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ORIGINAL ARTICLE

Early coagulation disorder after allogeneic stem cell transplantation is a strong prognostic factor for transplantation-related mortality, and intervention with recombinant human thrombomodulin improves the outcome: a single-center experience

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Abstract We retrospectively analyzed 60 cases of pediatric patients who received allogeneic stem cell transplantation (SCT) between 2000 and 2008, using the tentative scoring system for evaluation of early (<30 days) coagulation disorders. In the 41 patients who survived, D-dimer levels showed a transient increase 2 weeks after SCT and normalized thereafter, but these levels were persistently elevated in the 19 patients who died. Of 19 patients with a positive score, 11 died of transplantation-related complications [transplantation-related mortality (TRM) = 0.579] within 1 year, while none of the 41 with a negative score died during the same period. Since 2009, 12 of 30 patients had positive scores within 30 days after SCT. Intervention with recombinant human thrombomodulin (rhTM) was introduced for patients with a positive score, and 10 of these patients survived (TRM = 0.167) along with a dramatic improvement of D-dimer level. Although the effects of this treatment were observed in a limited number of patients, our observations suggest that early coagulation disorder after allogeneic SCT is a strong prognostic factor for TRM, and that intervention with rhTM improves TRM.

Keywords Thrombomodulin · Coagulopathy · Transplantation-related mortality · Stem cell transplantation

Abbreviations

rhTM	Recombinant human thrombomodulin
AT	Antithrombin
TMA	Thrombotic microangiopathy
VOD	Veno-occlusive disease
SCT	Stem cell transplantation
TRM	Transplantation-related mortality
SIRS	Systemic inflammatory syndrome
FDP	Fibrin degradation products
AML	Acute myeloid leukemia
ALL	Acute lymphocytic leukemia
CML	Chronic myeloid leukemia
SCID	Severe combined immunodeficiency
WAS	Wiskott-Aldrich syndrome
HIM	Hyper IgM syndrome
DKC	Dyskeratosis congenita
ID	Immunodeficiency
CAEBV	Chronic active EB virus infection
TBI	Total body irradiation
Bu	Busulfan
CY	Cyclophosphamide
ATG	Anti-thymocyte globulin
Flu	Fludarabine
LPAM	Melphalan
VP16	Etoposide
MSB	Matched sibling bone marrow
RMBM	Related mismatched bone marrow

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Introduction

Thrombotic microangiopathy (TMA) and veno-occlusive disease (VOD) are well-known transplantation-related complications induced by coagulation disorder [1–4]. The mortality rates are more than 60–70 % in patients with a severe condition [5]. Basically, patients with SCT are inevitably exposed to mucosal and endothelial damages by conditioning regimens such as irradiation and anti-cancer drugs, leukocytopenia complicated with infections, hypercytokinemia in an allogeneic immune reaction, and endothelial damages induced by a calcineurin inhibitor [6–8], which altogether lead to an increased susceptibility to a systemic coagulation disorder. Inflammation and coagulation are well known to be closely related and coagulation disorder aggravates graft-versus-host disease (GVHD) and vice versa. This multifactorial and systemic pathology is unique and different in various aspects from coagulation disorder observed in severe infection or malignancy. Recently, defibrotide (DF), a polydisperse oligonucleotide mixture, has been reported to be an effective therapeutic and prophylactic agent for VOD [9, 10]. However, specific strategies for management of coagulopathy in SCT are still not well exploited and remain to be established.

Recombinant human thrombomodulin (rhTM) is a newly developed drug, which works not only as an anticoagulant through its interaction with protein C, but also as an anti-inflammatory agent via its interaction with high-mobility group 1 (HMG1) protein and protease-activated receptor 1 (PAR1) [11–13]. In clinical practice, rhTM has shown marked effectiveness in coagulopathy occurring in severe infection, systemic inflammatory response syndrome (SIRS), and malignancy [14–17].

Early coagulopathy in SCT, including TMA and VOD, is considered as an important and central systemic pathology of transplantation-associated complication, which must be controlled in the initial stage.

To examine this issue, we retrospectively analyzed the cases of 60 patients who underwent SCT from the coagulopathy point of view and found that coagulopathy was a most significant prognostic factor of early transplantation-related mortality (TRM). We have used rhTM for the first time to prevent the progression of coagulopathy according to our tentative scoring system, which is used to predict the severity of coagulation disorder in SCT. Although the effect was observed in a limited number of patients, rhTM may be effective and safe in the management of coagulopathy observed in the early phase of SCT.

Patients and methods

Tentative scoring system

To evaluate the prognosis of coagulation disorder in SCT, we made an institutional tentative scoring system. We extracted GVHD (grade 3 and more), impairment of renal function, hyper-bilirubinemia, intestinal bleeding, and severe thrombocytopenia as important factors that were closely related with transplantation-related mortality by multivariate analysis of SCT cases performed in our institute. On the basis of these factors, we made a scoring system through discussion that is assessed by daily simple examination (presented in Japanese at the 52nd Annual Meeting of Japanese Pediatric Hematology Association in Osaka, Japan, 2010).

This scoring system consists of 5 clinical points: stem cell donor, GVHD and/or sign related to GVHD, increased platelet consumption, abnormal coagulation data, and laboratory data or clinical symptoms that suggest renal impairment, liver dysfunction, or intestinal ischemia. This score is calculated from simple, routine laboratory data and clinical symptoms, and easily evaluated on a daily basis. Patients were evaluated daily until 30 days after SCT, and those with a total score of 7 or more were determined to be "score positive". The precise scoring system is described in Table 1.

D-Dimer was measured by using latex-immune-aggregation kit (Mitsubishi Chemical Medicine; Tokyo, Japan) and normal value was below 1 μg/ml.

TRM (transplantation-related mortality) within 1 year after SCT is designated as early transplantation-associated death.

All of the score-positive patients were qualified as having DIC according to the diagnostic criteria for DIC established by the Japanese Association of Acute Medicine [18].

Patients

We retrospectively examined 60 pediatric patients who received allogeneic hematopoietic SCT (HSCT) between 2000 and 2008 at our institute. The transplant characteristics are summarized in Table 2. Nineteen patients were score positive and their profiles are listed in Table 3.

Of the 30 pediatric patients who received allogeneic HSCT between 2009 and 2011, 12 were prospectively treated with rhTM according to the tentative scoring system. The transplant characteristics of these patients are summarized in Table 4, and the profiles of score-positive patients are listed in Table 5.

