研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Ⅳ. 研究成果の刊行物・別刷

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Unrelated Cord Blood Transplantation for Severe Aplastic Anemia

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ABSTRACT

In the present study we evaluated the feasibility of unrelated cord blood transplantation (UCBT) in patients with severe aplastic anemia (SAA). The outcome of 31 SAA patients (median age 28; range: 0.9-72.3 years old) who received UCBT was analyzed. The cumulative incidences of the neutrophil and platelet recovery after UCBT were 54.8 and 72.2%, respectively (95% confidence interval [CI] = 36.0%-70.3% and 51.3%-85.3%, respectively). The cumulative incidences of grade ≥II acute and chronic graft-versus-host disease (aGVHD, cGVHD) were 17.1% (95% CI = 6.2%-32.8%) and 19.7% (95% CI = 6.2%-38.8%), respectively. Currently, 13 patients are alive, having survived for 33.7 months (median; range: 6-77 months) after UCBT. The probability of overall survival (OS) at 2 years was 41.1% (95% CI = 23.8%-57.7%). A conditioning regimen that included low-dose total body irradiation (TBI) (2-5 Gy), fludarabine, and cyclophosphamide resulted in a favorable OS (80%; 95% CI = 20.4%-96.9%). This result suggests that UCBT using the optimal conditioning regimen can be a salvage treatment for patients without a suitable bone marrow donor and warrants evaluation in further prospective studies.

KEY WORDS
Unrelated cord blood transplantation • Severe aplastic anemia

INTRODUCTION

Over the last 2 decades, the outcome of patients with severe aplastic anemia (SAA) has dramatically improved regardless of whether patients received immunosuppressive therapy (IST) or bone marrow transplantation (BMT) [1-3]. BMT from an HLA-matched sibling is curative in the majority of younger patients with SAA, and is currently recommended as first-line treatment [4]. IST, with a combination of antithymocyte globulin (ATG) and cyclosporine (CSA), has been an alternative therapy for patients without an HLA-matched sibling. BMT from an unrelated donor (UD) is used as a salvage therapy for patients who fail to respond to IST or who experience a relapse of the disease. However, in general, the results of UD-BMT have been inferior to those achieved with an HLA-matched sibling.

The report the Center for International Blood and Marrow Transplant Research (CIBMTR) on UD-BMT (n = 231), for the period 1988-1998, showed that the overall survival (OS) rates for matched and mismatched UD-BMT in patients with SAA were 39% and 36%, respectively [5]. The Japan Marrow Donor Program (JMDP) reported a favorable outcome with 56% survival rate in 154 patients with SAA who received UD-BMT between 1993 and 2000 [6]. In

the recent 2 reports from the European Group for Blood and Marrow Transplantation (EBMT) and the French Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC), the outcomes of UD-BMT for SAA before and after 1998 were compared. The results demonstrated improved OS rates of UD-BMT since year 1998 (32% versus 57% for EBMT and 29% versus 50% for SFGM-TC) [7,8]. The authors speculated that the better HLA matching because of the introduction of high-resolution HLA typing may have contributed to the improved outcomes. In pediatric series, 90% OS rates have been recently reported for UD-BMT patients, which is comparable to that observed for BMT from a matched sibling [9,10].

Treatment approaches for patients who lack a suitable unrelated bone marrow donor remain a great challenge. Cord blood has been used as an alternative source of HSCT, and it has the advantages of rapid availability on demand and a low incidence of graft-versus-host disease (GVHD). There were only a few reports on unrelated cord blood transplantation (UCBT), which included patients with SAA. The results showed poor outcome and high incidence of graft failure [11,12]. However, a few small series and case reports of successful UCBT for SAA have recently been reported [13-17]. Because of the possible reporting bias, the general efficacy of UCBT is still unknown. Therefore, we decided to further examine this procedure by using the database of the Japan Cord Blood Bank Network (JCBBN). We identified 31 patients with acquired SAA who received UCBT and analyzed the outcome.

PATIENTS AND METHODS

Patients

From September 1998 until February 2006, 53 patients with acquired SAA received UCBT through JCBBN. Twenty-two patients who received UCBT as a salvage therapy for the engraftment failure after previous HSCT were excluded, and the remaining 31 patients were included in this study. Patient characteristics and the cord blood units are summarized in Table 1. Patients were eligible for UCBT if they had no HLA-identical related or unrelated bone marrow donor. Patients who could not wait for UD-BMT because of unstable diseases were also considered to be eligible for UCBT. Cord blood units with 0 to 2 HLA locus mismatches by serology in HLA-A, HLA-B, and HLA-DRB1 were searched and then the unit with the largest cell dose was selected. At least 2.0×10^7/kg mononuclear cells (MNCs) were given in all patients.

The age of the patients ranged 0.9 to 72.7 years (median 27.9 years), and there were 8 patients older than 50 years of age. There were 25 patients who had been previously treated with IST, including ATG + CSA (n = 13), ATG only (n = 4), or CSA only (n = 8). In 4 patients, androgen had been given. The remaining 2 patients were given only supportive therapy. All patients or their guardians gave informed consent for transplantation and submission of the data to the JCBBN.

Table 1. *Patient and Donor Characteristics (n = 31)*

Characteristic	
Median patient age, years (range)	27.9 (0.8-72.7)
Sex (male/female)	
Patient (n)	11/20
Donor (n)	14/17
Etiology of aplastic anemia	
Idiopathic/hepatitis associated (n)	30/1
Disease duration before UCBT: median, days (range)	337 (31-5063)
1 year or less/ 1-3 year/3 year or more/unknown (n)	13/4/8/5
Red blood cell transfusions before UCBT	
Less than 20 times/20 or more times/unknown (n)	8/21/2
Platelet transfusions before UCBT	
Less than 20 times/20 or more times/unknown (n)	7/22/2
HLA mismatches (serologic): GVHD direction (n = 31)	
0/1/2 (n)	4/18/9
HLA mismatches (serologic): rejection direction (n = 31)	
0/1/2 (n)	6/17/8
HLA mismatches (DNA typing): GVHD direction (n = 22)	
0/1/2/3/4 (n)	2/6/6/6/2
HLA mismatches (DNA typing): rejection direction (n = 22)	
0/1/2/3/4 (n)	1/5/12/3/1

UCBT indicates unrelated cord blood transplantation; GVHD, graft-versus-host disease.

Recipient-Donor HLA Matching

Data were available for 31 patients with serology-based recipient-donor HLA matching and for 22 patients who underwent high-resolution DNA typing for class I-HLA-A, HLA-B, and DRB1 (Table 1). The HLA disparities for both GVHD and rejection directions are shown in Table 1.

