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Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation

Naofumi Matsuno,[1] Atsushi Wake,[1] Naoyuki Uchida,[1] Kazuya Ishiwata,[1] Hideki Araoka,[2] Shinsuke Takagi,[1] Masanori Tsuji,[1] Hisashi Yamamoto,[1] Daisuke Kato,[1] Yoshiko Matsuhashi,[1] Sachiko Seo,[1] Kazuhiro Masuoka,[1] Shigesaburo Miyakoshi,[1] Shigeyoshi Makino,[3] Akiko Yoneyama,[2] Yoshinobu Kanda,[4] and Shuichi Taniguchi[1]

[1]Department of Hematology, Toranomon Hospital, Tokyo; [2]Department of Infectious Diseases, Toranomon Hospital, Tokyo; [3]Department of Transfusion Medicine, Toranomon Hospital, Tokyo; and [4]Division of Hematology, Saitama Medical Center, Jichi Medical School, Saitama, Japan

Delayed engraftment or graft failure is one of the major complications after cord blood transplantation (CBT). To investigate factors impacting engraftment, we conducted a retrospective analysis of adult patients who underwent reduced-intensity CBT at our institute, in which preparative regimens mainly consisted of fludarabine, melphalan, and total body irradiation with graft-versus-host (GVH) disease prophylaxis using single calcineurin inhibitors. Among 152 evaluable patients, the cumulative incidence of neutrophil engraftment was 89%. High total nucleated cell and CD34$^+$ cell dose were associated with the faster speed and higher probability of engraftment. In addition, the degree of human leukocyte antigen (HLA) mismatch in the GVH direction was inversely associated with engraftment kinetics, whereas no statistically significant association was observed with the degree of HLA mismatch in the host-versus-graft direction. Similarly, the number of HLA class I antigens mismatched in the GVH direction, but not in the host-versus-graft direction, showed a negative correlation with engraftment kinetics. HLA disparity did not have significant impact on the development of GVH disease or survival. This result indicates the significant role of HLA disparity in the GVH direction in the successful engraftment, raising the novel mechanism responsible for graft failure in CBT. (Blood. 2009;114: 1689-1695)

Introduction

Recent studies have demonstrated cord blood transplantation (CBT) as a safe and feasible alternative to bone marrow (BM) or peripheral blood (PB) stem cell transplantation (SCT) in adults when no suitable related donor is available.[1-4] The incidence and severity of acute graft-versus-host disease (GVHD) after CBT have been low compared with those after unrelated donor BM transplantation,[1-4] permitting use of a mismatched unit as a graft. The use of CBT has also been increasing because of the potential advantage of rapid availability and the lower risk to donors. The development of reduced-intensity (RI) conditioning regimens for transplantation, 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 transplantation.[5,6] We and other groups have reported the feasibility of RI-CBT for adult patients with advanced hematologic diseases.[7-12]

Despite the obvious advantage of CBT, high treatment-related toxicity has been observed, which precludes the application of CBT as a primary graft source. One of the major complications of CBT is delayed engraftment or graft failure. Thus far, several factors have been found to impact engraftment, including total nucleated cell (TNC) dose, CD34$^+$ cell dose, and human leukocyte antigen (HLA) disparity.[13-15] Here, we report the results of a retrospective analysis of 163 adult patients who underwent RI-CBT at our institute, which revealed, for the first time, the importance of HLA disparity in the graft-versus-host (GVH) direction, adding a new viable factor in choosing cord blood (CB) units as transplantable grafts.

Methods

Study patients

This study included adult patients with hematologic malignancies who underwent RI-CBT as their first allogeneic SCT at Toranomon Hospital between January 2002 and December 2006 consecutively. Twenty-nine patients who had active serious infection or showed an Eastern Cooperative Oncology Group performance status of 3 or 4 before transplantation were not eligible for this study because of differences in transplantation procedures or supportive care resulting from serious organ dysfunction and active infection. Then, the remaining 163 consecutive patients were reviewed. All patients had diseases that were incurable with conventional treatments, lacked suitable sibling or unrelated donors, and were considered inappropriate for conventional allo-SCT as they were older than 50 years and/or had organ dysfunction (often attributable to previous intense chemotherapy and/or radiotherapy). Characteristics of the 163 patients are summarized in Table 1.

For disease status, those with hematologic malignancies in the first or second complete remission at the time of transplantation, those in the chronic phase or accelerated phase of chronic myeloid leukemia, and those with refractory anemia of myelodysplastic syndrome were defined as being at standard risk (n = 32), whereas those in other situations were defined as being at high risk (n = 131). All patients received a single CB unit. All patients provided written informed consent in accordance with the Declaration of Helsinki, and the study was conducted in accordance with the requirements of the Institutional Review Board of Toranomon Hospital.

Donor selection

CB units were obtained from the Japanese Cord Blood Bank Network. All CB samples, as well as the patient's blood samples, were serologically typed for HLA-A, -B and -DR antigens before transplantation. Alleles at the HLA-A, -B,

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Table 1. Patient and cord blood characteristics

Variable	Value
No. of patients	163
Median age, y (range)	55 (17-79)
Sex: male/female, no. of patients	98/65
Primary diseases, no. of patients	
Acute lymphoblastic leukemia	20
Acute myeloid leukemia	63
Chronic myelogenous leukemia	5
Myelodysplastic syndrome	12
Malignant lymphoma	39
Adult T-cell leukemia/lymphoma	18
Multiple myeloma	2
Others	4
Risk of underlying disease, no. of patients: standard/high	32/131
Preparative regimens, no. of patients	
Flu + Mel + TBI 2-8 Gy	135
Flu + BU + TBI 4-8 Gy	18
Flu + Mel	6
Flu + BU	4
Median no. of infused nucleated cells, 10^7/kg (range)	2.68 (1.82-4.83)
Median no. of infused CD34$^+$ cells, 10^5/kg (range)	0.76 (0.05-4.40)
Blood-type mismatch, no. of patients: match/mismatch	47/116
HLA antigen mismatch, no. of patients	
0	3
1	24
2	136
GVHD prophylaxis, no. of patients	
Cyclosporine A alone	73
Tacrolimus alone	90

Flu indicates fludarabine; Mel, melphalan; TBI, total body irradiation; and BU, busulfan.

and -DRB1 loci were identified by high-resolution DNA typing in 107 pairs because HLA typing of alleles was not routinely performed in Japanese CB banks. In 127 pairs, HLA-A and -B antigens were identified by serologic typing and HLA-DRB1 alleles were determined by high-resolution DNA typing. CB grafts had at most 2 mismatches for HLA-A, -B, and -DR antigens and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. Mismatch was counted separately in the GVH and host-versus-graft (HVG) direction, respectively. HLA mismatch in the GVH direction was defined when the recipient's antigens or alleles were not shared by the donor, whereas HLA mismatch in the HVG direction was defined when the donor's antigens or alleles were not shared by the recipient.

Transplantation procedures

Pretransplantation conditioning regimens varied and were determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. All patients received purine analog-based preparative regimens. The majority of patients (n = 119) received preparative regimens consisting of fludarabine 125 mg/m^2, melphalan 80 mg/m^2, and 4 Gy total body irradiation (TBI). Patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of melphalan. GVHD prophylaxis was carried out using a continuous infusion of cyclosporine A 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patients could tolerate oral administration.

Supportive care

All patients were treated in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis. Fluoroquinolone, azole, and acyclovir were administered to prevent bacterial, fungal, and herpes virus infection, respectively. Cytomegalovirus pp65 antigenemia was monitored weekly. Hemoglobin and platelet counts were maintained at more than 7 g/dL and at 10×10^9/L, respectively. Granulocyte colony-stimulating factor was administered intravenously from day 1 until neutrophil recovery became durable.

Definition of engraftment, GVHD, and survival

Date of engraftment was defined as the first of 3 consecutive days when the neutrophil counts exceeded 0.5×10^9/L. Patients who did not achieve this criterion at any time after transplantation were considered as primary graft failure. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10%. Acute and chronic GVHD was diagnosed and graded according to standard criteria.[16,17] Overall survival was calculated from the day of transplantation until death from any cause or last follow-up. Event-free survival was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. Final follow-up was conducted in December 2007, with a median follow-up of surviving patients being 29.0 months (range, 3.7-58.9 months).