Table 1 Tentative scoring system for evaluation of coagulopathy after SCT

	Score
1. High-risk donor for GVHD	0 or 1
Unrelated or mismatched donor	1
2. GVHD (either of below)	0 or 2 or 3
Active GVHD (≥II)	3
Fever (≥38 °C) or rash that may be related to alloreaction	2
3. Refractory to platelet transfusion or ≥50 % reduction of platelet within 24 h	0 or 3
$\leq 1.5 \times 10^4/\mu l$ despite of scheduled platelet transfusion※	3
4. Laboratory data (either of below)	0 or 2 or 3
Elevated FDP and/or D-dimer	2
Decreased AT (<80 %)	2
Sudden elevation of LDH and/or increased fragmented red blood cells	3
5. Suspected sign of organ dysfunction (either of below)	0 or 3
Increased creatinine[a]	3
Increased bilirubin[b]	3
Bloody diarrhea (sign of ischemic enteropathy)	3
Total score	0–13

Score more than 7 is determined to be score positive. For pediatric patients, baseline value of creatinine and bilirubin varies according to age

[a] When baseline creatinine is below 0.2 mg/dl, twofold increase is considered to be significant. When baseline creatinine is between 0.2 and 0.7 mg/dl, 1.75-fold increase is considered to be significant. When baseline creatinine is above 0.7 mg/dl, 1.5-fold increase is considered to be significant

[b] When baseline bilirubin is below 0.5 mg/dl, threefold increase is considered to be significant. When baseline bilirubin is between 0.5 and 1.0 mg/dl, twofold increase is considered to be significant. When baseline bilirubin is above 1.0 mg/dl, more than 2 mg/dl is considered to be significant

Table 2 Characteristics of the patients between 2000 and 2008

Age	8 years 1 month ± 7 years 3 months (4 months–22 years 8 months)
Gender	
Males	39 (12)
Females	21 (7)
Diagnosis	
Hematological malignancy	31 (10)
AML	12 (5)
ALL	13 (3)
CML	1 (0)
CAEBV	5 (2)
Non-hematological malignancy	29 (9)
WAS	10 (1)
HIM	7 (2)
SCID	5 (3)
ID	4 (2)
Others[a]	3 (1)
Stem cell source	
HLA-identical sibling	14 (2)
Related mismatched	6 (1)
Unrelated BM	21 (6)
Unrelated CB	19 (10)
Conditioning	
Myeloablative	49 (15)
Reduced intensity	11 (4)
TBI regimen	21 (5)
Non-TBI regimen	39 (14)
Bu-based	27 (10)
Non-Bu-based	12 (4)

Number in the parenthesis presents score-positive patients

[a] Osteopetrosis, adrenoleukodystrophy, hemophagocytic lymphohistiocytosis

GVHD prophylaxis consisted of cyclosporine (CSP) plus short-term methotrexate (MTX) for human leukocyte antigen (HLA)-identical related donors, and tacrolimus (TAC) plus short-term MTX for HLA-identical unrelated and HLA-mismatched related donors. For unrelated umbilical cord blood donors, CSP or TAC and short-term MTX were used. For prophylaxis of VOD, intravenous small molecule heparin and oral ursodeoxycholic acid were used for all patients. Intervention with conventional steroid (1–2 mg/kg/day) was used for patients with GVHD grade 2 and more.

Administration of rhTM

When the tentative score was 7 or more, 380 U/kg of rhTM (Asahi Kasei Pharma, Tokyo, Japan) was administered as a single daily infusion. Basically, rhTM was administered for 6–7 consecutive days except for cases 4 and 7, in which rhTM was administered for 11 consecutive days in case 4 and 19 consecutive days in case 7.

Statistical analysis

Log-rank test was used to assess differences between groups. P value of <0.05 was considered to be statistically significant. Multivariate analysis was performed using the Cox proportional hazards regression model. Variables analyzed included recipient age, donor type, disease type, GVHD, TMA, VOD, and score positivity.

Table 3 Transplant profiles of score-positive patients between 2000 and 2008

No.	Disease	Status	SCT	Stem cell	Conditioning	Age at SCT	Day (score)	aGVHD	TRM	ProteinC activity (%)	Complication	Cause of death
1	WAS		1st SCT	UCB	Bu + CY + ATG	1 year 1 month	19 (9)	Grade 4	*Dead (216 day)*	93	TMA	MOF
2	XHIM		1st SCT	MSB	TBI + CY	17 years 3 months	4 (11)	Grade 2	*Dead (69 day)*	n.a.	TMA	MOF
3	XHIM		1st SCT	MSB	Bu + CY	14 years 10 months	7 (10)	Grade 4	*Dead (89 day)*	n.a.	Aspergillosis	Aspergillosis
4	SCID		1st SCT	UCB	Bu + CY	9 months	14 (8)	Grade 3	Alive	n.a.	BCG sepsis	
5	SCID		1st SCT	UCB	BU + CY	8 months	3 (8)		*Dead (5 day)*	26	VOD	MOF
6	SCID		1st SCT	UCB	Flu + LPAM +ATG	3 years 9 months	10 (12)		*Dead (68 day)*	n.a.		RF
7	ID		1st SCT	UCB	Flu + LPAM +ATG	10 years 7 months	27 (13)	Grade 3	Dead[a] (590)	n.a.	TMA cGVHD	
8	ID		2nd SCT	UBM	Bu + CY	1 year 5 months	12 (8)	Grade 1	Alive	n.a.		
9	OP		1st SCT	UCB	Flu + LPAM +ATG	10 months	13 (9)	Grade 3	Alive	73	VOD	
10	CAEBV	Non-CR	1st SCT	RMBM	TBI + CY + VP16	14 years 7 months	19 (9)	Grade 3	*Dead (202 day)*	n.a.	VOD	MOF
11	CAEBV	Non-CR	1st SCT	UBM	Flu + LPAM +ATG	16 years 7 months	10 (9)	Grade 4	*Dead (59 day)*	47	TMA	MOF
12	AML	Non-CR	1st SCT	UCB	TBI + LPAM	4 years 10 months	6 (8)		*Dead (17 day)*	n.a.		PF
13	AML	1st CR	1st SCT	UBM	TBI + CY + LPAM	14 years 6 months	12 (9)	Grade 3	Alive	n.a.	cGVHD	
14	AML	Non-CR	1st SCT	UCB	Bu + CA + LPAM	1 year 1 months	6 (8)	Grade 3	*Dead (69 day)*	39		MOF
15	AML	1st CR	1st SCT	UBM	Bu + CY + LPAM	14 years 4 months	4 (9)	Grade 3	*Dead (335 day)*	66	VOD cGVHD	PF
16	AML	Non-CR	2nd SCT	UCB	Bu + LPAM	10 years 6 months	15 (8)	Grade 2	Dead[b] (609)	n.a.		
17	ph + ALL	2nd CR	2nd SCT	UBM	Bu + CY + LPAM	4 years 11 months	13 (9)		*Dead (60 day)*	43	VOD	MOF
18	ALL	1st CR	1st SCT	UBM	TBI + CY +VP16	11 year 8 months	12 (8)	Grade 2	Alive	n.a.	cGVHD	
19	ALL	1st CR	1st SCT	UCB	Bu + CY +VP16	8 months	9 (9)	Grade 2	Alive	n.a.	VOD	