Transplantation Procedure

Characteristics of the transplantation procedures are listed in Table 2. The conditioning regimens varied according to the individual centers used. The 3 most commonly used regimens were: TBI (4-5 Gy) + fludarabine (FLU; 120-175 mg/m^2) + Melphalan (MEL) (80-120/mg/m^2) (n = 12), TBI (2-4 Gy) + FLU (90-250/mg/m^2) and cyclophosphamide (CY; 50-100 mg/kg or 2250/mg/m^2) (n = 5), and TBI (10-12 Gy) + CY (120-200 mg/kg) + ATG (n = 3). Of the 25 patients given irradiation, 24 received TBI

Table 2. *Transplant Procedures (n = 31)*

	No. of Patients
Conditioning Regimen	
TBI (4-5 Gy) + MEL+ FLU	12
TBI (2-4 Gy) + CY + FLU	5
TBI (10-12 Gy) + CY + ATG	3
Others	11
Radiation	
TBI/TAI	25/1
No radiation	7
ATG	
Yes/No	7/24
GVHD prophylaxis	
CSA	6
CSA + others (MTX/steroid/MMF)	10
Tacrolimus	7
Tacrolimus + others (MTX/steroid)	8
MNC cell dose	
$\geq 2.0 \times 10^7$/kg, $< 3.0 \times 10^7$/kg	15
$\geq 3.0 \times 10^7$/kg	16
CFU-GM cell dose	
$< 2.0 \times 10^4$/kg	14
$\geq 2.0 \times 10^4$/kg	15
Unknown	2
CD34 cell dose	
$< 1.0 \times 10^5$/kg	10
$\geq 1.0 \times 10^5$/kg	15
Unknown	6

TBI indicates total body irradiation; TAI, thoracoabdominal irradiation; MEL, melphalan; FLU, fludarabine; CY, cyclophosphamide; ATG, antithymocyte globulin; CSA, cyclosporine; MTX, methotexate; MMF, mycophenolate mofetil; MNC, mononuclear cell; CFU-GM, colony-forming unit granulocyte-macrophage.

and 1 underwent thoracoabdominal irradiation. A total of 7 patients were administered with ATG, either horse ATG (Lymphoglobulin 30-75 mg/kg in 5 patients) or rabbit ATG (Thymoglobulin 10 mg/kg in 2 patients). GVHD prophylaxis also varied according to the individual centers (Table 2). To facilitate the recovery of neutrophils, all patients received recombinant human granulocyte colony-stimulating factor. The number of mononucleated cells, colony-forming units of granulocyte-macrophage (CFU-GM), and CD34-positive cells of the cord blood units at the time of freezing are shown in Table 2.

Definitions and Statistical Analysis

The status of all patients was evaluated based on the last follow-up report, which was performed using the standardized forms provided by the JCBBN. All results were analyzed as of June 2008.

Date of engraftment was defined as the first of the 3 consecutive days where the neutrophil recovery was $>0.5 \times 10^9$/L. Platelet recovery was defined as the first of the 3 consecutive days where the unsupported platelet count was $>50 \times 10^9$/L. Chimerism was evaluated in 12 patients, with fluorescent in situ hybridization for the Y chromosome performed in 6 sex-mismatched grafts and quantitative polymerase chain reaction analysis for microsatellite DNA markers performed in 6 sex-matched transplantations. Acute and chronic GVHD (aGVHD, cGVHD) were diagnosed and graded according to standard clinical criteria [18,19].

Probability of OS was estimated according to the Kaplan-Meier method. GVHD and engraftment were assessed using the cumulative incidence procedure, and death was the competing event. Univariate comparisons among various groups were made using the log-rank test. The variables evaluated included age of the patient, donor sex, sex mismatch, disease duration before UCBT, the number of pre-UCBT transfusions for red cells and platelets, IST before UCBT, HLA matching by serology and high-resolution DNA typing for both GVHD and rejection directions, the number of mononuclear cells, CFU-GM, CD34-positive cells of the cord blood units at the time of freezing, conditioning regimens, and the administration of ATG and GVHD prophylaxis (single agent versus ≥ 2 agents, MTX versus no MTX, or CSA versus tacrolimus). All statistical analyses were carried out with version 10 of the STATA software (StataCorp, College Station, TX).

RESULTS

Engraftment

Sustained engraftment was observed in 17 patients. The cumulative incidences of the neutrophil and platelet recovery after UCBT were 54.8 and 72.2%, respectively (95% confidence interval [CI] = 36.0%-70.3% and 51.3%-85.3%, respectively; Figure 1). The median times to achieve a neutrophil count $\geq 0.5 \times 10^9$/l and a platelet count $\geq 50 \times 10^9$/l were 19 days (range: 12-35 days) and 59 days (range: 39-145 days), respectively. Chimerism analysis results were available in 8 patients with sustained neutrophil engraftment. All of these patients showed complete donor chimerism with more than 99% donor cells. No mixed chimerism was observed. There were 7 patients who failed to achieve sustained engraftment among patients who survived more than 28 days after UCBT. Five patients did not achieve a primary engraftment. Although 3 of them underwent a second UCBT, all died of infections, with (n = 1) or without (n = 2) engraftment of the second graft. Autologous recovery was noted in 1 patient, which was proven by the chimerism analysis that demonstrated 100% recipient cells. One patient had achieved engraftment on day 19, but she suffered from late graft failure at day 176 and received second HSCT at day 203. The patient was still alive at the time of the last follow-up.

Results of the univariate analysis for engraftment are shown in Table 3. The GVHD prophylaxis with a single agent (CSA or tacrolimus) exhibited a significantly better engraftment rate than that seen for the other methods (75.0% versus 33.3%, $P = 0.02$).

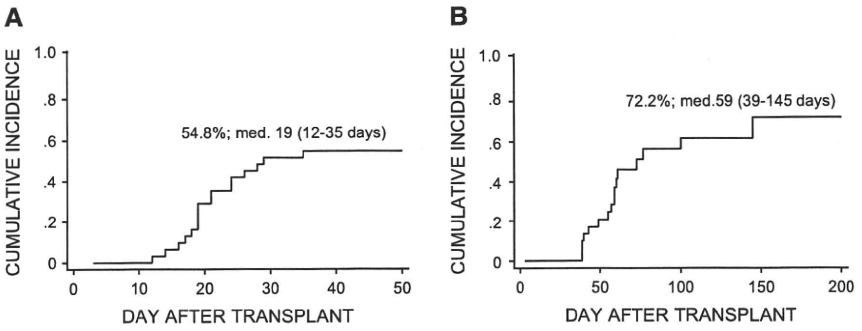

Figure 1. (A) Cumulative incidence of sustained donor neutrophil engraftment (>0.5 × 10^9/L) and (B) platelet engraftment (>50 × 10^9/L) after unrelated cord blood transplantation in patients with aplastic anemia.

