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Pre-Engraftment Syndrome after Unrelated Cord Blood Transplantation: A Predictor of Engraftment and Acute Graft-versus-Host Disease

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A B S T R A C T

Pre-engraftment syndrome (PES) is poorly characterized, and its clinical significance and the prognostic impact after unrelated cord blood transplantation (CBT) are unclear. To address these issues, we retrospectively analyzed the incidence, risk factors, and clinical outcomes of PES in unrelated CBT recipients. Data of 381 patients who received unrelated CBT from 18 medical centers in Korea were reviewed. PES was defined as unexplained fever >38.3°C not associated with infection, and/or unexplained skin rash with or without evidence of fluid retention before neutrophil recovery. PES developed in 102 patients (26.8%) at a median of 7 days after CBT. Of these patients, 74 patients (72.5%) received intravenous corticosteroid at a median dose of 1 mg/kg/day, and of these, 95% showed clinical improvement. Risk factors for developing PES included low risk disease, myeloablative conditioning, graft-versus-host disease (GVHD) prophylaxis without methotrexate or corticosteroid, and >5.43 x 10^7/kg infused nucleated cells. Absence of PES was one of the risk factors for graft failure in multivariate analysis. The cumulative incidence of grade II to grade IV acute GVHD by 100 days after CBT was higher in patients with PES than in those without PES (56.0% versus 34.4%, $P < .01$). PES was not associated with chronic GVHD, treatment-related mortality, relapse, or overall survival. PES seems to be common after CBT and may be associated with enhanced engraftment without significant morbidity.

INTRODUCTION

Cord blood transplantation (CBT) is a promising approach in patients for whom a sibling or matched unrelated donor is not available [1-3]. Post-transplantation immune disorders, including pre-engraftment syndrome (PES), engraftment syndrome, and acute graft-versus-host disease (GVHD) are problematic in CBT. The complex and intricate pathophysiology of post-transplantation immune disorders is a consequence of interactions between the donor and host innate and adaptive immune responses. PES, a clinical entity of unknown pathogenesis, has been described in patients receiving CBT [4-6]. Although a uniform definition is lacking, PES is commonly characterized by noninfectious fever and various other clinical findings before neutrophil engraftment, including skin rash, pulmonary infiltrates, diarrhea, jaundice, or weight gain. To date, PES is an entirely clinical entity with no known pathognomonic histopathologic changes or biochemical markers. Kishi et al. [4] were the first to report a pre-engraftment immune reaction, which occurred in 35 of 45 adult recipients of reduced-intensity conditioning CBT. However, PES still remains poorly characterized and its clinical significance and the prognostic impact after CBT are unclear. To address these issues, we retrospectively analyzed the incidence, risk factors, and clinical outcomes of PES in unrelated CBT recipients enrolled in a multicenter CBT trial.

MATERIALS AND METHODS

Patient and Transplantation Characteristics

The clinical data of 381 cases of unrelated CBT performed at 18 transplantation centers in Korea between 2000 and 2010 were reviewed

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retrospectively. Details about CBT from a given institution were registered in the Korean Cord Blood Transplantation Registry, and the data were verified by comparing the reports with the primary data sources. This study was approved by the Institutional Review Board of Hanyang University Medical Center. Informed consent was obtained from the patients and/or guardians before CBT. The characteristics of patients and transplantation are listed in Table 1. Reduced-intensity regimens were generally defined as reported previously [7,8].

Acute leukemia in first complete remission (CR), chronic myelogenous leukemia in the chronic phase, malignant lymphoma in CR, multiple myeloma in CR, myelodysplastic syndrome in refractory anemia, and nonmalignant diseases were defined as low risk, whereas all other malignant diseases were considered high risk. Patients who underwent previous hematopoietic stem cell transplantation were also classified in the high-risk group.