n.a. not applicable, *MOF* multiple organ failure, *PF* pulmonary failure

[a] Dead due to other than SCT complication or primary disease

[b] Dead due to relapse of AML

Table 4 Characteristics of the patients between 2009 and 2011

Age	6 years 10 months ± 5 years 1 month (6 months–17 years 7 months)
Gender	
Male	25 (9)
Female	5 (3)
Diagnosis	
Hematological malignancy	18 (9)
AML	6 (2)
ALL	12 (7)
Non-hematological malignancy	12 (3)
WAS	1 (0)
HIM	2 (0)
SCID	4 (1)
ID	4 (2)
Others[a]	1 (0)
Stem cell source	
HLA-identical sibling	7 (2)
Related mismatched	6 (3)
Unrelated BM	7 (2)
Unrelated CB	10 (5)
Conditioning	
Myeloablative	21 (9)
Reduced intensity	9 (3)
TBI regimen	8 (2)
Non-TBI regimen	22 (10)
Bu-based	15 (9)
Non-Bu-based	7 (1)

Number in the parenthesis presents score-positive patients

[a] Hemophagocytic lymphohistiocytosis

Results

Retrospective analysis

Forty-one patients have been alive for more than 5 years. D-Dimer levels increased transiently (>1 μg/ml) 2 weeks after SCT and subsequently returned to normal (Fig. 1) in these patients. In some of the 19 patients who died, the D-dimer levels were already slightly elevated before SCT. These levels increased at 2 weeks after SCT and then remained constant or increased further. This indicates that sustained coagulation disorder may be associated with the prognosis of SCT.

Our tentative scoring system indicated that 19 patients were score positive (Table 1). Among the score-positive patients, 11 died from transplantation-related complication within 1 year after SCT. Among 41 score-negative patients, none died within 1 year, and of the 6 patients who died, 5 died from GVHD-related complication. The detailed profiles of 19 score-positive patients are described in Table 3.

When patients with more than 1 μg/ml increase in D-dimer levels within 15 days after SCT were grouped as "D-dimer-positive patients", 48 out of 60 patients were D-dimer positive and the remaining 12 "D-dimer-negative" patients are alive with no complications.

TRM in score-positive patients (TRM = 0.579) was extremely higher than that in score-negative patients (TRM = 0.163) and D-dimer-positive patients (TRM = 0.239) (Fig. 2).

Multivariate analysis revealed that "score-positivity" was the most significant prognostic factor (OR 12.5; 95 % CI, 1.96–79.5) for early transplantation-associated death, which was only followed by GVHD grade 3 and more (OR 7.1; 95 % CI, 1.25–40.5). No significant difference was observed on the first day when the score turned positive between dead and alive patients among score-positive patients (11.07 ± 7.18 vs 12.00 ± 1.67 days).

These retrospective observations indicate that early coagulation disorder has a significant effect on the prognosis, positive score was strongly associated with early transplantation-associated death, and our scoring system seemed well effective to predict the prognosis.

Intervention of early coagulation disorder with rhTM

On the basis of the results of our retrospective analysis, we introduced an early intervention for coagulation disorder using rhTM for score-positive patients since 2009. Between January 2009 and 2011, 12 out of 30 patients were score positive 30 days after SCT. The detailed profiles of 12 patients are shown in Table 5.

TRM during 2009–2011 was 0.07 and significantly lower than that (TRM = 0.31) during 2000–2008. TRM in score-positive patients between 2009 and 2011 was much lower (TRM = 0.17) than that (TRM = 0.579) in patients between 2000 and 2008 (Figs. 3, 4).

In cases 1 and 2, rhTM was administered 2–3 days after the score turned positive, while administration of rhTM was initiated on the day when the score turned positive. Two patients (cases 2 and 7) died from TRM within 1 year. Figure 5 showed the changes of D-dimer and soluble IL-2R (sIL2R) values after administration of rhTM. In case 2 and 7, D-dimer values increased even after administration of rhTM, while in others who survived they dramatically decreased. On the contrary, sIL2R values were not significantly different between the two groups. This indicates that the immediate beneficial effect of rhTM was mainly based on the regulation of coagulation disorder and not of inflammation, including GVHD.

Table 5 Transplant profiles of score-positive patients between 2009 and 2011

No	Disease	Status	SCT	Stem cell	Conditioning	Age at SCT	Day (score)	aGVHD	TRM	ProteinC activity (%)	Complication	Cause of death
1	DKC		1st SCT	UBM	Flu + LPAM +ATG	9 years 3 months	28 (7)	Grade 3	Alive	72	TMA	
2	ALL	2nd Rel non-CR	1st SCT	UBM	TBI + CY +VP16	7 years 2 months	11 (8)		*Dead (31 days)*	19	TMA, Aspergillosis, encephalopathy	MOF
3	Ph + ALL	1st CR	1st SCT	MSB	TBI + CY +VP16	17 years 7 months	6 (7)	Grade 2	Alive	48	cGVHD	
4	AML	1st Rel non-CR	1st SCT	RMBM	Bu + LPAM	1 year 10 months	6 (8)	Grade 3	Alive	76	VOD	
5	ALL	1st CR	1st SCT	UBM	Bu + CY +VP16	8 years 11 months	5 (8)	Grade 3	Alive	n.a.	VOD	
6	Ph + ALL	2nd CR	1st SCT	MSB	TBI + CY +VP16	9 years 4 months	12 (8)	Grade 2	Alive	54		
7	ALL	3rd CR	2ndSCT	UCB	Bu + CY +VP16	5 years 4 months	7 (8)	Grade 4	*Dead (45 days)*	63	TMA, VOD, GVHD4	MOF
8	SCID		1st SCT	UCB	Bu + Flu	7 months	18 (10)		Alive	98	TMA	
9	AML	2nd CR	1st SCT	UCB	Bu + LPAM	9 years 10 months	9 (8)	Grade 2	Alive	38	VOD	
10	ALL	3rd CR	2ndSCT	RMBM	Bu + LPAM	10 years	13 (8)	Grade 1	Alive	n.a.		
11	ID		1st SCT	UCB	Bu + Flu + ATG	12 years 9 months	4 (7)		Alive	n.a.		
12	ALL	3rd CR	1st SCT	RMBM	Bu + LPAM	15 years 5 months	16 (9)	Grade 1	Alive	23	VOD	