When there was a lower number of transfusions (<20 times) of red cells and platelets prior to the HSCT, there was a trend for a better chance of successful engraftment compared to cases where there were higher number of transfusions (≥20 times), although this was not statistically significant. The number of infused MNCs, CFU-GM, and CD34 had no impact on the engraftment.

Table 3. *Outcome following Unrelated Cord Blood Transplantations for Aplastic Anemia: Univariate Analysis*

Covariates	2-Year-OS (%) (95% CI)	P	Engraftment (%) (95% CI)	P
Recipient age				
<20 year (n = 9)	44.4 (13.6-71.9)	.18	44.4 (13.6-71.9)	.76
20- 40 year (n = 12)	56.3 (24.4-79.1)		66.7 (33.7-86.0)	
>40 year (n = 10)	20.0 (3.0-47.5)		50.0 (18.4-75.3)	
Disease duration before UCBT				
<1 year (n = 13)	35.7 (13.0-59.4)	.34	57.1 (28.4-78.0)	.67
≥1 year (n = 12)	47.6 (18.2-72.4)		58.3 (27.0-80.1)	
RBC transfusions before UCBT				
<20 (n = 8)	62.5 (22.9-86.1)	.26	75.0 (31.5-93.1)	.08
≥20 (n = 21)	31.4 (13.1-51.7)		47.6 (25.7-66.7)	
Platelet transfusions before UCBT				
<20 (n = 7)	57.1 (17.2-83.7)	.28	85.7 (33.4-97.9)	.05
≥20 (n = 22)	35.0 (16.1-54.7)		45.4 (24.4-64.3)	
HLA matching by serologic typing (GVHD direction)				
0-1 mismatched (n = 22)	49.2 (27.3-68.0)	.34	63.6 (40.3-79.9)	.10
2 mismatched (n = 9)	22.2 (3.4-51.3)		33.3 (78.3-62.3)	
HLA matching by serologic typing (Rejection direction)				
0-1 mismatched (n = 23)	43.5 (23.3-62.1)	.64	52.2 (30.5-70.0)	.59
2 mismatched (n = 8)	37.5 (8.7-67.4)		62.5 (22.9-86.1)	
Conditioning regimen				
TBI + CY + FLU (n = 5)	80.0 (20.4-96.9)	.02	75.0 (40.8-91.2)	.17
TBI + MEL + FLU (n = 12)	46.9 (17.6-71.9)		80.0 (20.4-96.9)	
Others (n = 14)	21.4 (5.2-44.8)		28.6 (08.8-52.4)	
ATG				
No (n = 24)	48.9 (27.8-67.0)	.007	66.7 (44.3-81.7)	.19
Yes (n = 7)	14.3 (0.7-46.5)		14.3 (0.7-46.5)	
GVHD prophylaxis				
CSA or tacrolimus only (n = 13)	54.6 (27.4-75.3)	.07	75.0 (46.3-89.8)	.02
CSA or tacrolimus+others (n = 18)	26.7 (8.3-49.6)		33.3 (12.2-56.4)	
MTX				
No (n = 20)	38.5 (17.7-59.1)	.93	60.0 (35.7-77.6)	.24
Yes (n = 11)	45.5 (16.7-70.7)		45.5 (16.7-70.7)	
MNC				
2 × 10^7/kg-3 × 10^7/kg (n = 15)	45.0 (19.4-67.8)	.61	60.0 (31.8-79.7)	.70
≥3 × 10^7/kg (n = 15)	37.5 (15.4-59.8)		50.0 (24.-71.0)	
CD34				
<1 × 10^5/kg (n = 15)	45.7 (14.3-73.0)	.32	70.0 (32.9-89.2)	.52
≥1 × 10^5/kg (n = 15)	33.3 (12.2-56.4)		53.3 (26.3-74.4)	

GVHD indicates graft-versus-host disease; TBI, total-body irradiation; CY, cyclophosphamide; Mel, melpharan; Flu, fludarabine; ATG, antithymocyte globulin; CSA, cyclosporine; MTX, methotrexate; MNC, mononuclear cell; CFU-GM, colony-forming unit-granulocyte macrophage; UCBT, unrelated cord blood transplantation.

GVHD and Viral Infections

Acute GVHD (≥grade II) was observed in 5 patients (grade II; n = 4, grade III; n = 1) on days 8 through 56, and was lethal in the 1 patient with grade III aGVHD. Chronic GVHD was observed in 4 patients (extensive: n = 1, limited: n = 3; de novo n = 2, progression from aGVHD n = 2) on days 124 through 213. Figure 2 depicts the cumulative incidence of grade II-IV aGVHD (17.1%; 95% CI = 6.2%-32.8%) and cGVHD (19.7%; 95% CI = 6.2%-38.8%). Viral reactivations were commonly observed in this study. CMV reactivation was noted in 9 patients, and 1 of them developed CMV disease. Epstein-Barr virus (EBV) reactivation was noted in 1 patient, having developed cerebral infarction, which was considered to be related with EBV. Adenovirus induced cystitis occurred in 1 patient.

Survival

Of the 31 total patients, 13 are presently alive, with survival durations of 6 to 77 months (median 33.7 months) after the transplantations. The probability of OS at 2 years was 41.1% (95% CI = 23.8%-57.7%). The results of univariate analysis of the factors influencing survival are shown in Table 3. The conditioning regimen and the administration of ATG were the only factors that were significantly related to the survival. The conditioning regimen, which included low-dose TBI, FLU, and CY, resulted in better outcomes than were seen for the other regimens (Table 3 and Figure 3). The administration of ATG was associated with poor outcome (Table 3 and Figure 3). There were 5 out of 7 patients given ATG that died before engraftment because of infections (n = 3) or hepatic veno-occlusive disease (VOD) (n = 2). In the 2 other patients, 1 demonstrated autologous recovery, whereas the other patient has had sustained engraftment and is currently still alive. There tended to be a better outcome noted for GVHD prophylaxis with a single agent (either CSA or tacrolimus) compared to prophylaxis with 2 or more agents. The outcome for the patients aged 40 years and older was inferior to that seen for the younger patients, although this was not statistically significant.

In the 18 patients who died, the causes of death were graft failure (n = 7), bacterial/fungal infections (n = 3), EBV-related cerebral infarction (n = 1), VOD (n = 3), aGVHD (n = 1), acute respiratory distress syndrome (n = 1), encephalopathy (n = 1), and cardiac toxicity (n = 1).