Definitions

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count >0.5 x 10^9/L. Graft failure (GF) comprised 2 clinical entities: (1) failure to achieve an absolute neutrophil count of 0.5 x 10^9/L and marrow hypoplasia for fewer than 60 days with or without the existence of donor type hematopoiesis, and (2) complete loss of donor-type hematopoiesis at any time after transplantation. Late GF was defined as the loss of the graft during follow-up. Both acute and chronic GVHD were graded according to the previously published criteria [9,10]. Cytomegalovirus (CMV) infection was defined as positive pp65 CMV antigenemia, defined as ≥1 antigen-positive cell in a single slide. CMV disease was defined as end-organ disease, such as pneumonia, gastrointestinal disease, hepatitis, etc., with a documented CMV etiology [11]. Transplantation-related mortality (TRM) was defined as any death not the result of relapse, progression, or persistence of the underlying disease.

Following the criteria of Patel et al. [12], PES was defined as unexplained fever >38.3°C not associated with documented infection and/or an unexplained erythematous skin rash resembling that of acute GVHD, with either the fever or the rash occurring before or at neutrophil recovery. Specifically, fever attributed to PES was not associated with any clinical evidence of infection, with patients having both a negative infectious disease workup and a continued lack of response to broad-spectrum antimicrobial agents. Erythematous skin rash attributed to PES was not associated with any clinical suspicion of drug allergy. Weight gain was defined as a 3% increase in body weight between the day of CBT and the onset of PES. Noninfectious diarrhea was defined as passage of watery stools more than twice a day for at least 3 consecutive days with no evidence of infectious etiology. Treatment of the PES was at the physicians' discretion.

Table 1
Characteristics of Patients and Cord Blood Transplantation

Variables	Number of Patients (N = 381)
Age, years, median (range)	7.2 (0.3-65.4)
Weight, kg, median (range)	23.4 (6.1-89.7)
Gender, n	
M:F	221:160
Primary disease, n	
Malignant disease	314
Acute lymphoblastic leukemia	127
Acute myeloid leukemia	135
Myelodysplastic syndrome	13
Chronic myelogenous leukemia	20
Nonmalignant disease	67
Aplastic anemia	11
Others	56
Previous transplantation, n	33
Autologous:allogeneic	16:17
Preparative regimen, n	
Myeloablative:reduced-intensity	261:120
In vivo T cell depletion, n	
ATG	259
Other than ATG	14
None	108
CMV serostatus (n = 294)	
Positive:Negative	269:25
No. of infused nucleated cells, median (range), 10^7/kg	5.10 (0.27-104.4)
No. of infused $CD34^+$ cells, median (range), 10^5/kg	2.08 (0.08-61.6)
No. of donors	
One:Two	225:156
HLA match (A/B/DR, antigen level, lowest)*, n	
6/6	27
5/6	176
4/6	127
≤3/6	48

ATG indicates antithymocyte globlulin; CMV, cytomegalovirus.
* HLA match equals the poorest matched unit in recipients of 2 cord blood units.

Statistical Analysis

Categorical variables were compared using the chi-square test and continuous variables using the Mann-Whitney *U* test. The cumulative incidence rates of neutrophil recovery, GVHD, infections, relapse, and TRM were calculated and compared using Gray's method. For neutrophil engraftment, the competing risks were autologous recovery, infusion of a backup graft, or death. GF and death were the competing events for GVHD, whereas relapse was the competing event for TRM. Prognostic factors for the occurrence of PES, GF, GVHD, and survival were evaluated using Cox regression analysis. Overall survival (OS) was the time between day 0 of cord blood infusion and death from any cause, and living patients were censored at last follow-up. Survival was calculated by the Kaplan-Meier method and the difference in survival rates based on PES classification was determined using the log-rank test. Factors significant at the 0.1 level on univariate analysis were considered for multivariate analyses using backward elimination. Two-sided *P* values less than .02 were considered significant. All analyses were conducted using SPSS version 18.0 software and R version 2.10.1.