MOF multiple organ failure

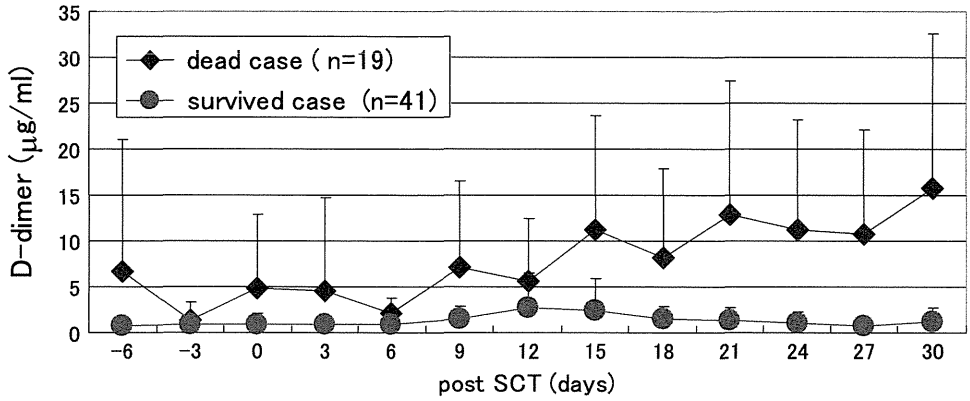

Fig. 1 The changes of D-dimer in patients who received allogeneic stem cell transplantation. D-Dimer in 41 patients who are alive and 19 patients who are dead (mean + SD)

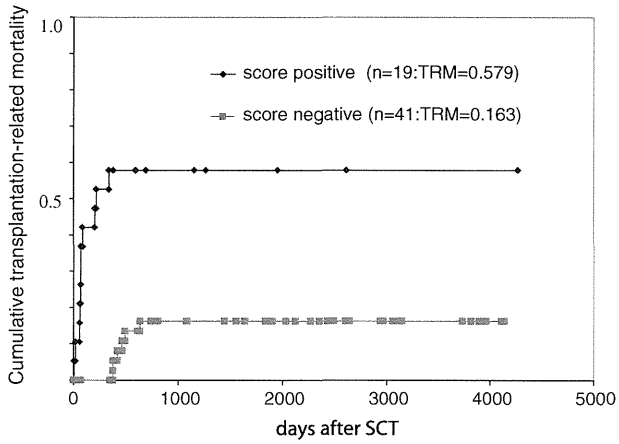

Fig. 2 Comparison of transplantation-related mortality between score-positive and -negative patients who received allogeneic stem cell transplantation between 2000 and 2008. TRM of score-negative patients was significantly lower than that of score-positive patients ($p < 0.001$)

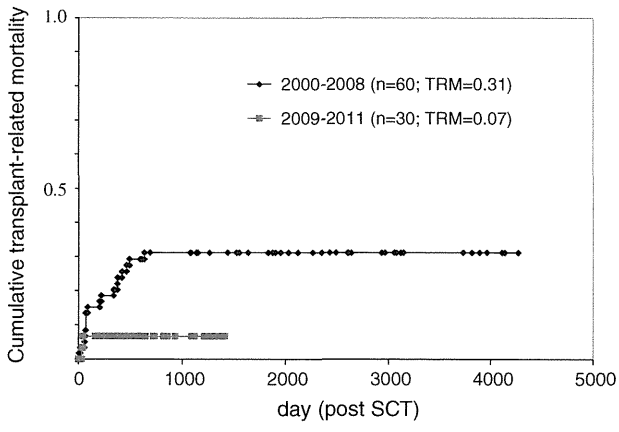

Fig. 3 Comparison of transplantation-related mortality in patients who received allogeneic stem cell transplantation in 2000–2008 and 2009–2011. TRM of patients who received allogeneic stem cell transplantation between 2009 and 2011 was significantly lower than that who received allo-SCT between 2000 and 2008 ($p = 0.024$)

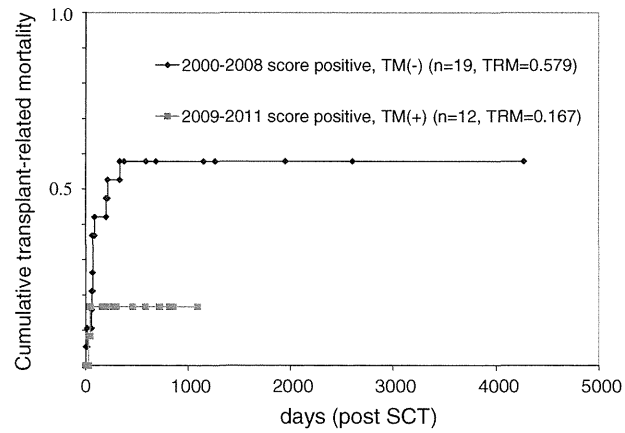

Fig. 4 Thrombomodulin reduces transplantation-related mortality in score-positive patients. The intervention with thrombomodulin reduced TRM of score-positive patients, although statistically not significant ($p = 0.060$)

In case 2, the patient relapsed shortly after second remission and received SCT in non-complete remission (CR) condition. She developed *Aspergillus* pneumonia and TMA and died with progressive organ failure. Protein C activity before rhTM administration was as low as 19 % and improvement of coagulopathy was minimal. In case 7, the patient received first cord blood SCT in the second CR and relapsed on day 105. Shortly after the third CR, she received second cord blood SCT. The interval between the first and second SCT was 7 months. She developed multidrug-resistant *Staphylococcus aureus* (MRSA) sepsis and TMA on day 5, which improved after administration of linezolid and rhTM. However, the progression of GVHD deteriorated TMA again. To control coagulation disorder, rhTM therapy was extended for 19 days, when intestinal bleeding was observed and rhTM was discontinued. With additional complication of *Aspergillus* infection, she died from multiple organ failure.