DISCUSSION

The outcome of 31 patients with SAA who received UCBT was analyzed in this study. This is the first report on a nationwide multicentral study that focused on UCBT for SAA as far as we know. The overall survival rate was 41%, which is comparative to the results of the large registry-based analysis of UD-BMT for SAA by CIBMTR [5], but inferior to the results of some recent reports of UD-BMT [6,20]. The incidence and the severity of aGVHD and cGVHD were considerably lower in this study, which is advantageous for UCBT. The major problem encountered, however, was still the high incidence of engraftment failure after UCBT. In the present study the conditioning regimen with the low-dose TBI, FLU, and CY resulted in better outcome (80% survival rate) compared to other regimens. This regimen and the selection of optimal donor with better HLA match and higher cell dose may improve the outcome of UCBT for SAA.

Previous reports on the conditioning regimen of UCBT for SAA are limited. Mao et al. [13] reported on 9 patients with SAA who were conditioned with ATG and CY (60 mg/kg) prior to undergoing UCBT. A total of 7 out of 9 of these patients survived with hematologic recovery. However, a donor-recipient mixed chimerism was present in all patients. There are a few case reports of UCBT for SAA using more intensified regiments, which resulted in successful engraftment along with complete chimerism [14-16,21].

Radiation-containing regimens are efficient in achieving better engraftments and widely used within the UD-BMT settings for patients with SAA, although these regimens are associated with significant early and late toxicities, including secondary malignancies [22]. Recent study by Deeg et al. [20] to define the optimal TBI dose in combination with CY (200 mg/kg) and

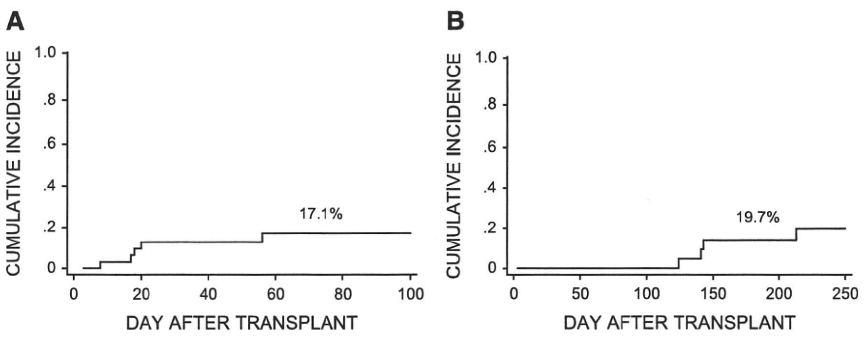

Figure 2. Cumulative incidence of ≥ grade II aGVHD (A) and cGVHD (B) in patients with aplastic anemia who received UCBT.

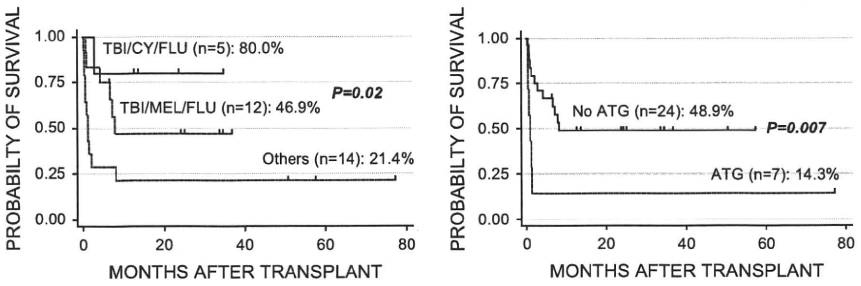

Figure 3. Probability of survival after conditioning regimens in patients with aplastic anemia, who received unrelated cord blood transplantation. TBI: total body irradiation, CY: cyclophosphamide, MEL: melphalan, FLU: fludarabine, ATG: antithymocyte globulin.

ATG for use with UD-BMT in patients with SAA showed that 2 Gy was sufficient to allow engraftment without increasing toxicities. This finding was also supported by a Japanese study on UD-BMT in patients with SAA, which reported that in a small group given a conditioning regimen of low-dose TBI (2-5 Gy), CY (200 mg/kg), and ATG, there was a 90% survival rate [7].

Fludarabine is currently widely used for nonmyeloablative transplants for a variety of diseases including SAA [23-26]. In the recent study on UD-SCT from the Severe Aplastic Anemia Working Party of the EBMT (SAA WP-EBMT), they designed a non-TBI regimen that used FLU (120 mg/m^2), CY (1200 mg/m^2), and ATG [27]. In this study, a total of 38 both pediatric and adults patients with SAA were included (36 BMT and 2 PBSCT patients) and the 2-year survival rate was 73%, with a low incidence of aGVHD and cGVHD. Therefore, this result suggests that a FLU containing regimen might be effective for use with UD-HSCT in SAA. The authors suggested that the conditioning regimen might need to be modified for adults through the addition of a low dose of TBI, as there was a significantly lower engraftment rate seen in the adult patients (82% overall, 68% in adults). Overall, these findings in previous reports and in this study suggest that the conditioning regimen that included the low-dose TBI and FLU resulted in favorable outcomes. In present study, the 7 patients given ATG were poor. Only 1 of them achieved engraftment and is alive. However, the number of patients given ATG was too small to reach any definitive conclusions and the benefit of ATG in UCBT for SAA should be evaluated in a large prospective study.

The GVHD prophylaxis using a single agent (CSA or tacrolimus) exhibited a better engraftment rate and a marginally better survival rate compared to that seen when 2 or more immunosuppressive agents were used. In the latter group, steroid, MTX, or mycophenolate mofetil (MMF) were given in addition to CSA or tacrolimus. Because of the limited number of patients and the highly heterogeneous regimen of the GVHD prophylaxis in this study, it is difficult to define the optimal GVHD prophylaxis based on the current results. However, the low incidence and severity of GVHD that we noted in our study suggests that a single agent, regardless of whether it is tacrolimus or CSA, may be effective enough to prevent GVHD in UCBT for SAA.

One of the most important factors that determine the success of UCBT is the cell dose in the CB [11,28-30]. In the present study, a minimum of 2 $\times$ 10^7/kg MNCs were infused in all patients. In this condition, the dose of MNCs, CFU-GM, and CD34 had no impact on engraftment and survival. One of the benefits of UCBT is that it can overcome the HLA barrier. Despite the HLA disparity in the majority of the patients, the incidence of GVHD was quite low in this study. There was a tendency for better HLA matching to result in a better outcome, although this was not statistically significant. Selection of the CB units with higher cell dose and better HLA match may be essential to improve the outcome of UCBT for SAA.