RESULTS

Incidence and Clinical Characteristics of PES

Of 381 patients, 102 patients (26.8%) fulfilled the diagnostic criteria for PES. Fever of otherwise unexplained etiology was present in 93.9% of transplantation patients diagnosed with PES, an erythematous skin rash in 81.8%, C-reactive protein elevation in 52.8%, diarrhea in 29.3%, weight gain >3% of baseline body weight in 27.3%, pulmonary edema in 13.3 %, and central nervous system symptoms in 8.0%. Hypoxia due to noncardiogenic pulmonary edema was identified in 8.7% of the PES patients. The first manifestation of PES was an unexplained fever with a median onset of 7 days (range, 3 to 41) after CBT, followed by skin rashes at day 10 (range, 2 to 34). The onset of PES occurred at a median of 11 days before neutrophil engraftment and was not significantly associated with any clinical variables. PES was also observed in 6 patients who had never engrafted.

Histologic examinations of skin were conducted in 10 patients (9.8%). Common findings were perivascular lymphocytic infiltration (n = 5) and vacuolization in the basal layer (n = 3).

Of all patients who developed PES, 28 patients (27.5%) showed clinical improvement with only supportive care, such as fluid restriction and the use of diuretics. Seventy-four patients (72.5%) received intravenous corticosteroid at a median dose of 1 mg/kg/day (range, 0.1 to 10) for a median of 7 days (range, 2 to 58). Compared to the patients whose PES resolved without corticosteroid therapy, those who received corticosteroid showed significantly earlier onset of fever (7 days versus 11 days, $P < .01$) and a higher incidence of weight gain >3% of baseline body weight (35.7% versus 10.7%, $P = .01$). Approximately 95% of the patients who received corticosteroid therapy because of PES showed clinical improvement. Three patients were steroid-refractory. These 3 patients died of PES itself, acute GVHD, or infection within 60 days of CBT.

Risk Factors for PES

Table 2 outlines the patient demographics and graft characteristics of the 102 patients with PES and the 279

patients who did not meet the PES criteria. In a multivariate analysis, low-risk disease, myeloablative conditioning, GVHD prophylaxis without methotrexate or corticosteroid, and infused total nucleated cells (TNC) $>5.43 \times 10^7$/kg were significant risk factors for PES (Table 3).

PES and Engraftment

GF occurred in 78 patients (20.5%): primary GF in 49 patients, late GF in 7 patients, and autologous recovery in 22 patients. Of the 102 patients with PES, 95 patients showed successful engraftment. The median time to neutrophil engraftment was 18 days (range, 7 to 84 days) for patients without PES and 19 days (range, 9 to 92) for those with PES. In a multiple logistic regression analysis, absence of PES was a significant risk factor for GF (RR, 5.50; 95% CI, 2.24 to 13.49, $P < .01$). Among the patients who experienced PES, there was no significant difference in the incidence of GF depending on steroid use ($P = .34$).

PES and GVHD

The cumulative incidence of grade II to grade IV acute GVHD in the entire group was 40.2%. PES was a significant risk factor for grade II to grade IV acute GVHD in multivariate analysis (RR, 1.84; 95% CI, 1.11 to 3.06, $P = .02$) after adjusting for age, sex, weight, conditioning regimen, HLA match, number of donors, and total cell counts.

The cumulative incidence rates of grade II to grade IV acute GVHD by 100 days after CBT in patients with and without PES were 56.0% and 34.4%, respectively ($P < .01$). Among the patients with PES, there was a negative correlation between the day of PES development and the incidence of acute GVHD ($P = .04$). That is, those in whom PES occurred earlier had a higher incidence of grade II to grade IV acute GVHD. Patterns of organ involvement were similar in PES and acute GVHD. Grade II to grade IV acute GVHD skin involvement was more frequent in patients with skin manifestations during the course of PES than in those without such manifestations (RR, 2.97; 95% CI, 1.03 to 8.51, $P = .04$). Patients who showed hyperbilirubinemia during the period of PES suffered a significantly higher incidence of acute liver GVHD ≥grade II (RR, 4.63; 95% CI, 1.36 to 15.7, $P = .01$). However, the incidence of acute gastrointestinal GVHD ≥grade II did not differ significantly in patients with and without diarrhea during the clinical course of PES.

The cumulative incidence of chronic GVHD was 20.9% by one year after CBT. The incidence and severity of chronic GVHD did not differ significantly in patients with and without PES.

