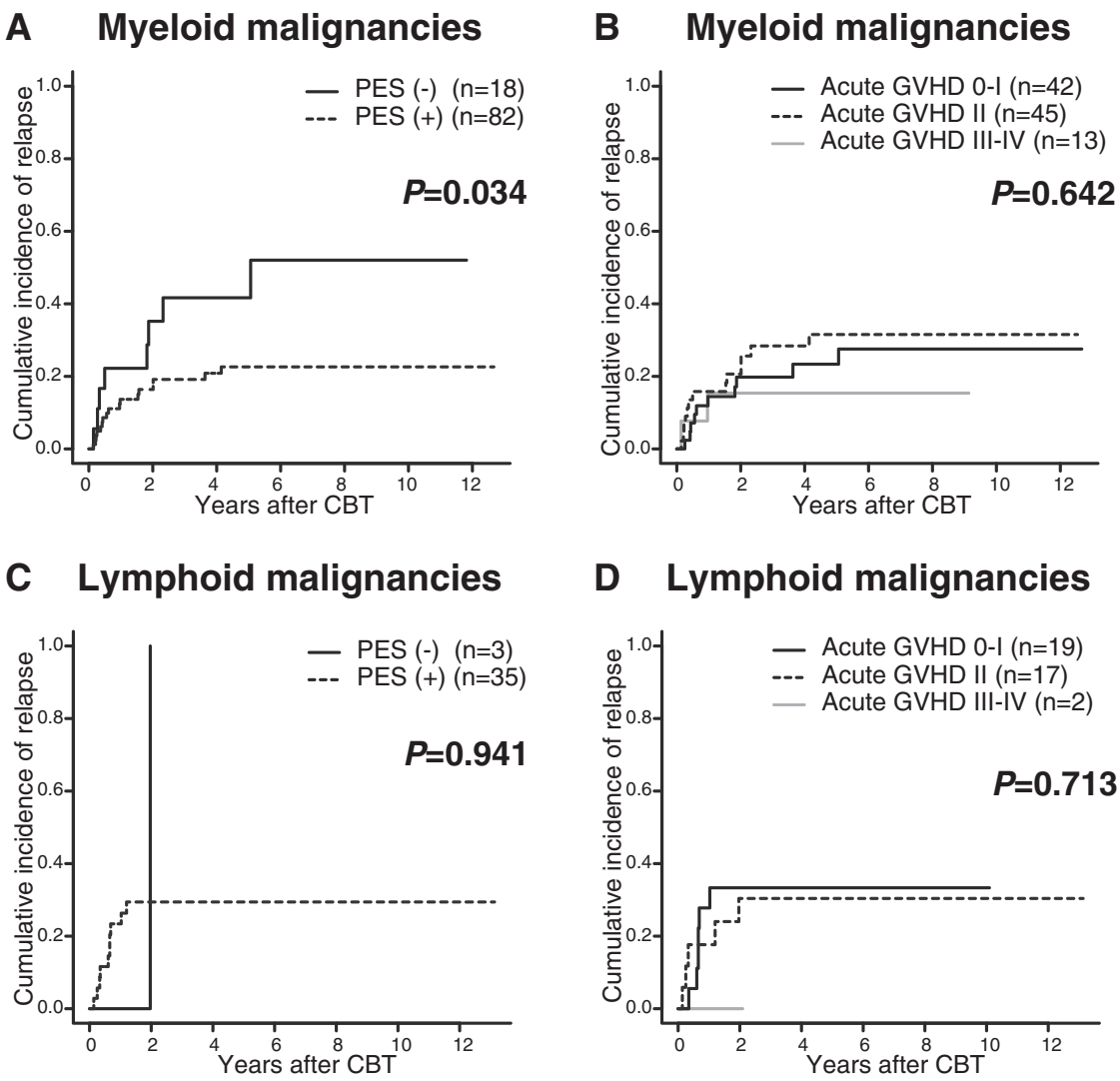

Figure 4. Cumulative incidence of relapse according to the development of PES and aGVHD in patients with myeloid malignancies (A and B) or lymphoid malignancies (C and D).

We also evaluated the relationship between a previous history of PES and development of aGVHD. On univariate and multivariate analyses, previous PES was not a significant risk factor for grade II-IV aGVHD or grade III-IV aGVHD (Supplementary Table 2).