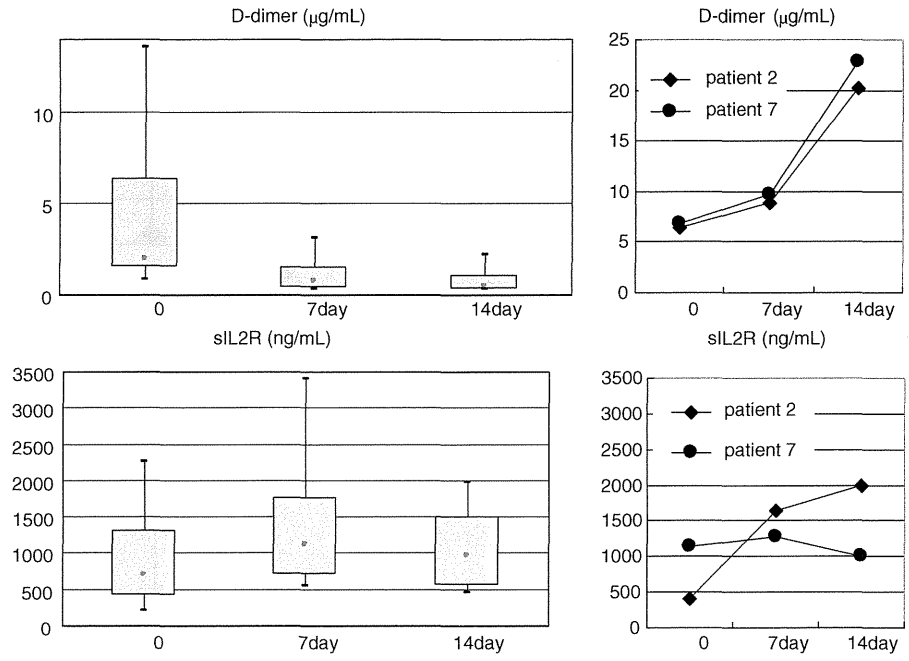

Fig. 5 Changes of D-dimer and sIL2R after administration of rhTM. Box plot shows D-dimer and sIL2R of 10 alive patients. Box plot presents maximum,75%, mean, 25%, and minimum value respectively.

Although all patients were thrombocytopenic (<1.0 × 10^4/μl) and refractory to platelet transfusion before administration of rhTM, no bleeding complication was observed except in case 7.

Discussion

Coagulation disorder induces organ dysfunction, which may sometimes be life threatening [5]. Furthermore, endothelial damage and coagulopathy trigger inflammation, which results in further exaggeration of GVHD [19]. In addition, GVHD induces endothelial damage and deteriorates pre-existing coagulopathy. This vicious cycle is one of the main pathologies of early TRM in SCT. There are internationally well-known diagnostic criteria for TMA and VOD [20–23]. Although these criteria are useful to classify and delineate the complicated and heterogeneous coagulation pathology in patients with SCT, they are not necessarily satisfactory from the therapeutic point of view. Occasionally, it will be too late to start therapeutic intervention on the basis of these diagnostic criteria. We developed a tentative scoring system to predict serious coagulation disorder that may lead to life-threatening complication. This score is based on simple laboratory data and clinical symptoms and can be evaluated on a daily basis. A score of 7 or more in 30 days after SCT is determined to be "positive."

We retrospectively analyzed the cases of 60 patients who received SCT between 2000 and 2008 on the basis of this scoring system. Nineteen patients were "score positive", and 11 out of them died because of TRM in 1 year. On the other hand, of the 41 score-negative patients, no patients died of TRM in the same period.

Our retrospective analysis showed that the D-dimer levels were transiently increased 2 weeks after SCT, and the possibility of TRM was high when these levels persisted. Our observation emphasizes that early coagulopathy should be considered as one of the most important prognostic factors in TRM and must be controlled as early as possible. Intervention and control of coagulopathy during the early phase of SCT could reduce TRM.

rhTM is a newly developed drug for the treatment of disseminated intravascular coagulopathy (DIC), and rhTM not only has anti-coagulation effect, but also anti-inflammatory effect [11–13]. Thrombomodulin activates thrombin activatable fibrinolysis inhibitor (TAFI) and then inhibits activation of plasminogen, C5a, bradykinin, and osteopontin [24]. Furthermore, thrombomodulin with thrombin interacts with RAR1 (protease activating receptor-1) on the endothelial cells and induces anti-inflammatory signals [25–27]. On the other hand, lectin-like domain at the N-terminal portion of thrombomodulin binds to and then sequesters HMG-1 protein, which is a potent inducer of inflammation via interaction with TLR2, 4, and receptor for advanced glycan end products (RAGE) [28, 29]. In this context, thrombomodulin may be useful to prevent the progression of GVHD. However, sIL2R value, which is a well-known biomarker of acute GVHD, was not affected by rhTM in our short-term observation (Fig. 5).

It has been reported that thrombomodulin-activated protein C improves the radiation-induced endothelial damages in mouse SCT model [30]. rhTM is designed from an endogenous active product and is safer than other anti-

DIC medicines. Clinically, rhTM has already been used widely for DIC encountered in infections and hematological malignancies and has shown outstanding effectiveness [20–23]. Contrary to activated protein C reagent, thrombomodulin activates protein C only in the presence of thrombin and thus theoretically prevents the excessive inhibition of coagulation and consequent bleeding tendency. Recently, the cases of a couple of patients have been reported, in which TMA or VOD after SCT was dramatically resolved using rhTM [31–34]. However, coagulation disorders in SCT are complicated and multifactorial as discussed before. We have to be careful in evaluating the effectiveness and safety of this new drug for coagulation disorder in SCT and prospective studies are necessary.

In this context, we have used rhTM for the first time in 12 patients with SCT on the basis of our tentative scoring system. Significant improvement in coagulopathy was observed in 10 out of 12 patients, and 10 patients survived. Although all patients were thrombocytopenic ($<1.0 \times 10^4/\mu l$) and refractory to platelet transfusion, significant adverse events such as deterioration of bleeding tendency were not observed.

rhTM may have an indirect and circumstantial role in patients with coagulation disorder after SCT. Removal and control of background complicated pathology, which causes and deteriorates endothelial damage, is essentially important. However, if coagulation disorder progresses fast and organ function worsens rapidly, the patient will be refractory to any treatment. In cases 1 and 2, rhTM administration was not initiated immediately after the score turned positive. Thereafter, rhTM administration was initiated as early as possible when the score turned positive. From the clinical point of view, only delaying rapid aggravation of coagulopathy and keeping moratorium time for essential treatment will be critical for survival of the patient with transplantation-related complication. Thus, rhTM seems quite promising in its efficacy and safety for the management of early coagulation disorder in SCT, although we performed a study with a limited number of patients.