PES and Transplantation Outcomes

Patients with PES had a higher incidence of early bacterial infection (≤28 days after CBT) than those without PES (21.0% versus 12.6%, $P = .05$). The incidence of early bacterial

Table 2
Comparison of Transplantation Characteristics in Patients with and Without Pre-Engraftment Syndrome (PES)

Variables	PES (n = 102)	No PES (n = 279)	Univariate *P*
Age, year, median (range)	6.3 (0.5-48.8)	7.5 (0.5-65.4)	.07
Weight, kg, median (range)	19.1 (6.2-73.0)	24.5 (6.1-89.7)	<.01
Double unit CBT, n	51	105	.03
Disease category, n			.36
Malignant disease	81	233	
Nonmalignant disease	21	46	
Salvage CBT, n	3	30	.014
Disease risk, n			<.01
High risk	22	121	
Low risk	79	151	
HLA match (A/B/DR, antigen level, lowest)*, n			.06
5-6/6	48	155	
4/6	38	89	
≤3/6	15	33	
Conditioning regimen, n			<.01
Myeloablative	86	175	
Reduced-intensity	16	104	
In vivo T cell depletion, n			<.01
Yes	62	211	
No	40	68	
Total body irradiation in conditioning, n			.54
Yes	36	90	
No	66	189	
GVHD prophylaxis with MTX, n			.04
Yes	4	30	
No	98	249	
GVHD prophylaxis with steroid, n			<.01
Yes	20	117	
No	82	162	
CMV serostatus (n = 294)			.08
Positive	65	204	
Negative	2	23	
No. of infused nucleated cells, (10^7/kg), median (range)	6.28 (1.45-31.3)	4.73 (0.27-104.4)	<.01
>5.43	58	110	
≤5.43	33	155	
No. of infused CD34$^+$ cells, (10^5/kg), median (range)	2.55 (0.4-26.9)	1.84 (0.08-61.6)	<.01
>1.60	76	149	
≤1.60	20	111	

CBT indicates cord blood transplantation; GVHD, graft versus host disease; MTX, methotrexate; CMV, cytomegalovirus.
* HLA match equals the poorest matched unit in recipients of 2 cord blood units.

Table 3
Significant Risk Factors for Pre-Engraftment Syndrome in the Multivariate Analysis

Variables	HR	95% CI	*P* Value
Disease risk			.02
High risk	1		
Low risk	2.00	1.01-3.64	
Conditioning regimen			<.01
Reduced-intensity	1		
Myeloablative	6.14	3.28-12.24	
GVHD prophylaxis with MTX			.03
Yes	1		
No	5.35	1.16-24.68	
GVHD prophylaxis with steroid			<.01
Yes	1		
No	5.01	2.63-9.54	
No. of infused nucleated cells, (10^7/kg)			<.01
≤5.43	1		
>5.43	2.08	1.21-3.59	

HR indicates hazard ratio; GVHD, graft-versus-host disease; MTX, methotrexate.

infection was not affected by the use of steroid to treat the PES ($P = .07$). No patients who received 1 mg/kg/day of intravenous corticosteroid for 7 days (median dose and duration of PES treatment demonstrated in this study) experienced early bacterial infection. However, higher dose ($P < .01$) and prolonged use ($P = .04$) of steroid were associated with early bacterial infection.

A higher incidence of CMV infection was found in patients with PES than in those without PES (57.4% versus 42.5%, $P = .01$). However, the prevalence of CMV disease did not differ between the 2 groups ($P = .27$).

After a median follow-up of 74 months, the 5-year OS of all patients was 49.2% ± 2.7%. In a multivariate analysis, factors associated with poor survival included total body irradiation-based conditioning regimen, CMV disease and low infused $CD34^+$ cell dose (Table 4). OS did not differ depending on the presence or absence of PES after CBT (Figure 1). Likewise, TRM by 3 years after CBT was not significantly different in the 2 groups (Figure 2). In patients with malignancies, the cumulative incidence of relapse 3 years after CBT was 31.7% in patients without PES and 16.9% in patients with PES (Figure 3). In summary, we could not identify any impact of the occurrence of PES on OS, TRM, or relapse.