Statistical methods

Cumulative incidence of neutrophil engraftment was calculated using the Gray method, treating death before engraftment or second transplantation as competing events.[18] Similarly, in the analysis of GVHD, death resulting from other causes or relapse leading to early withdrawal of immune suppression was considered competing risk. The probabilities of survival were estimated using the Kaplan-Meier method. Multivariate analysis was performed using the proportional hazards model. P values $< .05$ were considered statistically significant.

Results

Engraftment

Eleven of the 163 patients reviewed were not evaluable for the analyses of donor engraftment resulting from early death (before 28 days after

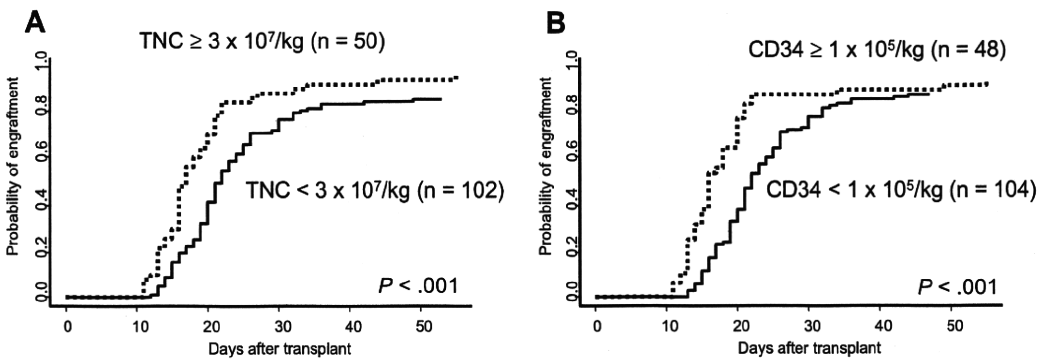

Figure 1. Cumulative incidence of neutrophil engraftment. (A) Effect of TNC dose. (B) Effect of CD34$^+$ cell dose.

transplantation) from disease progression (n = 1), infection (n = 6), and multiple organ failure (n = 4). Of 152 evaluable patients, 135 patients achieved neutrophil engraftment. The cumulative incidence of engraftment at day 60 was 89%, and the median time to engraftment was 20 days (range, 11-55 days). Chimerism analyses were performed in 125 of 135 patients who achieved engraftment using either PB or BM samples at the time of neutrophil recovery. All patients except for one who had residual leukemic cells in PB at the time of engraftment showed complete donor chimerism (> 90%). The median length of time required to donor chimerism was 22 days (range, 11-55 days).

Age, recipient sex, risk of underlying disease, blood type mismatch, and GVHD prophylaxis did not affect engraftment kinetics (data not shown). TNC more than or equal to 3×10^7/kg was associated with a significantly higher probability of engraftment ($P < .001$), with the median time to engraftment of 16.5 days (range, 11-55 days) compared with 21 days (range, 12-49 days) for those who received less than 3×10^7/kg (Figure 1A). Similarly, $CD34^+$ cell dose more than or equal to 10^5/kg was associated with a significantly faster engraftment ($P < .001$) than those who received less than 10^5/kg (Figure 1B).

The cumulative incidence of engraftment and the time to engraftment according to the degree of HLA mismatch are shown in Table 2. Patients who had 0 and 1 antigen mismatch with the grafts were combined, considering the small number of patients in 0 mismatch group and comparable rate of engraftment and time to neutrophil recovery between 0 and 1 antigen-mismatched group (Figure 2A-B), and were compared with those of 2 antigens mismatched. Although patients with 0 or 1 antigen mismatch showed a trend toward superior engraftment kinetics compared with patients with 2 antigens mismatched, the differences did not reach statistical significance (Figure 2A; Table 2). We further analyzed the influence of HLA disparity on engraftment in both the HVG and GVH direction. In the HVG direction, the cumulative incidence of engraftment at day 60 was 93% in 0 or 1 antigen mismatch and 87% in 2 antigens mismatched ($P = .4$, Table 2). In the GVH direction, however, the cumulative incidence of engraftment was 96% in 0 or 1 antigen mismatch and 85% in 2 antigens mismatched ($P < .001$, Figure 2B; Table 2), demonstrating that HLA antigen disparity in the GVH direction was significantly associated with engraftment kinetics. As shown in Figure 2C, HLA antigen disparity in the HVG direction did not contribute to engraftment kinetics in patients with 0 or 1 antigen mismatch in the GVH direction, as was also observed in those with 2 antigens mismatched in the GVH direction. Although the number of patients in each group was small, patients with 0 or 1 mismatch in the GVH direction but 2 mismatches in the HVG direction (n = 28) showed a trend toward superior engraftment kinetics compared with patients with 0 or 1 mismatch in the HVG direction but 2 mismatches in the GVH direction (n = 18; $P = .07$). This finding may indicate that HLA disparity in the GVH direction plays a greater role in engraftment than that in the HVG direction.

In addition to the degree of mismatch, we analyzed the significance of class I (HLA-A, -B) or class II (HLA-DR) mismatch (Table 2). The number of class I antigens mismatched in the GVH direction showed a negative correlation with the probability and the speed of engraftment ($P = .006$, Figure 2D), but not in the HVG or both directions. More specifically, the presence of HLA-B antigens mismatched in the GVH direction was significantly associated with inferior engraftment kinetics ($P = .04$). To the contrary, HLA-DR antigen mismatch did not influence engraftment kinetics in either the HVG or the GVH direction.

The cumulative incidence of engraftment was also assessed using 120 pairs who had HLA-A, -B antigens and -DRB1 allele information available (Table 2). Patients with 0 or 1 mismatch showed better engraftment kinetics compared with those with 2, 3, or 4 mismatches in the GVH direction, which was about to be significant statistically ($P = .05$), whereas HLA mismatch in the HVG direction did not show significant impact on engraftment.

HLA allele mismatch at the HLA-A, -B, and -DR was examined in 102 pairs. In the GVH direction, the cumulative incidence of engraftment was 94% in 0 or 1 allele mismatch, 88% in 2 alleles mismatched, and 80% in 3 to 5 alleles mismatched ($P = .05$), showing that alleles mismatched in the GVH direction could be inversely associated with engraftment kinetics (Table 2). In contrast, allele disparity in the HVG direction did not affect engraftment (Table 2). When HLA-A, -B, and -DR alleles were analyzed independently, no statistically significant differences were observed in any allele tested in either the GVH or HVG direction (data not shown).

Multivariate analyses revealed that low TNC dose ($< 3 \times 10^7$/kg) and HLA antigens mismatched in the GVH direction (0 or 1 vs 2 antigens mismatched) were significantly associated with inferior engraftment kinetics, when age, recipient sex, risk of underlying disease, GVHD prophylaxis, and blood type mismatch were included as covariates ($P = .002$ and $P = .004$, respectively).

Clinical features of graft failure

There were 17 patients who failed to achieve engraftment: 8 males and 9 females, median age of 55 years (range, 17-68 years), high-risk diseases in 12 patients. Median TNC dose of CB grafts was 2.36×10^7/kg (range, $2.01\text{-}3.40 \times 10^7$/kg), and median $CD34^+$ cell dose was 0.59×10^5/kg (range, $0.30\text{-}1.38 \times 10^5$/kg). Nine of them died before engraftment because of disease progression (n = 2), infection (n = 5), multiple organ failure (n = 1), and idiopathic pneumonia syndrome (n = 1). The remaining 8 patients received a second RI-CBT at a median of 34 days (range, 28-49 days) after first RI-CBT, and 3 of them were alive in remission.

Among those who did not achieve engraftment, chimerism analyses in the BM early after transplantation were performed on 8 patients (median, 12 days; range, 10-17 days). Of those, 4 achieved complete donor chimerism, one had mixed chimerism (60% donor type), and 3 patients showed recipient chimerism. Four of 5 patients with donor dominant chimerism showed hemophagocytosis in the BM. On the other hand, all 3 patients with recipient chimerism did not show hemophagocytosis.