Our tentative scoring system is preliminary and still open to argument, especially about the optimal timing for initiation of rhTM. In this study, we have introduced rhTM on a pre-emptive or emptive basis for early coagulation disorders in patients with SCT. To achieve better outcomes, preventive usage of rhTM could be considered in high-risk patients. Further clinical studies and discussion are required for more effective and suitable application of rhTM after SCT.

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Conflict of interest The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

B-cell function after unrelated umbilical cord blood transplantation using a minimal-intensity conditioning regimen in patients with X-SCID

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Abstract Patients with X-linked severe combined immunodeficiency (X-SCID) suffer from severe and persistent infections, and usually die early in life unless treated by hematopoietic stem cell transplantation. If a patient has an HLA-identical sibling donor, preparative conditioning is not necessary for T-cell engraftment and B-cell function. However, in the absence of such a donor, long-term reconstitution of full B-cell function is often problematic, leading in many cases to a lifetime requirement for immunoglobulin replacement therapy. Preparative myeloablative conditioning has been shown to improve long-term B-cell function, but may aggravate pre-existing infection and transplant-related toxicity. It is thus important to determine the minimum intensity of conditioning that assures immunoglobulin production. In the present study, we performed reduced-intensity conditioning (RIC), consisting of fludarabine 125 mg/m^2 and melphalan 80 mg/m^2, prior to unrelated umbilical cord blood transplantation (UCBT) for five patients with X-SCID, none of them had an HLA-identical donor. Four patients survived more than 4 years without sequelae, and none required long-term immunoglobulin replacement therapy. One patient succumbed to sepsis in conjunction with severe GVHD. Our result demonstrates that the RIC regimen described above in combination with UCBT is an effective and less toxic conditioning to correct B-cell function in patients with X-SCID.

Keywords X-SCID · Reduced-intensity conditioning · Umbilical cord blood transplantation · Fludarabine/melphalan

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Introduction

X-linked severe combined immunodeficiency (X-SCID), which accounts for approximately half the cases of SCID, is caused by mutations of the γc chain. Immunological characteristics of this disease include profound impairment of both cellular and humoral immunity due to the absence or diminished numbers of T cells and natural killer (NK) cells, and abnormal B-cell function in spite of normal or elevated numbers of B cells. Therefore, patients with X-SCID suffer from severe and persistent infections, including opportunistic pathogens, and usually die early in life unless treated by hematopoietic stem cell transplantation (HSCT) or gene therapy [1]. Previous reports demonstrated excellent results of HLA-identical BMT with

survival rate over 90 %, and full restoration of T- and B-cell function [2, 3]. Since most patients do not have an HLA-identical sibling donor, HLA-haploidentical bone marrow transplantation (BMT) was developed in the early 1980s, when better T-cell depletion methods became available. However, the survival rate was lower, at around 60–80 %, and about half of the patients required life-long immunoglobulin replacement therapy despite normal T-cell immunity with or without pre-transplant conditioning [2–4]. These results suggested that T-cell-depleted, HLA-haploidentical bone marrow cells might not be a suitable source of HSCT for correcting B-cell function. Another strategy to treat this condition is to use unrelated donor HSCT with conventional myeloablative conditioning regimens, which leads to stable reconstitution of T- and B-cell function [5]. However, this approach has been associated with significant treatment-related toxicities and aggravation of pre-existing infections. To avoid these problems, reduced-intensity conditioning (RIC) regimens have been developed. Recently, Rao et al. [6] reported that a RIC regimen using a total dose of 150 mg/m^2 of fludarabine, 140 mg/m^2 of melphalan, and Campath 1H or ATG resulted in an improved survival and reduced transplantation-related mortality, compared with myeloablative conditioning, in children with primary immunodeficiency (PID) undergoing unrelated BMT. They used the same regimen for patients with X-SCID who would be expected to need less intensive conditioning because their immune system is already profoundly impaired. Based on their report, we performed unrelated umbilical cord blood transplantation (UCBT) with pre-transplant conditioning using a further reduced dosage of fludarabine and melphalan, in the absence of Campath 1H or ATG, to investigate whether a minimally intensive conditioning regimen could assure correction of B-cell function in X-SCID patients.

Patients and methods

Patients

Five patients with typical X-SCID received unrelated UCBT because they had no HLA-matched sibling donor. As shown in Table 1, mutations in the γc chain gene were detected in all patients. Patient 3 suffered from pneumonia caused by *Pneumocystis jiroveci* at the time of diagnosis of X-SCID. All patients except for patient 3 were diagnosed with X-SCID at birth because their brothers had the same disease. Immunoglobulin replacement therapy was initiated once hypogammaglobulinemia was confirmed, and IgG trough levels were maintained over 500 mg/dL. This study was performed with the approval of Institutional Review Board at each university and with the written informed consents of the parents.

Conditioning regimen and GVHD prophylaxis

Pre-transplant conditioning for all patients consisted of fludarabine (25 mg/m^2 per day) from day −7 to day −3

Table 1 Patient characteristics

Patient	1	2	3	4	5
Age at diagnosis (months)	0	0	4	0	0
Age at UCBT (months)	3	3	10	3	3
Mutations in the γc chain	868 G > A	691 G > A	c.735 741*	IVS4 + 2 T > A	568A > G
HLA identity	6/6	5/6	6/6	5/6	5/6
Nucleated cell dose (×10^7/kg)	7.1	10.0	5.0	9.5	11.2
CD34+ cell dose (×10^5/kg)	1.09	3.65	ND	3.50	1.68
Hematological recovery					
Nt > 500/μL	30	20	36	20	12
Plt > 5 × 10^4/μL	10	16	95	17	16
Ret > 1 %	18	16	38	20	15
Complications at UCBT	None	None	Pneumonia	None	None
Additional infections during UCBT	None	None	Sepsis	CMV	Sepsis
GVHD					
Prophylaxis	CyA	FK + sMTX	FK	FK + sMTX	CyA + PSL
Acute (grade)	0	0	II	II	III
Chronic	–	–	Extensive	–	Extensive
Therapy	–	–	FK + mPSL	FK + PSL	FK + MMF + PSL

*c.735 741** c.735_741delAGCCACC→insGGGAGCAATACTT, *ND* not determined, *Nt* neutrophils, *Plt* platelets, *Ret* reticulocytes, *sMTX* short-term methotrexate

(total dose 125 mg/m^2) and melphalan (40 mg/m^2 per day) from day −4 to day −3 (total dose 80 mg/m^2). Neither ATG nor Campath 1H was included in the conditioning regimen.