DISCUSSION

To our knowledge, this is the largest study to date evaluating PES occurring after CBT. Many studies have reported that CBT recipients often develop immune reactions before neutrophil engraftment [6,12,13]. Recently, several groups

Table 4
Significant Prognostic Factors for Overall Survival in the Multivariate Analysis

Variables	HR	95% CI	*P* Value
Total body irradiation in conditioning			<.01
No	1		
Yes	1.74	1.27-2.38	
CMV disease			.01
No	1		
Yes	1.60	1.09-2.34	
No. of infused $CD34^+$ cells, (10^5/kg)			.02
>1.60	1		
≤1.60	1.23	1.00-1.58	

HR indicates hazard ratio; CMV, cytomegalovirus.

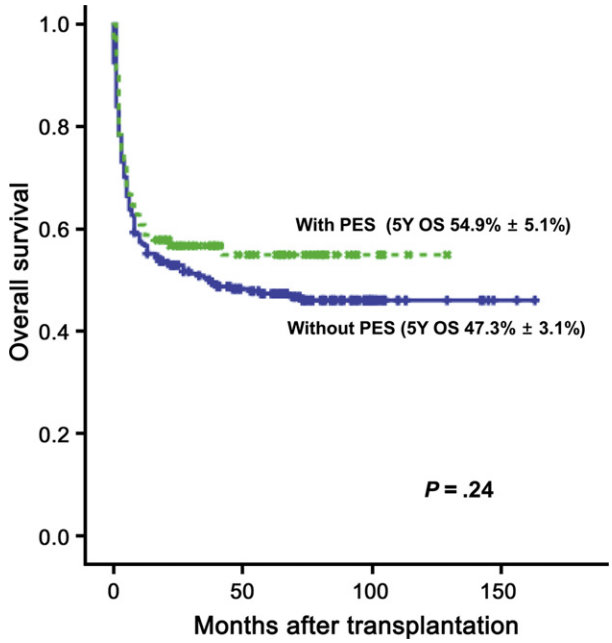

Figure 1. Overall survival in patients with and without pre-engraftment syndrome.

classified post-CBT immune reactions in relation to the time to neutrophil engraftment as follows: PES or pre-engraftment immune reactions, engraftment syndrome, and acute GVHD (postengraftment immune reaction) [4,6,14]. The incidence of PES following CBT has been reported as 20% to 70% [4,13]. Although PES is a clinical entity described in patients receiving CBT, Lee et al. [5] have reported that its incidence does not depend on the source of the graft. In our study, PES occurred in 26.8% of patients. Onset of PES in our CBT patient series was a median of 11 days before neutrophil recovery, clearly justifying the term "pre-engraftment". Interestingly, PES also developed in some

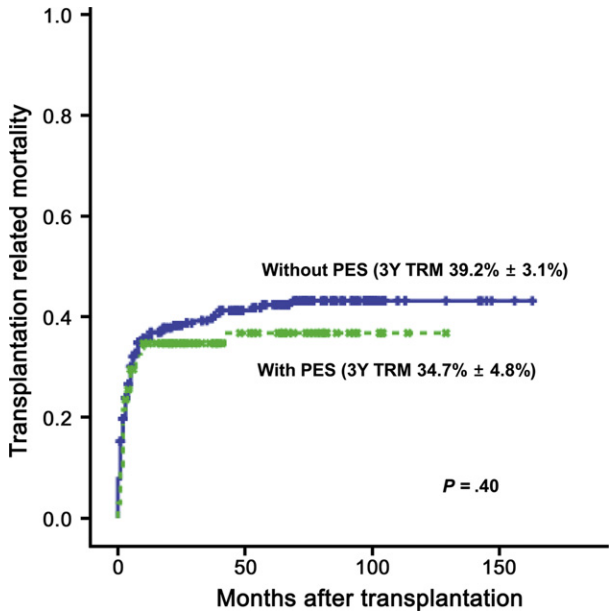

Figure 2. Treatment related mortality in patients with and without pre-engraftment syndrome.

patients who had not achieved engraftment, suggesting that the mechanism of PES differs from that of engraftment syndrome or GVHD. Furthermore, the median time to neutrophil engraftment did not differ in patients with and without PES. On the basis of these findings and other studies [15,16], we suggest that PES is induced by cytokine storms due to cytokines that already existed in the donor cord blood cells. That is, PES may be a response of recipients to the infused cord blood cells, whereas the known mechanism of GVHD involves an interaction of engraftment-driven lymphocytes with recipient tissues.