GVHD and survival

Among 134 evaluable patients, the cumulative incidence of acute GVHD of grade II to IV was 43%. The incidence of acute GVHD according to HLA disparity in the GVH direction was summarized in Table 3. Patients with 2 antigens mismatched showed a trend toward higher incidence of acute GVHD II-IV ($P = .08$). The number of class I or class II antigens mismatched had no correlation with the incidence of acute GVHD. Similarly, HLA disparity in the allele level was not significantly associated with the incidence of acute GVHD. Among 66 evaluable patients, the cumulative incidence of chronic GVHD was 51%. The degree of HLA mismatch was not significantly associated with the incidence of chronic GVHD (data not shown). Other pretransplantation factors, including age, infused cells, and GVHD prophylaxis, did not affect the incidence of GVHD. Overall survival and event-free survival at 2 years were 35% and 30%, respectively. HLA disparity in the GVH direction, as well as in the HVG direction, did not influence overall survival and event-free survival (Table 3; and data not shown).

Table 2. Univariate analyses of engraftment kinetics according to HLA disparity

	Neutrophil engraftment				
No. of HLA mismatches	**n**	**Cumulative incidence, %**	**Median day**	**Range**	***P***
HLA-A, -B, -DR (antigen)					.09
0 + 1	23	91	17	11-30	
2	129	89	20	11-55	
HLA-A, -B, -DR (antigen, HVG)					.4
0 + 1	43	93	19	11-55	
2	109	87	20	11-49	
HLA-A, -B, -DR (antigen, GVH)					< .001
0 + 1	53	96	19	11-36	
2	99	85	20	11-55	
HLA-A, -B (class I antigen)					.1
0	13	92	17	12-30	
1	86	91	20	11-44	
2	53	85	20	11-55	
HLA-A, -B (class I antigen, HVG)					.4
0	22	96	18	12-36	
1	86	89	20	11-55	
2	44	84	20	11-49	
HLA-A, -B (class I antigen, GVH)					.006
0	23	95	17.5	11-36	
1	88	91	20.5	11-44	
2	41	81	20	12-55	
HLA-A (antigen)					.7
0	87	89	19	11-44	
1 + 2	65	89	20	11-55	
HLA-A (antigen, HVG)					.8
0	96	89	20	11-55	
1 + 2	56	89	20	11-49	
HLA-A (antigen, GVH)					.2
0	103	90	19	11-44	
1 + 2	49	86	20	13-55	
HLA-B (antigen)					.07
0	36	94	19	12-34	
1 + 2	116	87	20	11-55	
HLA-B (antigen, HVG)					.06
0	45	95	19	12-36	
1 + 2	107	86	20	11-55	
HLA-B (antigen, GVH)					.04
0	42	95	18.5	11-36	
1 + 2	110	86	20	11-55	
HLA-DR (antigen)					.4
0	70	87	20	11-55	
1 + 2	82	90	19.5	11-44	
HLA-DR (antigen, HVG)					.7
0	76	88	20	11-55	
1 + 2	76	89	20	11-44	
HLA-DR (antigen, GVH)					.8
0	83	88	20	11-55	
1 + 2	69	90	20	11-44	
HLA-A, -B (antigen), -DR (allele)					.5
0 + 1	13	92	18	14-30	
2	63	84	20	11-47	
3 + 4	44	86	20	11-49	
HLA-A, -B (antigen, HVG), -DR (allele, HVG)					.2
0 + 1	25	96	18	11-32	
2	54	80	20	11-44	
3 + 4	41	90	20	11-49	
HLA-A, -B (antigen, GVH), -DR (allele, GVH)					.05
0 + 1	26	96	18	11-36	
2	57	84	19.5	11-49	
3 + 4	37	84	20	11-34	

Table 2. Univariate analyses of engraftment kinetics according to HLA disparity (Continued)

	Neutrophil engraftment				
No. of HLA mismatches	**n**	**Cumulative incidence, %**	**Median day**	**Range**	***P***
HLA-A, -B, -DR (allele)					.4
0 + 1	10	90	18	14-30	
2	36	86	20	11-44	
3 + 4 + 5	56	84	19	11-49	
HLA-A, -B, -DR (allele, HVG)					.3
0 + 1	19	94	19	11-32	
2	34	79	20	13-44	
3 + 4 + 5	49	86	21	11-49	
HLA-A, -B, -DR (allele, GVH)					.05
0 + 1	16	94	17	11-30	
2	40	88	20	11-44	
3 + 4 + 5	46	80	20	11-49	

Discussion

Delayed hematopoietic recovery and graft failure are significant concerns in adult CBT. In the present study, median time to engraftment was 20 days, which was comparable with that reported in previous studies.[1,4,7,19] These data indicate that our pretransplantation conditioning regimens, consisting mainly of fludarabine, melphalan, and 4 Gy TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert reasonable immunosuppressive effects that allow rapid hematopoietic recovery after CBT. The engraftment was durable except for disease progression.

Almost all reports on CBT have demonstrated the profound impact of infused cell dose on engraftment.[13,14,20] We showed that both high numbers of TNCs and $CD34^+$ cells were favorably associated with time to engraftment and the probability of engraftment, confirming previous findings on the association of cell dose with neutrophil recovery. Considering that $CD34^+$ cell dose reflects stem cell contents in the CB unit, stem cell dose is one of the major determinants of successful engraftment, as has been observed in the xenogeneic transplantation model.[21-23]

Although our results, demonstrating that HLA disparity in the GVH direction affected engraftment kinetics more than HLA disparity in the HVG direction, may seem paradoxical to the former notion of graft failure that results from graft rejection in most cases, they suggest a novel mechanism of graft failure in CBT. Previously, we have reported that a high incidence of noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9 in more than 50% of the patients receiving CBT.[8,24] We regarded this reaction as early onset of acute

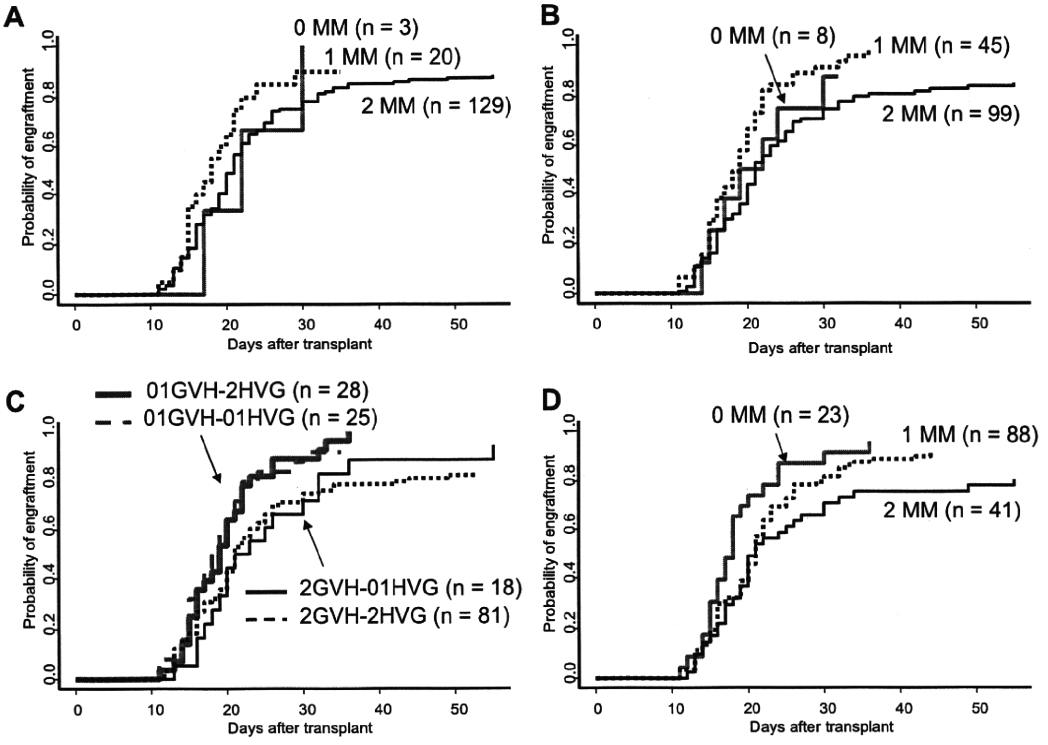

Figure 2. Cumulative incidence of neutrophil engraftment. MM indicates mismatch. (A) Effect of HLA antigen mismatch. (B) Effect of HLA antigen mismatch in the GVH direction. (C) Effect of HLA antigen mismatch according to mismatch both in the GVH and the HVG directions. 2GVH indicates 2 antigens mismatch in the GVH direction; 2HVG, 2 antigens mismatch in the HVG direction; 01GVH, 0 or 1 antigen mismatch in the GVH direction; 01HVG, 0 or 1 antigen mismatch in the HVG direction. (D) Effect of HLA class I antigen mismatch in the GVH direction.

