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ORIGINAL ARTICLE

Peri-engraftment syndrome in allogeneic hematopoietic SCT

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Engraftment syndrome (ES) and pre-engraftment syndrome (pre-ES) are both inflammatory conditions that occur after hematopoietic SCT (HSCT) and are characterized by non-infectious fever and skin rash. Although the pathogenesis is not fully understood, both syndromes are similar, and could be defined as a new clinical syndrome, named as peri-engraftment syndrome (peri-ES). We retrospectively analyzed the clinical records in 176 pediatric patients, following allogeneic HSCT. We utilized the definition of ES by Spitzer as the diagnostic criteria, excluding 'within 96 h of engraftment' criteria. Thirty cases developed peri-ES with a cumulative incidence of 17.0%. High cumulative incidence (50%) was seen in patients who underwent a double-unit cord blood transplantation (DUCBT; $P<0.01$). Clinical findings of peri-ES are similar, regardless of the onset day, and encephalopathy was the most severe complication. In the DUCBT cohort, the use of TBI and early complete chimerism ($\leqslant$day 21) were identified as risk factors that predispose the development of peri-ES. We determined that both, ES and pre-ES, might have similar causes, which could be included in peri-ES. Particularly, it occurred more in DUCBT patients, which means that not only neutrophil engraftment but also immune reactions within the two units might contribute to peri-ES.

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INTRODUCTION

Engraftment syndrome (ES) is a complication seen during neutrophil recovery following hematopoietic SCT (HSCT), characterized by non-infectious fever, skin rash, pulmonary infiltrates and other various clinical findings. The pathogenesis of ES is not clearly revealed, but it is thought that overproduction of pro-inflammatory cytokines and cellular interactions may have a major role in the pathogenesis of ES.[1]

It has been reported in patients who underwent both autologous and allogeneic HSCT. Because of the various diagnostic criteria of ES, however, the incidence, risk factors and clinical outcomes of the ES remain multifarious.[2–7] Furthermore, pre-engraftment syndrome (pre-ES), which is more emphasized with a fever, rash and an occurrence in the pre-engraftment period, has been described and considered different from ES.[8–11]

We retrospectively analyzed the clinical records of 176 patients following allogeneic HSCT. Furthermore, in a comparative study of the clinical features of pre-ES and ES patients, we found that both clinical syndromes were similar, which could be defined as a new entity of a clinical syndrome, termed as peri-engraftment syndrome (peri-ES). We specifically determined the incidence, risk factors and clinical outcomes of peri-ES, which markedly occurred in patients who underwent a double-unit cord blood transplantation (DUCBT).

PATIENTS AND METHODS

Study population

All consecutive patients who underwent allogeneic HSCTs at the Seoul National University Children's Hospital between October 2004 and January 2010 were analyzed retrospectively. The disease and transplant characteristics of the patients are shown in Table 1. Parent's informed consent for HSCT was obtained in all cases. This study was performed after obtaining approval from the Institutional Review Board at our institution (H-1108-102-374).

Conditioning regimen and supportive care

Conditioning regimens were different, according to the underlying disease and type of transplantation (Table 1). In DUCBT, BU, fludarabine and antithymocyte globulin ($\pm$Etoposide) were mostly used (63.0%). Followed by a TBI-based regimen (25.9%), five were with fludarabine and cytarabine, five were with CY and cytarabine and four were with fludarabine and CY.

Transplant procedures were performed in a room with HEPA-filtered air. Infectious prophylaxis with acyclovir and oral itraconazole was given, and G-CSF was administered after transplantation. Febrile neutropenia was treated with empirical broad-spectrum antibiotic therapy, and parenteral itraconazole (5 mg/kg/dose twice a day for 2 days, then the same dose once daily) was given for patients with persistent 3–5-day fevers.[12] Blood and urine cultures were taken at the onset of fever and repeated every 48 h, when a fever persisted. If patients presented with respiratory symptoms, respiratory virus PCR, including influenza, respiratory syncytial and adeno viruses, was performed by nasal aspiration.

Platelets were transfused if platelet counts decreased to $<20\times10^9$/L, or for patients with bleeding tendencies. Red blood cells were given to maintain a hemoglobin level >8 g/dL. Supportive care was performed, according to the guidelines for HSCT of our center.[13] All patients who developed peri-ES received methylprednisolone (1 mg/kg/day) for 3–5 days, without tapering off.