In our analysis, high numbers of $CD34^+$ cells/kg and TNC/kg were significantly associated with PES. In multivariate analysis, high TNC count remained significant, but number of $CD34^+$ cells did not. This finding also supports a role of CB mononuclear cells such as lymphocytes in the development of PES.

As shown in this study, the fact that GVHD prophylaxis can influence the incidence of PES suggests that PES may be caused by immunologic reactivity, and this interpretation is supported by the fact that PES patients who were treated responded very well to intravenous corticosteroid. Furthermore, steroid prophylaxis decreased the incidence of PES almost 5-fold in our study. The beneficial effects of steroid therapy may derive from its immunosuppressive effect on cytokine reactions as well as its anti-inflammatory action. PES supposedly develops in conjunction with cytokine storms or proliferation of naïve T cells in cord blood [4,17].

Parenchymal tissue may be sensitized to the potentially toxic effects of transplantation conditioning by previous therapy, which would tend to aggravate inflammation. Immunological phenomena during the pre-engraftment period may be influenced by proinflammatory cytokines produced soon after the conditioning therapy. At the time of transplantation, patients with advanced disease may be relatively depleted of important cellular populations (eg, tissue macrophages, B cells) that contribute to inflammation during transplantation and thus may be at decreased risk of producing inflammatory cytokines during conditioning [18,19]. This concept is supported by the evidence for lower post-transplantation inflammatory cytokine levels in the recipients pretreated with chemotherapy [18] and the enhanced inflammatory cytokine levels seen in chemotherapy-naïve patients undergoing transplantation [20,21]. Therefore, low-risk patients with enhanced inflammatory cytokine levels could provoke more severe inflammatory response to the infused CB cells than high-risk patients. This hypothesis supports our finding that patients with low-risk diseases are at risk of PES.

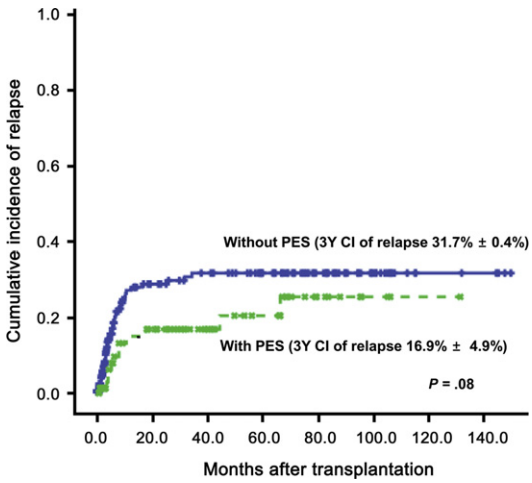

Figure 3. Cumulative incidence of relapse in patients with and without pre-engraftment syndrome.

In our study, patients who did not experience PES had a higher incidence of GF than patients with PES. Wang et al. [22] similarly reported that the cumulative incidence of engraftment in patients with PES was 91.9% compared with 76.7% in those without PES. Frangoul et al. [6] also reported that the cumulative incidence of neutrophil engraftment in patients with PES was 84% compared with 77% in those without PES. Although the rates of engraftment in patients with PES in these 2 studies were higher, the corresponding *P* values did not reach statistical significance, perhaps due in part to small sample sizes or baseline differences in transplantation characteristics. The complex interplay of the bone marrow microenvironment with the cytokines of the interacting cells, which occurs during PES, may be associated with neutrophil regeneration during the early post-transplantation period [14,23]. Recently, Takahashi et al. [15] found that the levels of various proinflammatory cytokines such as TNFα, IL-1β, IL-6, and IFNγ, are strongly interrelated. Interestingly, concentrations of IL-8, which is integral to neutrophil regeneration and function [24,25], were highly correlated with levels of proinflammatory cytokines and G-CSF. Furthermore the levels of these proinflammatory cytokines were also strongly correlated with those of growth factors (eg, G-CSF and GM-CSF). In addition, accumulating evidence supports an essential role of IL-6 in the development, differentiation, and regeneration of stem cells [26,27]. These findings support the hypothesis that PES affects engraftment by an indirect mechanism.