Impact of PES and aGVHD on Relapse and NRM

In the entire cohort, the cumulative incidence of relapse at 5 years was 27.2% (95% CI, 19.7% to 35.2%). In univariate analysis, the development of PES (Gray's test, $P = .056$) or aGVHD (Gray's test for the entire group, $P = .531$) was not significantly associated with relapse (Figure 2A and B). In multivariate analysis, the development of PES (HR, .58; 95% CI, .27 to -1.28; $P = .177$), grade II-IV aGVHD (HR, .82; 95% CI, .41 to 1.64; $P = .574$), and grade III to IV aGVHD (HR, .48; 95% CI, .11 to 2.06; $P = .321$) had no significant effect on relapse (Table 2).

The cumulative incidence of NRM at 5 years was 10.0% (95% CI, 5.4% to 16.2%). In univariate analysis, the development of aGVHD was significantly associated with NRM (Gray's test for the entire group, $P < .001$), but the development of PES was not (Gray's test, $P = .431$) (Figure 2C and D). In multivariate analysis, the development of grade III-IV aGVHD (HR, 46.52; 95% CI, 7.32 to 295.60; $P < .001$) remained independent factors for higher NRM, but the development of PES (HR, 1.59; 95% CI, .20 to 12.50; $P = .661$) and grade II-IV aGVHD (HR, 1.53; 95% CI, .45 to 5.15; $P = .493$) had no significant effect on NRM. According to the target organs, stage ≥ 1 gut aGVHD (HR, 3.15; 95% CI, 1.01 to 9.78; $P = .046$) and stage ≥ 1 liver aGVHD (HR, 8.26; 95% CI, 1.74 to 39.20; $P = .008$) were significantly associated with a higher incidence of NRM in multivariate analysis (Table 2).

Impact of PES and aGVHD on OS and DFS

In the entire cohort, the probability of OS at 5 years was 71.7% (95% CI, 62.5% to 79.0%). In univariate analysis, the development of PES (log-rank test, $P = .844$) or aGVHD (log-rank test for the entire group, $P = .055$) was not significantly associated with OS (Figure 3A and B). In multivariate analysis, the development of PES (HR, 1.16; 95% CI, .44 to 3.04; $P = .764$) and grade II-IV aGVHD (HR, .67; 95% CI, .35 to 1.32; $P = .249$) had no significant effect on overall mortality, but a trend toward higher overall mortality was observed in patients who developed grade III-IV aGVHD (HR, 2.26; 95% CI, .90 to .69; $P = .084$) (Table 2).

Table 3
Univariate and Multivariate Analyses of Transplantation Outcomes According to the Development of PES and aGVHD in AML

Outcome		Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Relapse*					
	PES	.32 (.12-.83)	**.020**	.24 (.07-.88)	**.032**
	Grade II-IV aGVHD	1.13 (.45-2.84)	.794	.98 (.35-2.71)	.962
	Grade III-IV aGVHD	.91 (.21-3.94)	.903	.78 (.18-3.47)	.744
	Stage ≥3 skin aGVHD	1.25 (.52-3.02)	.620	1.05 (.41-2.72)	.918
	Stage ≥1 gut aGVHD	.70 (.23-2.09)	.522	.48 (.15-1.54)	.220
	Stage ≥1 liver aGVHD	2.00 (.26-15.4)	.503	.96 (.11-8.38)	.974
Overall mortality†					
	PES	.90 (.27-3.06)	.872	.87 (.20-3.90)	.860
	Grade II-IV aGVHD	.74 (.32-1.69)	.470	.60 (.25-1.46)	.264
	Grade III-IV aGVHD	1.16 (.34-3.90)	.814	.93 (.23-3.68)	.916
	Stage ≥3 skin aGVHD	.75 (.33-1.72)	.500	.74 (.31-1.75)	.495
	Stage ≥1 gut aGVHD	.93 (.36-2.35)	.872	.62 (.22-1.75)	.367
	Stage ≥1 liver aGVHD	1.58 (.21-11.8)	.654	.63 (.07-5.32)	.669
Treatment failure†					
	PES	.45 (.18-1.12)	.086	.29 (.09-.98)	**.046**
	Grade II-IV aGVHD	1.01 (.46-2.22)	.975	.90 (.39-2.07)	.800
	Grade III-IV aGVHD	1.45 (.50-4.19)	.500	1.36 (.44-4.26)	.596
	Stage ≥3 skin aGVHD	1.10 (.52-2.34)	.804	1.07 (.48-2.37)	.876
	Stage ≥1 gut aGVHD	.94 (.40-2.24)	.895	.66 (.26-1.69)	.381
	Stage ≥1 liver aGVHD	3.29 (.75-14.3)	.113	1.53 (.31-7.52)	.599