Chimerism analysis

Hematopoietic chimerism was evaluated by serial analysis of short tandem repeats (STR) of BM aspirates at 1, 3 and 6 months and 1 year after the allogeneic HSCT other than cord blood transplantation (CBT). For CBT, hematopoietic chimerism was analyzed more frequently at 1, 2 and 3 weeks with peripheral blood and 1, 3, 6 months and 1 year with

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BM aspirate by the same method.[14] The method was based on the quantitative amplification of informative polymorphic STR regions in the recipient and donor, using the AmpFlSTR profiler PCR amplification kit (Applied Biosystems, Foster City, CA, USA), as per the manufacturer's instructions.

Definitions

We defined peri-ES, according to the diagnostic criteria by Spitzer,[1] but uncoupling the timing between clinical symptoms and neutrophil recovery. The major criteria were as follows: (i) fever of $\geqslant 38.3\,^{\circ}C$ without identifiable infectious etiology, (ii) erythrodermatous rash involving $>25\%$ of the body surface area and not attributed to medication and (iii) non-cardiogenic pulmonary edema manifested as diffuse pulmonary infiltrates and hypoxia. The minor criteria were as follows: (i) hepatic dysfunction with either total bilirubin $\geqslant 2$ mg/dL or transaminase levels greater than or equal to double the normal level, (ii) renal insufficiency (serum creatinine of greater than or equal to double the baseline value), (iii) weight gain $\geqslant 2.5\%$ of the baseline body weight and (iv) transient encephalopathy that was unexplained by other causes. A diagnosis of peri-ES was established based on the presence of all three major criteria, or of two major criteria and one or more of the minor criteria, without clinical and pathological symptoms and signs of GVHD or infection.

Due to the importance of HLA matching in the unrelated donor HSCT,[15] we defined the high-risk group of HLA disparity in DUCBT as patients for whom HLA mismatching between each cord blood unit and the recipient (serological typing of HLA-A, -B and allele typing of HLA-DR) is greater than or equal to the two loci.

Neutrophil engraftment was defined as the first day of the three consecutive days with ANC $>0.5\times10^9$/L and both acute and chronic GVHD were graded according to previously published criteria.[16,17]

Table 1. Patient characteristics

	n = 176
Patient age, years, median (range)	7.6 (0.3–19.1)
Gender, male/female, no. (%)	94 (53.4)/82 (46.6)
Dignosis, no. (%)	
ALL	56 (31.8)
AML	53 (30.1)
ABL	13 (7.4)
AA	26 (14.8)
Others (MDS, JMML, HLH, etc.)	28 (13.1)
Stem cell source, no. (%)	
BMT, related/unrelated	20 (11.4)/35 (19.9)
PBSCT, related/unrelated	18 (10.2)/38 (21.6)
CBT, single/double-unit	9 (5.1)/54 (30.7)
Haploidentical	2 (1.1)
Conditioning regimen, no. (%)	
TBI based	44 (25.0)
BU based	104 (59.1)
TBI/BU based	5 (2.8)
Others	23 (13.1)
Use of ATG, no (%)	
Yes	131 (74.4)
No	45 (25.6)
GVHD prophylaxis, no. (%)	
CsA + MMF	60 (34.1)
CsA + steroid	31 (17.6)
CsA + MTX	18 (10.2)
Tacrolimus + MTX	63 (35.8)
Others	4 (2.3)

Abbreviations: AA = aplastic anemia; ABL = acute biphenotypic leukemia; ATG = antithymocyte globulin; CBT = cord blood transplantation; HLH = hemophagocytic lymphohistiocytosis; JMML = juvenile myelomonocytic leukemia; MDS = myelodysplastic syndrome; MMF = mycophenolate mofetil.