Prophylaxis for acute GVHD was performed with either cyclosporine A (CyA) with/without prednisolone or FK506 with/without short-term methotrexate as shown in Table 1.

Graft characteristics

As shown in Table 1, UCB units were either serologically full-matched or one locus mismatched at 6/6 (A, B, DR) HLA loci. Infused nucleated cell doses were 5.0 × 10^7/kg–11.2 × 10^7/kg (mean 8.6 × 10^7/kg), which contained CD34+ stem cells, ranging from 1.09 × 10^5/kg to 3.65 x 10^5/kg (mean 2.48 × 10^5/kg) except for patient 3, whose information on CD34+ cells was not available.

Chimerism studies

Hematological recovery was defined as achievement of absolute neutrophil count (ANC) >500/μL for 3 consecutive days and a platelet count >5.0 × 10^4/μL for 7 consecutive days without need for further transfusion. Chimerism was analyzed at Human Leukocyte Antigen Laboratory (Kyoto, Japan) as described previously [7]. Briefly, T cells, B cells and NK cells were separated by anti-CD3, anti-CD19 and anti-CD56 microbeads (Invitrogen Dyanl AS, Oslo, Norway), respectively. Donor- and recipient-specific polymorphic short tandem repeats (STR) were amplified by PCR, and subsequently analyzed by SDS-PAGE.

Immunological reconstitution studies

Immunological reconstitution status after transplantation was monitored by serum immunoglobulin levels (IgG, IgA, IgM and IgE), isohemagglutinin, and specific antibodies, and by flow cytometry analyses of peripheral mononuclear cells for CD3, CD4, CD8, CD19, CD16 and CD56.

Results

The age at transplantation was 3 months in four patients and 10 months in one patient (Table 1). All patients received UCBT using fludarabine (125 mg/m^2) and melphalan (80 mg/m^2) as a pre-transplant conditioning. They all achieved engraftment of ANC > 500 μL and platelets > 5.0 × 10^4/μL at a mean of 23.6 days (range 12–36 days) and 30.8 days (range 10–95 days), respectively. All but one survived more than 4 years without complication. One patient, patient 5, succumbed to sepsis in conjunction with severe GVHD.

Infections

Patient 3 suffered pneumonia due to *P. jiroveci* infection prior to admission and intravenous trimethoprim/sulfamethoxazole therapy was initiated. The pneumonia resolved with the engraftment of donor cells. He also experienced an episode of sepsis due to enterococci after UCBT, which was cured by appropriate antibiotics. Patients 1, 2, 4, and 5 were diagnosed with X-SCID at birth by sequencing of the γc chain because their brothers had the same disease. They had been protected in a clean environment soon after birth and they did not experience any infection until UCBT. Patient 5 developed sepsis due to a catheter infection, which was the cause of death at day 491 after UCBT.

Regimen-related toxicity and GVHD

Mild mucositis and myelosuppression were observed with this reduced-intensity conditioning, and no other regimen-related toxicity was noted.

Patient 3 developed acute GVHD grade II (skin stage 3) and extensive chronic GVHD, while patient 4 developed acute GVHD grade II (skin stage 3, liver stage 1 and gut stage 1). Symptoms in both cases resolved on prednisolone and FK506. Patient 5 developed acute GVHD grade III (skin grade 1, liver grade 3 and gut stage 3), followed by extensive chronic GVHD. He succumbed to sepsis in conjunction with uncontrolled GVHD, although he was treated with prednisolone, FK506 and mycophenolate mofetil (MMF).

Chimerism

Median follow-up was 68 months (range 48–73 months). As shown in Table 2, all survivors had complete donor T-cell chimerism. One survivor, patient 3, also had complete lymphocyte and granulocyte chimerism, which was confirmed by day 52. The others demonstrated mixed chimerism in these cell lineages. The percentage of the donor cells in each cell lineage had been stable since day 168 after UCBT in patient 1. In patients 2 and 4, detailed chimerism using fractionated cells was analyzed only the date indicated in Table 2. Donor cells of patient 5 constituted only 5 % of his peripheral blood nucleated cells at day 420 after UCBT, although T cells were 100 % of donor origin.

Immune reconstitution

Table 3 shows the results of immunologic evaluation at the most recent follow-up after UBCT in all survivors. Absolute numbers of lymphocytes were normal after

Table 2 Leukocyte chimerism

Patient	1		2		3		4		5
Days after UCBT[a]	168	1620	60	1098	52	2021	90	2078	420
T cell (donor %)	100	>95	90	94		100	100	92	100
B cell (donor %)	24	20	20	8		100	70	50	ND
NK cell (donor %)	55	69	15	33		100	90	84	ND
Granulocyte (donor %)	65	59	18	48	>95	100	20	13	ND
Lymphocyte (donor %)					>95				

ND not determined

[a] Days after UCBT when chimerism was determined

Table 3 Immune reconstitution

Patient	1	2	3	4	5 (at day 470)
WBC (/μL)	7700	7400	4710	9200	1000
Lymphocyte (/μL)	3700	4370	4120	4100	400
CD3 (%)	82.2	60.4	63.7	81.3	38.6
CD4 (%)	48.4	24.4	35.3	42.0	33.0
CD8 (%)	27.8	28.7	25.7	32.2	11.5
CD19 (%)	13.4	37.9	32.0	15.1	0.0
CD16/56 (%)	1.8	0.6	4.0	2.7	13.0
B-cell function					
IgG (mg/dL)	937	531	692	1157	660 (under i.v.Ig)
IgA (mg/dL)	58	32	55	101	89
IgM (mg/dL)	117	77	115	231	112
IgE (IU/mL)	37	<3	4.2	1	ND
Isohemagglutinin	+	+	−	+	−
Specific antibody	−	+	+	+	ND
T-cell function					
PHA stimulation (SI)	164.4	243.6	220.9	1213.4	1.1
ConA stimulation (SI)	897.7	322.1	225.5	713.1	1.1
NK activity (%)	15	4	10	19	ND

Normal values; PHA stimulation (SI) >100, ConA stimulation (SI) >75, NK activity 18–40 %

ND not determined, *SI* stimulation index

transplantation (Table 3). Numbers of CD3+, CD4+, CD8+ T cells and CD19+ B cells were within normal ranges, and T-cell function was normal by assessment with PHA and ConA stimulation. Immunoglobulin serum levels were within normal ranges of age-matched controls in all four patients, and none requires IgG substitution (Tables 3, 4). Also each patient had a positive antibody response. NK activity was lower than normal in all but patient 4.