In our study there were also 8 patients who were older than 50 years of age, which is generally considered to be over than the cutoff age for transplantation. Because of the poor outcome of UCBT in older patients (OS = 20% in group with age >40 years old), UCBT cannot be recommended for older patients at present, and repeated IST should be considered in these patients [31,32].

In summary, this first multicentral study focused on the UCBT for SAA suggests that UCBT can be an alternative treatment for SAA patients who failed to IST and have no suitable bone marrow donor. The results may be improved by using the optimal conditioning regimen such as low-dose TBI, FLU, and CY and by donor selection of better HLA match and higher cell dose.

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Umbilical Cord Blood Transplantation after Reduced-Intensity Conditioning for Elderly Patients with Hematologic Diseases

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ABSTRACT

Although allogeneic hematopoietic stem cell transplantation is a potentially curative approach for advanced hematologic diseases, its application to elderly people is limited because of their comorbid physical conditions and lower chance of finding suitable related donors. Umbilical cord blood transplantation with reduced-intensity pretransplant conditioning (RI-UCBT) is 1 way to avoid these obstacles. We analyzed elderly patients aged 55 years and older with hematologic diseases who underwent RI-UCBT at our institute to assess feasibility and effectiveness of this treatment approach. Among the 70 patients included, 50 died, 74% of them from nonrelapse causes. Infection was the primary cause of death. Estimated overall survival and progression-free survival at 2 years were both 23%. In multivariate analyses, standard-risk diseases, age younger than 61 years, grade 0-II acute graft-versus-host disease, and the absence of preengraftment immune reaction were significantly associated with better overall survival. RI-UCBT is a potentially curative and applicable approach for elderly patients. Higher mortality, especially from nonrelapse causes, is the biggest problem to be solved to increase the feasibility of this approach.

KEY WORDS
Cord blood transplantation • Reduced intensity • Elderly patients • Hematologic diseases

INTRODUCTION

Although morbidity associated with hematologic malignant diseases in elderly patients is higher than that in younger patients [1], elderly patients are less likely to be candidates for allogeneic stem cell transplantation, because of the fact that they are more likely to have comorbid organ conditions, either clinically or subclinically, which result in a higher rate of procedure-related mortality [2], and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity conditioning (RIC) for transplants, which results in less toxicity and depends largely on graft-versus-tumor effects rather than high-dose therapy to eliminate malignant cells, has been shown to allow elderly patients to undergo allogeneic transplants [3-5]. The use of umbilical cord blood transplantation (UCBT) has been increasing because of the potential advantage of rapid availability and the lower risk of graft-versus-host disease (GVHD), thus permitting less stringent HLA matching [6,7]. The outcome of UCBT has been reported to be similar to unrelated bone marrow in the myeloablative setting [8-10]. UCBT with reduced-intensity pretransplant conditioning (RI-UCBT) for adults, mostly younger than 55 years old, has been increasingly reported, and has been shown to be applicable even in patients with a relatively low number of nucleated cells for their body weight [11-16]. However, little information has been available on whether elderly patients can tolerate slower engraftment, more infectious complications [17], and the unique preengraftment immune reaction (PIR) associated with UCBT [18,19]. PIR has been described by us and others [18,19], characterized

by the symptoms induced possibly by hypercytokinemia, which sometimes cause severe organ damage and fatal outcome. We therefore retrospectively evaluated the use of the RI-UCBT in patients aged 55 and older by analyzing engraftment, nonrelapse mortality (NRM), GVHD, progression-free (PFS), and overall survival (OS) to address the feasibility and effectiveness of this method in older patients.

PATIENTS, MATERIALS, AND METHODS

Patients

This study included patients aged 55 and older who underwent RI-UCBT at our institute from July 18, 2002 through October 28, 2005. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia (AA) refractory to standard immunosuppressive therapy, as well as if they were unable to find suitable related or unrelated bone marrow (BM)/peripheral blood (PB) donors within reasonable time periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse because of adverse cytogenetic abnormalities, have a prior hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia with excess of blasts or chronic myelomonocytic leukemia, or have refractory anemia with transfusion dependency and/or severe neutropenia. Patients with chronic myeloid leukemia (CML) had to be beyond the first chronic phase. Lymphoma patients had to be beyond the first remission except those with acute or lymphoma type adult T cell leukemia. Patients who had end-stage organ dysfunction (DLco <30% predicted or LVEF <35%), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent, and the study was approved by the appropriate institutional review boards.

Donor Selection

UCB units were obtained from Japanese Cord Blood Bank Network. HLA-A and HLA-B antigens were identified by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using polymerase chain reaction (PCR) sequence-specific primers. UCB grafts had at least 4 of 6 HLA-A, B antigens, and DRB1 alleles that were matched to the recipient and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. The median total nucleated cell number and median $CD34^+$ cell number were 2.8 (range: 1.8–5.2) $\times$ 10^7/kg and 0.84 (0.11-3.28) $\times$ 10^5/kg, respectively.

Patient Characteristics

Seventy consecutive patients were included in this study. Their characteristics are shown in Table 1.

Table 1. *Patient and Donor Umbilical Cord Blood Characteristics*

Characteristic	No. (%) of Patients
Sex	
Male	45 (64)
Female	25 (36)
Age (years)	
Median (range)	61 (55-79)
Age distribution (years)	
55 to 59	31 (44)
60 to 64	16 (23)
65 to 69	17 (24)
At least 70	6 (9)
Diagnosis	
AML	28 (40)
MDS	3 (4)
CML	4 (6)
ALL	11 (16)
NHL	8 (11)
ATL	12 (17)
MM	1 (1)
PCL	1 (1)
AA	2 (3)
HCT-CI	
0	24 (34)
1	25 (36)
2	11 (16)
3 or greater	10 (14)
History of prior chemotherapy	
Yes	59 (84)
No	11 (16)
History of prior documented infections	
Yes	15 (21)
No	55 (79)
Disease status	
Standard risk	15 (21)
High risk	55 (79)
Conditioning regimen	
Flu/Mel/TBI	65 (93)
Flu/Bu/TBI	4 (6)
Others	1 (1)
GVHD prophylaxis	
Cyclosporine A alone	37 (53)
Tacrolimus alone	33 (47)
HLA disparity to UCB	
5/6	9 (13)
4/6	61 (87)
Sex mismatch to UCB	
Yes	51 (73)
No	19 (27)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; MM, multiple myeloma; PCL, plasma cell leukemia; AA, aplastic anemia; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; UCB, umbilical cord blood: HCT-CI, hematopoietic cell transplantation-specific comorbidity index.