Statistical analysis

The categorical variables and continuous variables related to the patients were compared using a chi-squared test and Mann–Whitney test, respectively, and a multivariate analysis was performed, using a logistic regression model. Peri-ES and acute GVHD were computed using the cumulative incidence function. For both, the competing risks were graft failure and death. OS and EFS were performed using the Kaplan–Meier method. The difference in cumulative incidence curves was based on Gray's test; the difference in survival rates based on peri-ES was determined using the log-rank test. P values <0.05 were considered statistically significant. For statistical analysis 'R' version 2.6.1 (R Development Core Team, Vienna, Austria) and SPSS 17.0 (SPSS, Chicago, IL, USA) were used.

RESULTS

Cumulative incidence of peri-ES in all the patients

To assess the cumulative incidence of peri-ES in allogeneic HSCT recipients, we applied the above-mentioned peri-ES criteria. Of the 176 patients, 30 patients developed peri-ES with an estimated cumulative incidence of 17.0%. A particularly, high cumulative incidence (50%) was shown in patients who underwent a DUCBT (27 of 54 patients, $P<0.01$). Two patients with unrelated peripheral blood stem cell transplantation (PBSCT) and one with related PBSCT were also diagnosed (Figure 1). There was no incidence in patients with related/unrelated BMT and single-unit cord blood transplantation (SUCBT).

Clinical manifestations of peri-ES

There were patients who presented with unexplained fever (100%), maculopapular rash (100%), pulmonary infiltrates and hypoxia (57%), body weight gain (50%), hepatic dysfunction (23%), renal dysfunction (10%) and unexplained encephalopathy (20%). The median day for the onset of peri-ES was 13 days after transplantation, with a median length of duration of 3 days (range, 1–22 days). The median number of days for neutrophil recovery was 16 (range 9–40 days).

There was no proven pathogen in patients with peri-ES, including respiratory viruses in patients with respiratory symptoms.

The periods during which each patient fulfilled the peri-ES criteria are shown in Figure 2, setting the first day of neutrophil engraftment (shown as a midline in Figure 2a) and the stem cell infusion day (shown as a vertical line in Figure 2b).

Of the total 30 patients who developed peri-ES, 27 patients underwent DUCBT. In a comparative study of the clinical features of peri-ES patients in the DUCBT cohort, dividing them into

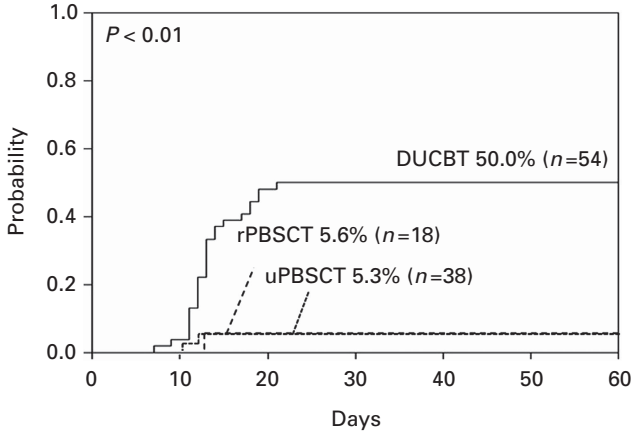

Figure 1. Cumulative incidence of peri-engraftment syndrome in patients who underwent double-unit CBT (DUCBT), related PBSCT (rPBSCT) and unrelated PBSCT (uPBSCT). There was no incidence in patients with related/unrelated BMT and single-unit CBT (SUCBT).