Table 3. Univariate analyses of acute GVHD and survival according to HLA disparity in the GVH direction

	Acute GVHD II-IV			2-year overall survival		
No. of HLA mismatches in the GVH direction	n	Cumulative incidence, %	P	n	Survival rate, %	P
HLA-A, -B, -DR (antigen)			.08			.5
0 + 1	50	33		59	36	
2	84	48		104	35	
HLA-A, -B (class I antigen)			.5			.2
0	22	36		24	54	
1	80	42		96	32	
2	32	46		43	32	
HLA-DR (class II antigen)			.5			.9
0	71	38		91	32	
1 + 2	63	47		72	38	
HLA-A, -B (antigen), -DR (allele)			.4			1.0
0 + 1	25	32		29	38	
2	48	51		60	38	
3 + 4	30	44		38	39	
HLA-A, -B, -DR (allele)			.3			.4
0 + 1	15	27		16	56	
2	35	49		41	37	
3 + 4 + 5	36	51		50	35	

GVHD in which activated donor T cells secreted various cytokines.[25] HLA disparity in the GVH direction may augment alloimmune reactions, which evoke hypercytokinemia and macrophage activation and occasionally result in establishment of hemophagocytic syndrome, one of the major complications directly related to graft failure in recipients.[26-28] Indeed, a considerable number of patients showed hemophagocytosis in the BM with donor dominancy, leading to graft failure, even though we cannot exclude the possibility of graft rejection caused by recipient lymphocytes in some cases. In addition, among those who achieved donor cell engraftment, delayed neutrophil recovery was prominent for those with more HLA mismatch in the GVH direction rather than in the HVG direction. Myelosuppression is commonly observed during acute or chronic GVHD, indicating that GVHD can negatively affect hematopoietic function of the graft, possibly because of an attack on the hematopoiesis-supporting recipient stromal cells[29] or production of cytokines from immune cells, such as transforming growth factor-β, known to regulate hematopoiesis negatively.[30] The delayed engraftment observed in our study may have been caused by similar mechanisms during the recovery of donor cells. Furthermore, our results demonstrated that HLA class I antigen mismatch in the GVH direction was associated with inferior engraftment. Higher impact of HLA class II disparity on the development of acute GVHD has been reported in National Marrow Donor Program data.[31] On the contrary, the Japan Marrow Donor Program registry data showed that mismatch in class I had higher impact than that in class II.[32] The discrepancy may be explained by unique ethnic background of the Japanese population. The observation shown here may further strengthen our hypothesis that GVH reactions play a crucial role in engraftment process. In the analysis using allele data, the statistical power of HLA disparity in the GVH direction on engraftment had decreased. This discrepancy probably results from the small sample size in each mismatched category but may be suggestive of more powerful immunogenicity of mismatch in antigen rather than allele level.

In the Eurocord registry data, which includes 550 CBTs, HLA disparity was shown to have a negative impact on engraftment, although the effect of direction of mismatch was not described.[14,33] More specifically, it was reported from the Düsseldorf Cord Blood Bank and Eurocord-Netcord Registry that HLA-A locus high-resolution typing in the HVG direction was associated with reduced cumulative incidence of engraftment in 122 patients receiving CBT.[34] Several reasons may explain this discrepancy from our observations. First, patients included in our study received relatively uniform pretransplantation conditioning regimens consisting mainly of fludarabine, melphalan, and TBI, whereas those in the Eurocord database had more variable pretransplantation conditioning regimens. Second, all of our patients had GVHD prophylaxis using single calcineurin inhibitors, whereas most of those in the Eurocord Registry received additional chemicals or anti-thymocyte globulin. Many institutes use methotrexate,[35,36] mycophenolate mofetil,[19,37] corticosteroids,[13] or anti-thymocyte globulin[38,39] in combination with a calcineurin inhibitor as GVHD prophylaxis in CBT. Narimatsu et al demonstrated that use of short-term methotrexate was associated with a lower rate of posttransplantation immune reactions without compromising engraftment.[36] Thus, more intensive immunosuppression may be beneficial for controlling early immune reactions and overcoming the issue of HLA mismatch. In addition, the unavoidable high incidence of gastrointestinal tract damage caused by TBI or melphalan in preparative regimens may have increased the chance of triggering GVH reactions.[40]

In the present study, HLA disparity had little association with the development of GVHD and survival, despite its obvious impact on engraftment. According to the Eurocord Registry data, better HLA match was not associated with better outcome in hematologic malignancies receiving CBT.[20] Further analyses are required to determine whether this is the result of the unique immunologic immaturity of CB or to the heterogeneous patient population with the majority being in the high-risk disease status.

In conclusion, HLA disparity in the GVH direction, especially class I disparity, was found to have a significant impact on engraftment. These results shed light on a novel mechanism responsible for graft failure in CBT and add a valuable clue for choosing a better CB unit to avoid graft failure.

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Authorship

Contribution: N.M. and A.W. performed research and extracted data; A.Y. reviewed histopathologic methods; N.M. and Y.K. performed statistical analysis; N.U. and S. Taniguchi reviewed study design and methods; and K.I., H.A., S. Takagi, M.T., H.Y., D.K., Y.M., S.S., K.M., S. Miyakoshi, and S. Makino contributed to the writing of the paper.

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Correspondence: Naoyuki Uchida, 2-2-2 Toranomon, Minato-Ku, Tokyo 105-8470; e-mail: nuchida@toranomon.gr.jp.

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Incidence and Risk Factors of Early Bacterial Infections after Unrelated Cord Blood Transplantation

Makoto Yazaki,[1] Yoshiko Atsuta,[2] Koji Kato,[3] Shunichi Kato,[4] Shuichi Taniguchi,[5] Satoshi Takahashi,[6] Hiroyasu Ogawa,[7] Yasuji Kouzai,[8] Takeshi Kobayashi,[9] Masami Inoue,[10] Ryoji Kobayashi,[11] Tokiko Nagamura-Inoue,[12] Hiroshi Azuma,[13] Minoko Takanashi,[14] Shunro Kai,[15] Masao Nakabayashi,[16] Hidehiko Saito[17] for the Japan Cord Blood Bank Network

Incidence and characteristics of early bacterial infection within 100 days after unrelated cord blood transplantation (UCBT) were assessed for 664 pediatric and 1208 adult recipients in Japan. Cumulative incidence of early bacterial infection at day 100 post-UCBT was 11% (95% confidence interval [CI], 8%-13%) for children and 21% (CI, 19%-24%) for adults ($P < .0001$). Early bacterial infection in adults had a significant impact on mortality (hazard ratio [HR] = 2.1, CI, 1.7-2.6; $P < .0001$), although no significant risk factors were identified. Multivariate analysis identified older age group (6-10, and 11-15 years versus 0-5 years of age) at transplant (HR = 2.0 and 2.7, CI, 1.1-3.5 and 1.4-4.9; $P = .020$ and .002, respectively) as an independent risk factor of early bacterial infection for children. Early bacterial infection in children did not have a significant impact on mortality when adjusted. Of 315 bacteremia, 74% were caused by Gram-positive microorganisms. Pneumonia occurred in 39 patients including 13 cases of *Stenotrophomonas maltophilia* pneumonia. Early bacterial infection had a negative effect on survival for adults and the median day of development was 10 days after transplant, suggesting that the prevention of bacterial infection in the very early post-UCBT phase is important.