P values in bold type are statistically significant (<.05).

* High or very high risk by the refined Disease Risk Index was the sole significant variable for relapse in the multivariate analysis according to each analysis of PES, grade II-IV aGVHD, grade III-IV aGVHD, stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, or stage ≥1 liver aGVHD.

† Except for the development of PES as a significant variable for treatment failure, there were no significant variables for overall mortality and treatment failure in the multivariate analysis according to each analysis of PES, grade II-IV aGVHD, grade III-IV aGVHD, stage ≥3 skin aGVHD, stage ≥1 gut aGVHD, or stage ≥1 liver aGVHD.

The probability of DFS at 5 years was 61.4% (95% CI, 52.1% to 69.4%). In univariate analysis, the development of PES (log-rank test, $P = .217$) or aGVHD (log-rank test for the entire group, $P = .207$) was not significantly associated with DFS (Figure 3C and D). In multivariate analysis, the development of PES (HR, .68; 95% CI, .33 to 1.38; $P = .286$), grade II-IV aGVHD (HR, .94; 95% CI, .53 to 1.69; $P = .844$), and grade III-IV aGVHD (HR, 1.79; 95% CI, .79 to 4.06; $P = .167$) had no significant effect on the treatment failure of DFS (Table 2).

Subset Analysis of Disease Type

Given that the strength of the GVL effect may vary according to underlying disease type, we performed subgroup analyses stratified by disease type. In univariate analysis, the development of PES was significantly associated with decreased relapse in myeloid malignancies (Gray's test, $P = .034$), but not in lymphoid malignancies (Gray's test, $P = .941$) (Figure 4A-C). The development of aGVHD had no significant effect on relapse in myeloid and lymphoid malignancies (Figure 4B and D). In multivariate analysis, the development of grade III-IV aGVHD alone was significantly associated with inferior OS and DFS in myeloid malignancies (data not shown).

In the patients with AML, the development of PES was also significantly associated with decreased relapse in univariate analysis (Gray's test, $P = .016$), but aGVHD was not (Gray's test, $P = .789$) (Figure 5A and B). The development of PES was associated with better DFS in univariate analysis, but the association was not significant (log-rank test, $P = .077$) (Figure 5C and D). In multivariate analysis, the development of PES had a significant effect on decreased relapse and better DFS in AML (Table 3).

DISCUSSION

Previous studies have evaluated the impact of PES on outcomes after CBT [1-7]. These studies demonstrated that the development of PES was significantly associated with the development of aGVHD [3,4,6] and chronic GVHD [3], but not with relapse [2,5,6], NRM [1,2,4,6], or OS [1,3-7]. However, these studies included both children and adults who received both single and double cord blood units. Meanwhile, several other studies also demonstrated that aGVHD was associated with a significantly decreased rate of relapse after CBT, indicating that CBT could confer a meaningful GVL effect accompanied by GVHD [15,23-25]. However, among these studies, the development of aGVHD began at 3 to 7 days after CBT [23-25], indicating that some cases of aGVHD might have included PES in the evaluation of the effect of aGVHD on outcomes after CBT. Thus, in the present study, we retrospectively compared the effects of PES and aGVHD on outcomes after single-unit CBT in 138 adult patients with hematologic malignancies. Our data clearly demonstrate that the development of grade III-IV aGVHD was significantly associated with a higher incidence of NRM after single-unit CBT, but PES and grade II-IV aGVHD were not. Interestingly, the development of PES, but not of aGVHD, had a significant impact on the decreased rate of relapse of AML after single-unit CBT in adults. These data suggest that the GVL effects might be induced by the development of PES after single-unit CBT, particularly in patients with AML.