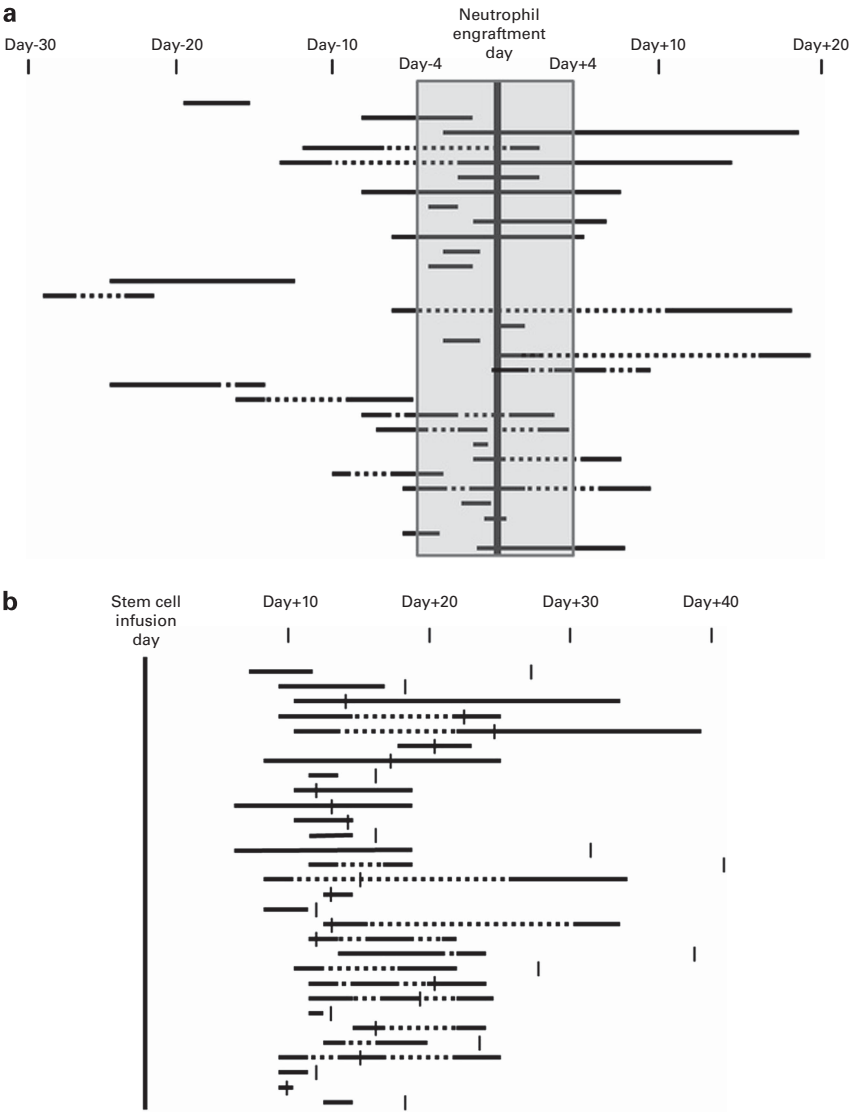

Figure 2. The period of fulfillment of the peri-engraftment syndrome criteria for each patient is shown as a solid line, whereas the period during which symptoms subsided by methylprednisolone treatment in relapsed patients is represented as a dotted line, (**a**) setting the first day of neutrophil engraftment as a midline. (**b**) The long thick vertical line on the left represents the day of stem cell infusion and the short thin vertical line represents the first day of neutrophil engraftment for each of the patients.

a pre-ES and an ES group by the initial onset time, there was no difference in clinical symptoms and signs in both the groups (Figure 3).

Risk factors of peri-ES

As mentioned above, DUCBT was identified as the most significant risk factor of peri-ES. The use of TBI also indicated a trend toward increased incidence of peri-ES in all the patients (12 of 30 patients vs 34 of 146 patients, $P = 0.06$). In terms of GVHD prophylaxis, the 31 patients who received steroids (19 of them underwent related BMT, 10 related PBSCT, 1 SUCBT and 1 DUCBT) did not develop peri-ES. The majority of patients (29 of 31 patients) who received steroids for GVHD prophylaxis were those who underwent related SCT (BMT, 19 patients; and PBSCT, 10 patients). Of the total related SCT group (BMT, $n = 20$; and PBSCT, $n = 18$), 9 patients did not receive steroids as part of the GVHD prophylaxis regimen. Because we came across only one peri-ES case in related PBSCT patients, it was difficult to compare the preventive effects of steroids in this group (peri-ES in the steroids group, 0 of 29 patients; and non-steroids group, 1 of 9 patients; $P = 0.24$).