Growth and psychomotor development

As shown in Table 4, all survivors have shown normal height, body mass index (BMI), psychomotor development and performance status to date.

Discussion

We report the outcome of unrelated UCBT in five patients with X-SCID using a RIC regimen. The most important result of this study is all four survivors are free from immunoglobulin replacement therapy.

Previous studies showed that about two-thirds of SCID patients required immunoglobulin replacement therapy after T-cell-depleted, HLA-haploidentical BMT from related donors without pre-transplant conditioning [2, 8]. In Europe, about half of SCID patients who received HLA-haploidentical related marrow cells were conditioned mostly with busulfan (8 mg/kg) and cyclophosphamide (200 mg/kg) [3]. However, the mortality rate for this type of conditioning was higher than that of patients without conditioning. Further, pre-transplant conditioning in combination with HLA-haploidentical related marrow cells did not always result in correction of B-cell function, and about one-third of the SCID patients continue to require immunoglobulin replacement therapy. In contrast, all surviving SCID patients, who had received bone marrow cells from unrelated donors after conventional conditioning with busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg), did not require immunoglobulin replacement therapy [5, 6]. This conventional conditioning regimen, however, has been associated with a significant mortality rate due to treatment-related toxicities such as profound pancytopenia, severe organ toxicity, and exacerbation of pre-existing infections. In addition, children treated with myeloablative regimens often suffer from delayed effects such as infertility, hormonal dysfunction, growth failure and secondary malignancies [9]. Recently, Rao et al. [6] reported the outcome of 33 patients with primary immunodeficiency (PID) [SCID ($n = 6$) and non-SCID ($n = 27$)] who received unmodified unrelated donor marrow grafts following reduced-intensity conditioning consisting of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (Campath 1H) or anti-thymocyte globulin (ATG). All patients had primary engraftment, and most patients achieved normal immunoglobulin production and B-cell function, although it is not clear whether patients with SCID were on immunoglobulin replacement therapy or not. From these

Table 4 Current status

Patient	1	2	3	4	5
Clinical status	Alive	Alive	Alive	Alive	Dead (at 17 months)
Follow-up (months)	68	48	73	69	17 months
Last i.v.Ig (months)	44	32	8	3	17 months
i.v.Ig at present	Off	Off	Off	Off	NA
Height	−1.0 SD	+1.92 SD	−1.0 SD	−0.2 SD	Short stature
Body mass index	15.9	14.5	14.5	15.2	BW 6 kg
Mental status	Normal	Normal	Normal	Normal	Normal
Karnofsky performance status	100 %	100 %	100 %	100 %	30 %

i.v.Ig intravenous immunoglobulin, *NA* not applicable, *SD* standard deviation, *BW* body weight

results, we speculated that T-cell depletion might interfere with B-cell engraftment and function. In this context, it is interesting to note that patients in our study who had acute GVHD complications showed higher B-cell chimerism and early immunoglobulin production after UCBT. However, one of our patients succumbed to sepsis in conjunction with severe GVHD. Unlike patients with hematologic malignancies, who benefit from the graft-vs-leukemia effect of donor cells, there is no such benefit from GVHD in patients with PID [10]. Thus, it is inevitable to use immunosuppressive drugs to prevent GVHD, and modifications such as the addition of ATG to our protocol to reduce the risk of GVHD will need to be evaluated in a future study [11]. Of note, two of our patients who did not develop acute GVHD gradually corrected their B-cell function, and immunoglobulin replacement therapy could be discontinued 32 and 44 months after UCBT. These results suggest that the RIC regimen described here may provide a minimal-intensity conditioning regimen in combination with UCB, which can assure sufficient production of immunoglobulin.

Some reports have raised concern about cardiac toxicity associated with high-dose melphalan and fludarabine used in combination [12, 13]. However, patients with this adverse event had been suffering from advanced hematologic malignancies and had been heavily treated with cytotoxic drugs including anthracyclines prior to pre-transplantation conditioning, and the total dosage of fludarabine (150 mg/m^2) and melphalan (140 mg/m^2) used for conditioning was much higher than the present study. In addition, reduction of melphalan from 140 to 80 mg/m^2 is expected to result in a lower frequency of cardiac toxicity. We only observed mild myelosuppression and mucositis as adverse events of the RIC regimen. Engraftment of unrelated cord blood cells, which might not be achieved with lower concentration of melphalan, was observed in all patients in our study. To date, none of our patients has shown any delay in growth or mental development. Long-term follow-up is necessary to validate the efficacy and safety of this RIC regimen.

Regarding B-cell engraftment and function, T-cell depletion from related donor bone marrow cells may not be a suitable source of HSCT for PID patients who do not have an HLA-identical sibling donor as described above. Recently, it was reported that UCB from unrelated donors could be used successfully for patients with PID [14, 15]. As UCB contains T cells, faster emergence of donor T cells is expected even though the infused T cells are functionally naïve. UCB recipients were able to discontinue immunoglobulin replacement therapy sooner and more frequently compared with T-cell-depleted bone marrow recipients although the estimated 5-year over all survival rates were comparable when UCB recipients received a myeloablative conditioning regimen [15]. In addition, UCBT is more tolerant of HLA disparity because the incidence and severity of GVHD is lower than for unrelated BMT. These results together with ours support the application of UCBT for patients with X-SCID who do not have an HLA-identical sibling donor.

Another risk factor for a poor outcome using HSCT for SCID is a pre-existing infection [8]. In our patients, all but one were diagnosed with X-SCID at birth from their family histories, and they had been kept in a protective environment for 3 months until they received UCBT. There are two reasons why we performed UCBT at the age of 3 months. One is to minimize regimen-related toxicities because infants are more susceptible to cytotoxic drugs, and the other is to expect higher survival rate after transplantation in the first 3.5 months of life as described previously [2, 16]. Early diagnosis before any infectious episodes is necessary for safe HSCT in the patients with SCID. Recently, screening of newborns for SCID has been recommended [17], and the RIC regimen described above in combination with UCBT is an alternative to HLA-haploidentical BMT for such patients.

In conclusion, our regimen in combination with UCBT is well tolerated and resulted in normal immunoglobulin production and B-cell function in our patients. Future studies with a modification of GVHD prophylaxis for patients with X-SCID who do not have an HLA-matched

sibling donor will be needed to further improve the outcome.

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Conflict of interest The authors declare no conflict of interest.

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重症複合免疫不全症に対する臍帯血ミニ移植後の混合キメリズムの遷延

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