Of these 70 patients, 25 were women and 45 were men. Their median age was 61 years (range: 55-79 years). The patients' diagnoses included acute myeloid leukemia (AML; n = 28), acute lymphoblastic leukemia (ALL; n = 11), MDS (n = 3), CML (n = 4), non-Hodgkin lymphoma (NHL; n = 8), adult T cell

leukemia (n = 12), plasma cell leukemia (n = 1), multiple myeloma (n = 1), and AA (n = 2). Three patients had previous autologous hematopoietic cell transplantation. For disease status, those with hematologic malignancies in first or second complete remission at the time of transplant, those in the chronic phase or accelerated phase of CML, those with refractory anemia of MDS, and those with nonmalignant diseases were defined as being at standard risk (n = 15), whereas those in other situations were defined as being at high risk (n = 55). Patients were assessed for their comorbidity by the previously reported scoring system [20].

Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditioning varied, and was determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. Sixty-five patients underwent conditioning regimens with 125-180 mg/m^2 of fludarabine (Flu; 25 mg/m^2 for 5 days or 30 mg/m^2 for 6 days), along with 80 mg/m^2 of melphalan (Mel; 40 mg/m^2 for 2 days) and total-body irradiation (TBI) at a total dose of 4 Gy for 63 and 2 Gy for 2. Four patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of Mel. One patient underwent a conditioning regimen with thiotepa (5 mg/kg for 2 days) in addition to 125 mg/m^2 of Flu and 80 mg/m^2 of Mel, because of the urgent transplant schedule that did not allow access to TBI. Valproate sodium (300 mg/day) was administered to all patients who received Bu. Immunosuppressive therapy with cyclosporine A (CsA, 3 mg/kg continuous infusion, aiming for a serum concentration of 250-400 ng/mL) or tacrolimus (Tac, 0.03 mg/kg continuous infusion, aiming for 12-17 ng/mL) was started on day −1. CsA was used for patients in the early phase of this study, and, based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA.

Supportive Care

Prophylactic antibiotics, including fluorquinolone, fluconazole, and acyclovir, were used routinely. Patients received ganciclovir or foscarnet for any sign of a cytomegalovirus reactivation, such as isolation of CMV or detection of viral proteins (pp65) or nucleic acid in any body fluid or tissue specimen. *Pneumocystis jiroveci* prophylaxis included trimethoprim-sulfamethoxazole as first-line therapy.

Definition of Engraftment, Preengraftment Immune Reaction, and End Points

OS ands PFS were computed from the date of transplantation. Engraftment was defined as absolute neutrophil count >0.5 × 10^9/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-mismatched pairs, PCR for variable number of tandem repeats was used with donor cells detected at a sensitivity of 10%. Whole blood or BM cells were assessed at the time of granulocyte engraftment. PIR was characterized by the presence of at least 2 of the following symptoms with no direct consequences of infection or adverse effects of medication 6 or more days before engraftment, as described previously [12,18]; a high fever (>38.5°C), skin eruptions, diarrhea, jaundice (serum levels of total bilirubin >2.0 mg/dL), or body weight gain >10% of baseline. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for AML, ALL, CML, or MDS was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for NHL was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD (aGVHD, cGVHD) were defined and graded by standard criteria [21]. The following factors were considered potential predictors of outcomes: recipient's age, disease risk (standard versus high), ECOG performance status, HCT-specific comorbidity index score, history of prior chemotherapy (all cytoreductive chemotherapy excluding hydroxyurea and imatinib mesylate), history of prior documented infections (infectious episode with positive culture results for bacterial or yeast infections, and at least probable diagnosis of mold infection by EORTC/NIH-MSG criteria [22]), number of total nucleated cord blood cells, number of CD34$^+$ cells, HLA disparity, conditioning regimen, GVHD prophylaxis, grade of aGVHD, and the presence or absence of PIR.

Statistical Methods

OS was calculated from the day of transplantation until death from any cause or last follow-up. Disease-free survival (DFS) was calculated from the day of transplantation until relapse or death from any cause or last follow-up. The probabilities of survival and DFS were estimated and plotted using the Kaplan-Meier method [23]. Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks [24]. Similarly, in the analysis of GVHD rates, death because of other causes or relapse leading to early withdrawal of immune suppression were considered competing risks. The effect

of various patient and disease categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. Cumulative incidence curves were drawn using Gray's method [25].

RESULTS

Engraftment

Ten of the 70 patients were not evaluable for donor engraftment because of early death (before 28 days posttransplant) from disease progression (n = 1), infection (n = 7), and complications of central nervous system (n = 2). Of the 60 evaluable patients, the cumulative incidence of primary donor engraftment was 92% at a median of 18 days after transplantation (range: 11-53 days). Platelet recovery $>20 \times 10^9$/L was observed in 38 patients (63%), at a median of 35 days (range: 25-95 days). All patients required transfusions of platelets and red blood cells. Recovery of neutrophil counts $>0.5 \times 10^9$/L did not occur in 5 patients who survived beyond 28 days posttransplant; these patients were classified as primary graft failures. Two of these patients received secondary RI-UCBT and died of infection. The remaining 3 patients died of infection. All engrafting patients without BM relapse were complete donor chimeras beyond 1 month after transplantation (data not shown). Remarkably, all 3 evaluated patients of 10 who died before day 28 showed complete donor chimerism (94%, 100%, and 94.6% on days 12, 15, and 20 posttransplant, respectively).

PIR and GVHD

Forty-three patients experienced clinical symptoms defined as PIR, as described previously [12,18]. Patients who received Tac as GVHD prophylaxis tended to have a lower chance of experiencing PIR compared with those who received CsA, although differences were not statistically significant (53% versus 72%, respectively; $P = .1$).

Among 54 evaluable patients, 33 patients (61%) developed aGVHD of grade II or higher, including 23 patients (43%) who developed that of grade III or IV. Of the 30 patients who survived longer than 100 days posttransplant, 12 (40%) developed cGVHD, including 7 with limited and 5 with extensive form (Table 2).

Survival, Disease Progression, and NRM

At the time of analysis, 20 of 70 patients survived a median of 512 days (range: 103-1213 days) after transplantation. The Kaplan-Meier estimates of OS and PFS at 2 years were both 23% (Figure 1). The median OS time was 114 days (range: 7–1213 days), and the median PFS time was 92 days (range: 7–1213 days).

Table 2. *The Incidence and Severity of Graft-versus-Host Disease (GVHD)*

	Patients (n = 54)
	No. (%)
Acute GVHD	45 (83)
Grade II-IV	33 (61)
Grade III-IV	23 (43)
	Patients (n = 30)
	No. (%)
Chronic GVHD	12 (40)
Limited	7 (23)
Extensive	5 (17)

Eighteen patients (26%) showed progression of the underlying disease at a median of 134 days (range: 13–785 days) after transplantation, and 15 of these patients died of their disease.