Treatment and outcomes of peri-ES

The peri-ES patients were treated by i.v. methylprednisolone 1 mg/kg daily for a median of 5 days (range, 2–5 days) through each cycle until resolution of symptoms. Steroids then were discontinued without taper. For patients with recurrent symptoms, steroids would be reinitiated. The fever subsided within 24 h after receiving the first dose of methylprednisolone in 21 (68%) patients and 13 patients received a second cycle of i.v. methylprednisolone, owing to a relapse (one patient required three cycles). The median number of days from the end of the first steroid treatment until relapse was 2 (range, 1–7 days). Three patients, all of whom underwent DUCBT, showed peri-ES for >10 days after neutrophil engraftment, without developing either acute GVHD or overt proven infection. Of them, one patient improved promptly by the second cycle of methylprednisolone, another patient improved

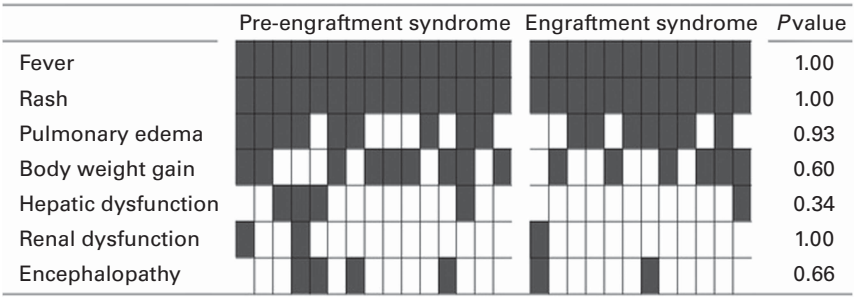

Figure 3. Peri-engraftment syndrome patients in the double-unit CBT cohort were classified into two groups: pre-engraftment syndrome (onset before 96 h of engraftment) and engraftment syndrome (onset within 96 h of engraftment). A comparison of diagnostic criteria between both the groups showed no significant difference.

after 7 days from a second cycle of methylprednisolone, and the one other remaining patient improved spontaneously.

Among all patients, there were no differences in OS (76.5% vs 66.8%, $P = 0.52$) and EFS (76.7% vs 63.8%, $P = 0.36$) between patients with and without peri-ES. There were no patients who died owing to peri-ES. However, encephalopathy, which was the most severe complication, had developed in six patients with symptoms such as seizures, altered mentality or motor weakness. Magnetic resonance imaging scan of the brain was taken in all those patients at the time of peri-ES, which showed nonspecific findings except in one patient with a focal diffusion restriction lesion. Cerebrospinal fluid tapping was available in two patients, of which results showed no pleocytosis with negative for microbiological culture, including HSV and enterovirus. All those patients fulfilled the diagnostic criteria of peri-ES, initially, without encephalopathy. Four of them, who were not treated with methylprednisolone, owing to an uncertain diagnosis at that time because of an accompanying infection or veno-occlusive disease, developed an encephalopathy median 4 days later (range 1–10 days). The other two patients were managed successfully with methylprednisolone at the first event, but encephalopathy developed with the second and third recurrent events, respectively. Of the six patients with encephalopathy, neurological sequalae remained in four patients until the last follow-up (three patients treated with anti-epileptic drugs, one patient developed a bed-ridden state).

Peri-ES in DUCBT patients

In the CBT group, the patients with SUCBT did not develop peri-ES. There were no differences in the total numbers of $CD34^+$ cells and total nuclear cells between the DUCBT and SUCBT groups (mean $CD34^+$ cells 3.24×10^5 vs 3.53×10^5/kg; $P = 0.22$, mean number of total nuclear cells 8.70×10^7 vs 6.34×10^7/kg; $P = 0.80$). Therefore, of the 54 patients who underwent DUCBT, a subanalysis was conducted for 48 patients without engraftment failure or early death.

The patient demographics and transplantation characteristics of the 27 peri-ES patients and 21 non-peri-ES patients are compared in Table 2. The use of TBI as a conditioning regimen was significantly associated with peri-ES in univariate and multivariate analysis, whereas the use of antithymocyte globulin was not. Peri-ES for patients who received TBI tended to develop earlier than 96 h before neutrophil engraftment (9 of 11 patients, $P = 0.02$). The total count and cell dose difference (cell dose difference/cell dose of the larger unit)[18] of $CD34^+$ and total nuclear cells were not statistically different in both groups. Moreover, those of $CD3^+$ cells were also not significantly associated with the development of peri-ES.