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KEY WORDS: Early bacterial infection, Cord blood transplantation, The Japan Cord Blood Bank Network, Risk factor for infection, Urelated donor

INTRODUCTION

Infection is 1 of the major causes of morbidity and mortality for patients undergoing bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT) [1,2]. Recently, use of cord blood transplantation (CBT) from unrelated donors has increased for patients who do not have suitable donors for BMT or PBSCT, yielding promising results [3-7]. However, neutrophil recovery has been significantly delayed in unrelated CBT patients compared to unrelated BMT patients. Bacterial infection remains 1 of the most common problems after unrelated cord blood transplantation (UCBT) [5,8-10].

From the [1]Department of Pediatrics, Nagoya City East Medical Center, Nagoya, Japan; [2]Department of HSCT Data Management, Nagoya University School of Medicine, Nagoya, Japan; [3]Division of Hematology/Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [4]Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; [5]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [6]Division of Molecular Therapy, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [8]Department of Transfusion Medicine, Tokyo Metropolitan Fuchu Hospital, Fuchu, Japan; [9]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [10]Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; [11]Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [12]Division of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [13]Hokkaido Red Cross Blood Center, Sapporo, Japan; [14]Blood Preparation Department, The Japanese Red Cross Tokyo Blood Center, Tokyo, Japan; [15]Department of Blood Transfusion, Hyogo College of Medicine, Nishinomiya, Japan; [16]Department of Obstetrics/Gynecology, Maternal and Child Health Center Aiiku Hospital, Tokyo, Japan; and [17]Nagoya Central Hospital, Nagoya, Japan.

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Correspondence and reprint requests: Makoto Yazaki, MD, Department of Pediatrics, Nagaya City East Medical Center, 1-2-23 Wakamizu, Chikusa-ku, Nagoya, Aichi 464-8547 Japan (e-mail: mhyazaki@sb.starcat.ne.jp).

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In this paper, we report the results of our analysis of early bacterial infections before day 100 following UCBT in 1872 Japanese patients. We conducted this analysis to investigate the incidence and timing of infections, causative micro organisms, potential risk factors of infections, and the influence of infection on outcome.

PATIENTS AND METHODS

Patients

Between September 1997 and September 2005, 2362 UCBT procedures were performed using a single cord blood (CB) in 175 transplantation centers with 221 transplantation units supported by 11 CB banks affiliated with the Japan Cord Blood Bank Network (JCBBN) in Japan. The subjects analyzed were 1872 patients whose initial clinical report forms (CRFs), completed 100 days after UCBT, were submitted to the JCBBN. The clinical protocols for UCBT were approved by the institutional review board of the respective institutions. Patients underwent UCBT if they had no human leukocyte antigen (HLA)-identical, 1 locus mismatched relative or an HLA-matched unrelated BM donor could not be identified within 6 to 8 weeks [11]. The patients or their parents gave their consent for UCBT after being informed of the potential risks and benefits of the procedure. All patients received conditioning chemotherapy in the sterile unit with high-efficiency particulate air filtration. The conditioning regimen, acute graft-versus-host disease (aGVHD) prophylaxis and prevention of bacterial infections varied according to the institute's policy and type of disease, although most of the institutions used oral polymyxin B or fluoroquinolone with intravenous antibiotics to prevent bacterial infections.

Selection of Grafts

Searches for unrelated CB units were processed through the JCBBN, where 25,803 CB units were available in August 2006. Suitable CB in JCBBN was selected by cell count of nucleared cell before freezing and HLA compatibility between CB and patients. Preferred unrelated CB units were those that matched at least 4 of 6 HLA antigens, based on serologic typing for class I HLA-A and HLA-B, antigens and low-resolution DNA typing for class II HLA-DR and contained a minimum cell count of 2×10^7/kg nucleated cells of the recipient's body weight before freezing.

Bacterial Infections

We analyzed bacterial infections reported in the JCBBN 100-day CRF with clinical symptoms and pathogenic micro-organisms were discovered, because it is not easy to distinguish bacteremia or pneumonia without microbiologically documented infection from preengraftment fever or capillary leak syndrome in the early post-UCBT phase.

Early bacterial infections were defined as those occurring within the first 100 days after graft infusion. If a second episode with the same organism occurred within 7 days, it was counted as a single infection episode [10].

Collection of Data

Detailed patient and clinical variables were collected by the JCBBN CRF. Its 100-day CRFs were submitted by transplantation centers or units to the 11 CB banks and checked by a data manager of each bank for missing data and inconsistent data. After the data cleaning, all CRFs were submitted from CB banks to the data center of JCBBN. Annual follow-up for each transplant case is performed to update the data on engraftment, relapse, survival, and complications. The final data set used for the analyses was fixed in March 2006.

Statistical Analysis

Because preliminary study of all patients revealed that 16 years of age and older was the sole significant variable in multivariate analysis, separate analyses were performed for children (younger than 16 years of age) and adults (16 years of age and older) to find the risk factors and to investigate the impact of infection on survival. All episodes of infection were included in the analyses to identify causative micro-organisms of infections. Various clinical factors were evaluated as potential risk factors for early bacterial infection in univariate and multivariate analyses combined with the Cox proportional-hazards regression model. Factors found to be significant ($P < .05$) or marginally significant ($P < .1$) in univariate analysis were included in the multivariate analysis using a forward stepwise method. The categorization for the analyses of risk factors was based on the rule that the smaller group of variable needed to contain at least 10% of the patients. The proportional hazards regression model with early bacterial infection as a time-dependent covariate was used to determine the effect of early bacterial infection on survival. Survival distributions were estimated with the method of Kaplan and Meier. Probabilities of early bacterial infection were calculated by means of cumulative incidence curves treating death without early bacterial infection as competing risks. Statistical analyses were performed with Stata software version 9.0 (Stata Corp., College Station, TX).

RESULTS

Characteristics of Patients

Table 1 shows the characteristics of 664 pediatric (age <16 years) and 1208 adult (age ≥16 years) patients who underwent UCBT in Japan. In the child cohort,

Table 1. Characteristics of Pediatric and Adult Patients Who Received Unrelated Cord Blood Transplantation

Variable	Child (Age <16) No. Eval (n = 664)			Adult (age ≥16) No. Eval (n = 1208)		
Sex—no. (%)						
Male	664	403	(61)*	1208	662	(55)
Female		261	(39)		546	(45)
Age group—no. (%)						
0-15	664	664		1208		
16-30					270	(22)
31-45					338	(28)
≥46					600	(50)
Disease—no. (%)						
Acute lymphoblastic leukemia	664	279	(42)	1207	211	(17)
Acute myelogenous leukemia		151	(23)		490	(41)
Adult T cell leukemia		0			65	(5)
Chronic myelogenous leukemia		8	(1)		69	(6)
Chronic lymphocytic leukemia		0			3	
Myelodysplastic syndrome		15	(2)		103	(9)
MDS/MPD		20	(3)		7	(1)
Lymphoma		28	(4)		188	(16)
Myeloma		0			32	(3)
Solid tumor		27	(4)		6	
Aplastic anemia		14	(2)		24	(2)
Immunodeficiency		47	(7)		1	
Metabolic disease		25	(4)		0	
Others		50	(8)		8	(1)
History of previous transplantation—no. (%)						
No	664	556	(84)	1208	914	(76)
Yes		108	(16)		294	(24)
Conditioning regimen—no. (%)						
Myeloablative	664	545	(82)	1208	579	(48)
Nonmyeloablative		99	(15)		621	(51)
Unknown		20	(3)		8	(1)
Total-body irradiation	664	350	(53)	1208	928	(77)
ATG/ALG	664	75	(11)	1208	38	(3)
Prophylaxis against GVHD						
Cyclosporine based	630	401	(64)	1172	846	(72)
Tacrolimus based		199	(32)		312	(27)
Others		30	(5)		14	(1)
Methotrexate used	630	362	(57)	1172	582	(50)
Prednisolone used	630	161	(26)	1172	47	(4)
Mycophenolate mofetil used	630	2		1172	78	(7)
Nucleated cell dose/kg body weight—$\times 10^{-7}$						
Median	664	5.10		1208	2.53	
Range		1.18-24.91			1.02-6.42	
HLA compatibility(GVHD direction)—no./total no. (%)						
Matched	656	162	(25)	1187	129	(11)
One-antigen mismatch		380	(58)		457	(39)
Two-antigen mismatch		106	(16)		577	(49)
Three-antigen or more mismatch		8	(1)		24	(2)

MDS/MPD indicates myelodyplasticsyndrome/myeloproliferative disease; ATG, antithymocyte globulin; ALG, antilymphocyte globulin; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
*Figures in parentheses show percentages.