We observed a close relation between PES and the development of grade II to grade IV acute GVHD but not chronic GVHD. This association raises the possibility that PES represents an early form of acute GVHD. The propensity of acute GVHD in PES to target organs with environmental interfaces and thus potentially with previously damaged organs may not be coincidental. Since cytokine release and hyperinflammation are hallmarks of both PES and acute GVHD, one may speculate that there is some overlap between the 2 syndromes. However, the association of PES with chronic GVHD seems different from its relationship with acute GVHD. It appears that autoantibodies and B cells contribute to chronic GVHD process through the simulation of fibrosis [28,29]. Chronic GVHD itself does not appear to be proinflammatory state, unlike acute GVHD and PES. Therefore, one would not anticipate any significant association between PES and chronic GVHD.

We observed that PES was associated with increased rates of early bacterial infection and CMV infection. The increased risk of clinical infection in PES may be associated with cytokine dysregulation and impaired immunomodulatory function. In addition, immunosuppressive therapy may increase the risk of infection. Where PES is clinically severe, additional steroid treatment may be inevitable in some patients. We found that corticosteroid use due to PES was not a significant risk factor for infection in CBT. However, higher doses or prolonged use of steroid can be significant risk factors for infection. Prompt recognition of PES and treatment with a short-course corticosteroid regimen, if needed, could help to avoid unnecessarily long, empiric courses of treatment that could promote opportunistic infections.

In this study, as in others [6,12,22], PES did not have a negative impact on survival or transplantation–related death. This contrasts with the adverse survival impact of hyperacute or pre-engraftment GVHD in unrelated bone marrow or peripheral blood stem cell transplantation [30,31]. It seems that there are some differences between PES after CBT and hyperacute or pre-engraftment GVHD after transplantation using bone marrow or peripheral blood. Factors that may contribute to the relatively benign clinical course of PES after CBT may include reduced graft lymphocyte numbers, fewer T cells interacting with recipient's antigen-presenting cells, and limited responses of naïve T cells to recipient alloantigen [32-34]. Other explanations may include the possibility that the PES was sufficiently manageable to not affect survival in cases of CBT. In our series, most of patients with PES responded well to steroid. Nevertheless, close observation of patients with PES is required because some patients deteriorate and are refractory to steroids, as shown in this study.

In conclusion, PES seems to be common after CBT and may be associated with enhanced engraftment. Although PES is closely associated with acute GVHD, PES after CBT is not associated with significant morbidity and is easily manageable with intravenous steroid. Because failure to recognize PES in CBT recipient risks unnecessary complications of this syndrome and unnecessarily long, empirical treatment, physicians should be aware of the possible occurrence of PES after CBT, especially in patients who have relevant risk factors for developing PES.

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APPENDIX

This study was conducted at the following institutions: College of Medicine, Chungbuk National University, Cheongju; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Hanyang University Medical Center, Hanyang University College of Medicine, Seoul, Korea; Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea; Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea; Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Gwangju, Korea; Yeungnam University, Daegu; Chungnam National University, Daejon, Korea; Ajou University School of Medicine, Suwon, Korea; CHA Bundang Medical Center, CHA University, Seongnam; Mokdong Hospital, Ehwa Women's University, Seoul, Korea; Yonsei University, College of Medicine, Seoul, Korea; Gyeonsang National University School of Medicine, Jinju, Korea; Pusan National University, School of Medicine, Pusan; National Cancer Center, Goyang; Konkuk University College of Medicine, Seoul, Korea; Gachon University Gil Hospital, Gachon University School of Medicine, Incheon, Korea; and Daegu Fatima Hospital, Daegu, Korea.