Whether different immunologic profiles are involved in the development of PES and aGVHD remains unclear. Matsuno

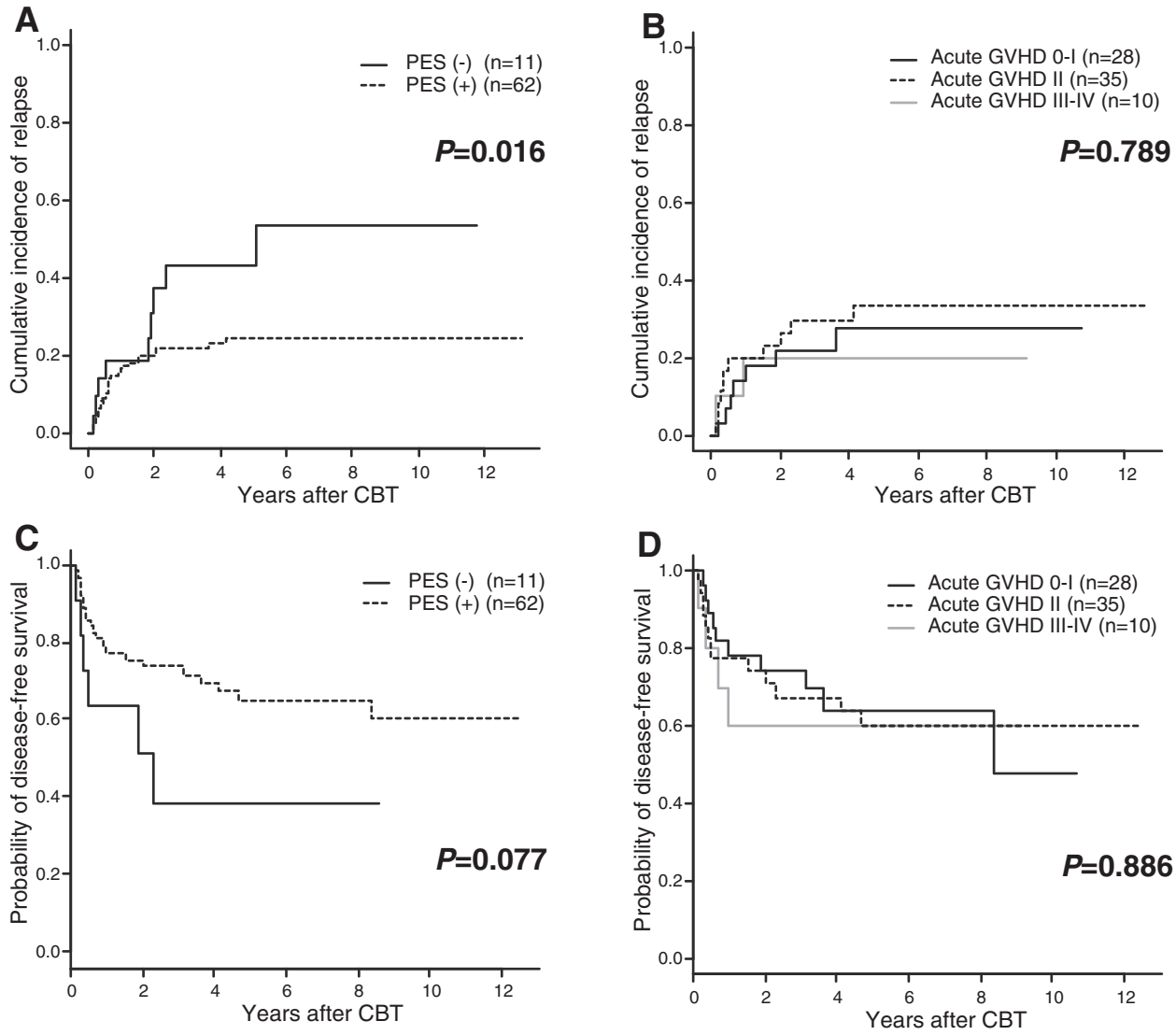

Figure 5. Cumulative incidence of relapse (A and B) and probability of DFS (C and D) according to the development of PES and aGVHD in patients with AML.