108 patients (16%) had a history of previous transplantation. Myeloablative conditioning regimen was administered to 545 patients (82%). Total body irradiation (TBI) was administered to 350 of 664 patients (53%) and 311 of 545 patients (57%) who received a myeloabalative condition regimen. For GVHD prophylaxis, cyclosporine (CsA)-based prophylaxis was administered to 401 patients (64%), and tacrolimus-based prophylaxis to 199 (32%). Methotrexate (MTX) was used for GVHD prophylaxis for 362 patients (57%), and prednisolone for 161 (26%). The median dose of nucleated cells per kilogram of patient's body weight was 5.10×10^7. In the adult cohort, 600 patients (50%) were 46 years old or older, and 294 patients (24%) had a history of previous transplantation. TBI was administered to 998 of 1208 patients (77%) and 504 of 579 patients (87%) who received a myeloabalative condition regimen, and 621 patients (51%) were given a nonmyeloablative conditioning regimen [12-15]. CsA-based GVHD prophylaxis was administered to 846 patients (72%), and tacrolimus-based prophylaxis to 312 patients (27%). The median dose of nucleated cells per kilogram of patient's body weight was 2.53×10^7.

Incidence and Timing of Early Bacterial Infection

In the child cohort, 77 patients (12%) developed early bacterial infection with a cumulative incidence of 9% (95% confidence interval [CI] 7%-11%) at 50

days and 11% (CI 8%-13%) at 100 days posttransplant (Figure 1). The median day of bacterial infection development was 8 days (range: 0-100) posttransplant. Seventy-five percent of early infection in children occurred within 31 days post-UCBT. In the adult cohort, the cumulative incidence of early bacterial infection was 19% (CI 17%-21%) at 50 days and 21% (CI 19%-24%) at 100 days after UCBT. Early bacterial infection on median day 10 (0-97) posttransplant occurred in the 260 adult recipients (22%) with 75% of the events occurring within 25 days. Statistical analysis demonstrated that the cumulative incidence of early bacterial infection in adults was significantly higher than that in children ($P < .0001$).

The majority of early bacterial infections developed during neutropenia (in 80% of children and 80% of adults). The median day of early bacterial infection development during neutropenia was 7 days (range: 0-80 days) for children and 8 days (0-80 days) for adults respectively, whereas the corresponding figures for early bacterial infections after neutrophil recovery were 55 days (20-100 days) and 46 days (14-97 days), respectively.

Types of Infections

Of the total of 1872 patients, 337 (18%) suffered from bacterial infections between day 0 and day 100 after UCBT, with 12% of children and 22% of adults suffering from early bacterial infections. As shown in Table 2, bacteremia was the most common infection within the first 100 days. In the child cohort, 68 cases of bacteremia, 2 of pneumonia, and 4 of colitis (2 of *Clostridium difficile* colitis) developed, and in the adult cohort, 247 cases of bacteremia, 38 of pneumonia, 2 of colitis (one of *Clostridium difficile* colitis), 2 each of urinary infection and sinusitis, and 1 each of *Bacillus cereus* meningitis and catheter infection. Of the 218 adults with early bacteremia, 192 patients had 1 infection episode, 23 had 2, and 3 had 3 infection episodes.

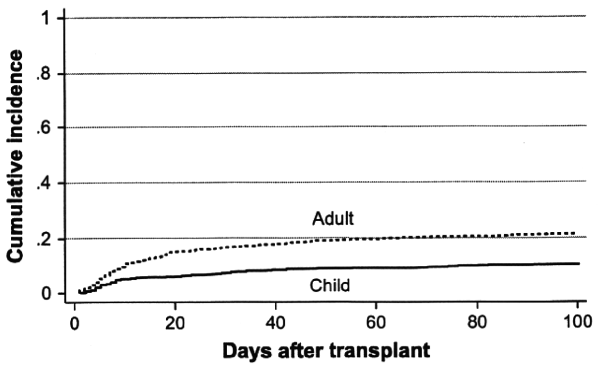

Figure 1. Cumulative incidence of early bacterial infection within 100 days following unrelated cord blood transplantation was 9% (95% CI 7%-11%) at 50 days and 11% (CI 8%-13%) at 100 days posttransplant for children. The corresponding values were 19% (CI 17%-21%) and 21% (CI 19%-24%) for adults.

Table 2. Patients with Early Bacterial Infections Who Received Cord Blood Transplantation

	Child (Age <16)		Adult (Age ≥16)	
	Patients	Episodes	Patients	Episodes
Bacteremia	65	68	218	247
Pneumonia	2	2	37*	38
Colitis	4	4	2	2
Urinary infection	0	0	2	2
Sinusitis	0	0	2	2
Meningitis	0	0	1	1
Catheter infection	0	0	1	1
Others	6	6	7	7

Total patients with infection in children and in adults were 77 and 260, respectively.
Number *Clostridium difficile* colitis 3.
*Ten patients with bacteremia developed pneumonia as the second bacterial infection.

Causative Micro organisms of Bacteremia

Of the 315 episodes of bacteremia, Gram-positive micro organisms accounted for 234 (74%), and Gram-negative micro organisms for 81 (26%) of the cases (Table 3). *Staphylococcus* species (spp) were the most common Gram-positive pathogens responsible for 147 of the bacteremia cases (47%), with coagulase-negative *Staphylococcus* (CNS) detected in 111 of these cases (76%). *Staphylococcus epidermidis* was the most

Table 3. Causative Micro organisms of the Early Bacteremia following Cord Blood Transplantation

	No. episodes		(%)
Bacteremia	315		
Gram-positives	234		(74)
Staphylococcus spp.		147	(47)
Enterococcus spp.		56	(18)
Sreptococcus spp.		19	(6)
Bacillius spp.		8	(3)
Corynebacterium spp.		2	
Clostridium spp.		1	
Mycobacterium tuberculosis		1	
Gram-negatives	81		(26)
Pseudomonas aeruginosa		34	(11)
Acinetobacter spp.		7	
Enterobacter cloacae		7	
E. coli		5	
Stenotrophomonas maltophilia		5	
Burkholderia cepacia		4	
Klebsiella pneumoniae		3	
Chyseobacterium spp.		3	
Alcaligenes xylosoxidans		2	
Salmonella spp.		2	
Serratia spp.		1	
Morganella morganii		1	
Leuconostoc spp.		1	
Micrococcus spp.		1	
Aeromonas hyrophila		1	
Capnocytophaga spp.		1	
Bacteroides fragilis		1	
Provotella oralis		1	
Fusobacterium necrophorum		1	

common CNS micro organism and was isolated in 94 cases. *Staphylococcus aureus* was isolated in 32 of the 147 cases, and was reported as methicillin-resistant *Staphylococcus aureus* (MRSA) in 25 of 32 (78%). *Enterococcus* spp was found in 56 cases (18% of all bacteremia cases), with 23 cases of *Enterococcus faecalis*, 21 cases of *Enterococcus faecium*, 1 case of *Enterococcus gallinarum*, and 11 unidentified cases. *Streptococcus* spp was the third most common Gram-positive pathogen (19 cases), with 15 cases of alpha-*Streptococcus*, 3 of *Streptococcus agalaciae*, and 1 unidentified case. *Streptococcus mitis* was found in 9 of the 15 alpha-*Streptococci* cases (60%). Of the 81 cases with Gram-negative microorganisms, *Pseudomonas aeroginosa* was found in 34 cases, accounting for 11% of all bacteremia cases, and *Stenotrophomonas maltophillia* in 5 cases. Anaerobic Gram-negative organisms, such as *Bacteroides fragilis* (n = 1), *Provotella oralis* (n = 1), and *Fusobacterium necrophorum* (n = 1), were also isolated.