No association was noted in the cumulative incidence of acute GVHD, chronic GVHD, veno-occlusive disease, CMV infection, and rapidity of neutrophil and platelet engraftment between patients with and without peri-ES in the DUCBT group. With a median follow-up of 29.7 months (range 1–76 months), OS (77.8% vs 65.9%, $P = 0.53$), EFS (77.8% vs 66.7%, $P = 0.51$) and non-relapse mortality (NRM) (22.2% vs 23.8%, $P = 0.99$) did not differ in both the peri-ES and non-peri-ES groups. However, the weekly chimerism analysis, which was performed for the 38 patients, revealed that early complete chimerism ($>90\%$) achievers ($\leqslant$day 21) developed peri-ES more frequently than patients who achieved late complete chimerism (23 of 30 patients vs 3 of 8 patients, $P = 0.03$). The median number of days of donor-type complete chimerism achievement was 14 (range 14–90 days).

DISCUSSION

ES is a post-HSCT complication that is described as an entity of clinical symptoms and signs, including fever, skin rash and pulmonary infiltrates with hypoxemia. However, variously defined diagnostic criteria make the diagnosis difficult.[1–7] Spitzer[1] previously proposed a uniform definition of ES composed of three major and four minor criteria, which should occur within 96 h of neutrophil engraftment. Maiolino *et al.*[2] recommended less strict criteria for a better sensitivity and early treatment, which were fever accompanied by any of the following: cutaneous rash, pulmonary infiltrates and diarrhea. Furthermore, other unique criteria were also applied, owing to the diversity of clinical findings of ES.[3,5,6] For this reason, it is important to know which criteria were used to evaluate ES.

Furthermore, pre-ES, which is distinct from ES, was described as unexplained fever, not associated with documented infection and unresponsive to antimicrobial manipulations, and/or unexplained erythematous skin rash, particularly in CBT.[8–11] Although the pathogenesis was poorly understood, pre-ES could have developed long before neutrophil engraftment, of which the cumulative incidence was generally higher than that of ES, due to less stringent diagnostic criteria.

In our study, however, we used new diagnostic criteria, which were based on Spitzer's criteria for a more strict application, except for the concept of time to cover the above-mentioned longer period that was described as peri-ES. Because the clinical features were not different, whether this syndrome developed long before engraftment or within 96 h following engraftment, the pathophysiology of both pre-ES and ES might not have been divergent. Thus, both syndromes could be included in peri-ES, owing to similar clinical manifestations and outcomes. In particular, encephalopathy occurred in the peri-ES patients more frequently (20%, 6 of 30 patients) than in the previous studies (0–11%).[2–5] Moreover, encephalopathy tends to develop later, after the fulfillment of the diagnostic criteria, which means that early detection and prompt treatment were crucial to minimizing the morbidity of neurological problems.

Table 2. Risk factors of peri-engraftment syndrome in double-unit CBT patients

Variable	*ES*	*No ES*	*Univariate*		*Multivariate*	
	n = *27*	n = *21*	P *value*	*OR*	P *value*	*Exp(B)*
Female gender	15	7	0.125	2.5	0.235	2.2
Median age (years)	7.4 (0.9–15.5)	8.0 (1.1–16.9)	0.683			
Body weight (kg)	24.5 (9.6–63.0)	29.1 (9.6–65.7)	0.286			
Use of TBI	11	3	0.045	4.13	0.043	4.9
Use of ATG	16	12	0.883	1.09		
Infused $CD3^+$ cells, 10^7/kg[a]	1.37 (0.6–2.8)	1.24 (0.4–3.4)	0.628			
infused $CD3^+$ difference (%)[a,b]	35.6 (2.7–79.6)	32.1 (1.2–61.4)	0.572			
Infused $CD34^+$ cells, 10^5/kg	3.3 (1.6–6.1)	3.7 (0.9–8.0)	0.494			
Infused $CD34^+$ difference (%)[b]	45.5 (3.0–88.2)	39.3 (1.6–85.5)	0.391			
Infused TNC, 10^7/kg	9.8 (3.3–21.7)	8.7 (0.3–28.6)	0.464			
Infused TNC difference (%)[b]	32.4 (3.3–72.7)	24.0 (3.1–53.3)	0.15		0.096	1.03
IRF detection $\leqslant$9 days	15	10	0.585	1.38		
HLA disparity, high risk	13	11	0.771	0.84		

Abbreviations: ATG = antithymocyte globulin; ES = engraftment syndrome; IRF = immature reticulocyte fraction; OR = odds ratio; TNC = total nucleated cell.
[a]Available in 40 patients. [b](cell counts of the larger unit − cell counts of the smaller unit)/cell counts of the larger unit.