et al [10] observed donor-derived memory T cell expansion in patients who developed PES after CBT. Our previous study demonstrated a similar behavior of cytokine profiles and T cell subsets in both PES and aGVHD after CBT [11]. Moreover, the addition of methotrexate (MTX) to a calcineurin inhibitor for GVHD prophylaxis reportedly reduced the incidence of PES and aGVHD after CBT [8,34,35], although our study failed to show significant associations between the addition of MTX for GVHD prophylaxis and the development of PES ($P = .640$). In our study, the addition of MTX for GVHD prophylaxis also did not affect the time to neutrophil engraftment ($P = .291$) or the recovery of ALCs in peripheral blood at 1 month ($P = .124$) or 3 months ($P = .286$) after CBT. These findings suggest the possibility that a similar mechanism might be involved in the development of PES and aGVHD. Thus, PES might be distinguished from aGVHD based solely on the timing of onset, and an early immune reaction before neutrophil engraftment might contribute to the GVL effect after CBT.

Sensitivity to the GVL effect might differ depending on disease type, donor source, or HLA mismatch. Our data show that myeloid malignancies were more sensitive to an early immune reaction than lymphoid malignancies. This is consistent with previous reports of a more protective relapse effect associated with GVHD [16,17,21] and greater effectiveness of donor lymphocyte infusion for myeloid malignancies than for lymphoid malignancies [36]. However, in our series, the number of patients with diseases other than AML was too small to allow for such an evaluation. In addition, exploitation of an HLA mismatch to reduce relapse after HLA-mismatched CBT may be a attractive strategy [37]. Several studies have demonstrated associations between lower relapse incidence and a specific HLA locus mismatch [38,39] and an increased number of HLA mismatches [40,41] after CBT. Moreover, recent studies have suggested that the GVL effects after CBT might be stronger than those after matched related or unrelated conventional adult donor HCT [42,43]. Although our study could not confirm whether HLA disparities affected the decreased relapse incidence after CBT, further studies are warranted to determine the enhancement of the GVL effect accompanied by HLA-mismatched CBT.

In our cohort, the development of PES did not contribute to NRM or mortality after CBT, which is consistent with previous studies [1-7]; however, grade III-IV aGVHD, stage ≥ 1 gut aGVHD, and stage ≥ 1 liver aGVHD significantly contributed to

NRM, although grade II-IV aGVHD did not. Although Murata et al [44] reported a significantly higher response rate to systemic corticosteroids for grade II-IV aGVHD in CBT compared with conventional adult donor HCT, gut or liver involvement of GVHD has been associated with lower response rates to systemic corticosteroids after conventional adult donor HCT [44-46]. Moreover, in our cohort, 43 of the 62 patients (69%) who developed grade II-IV, stage 3 skin aGVHD were not treated with systemic corticosteroids. Taken together, these findings indicate that gut or liver involvement of aGVHD itself and immunosuppressive treatment for aGVHD may contribute to increased NRM.

Our study has several limitations. First, this was a retrospective analysis at a single institution in Japan with a small number of patients, and thus our results should be interpreted with caution. Further studies with larger numbers of patients are warranted to confirm the impact of PES and aGVHD on transplantation outcomes in adult patients after CBT. Second, although previous studies have demonstrated that HLA-DPB1 mismatch [47], killer cell immunoglobulin-like receptor ligand incompatibility [48], and noninherited maternal antigen match [49] are associated with a lower relapse rate after single-unit CBT, we were unable to use these data owing to insufficient data in the medical records. Third, double-unit CBT has been reported to be significantly associated with a lower relapse rate compared with single-unit CBT owing to an enhanced GVL effect [50-52], although prospective studies have shown comparable relapse rates with single- and double-unit CBT [53,54]. Thus, a stronger GVL effect using double-unit CBT is a matter for future investigation.

In conclusion, our data demonstrate that grade III-IV aGVHD, particularly with gut or liver involvement, contributed to a higher NRM, resulting in higher overall mortality, but PES and grade II-IV aGVHD did not. PES was associated with a reduced relapse rate in myeloid malignancies and provided better DFS in patients with AML, indicating that the early immune reaction before neutrophil engraftment may serve a unique GVL effect after single-unit CBT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.02.007.

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