We also investigated causative micro organisms of early bacteremia that developed in children and adults either during neutropenia or after neutrophil recovery. The distribution of these micro organisms in all groups was similar except for that of *Stenotrophomonas maltophillia* and *Enterococcus* spp, with all 5 *Stenotrophomonas maltophilia* bacteremias that had developed in adults during neutropenia, the percentage of *Enterococcus* spp. bacteremia having developed in adults during neutropenia being 3.8 times higher than in children.

Causative Micro organisms of Pneumonia

Thirty-seven adults and 2 children developed bacterial pneumonia within the first 100 days after UCBT. Bacterial pneumonia developed as the first infection in 29 of these patients and 10 developed bacterial pneumonia following bacteremia, 4 of them because of the same micro organism that caused bacteremia and 6 because of a different micro organism. One patient developed secondary pneumonia of *Stenotrophomonas maltophilia* following MRSA pneumonia, which accounted for a total of 40 episodes of early bacterial pneumonia. Gram-positive and Gram-negative micro organisms accounted for 50% each of the cases of bacterial pneumonia. The causative micro organisms of pneumonia in adults were identified as *Staphylococcus aureus* (n = 6), CNS (n = 8), *Enterococcus* spp. (n = 3), *Corynebacterium* spp (n = 1), *Pseudomonas aeruginosa* (n = 5), *Burkholderia cepacia* (n = 2), and *Stenotrophomonas maltophilia* (n = 13). Eleven of the *Stenotrophomonas maltophilia* pneumonias developed during neutropenia.

Outcome of Patients with Early Bacterial Infection

Of the 43 patients who developed bacteremia with shock, 32 (74%) died as did 143 of 240 (60%) of those who developed bacteremia without shock. For patients who developed early bacteremia, bacterial infection was the main cause of death for 20 (47%) of the patients with bacteremia with shock, and 55 (23%) of the patients with bacteremia without shock. *Pseudomonas aeroginosa* bacteremia caused a higher mortality, because 73% of the patients with this type of bacteremia died. Bacterial infection was the main cause of death for 53% patients who developed *Pseudomonas aeroginosa* bacteremia. Twenty-six (70%) of the 37 adults who developed bacterial pneumonia died, as did 18 (49%) of the 37 adults who developed early bacterial pneumonia because of bacterial infection.

Risk Factors for Early Bacterial Infection after UCBT

Among the factors assessed as risk factors for early bacterial infection for children, older age group (6-10 years, and 11-15 years versus 0-5 years of age) at transplant (hazard ratio [HR] = 1.9 and 2.8, CI 1.1-3.3 and 1.6-5.1; $P = .024$ and $P < .0001$, respectively), presence of prior hematopoetic stem cell transplantation (HR = 1.8, CI 1.1-3.1; $P = .032$), infusion of $<5.10 \times 10^7$ nucleated cells per kilogram of patient's body weight (HR = 1.6, CI 1.0-2.6, $P = .049$), and use of nonmyeloablative conditioning regimen (HR = 1.8, CI 1.0-3.2; $P = .039$) were identified as significant in univariate analysis (Table 4). Use of prednisolone for GVHD prophylaxis was identified as a marginal risk factor (HR = 1.6, CI, 1.0-2.7; $P = .070$) in univariate analysis. Multivariate analysis identified older age group (6-10 years, and 11-15 years versus 0-5 years of age) at transplant (HR = 1.96 and 2.66, CI, 1.11-3.47 and 1.44-4.91; $P = .020$ and .002, respectively) as an independent risk factor of early bacterial infection for children. Use of prednisolone for GVHD prophylaxis was also identified as a marginal risk factor (HR = 1.63, CI 0.98-2.71; $P = .062$).

In the adult cohort, use of nonmyeloablative conditioning regimen was not significant. Univariate analysis results identified the use of tacrolimus for GVHD prophylaxis as a marginal risk factor (HR = 1.31, CI 1.0-1.7; $P = .055$) compared to the use of CsA for GVHD prophylaxis (Table 5). The cumulative incidence of early bacterial infection tended to be higher for patients in the adult cohort who received tacrolimus-based GVHD prophylaxis compared to those who received nontacrolimus GVHD prophylaxis (25%, 95% CI, 20%-30% versus 20%, 95% CI, 17%-24% at 100 days posttransplant, $P = .088$). No significant risk factor for early bacterial infection was identified in univariate analysis, so that multivariate analysis was not performed. The risk of early bacterial infection did not increase with age in the adult cohort (Table 5).

Effect of Early Bacterial Infections on Survival

The probability of survival of children 6 months and 2 years after UCBT was 70% (CI 66%-73%)

Table 4. Univariate Analysis for Risk of Early Bacterial Infection in Children

Factor	n	HR	95% CI	P value
Age at transplant (Years)				
0-5	392	1.00		
6-10	164	1.90	1.09-3.33	.024
11-15	107	2.84	1.60-5.05	<.0001
Prior HSCT				
≥1 prior HSCT	108/664	1.82	1.05-3.14	.032
Disease				
Acute myelogenous leukemia	151	1.00		
Acute lymphoblastic leukemia	279	0.89	0.49-1.62	.701
HLA disparity				
≥Two-antigens mismatch for GVHD direction	114/656	0.82	0.42-1.61	.574
≥Two-antigens mismatch for rejection direction	121/655	0.76	0.39-1.48	.415
Number of cells infused*				
$CD34^+$ cell $<1.42 \times 10^5$/kg	244/487	1.21	0.70-2.09	.494
Nucleated cell $<5.10 \times 10^7$/kg	332/664	1.62	1.00-2.63	.049
Conditioning regimen				
Myeloablative	545/664	1.00		
Nonmyeloablative	99/664	1.82	1.03-3.23	.039
Myeloablative condition with total body irradiation	331/664	1.24	0.78-1.99	0.368
Antithymocyte globulin/antilymphocyte globulin	75/664	1.58	0.83-3.01	.164
GVHD prophylaxis				
Cyclosporine based	401	1.00		
Tacrolimus based	199	1.23	0.72-7.08	.451
Prednisolone not used	469	1.00		
Prednisolone used	161	1.60	0.96-2.66	.070
Methotrexate not used	268	1.00		
Methotrexate used	362	0.87	0.53-1.42	.580
Disease status of malignant disease				
Standard disease†	215	1.00		
Advanced disease‡	308	1.03	0.61-1.76	.907

HSCT indicates hematopoietic stem cell transplantation; GVHD, graft-versus-host diseasel; CI, confidence interval; HR, hazard ratio.
*Number of cells at freezing.
†Standard disease means first complete remission or first chronic phase of maliganant disease.
‡Advanced disease means all others except standard disease.