Although human herpes virus 6 reactivation was known as a cause of opportunistic infections after HSCT,[19] we could not screen for this virus in cerebrospinal fluid routinely, due to the patients' unstable clinical condition. However, patients who had encephalopathic symptoms improved after steroids treatment, implying the low possibility of human herpes virus 6 infection.

The authors had observed a higher incidence, along with more severe clinical signs and symptoms of peri-ES in patients who underwent DUCBT. Moreover, TBI as a conditioning regimen was found to be a risk factor for developing peri-ES in the DUCBT group. Severe inflammation provoked by TBI could have been one of the causes of peri-ES; however, 16 of 27 patients who developed peri-ES in DUCBT received a BU and antithymocyte globulin-based conditioning regimen without TBI, which means that not only TBI but also other risk factors such as DUCBT might have a role in its development.

DUCBT, which is the most important risk factor for peri-ES in our study, has been performed to prevent cell dose limitation and enhance engraftment, which is being increasingly used and is considered a promising modality.[20] However, the biological basis of graft-to-graft interaction and single-unit dominance has not been fully understood. Barker *et al.*[21] reported previously that a higher $CD3^+$ cell dose was associated with the cord blood unit that would predominate in adult patients. In our previous report, comparison of each unit in patients with DUCBT revealed that the CFU-GM (colony-forming unit granulocyte macrophage) dose was a significant factor that influences dominancy.[18] A recent *in vivo* study demonstrated that immune rejection mediated by effector $CD8^+$ T cells derived from the dominant cord blood unit developing after DUCBT is responsible for the engraftment failure of the other unit.[22] These studies could provide evidence that graft-vs-graft immune reaction is a complex process, which is distinct from that of the other HSCTs, and could provoke the production of pro-inflammatory cytokines and the activation of various inflammatory processes that cause peri-ES more frequently than the other types of HSCT. Moreover, our data show that early achievement of complete chimerism might be associated with the development of peri-ES, which means that early complete chimerism could be responsible for a stronger immune reaction, possibly graft-vs-graft. Because DUCBT can also potentially augment the GVL effect and reduce the risk of relapse,[23] peri-ES might be the manifestation of these effects, and could be a possible explanation of better EFS, although it was not statistically significant in our study. This hypothesis needs to be validated in a much larger population in a future study.

The use of steroids for GVHD prophylaxis in DUCBT could be considered for the prevention of peri-ES. At our center, cyclosporine and methylprednisolone were used during the early period for GVHD prophylaxis in DUCBT patients. However, it was changed to the combination of cyclosporine and mycophenolate mofetil in all subsequent patients with DUCBT in accordance with the experience of the Minnesota group.[21] Therefore, we could not evaluate the preventive effect of steroids for peri-ES in DUCBT. Taken together, the patients with DUCBT who received cyclosporine and mycophenolate mofetil as GVHD prophylaxis need to be monitored for peri-ES.

In conclusion, we described a new entity of clinical syndrome, peri-ES, which could include two independent, previously researched clinical syndromes, ES and pre-ES. Peri-ES is developed more in patients who underwent DUCBT, which means that one of the causes of peri-ES might be a graft-vs-graft immune reaction, which is marked in patients who achieved early complete chimerism. Owing to the high incidence of encephalopathy, clinicians should monitor for early detection and subsequent early treatment. Further multicenter investigations are warranted for better understanding of this syndrome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **27**: 893–898.
2 Maiolino A, Biasoli I, Lima J, Portugal AC, Pulcheri W, Nucci M. Engraftment syndrome following autologous hematopoietic stem cell transplantation: definition of diagnostic criteria. *Bone Marrow Transplant* 2003; **31**: 393–397.
3 Nishio N, Yagasaki H, Takahashi Y, Hama A, Muramatsu H, Tanaka M *et al.* Engraftment syndrome following allogeneic hematopoietic stem cell transplantation in children. *Pediatr Transplant* 2009; **13**: 831–837.
4 Schmid I, Stachel D, Pagel P, Albert MH. Incidence, predisposing factors, and outcome of engraftment syndrome in pediatric allogeneic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2008; **14**: 438–444.