Thirty-seven patients died of nonrelapse causes (Table 3). Nineteen of them were from infections, which was the leading cause of NRM. Among 33 deaths observed before day 100 posttransplant, 30 were from nonrelapse causes and 3 from disease progression. The cumulative incidences curves of NRM and disease progression are shown in Figure 2.

Factors Contributing to OS and NRM

In univariate analyses, survival was associated with recipient's age ($P = .01$), disease risk ($P < .01$), aGVHD ($P < .01$), and PIR ($P < .01$), with favorable outcomes in younger recipients (<61 years), those with standard risk, those with lower grade aGVHD (grade 0-II), and those without PIR (Figure 3A-D). Potential risk factors such as ECOG performance status, HCT-specific comorbidity index score, history of prior documented infection, history of prior chemotherapy, HLA disparity,

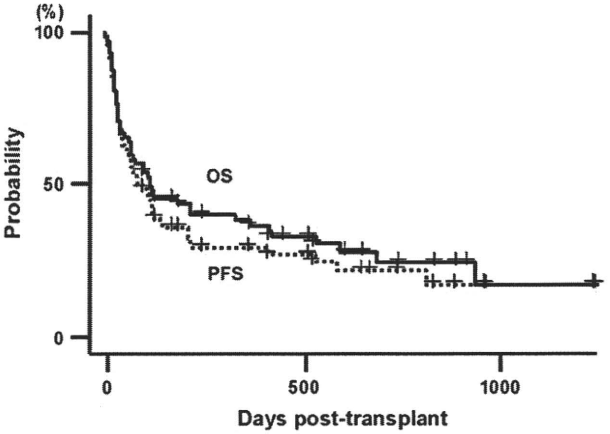

Figure 1. OS and PFS estimates for 70 patients with hematologic diseases treated with RI-UCBT.

Table 3. *Causes of Death*

	Patients (n = 70)
	No. (%)
NRM	37 (53)
Infection	19 (27)
GVHD	9 (12)
IP	4 (6)
TMA	3 (4)
Others	2 (3)
Relapse	13 (19)
Total	50 (71)

NRM indicates nonrelapse mortality; GVHD, graft-versus-host disease; IP, interstitial pneumonia; TMA, thrombotic microangiopathy.

sex mismatch, number of infused cells, number of infused $CD34^+$ cells, and cGVHD did not reach statistical significance.

In the Cox regression analyses, recipient's age equal to or older than 61 (hazard ratio [HR] = 3.33; 95% confidence interval [CI] = 1.39-7.14; $P = .006$), high risk disease (HR = 3.33; 95% CI = 1.01; 8.33 $P = .049$), grade III-IV aGVHD (HR = 2.5; 95% CI = 1.28; 5.88 $P = .0002$), and the presence of PIR (HR = 2.5; 95% CI = 1.14; 6.25 $P = .023$) were associated with statistically worse OS (Table 4). No other factors were significantly or suggestively associated with OS.

Regarding toxicity, multivariate analyses revealed that GVHD prophylaxis (HR = 3.9, 95% CI = 1.3-11.6 for CsA versus Tac; $P = .01$) and aGVHD (HR = 5.7, 95% CI = 2.1-15.7 for grade III–IV versus 0-II; $P = .001$) were associated with NRM.

DISCUSSION

This study was undertaken to evaluate engraftment and toxicities in elderly patients with advanced hematologic diseases who received UCBT matched for at least 4 loci of HLA-A, -B, and –DRB1 using a nonmyeloablative regimen.

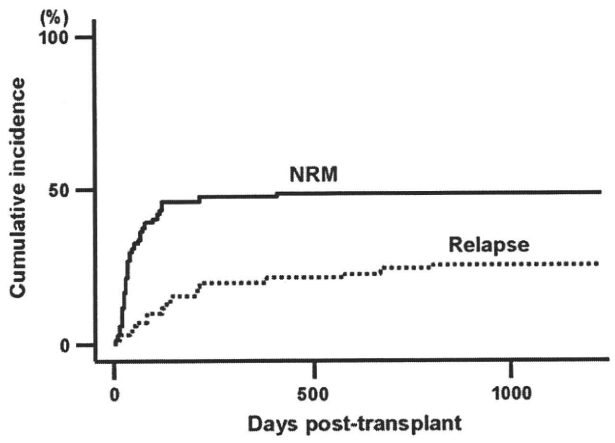

Figure 2. NRM and disease progression. Cumulative incidence estimates of NRM and disease progression for all 70 patients.

Several observations were made. First and foremost, RI-UCBT was a feasible treatment strategy for elderly patients with a successful engraftment rate of 92% without secondary graft failure except disease progression. The average interval between transplant and neurtophil recovery to 500/μL was 18 days, which is comparable to previously reported in RIC [11,12]. The chimerism study confirmed rapid engraftment of donor cells in all engrafted patients. Together with the fact that all 3 evaluated patients who died before day 28 already achieved complete donor chimerism, these data indicate that our pretransplant conditioning regimens, mainly consisting of Flu, Mel, and TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert sufficient immunosuppressive effects that allow engraftment of CB cells. Compared to the conditioning regimen containing cyclophosphamide reported from Minnesota group [11], which allow mixed chimeric state especially for myeloid lineages during the early period of posttransplant, our conditioning is more powerful in eradicating host myeloid cells as well, which may have beneficial effect for rapid control of myeloid malignancies. The OS and PFS were estimated as both 23% at 2 years posttransplant, almost comparable to or slightly less than the data reported previously [15,16,26], which can be reasonably explained by higher age range and poor disease status before transplant in this study cohort, which can be further supported by the result of subgroup analysis indicating those with standard disease status showed much better outcome (Figure 3B).

UCBT has been associated with lower incidence of aGVHD, possibly because of the immunologic naïvety of transplanted lymphocytes; however, this naïvety raises a concern about whether transplanted cells will have sufficient antimalignant activity. Several reports indicate the in vivo antimalignancy effect of cord blood cells [27-30]. Cumulative incidence of disease progression at 2 years posttransplant in our series was 24%, which is comparable to those previously reported [15,16,26]. It plateaued later than 795 days, indicating that our RI-UCBT treatment protocol offered fairly good disease control.

The incidence of GVHD was higher than previous reports in RIC [11,12], and was almost comparable to those of BM transplants, PB cell transplants, or UCBT with conventional conditioning [8-10,31-36]. Because of the poor disease status of the majority of patients included in this study, GVHD prophylaxis was initially planned to be less intensive with single calcineurin inhibitors. Older patients' age [37] or high incidence of infectious complications, which possibly induced excessive inflammatory cytokine secretions, could have been relevant to this result [38].

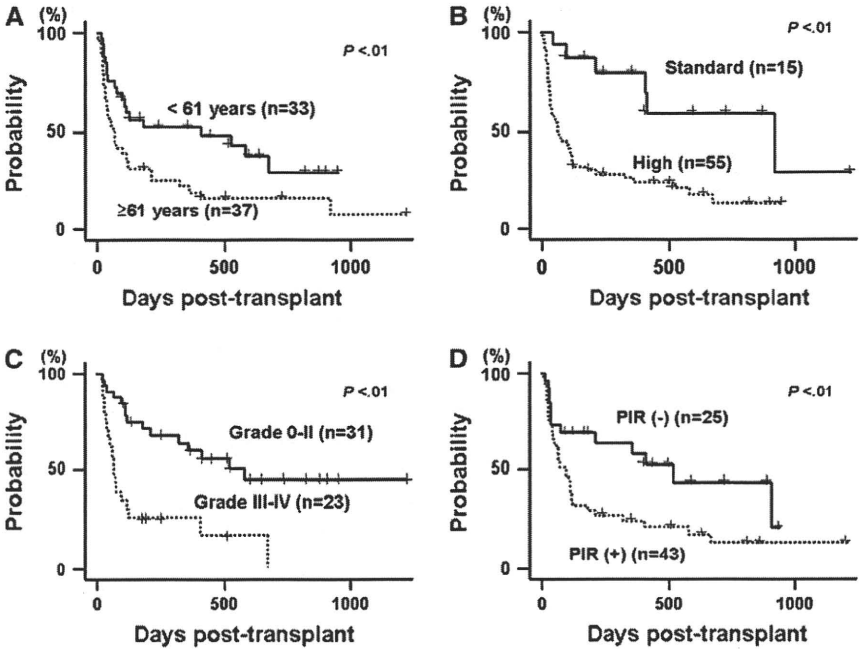

Figure 3. OS estimates after RI-UCBT (n = 70). (A) Effect of age. (B) Effect of disease status. (C) Effect of severity of aGVHD. (D) Effect of PIR.

Although RI-UCBT has been a feasible approach in terms of engraftment, a significant number of patients died from treatment-related complications. NRM was close to 3 times higher than mortality from relapse or disease progression, and most NRM occurred within 100 days posttransplant. Of 37 deaths because of NRM, 19 were from infection. Delayed engraftment relative to other stem cell sources such as BM or PB cells has been suggested to account for the higher rate of infectious complications after UCBT [32,39,40], but the time to engraftment in our series of patients was not delayed. Higher grade of aGVHD and the presence of PIR were found to be significantly associated with poor OS in multivariate analysis, indicating that immune-mediated events have strong impact on patients' outcome (Table 4). PIR is the syndrome observed in our setting of RI-UCBT. Although the mechanism behind PIR has not been investigated extensively yet, it is assumed to be reflecting alloimmune event, given our experience that more intensive GVHD prophylaxis with Tac had tendency to decrease the incidence of PIR. Moreover, development of PIR may have been suppressed in reported cases from other institutes that utilized additional agents to calcineurin inhibitors, such as methotrexate [10,19], antithymocyte globulin [31], or mycophenolate mofetil [16]. There has been a similar early immune reaction-like syndrome reported as "hyperacute GVHD" observed following BM or PBSC transplant, and responded poorly to corticosteroids compared to traditional aGVHD [41,42]. The incidence of PIR was higher than that of hyperacute GVHD, and further investigation on biologic mechanisms may help us define PIR more precisely relative to other immune-mediated diagnosis and develop optimal treatment approach. The presence of PIR was shown to cause more NRM than the absence in univariate analysis (P = .02), although it did not reach statistical significance in multivariate analysis. Thus, better management of immune-mediated complications will be the key to reduce NRM and improve OS. Based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA, because Tac was shown to be more potent than CsA in BM transplant [43-45]. Patients who received Tac as GVHD prophylaxis had less chance of experiencing PIR compared with those who received CsA and had less NRM, indicating the potential benefit of using Tac as a standard agent for GVHD prophylaxis. Adding methotrexate, mycophenolate mofetile, or sirolimus to the calcineurin inhibitor may further improve the final outcome [10,11,46,47]. Older age was another factor that influenced OS with statistical significance, even within the age range studied (Figure 3A and Table 4). Patients aged 61 years and older experienced more NRM than patients younger than 61 years (65% versus 39%), whereas their death rate because of disease progression was comparable (19% versus 18%), suggesting the vulnerability of higher aged population to procedure toxicity. Although the possible impact of slight variation in conditioning regimen to the outcome cannot be excluded, it is unlikely, because the great majority (93%) were conditioned with Flu/Mel/TBI regimen fairly uniformly, and comparison between Flu/Mel/TBI and others did not reach statistical significance.

Table 4. *Cox Regression Analyses of Factors Potentially Associated with OS and NRM after RI-UCBT*

Variables	HR	95% CI	P
OS			
Age			
Less than 61 years (n = 33)	**0.3**	**0.14-0.72**	**.006**
At least 61 years (n = 37)	**1.0**		
Disease risk			
Standard (n = 15)	**0.3**	**0.12-0.995**	**.049**
High (n = 55)	**1.0**		
PIR			
No (n = 25)	**0.4**	**0.16-0.88**	**.023**
Yes (n = 43)	**1.0**		
Acute GVHD			
Grade 0-II (n = 31)	**0.4**	**0.17-0.78**	**.0002**
Grade III-IV (n = 23)	**1.0**		
NRM			
GVHD prophylaxis			
CsA (n = 37)	**3.9**	**1.3-11.6**	**.01**
Tac (n = 33)	**1.0**		
Acute GVHD			
Grade 0-II (n = 31)	**1.0**		
Grade III-IV (n = 43)	**5.7**	**2.1-15.7**	**.001**

GVHD indicates graft-versus-host disease; CsA, cyclosporine A; Tac, tacrolimus; NRM, nonrelapse mortality; CI, confidence interval; HR, hazard ratio; OS, overall survival.

In conclusion, this is the first study specifically focusing on elderly patients aged 55 years and older with advanced hematologic diseases to show the feasibility of RI-UCBT. Older age per se cannot be considered to be contraindication to RI-UCBT, although a high NRM has been observed. Further optimization of the treatment protocol, such as immunosuppressive therapy for GVHD prophylaxis, is warranted to establish the safety of this promising treatment strategy for elderly patients with advanced hematologic diseases.

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