5 González-Vicent M, Ramírez M, Sevilla J, Pérez A, Fernández S, Madero L *et al.* Engraftment syndrome after autologous peripheral blood progenitor cell transplantation in pediatric patients: a prospective evaluation of risk factors and outcome. *Bone Marrow Transplant* 2004; **34**: 1051–1055.
6 Foncillas MA, Diaz MA, Sevilla J, Gonzalez Vicent M, Fernandez-Plaza S, Perez A *et al.* Engraftment syndrome emerges as the main cause of transplant-related mortality in pediatric patients receiving autologous peripheral blood progenitor cell transplantation. *J Pediatr Hematol Oncol* 2004; **26**: 492–496.
7 Carreras E, Fernández-Avilés F, Silva L, Guerrero M, de Larrea CF, Martínez C *et al.* Engraftment syndrome after auto-SCT: analysis of diagnostic criteria and risk factors in a large series from a single center. *Bone Marrow Transplant* 2010; **45**: 1417–1422.
8 Patel KJ, Rice RD, Hawke R, Abboud M, Heller G, Scaradavou A *et al.* Pre-engraftment syndrome after double-unit cord blood transplantation: a distinct syndrome not associated with acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2010; **16**: 435–440.
9 Frangoul H, Wang L, Harrell FE, Ho R, Domm J. Preengraftment syndrome after unrelated cord blood transplant is a strong predictor of acute and chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2009; **15**: 1485–1488.
10 Wang X, Liu H, Li L, Geng L, Ding K, Liu X *et al.* Pre-engraftment syndrome after unrelated donor umbilical cord blood transplantation in patients with hematologic malignancies. *Eur J Haematol* 2012; **88**: 39–45.
11 Lee YH, Lim YJ, Kim JY, Kim YD, Lee SW. Pre-engraftment Syndrome in Hematopoietic Stem Cell Transplantation. *J Korean Med Sci* 2008; **23**: 98–103.
12 Kim H, Shin D, Lee JW, Jang MK, Park KD, Shin HY *et al.* Successful empirical antifungal therapy of intravenous itraconazole with pharmacokinetic evidence in pediatric cancer patients undergoing hematopoietic stem cell transplantation. *Blood (ASH Annual Meeting Abstracts)* 2011; **118**: 3019.
13 Kang HJ, Shin HY, Choi HS, Ahn HS. Fludarabine cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. *Bone Marrow Transplant* 2004; **34**: 939–943.
14 Kang HJ, Kho SH, Jang MK, Lee SH, Shin HY, Ahn HS. Early engraftment kinetics of two units cord blood transplantation. *Bone Marrow Transplant* 2006; **38**: 197–201.
15 Park M, Koh KN, Kim BE, Im HJ, Park KD, Kang HJ *et al.* The impact of HLA matching on unrelated donor hematopoietic stem cell transplantation in Korean children. *Korean J Hematol* 2011; **46**: 11–17.
16 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
17 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250–259.
18 Kang HJ, Yoo KH, Lee JW, Kim H, Lee SH, Sung KW *et al.* Double umbilical cord blood transplantation for children and adolescents. *Ann Hematol* 2010; **89**: 1035–1044.
19 de Pagter PJ, Schuurman R, Meijer E, van Baarle D, Sanders EAM, Boelens JJ. Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation. *J Clin Virol* 2008; **43**: 361–366.
20 Sideri A, Neokleous N, De La Grange PB, Guerton B, Le Bousse Kerdilles MC, Uzan G *et al.* An overview of the progress on double umbilical cord blood transplantation. *Haematologica* 2011; **96**: 1213–1220.
21 Barker JN. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 2004; **105**: 1343–1347.
22 Gutman JA, Turtle CJ, Manley TJ, Heimfeld S, Bernstein ID, Riddell SR *et al.* Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. *Blood* 2009; **115**: 757–765.
23 Majhail NS, Brunstein CG, Wagner JE. Double umbilical cord blood transplantation. *Curr Opin Immunol* 2006; **18**: 571–575.