Table 5. Univariate Analysis for Risk of Early of Early Bacterial Infection Adults

Factor	n	HR	95% CI	P Value
Age at transplant (years)				
16-30	270	1.00		
31-45	338	0.85	0.59-1.21	.355
46-60	445	1.04	0.75-1.43	.834
≥61	155	1.05	0.69-1.59	.838
Prior HSCT				
≥1 prior HSCT	294/1208	1.02	0.76-1.37	.881
Disease				
Acute myelologenous leukemia	490	1.00		
Aute lymphoblastic leukemia	211	0.90	0.63-1.29	.572
Lymphoma	188	1.23	0.87-1.77	.233
HLA disparity				
≥Two-antigens mismatch for GVHD direction	601/1187	0.99	0.77-1.27	.937
≥Two-antigens mismatch for rejection direction	623/1187	0.92	0.71-1.17	.485
Number of cell infused *				
$CD34^+$ cell $<0.80 \times 10^5$/kg	560/1130	1.18	0.90-1.51	.240
Nucleated cell $<2.53 \times 10^7$/kg	608/1208	1.17	0.91-1.49	.224
Conditioing regiman				
Myeloablative	579/1208	1.00		
Nonmyeloablative	621/1208	1.21	0.95-1.55	.125
Myeloablative condition with total body irradiation	504/1208	1.21	094-1.55	0.145
GVHD prophylaxis				
Cyclosporine based	846	1.00		
Tacrolimus based	312	1.31	0.99-1.72	.055
Methotrexate not used	590	1.00		
Methotrexate used	582	0.84	0.66-1.08	.182
Disease status malignant disease				
Standard disease†	427	1.00		
Advanced disease‡	739	1.10	0.58-1.44	.469

HSCT indicates hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; CI, confidence interval; HR, hazard ratio.
*Number of cell at freezing.
†Standard disease means first complete remission or first chronic phase of maliganant disase.
‡Advanced disease means all other except standard disease.

and 52% (CI 48%-56%), respectively. The median follow-up of survivors was 2.1 years (range: 0.07-7.5). Bacterial infection was the main cause of death in 12 of the 77 pediatric recipients (16%) with early bacterial infection. Evaluation of early bacterial infection as a time-dependent covariate for patient's survival showed statistical significance (HR = 1.6, CI 1.2-2.2; $P = .005$) in univariate analysis. When adjusted for patient age, sex, disease status, presence of previous transplant, transplanted cell dose, HLA disparity, conditioning regimen, and GVHD prophylaxis, this factor showed no significance (HR = 1.5, CI 0.9-2.4; $P = .111$) for children.

In the adult cohort, the probability of survival 6 months and 1 year posttransplant was 50% (CI 47%-53%) and 41% (CI 38%-44%), respectively. The median follow-up of survivors was 1.0 year (range: 0.05-6.2). Bacterial infection was the main cause of death in 79 of the 260 adult recipients (30%) with early bacterial infection. The analysis of the effects of early bacterial infection showed statistical significance in univariate analysis (HR = 2.1, CI 1.7-2.5; $P < .0001$) as well as in multivariate analysis (HR = 2.1, CI 1.7-2.6; $P < .0001$) adjusted for the same variables as for the child cohort.

Overall survival (OS) rates of children who developed bacterial infection during neutropenia 100 and 365 days after infection were 58% (95% CI 44%-70%), and 40% (95% CI 27%-53%), respectively. The corresponding rates for children who developed bacterial infection after neutrophil recovery were 67% (95% CI,38%-85%) and 67% (95% CI 38%-85%), respectively. In the adult cohort, the corresponding rates were 40% (95% CI 33%-47%) and 27% (95% CI 20%-34%), for bacterial infection having developed during neutropenia and 49% (95% CI 34%-63%) and 38% (95% CI 23%-52%) for after neutrophil recovery.

Because early bacterial infection, neutrophil recovery, and aGVHD occur during the early phase after transplant, we performed multivariate analyses by treating these variables as time-dependent variables for the analysis of early bacterial infection in terms of status of neutrophil recovery and status of aGVHD.

Multivariate analyses revealed that early bacterial infection remained a significant risk factor for overall mortality of adults (HR = 2.05, CI 1.68-2.49; $P < .0001$). However, early bacterial infection did not affect child mortality (HR = 1.32, CI 0.81-2.15; $P = .27$). Neutrophil recovery was a significant risk factor for overall child mortality (HR = 0.43, CI 0.28-0.67; $P < .0001$) and adults (HR = 0.47, CI 0.38-0.59; $P < .0001$) after adjustment for patient and transplant characteristics. However, grade ii to iv aGVHD did not have an effect on child mortality (HR = 1.00, CI 0.72-1.38; $P = .98$) and adults (HR = 1.08, CI 0.89-1.32; $P = .43$). These findings suggest that early bacterial infection is an independent risk factor for overall mortality of adults.

DISCUSSION

Bacterial infections remain a major complication following UCBT. To the best of our knowledge, this study of 664 pediatric and 1208 adult patients represents the largest study reported to date for the examination of early bacterial infection following UCBT. The incidence of early bacterial infection for adult patients was significantly higher than that for pediatric patients. The median day of bacterial infection development was 8 days in children and 10 days in adults posttransplant, respectively.

Gram-positive organisms were predominant (74%) in the cases of early bacteremia examined in our study. Previous studies also reported that Gram-positive organisms were prominent in bacteremia following UCBT [8,12]. In our large-scale study, *Staphylococcus epidermidis* was the most common organism isolated in 94 of the 147 *Staphylococcus* spp. cases, whereas *Staphylococcus aureus* was isolated in 32 cases. Among patients with bacteremia of *Staphylococcus aureus*, MRSA was found in 78% of the patients. *Enterococcus* spp. was the second most common Gram-positive pathogen, with 23 cases of *Enterococcus faecalis* and 21 of *Enterococcus faecium*. The percentage of *Enterococcus* spp. bacteremia in adults was 3.8 times higher than that in children during neutropenia. Because carbapenems or vancomycin has been used in the past, vancomycin-resistant *Enterococcus faecalis* or *Enterococcus faecium* was found in some patients. *Streptococcus* spp. was the third most common Gram-positive pathogen (19 cases), 79% of which were accounted for by alpha-*Streptococcus*, with *Streptococcus mitis* being the most common pathogen. *Pseudomonas aeruginosa* was the most frequently occurring bacterium in Gram-negative organisms.

Stenotrophomonas maltophilia was found in 13 of 37 (35%) adults who developed early bacterial pneumonia, and the condition of 75% of these patients deteriorated in spite of intensive therapy. Eleven of 13 *Stenotrophomonas maltophillia* adult pneumonias developed during neutropenia. *Stenotrophomonas maltophilia* is naturally resistant to penicillins, cephems except ceftazidim and cefpiramide, aminoglycosides, and carbapenems, therefore, antibiotics must be carefully selected for the treatment of patients with bacteremia or pneumonia caused by this bacterium.

For children, use of nonmyeloablative conditioning regimen was identified to be significant in univariate analysis. It was somewhat surprising that the use of nonmyeloablative conditioning in children was associated with a higher frequency of infections than in the myeloablative treated patients. Because the standardized JCBBN 100-day CRF do not include items for identifying information on comorbidity such as the recently introduced comorbidity index by Sorror et al [16], we could not make adjustments for patients' comorbidity status at transplant. We therefore cannot rule out the possibility that high-risk patients with organ failure and poor infectious defense were more likely to have been treated with nonmyeloablative conditioning, increasing risk of bacterial infections.

Multivariate analysis identified older age (6-10 years, and 11-15 years versus 0-5 years of age) at transplant as an independent risk factor for early bacterial infection in children, whereas univariate analysis revealed that older age at transplant and infusion of $<5.10 \times 10^7$ nucleated cells per kilogram of patient's body weight were identified as significant. These findings suggest that older age of children was a stronger risk factor than the number of infused nucleated cells per kilogram of patient's body weight. Higher activity of cell reproduction in younger children may be associated with a low incidence of mucosal toxicity, thus contributing to a lower risk of bacterial infection.

In adults, the incidence of early bacterial infection was almost twice as high, 21% at 100 days posttransplant compared to 11% for children. Although no specific risk factor was identified in adults, the prognostic significance of early bacterial infection was clearly identified in our analysis, thus indicating the importance of the prevention of early bacterial infection. No tendency for risk to increase with age was observed in individuals 16 years or older.

In conclusion, we analyzed the incidence of early post-UCBT bacterial infection in pediatric and adult patients. The incidence of early bacterial infection for adult patients was significantly higher than that for pediatric patients. The risk of early bacterial infection increased with age for individuals younger than 16 years, but not for those 16 years or older. Early bacterial infection had a negative effect on survival, especially in adults for whom the incidence of early bacterial infection was 21% and the median day of development was 10 days post transplant. These findings suggest that the prevention of bacterial infection during conditioning and the very early post-UCBT phase is especially important. Prospective clinical

studies are needed to establish the better prophylaxis against early bacterial infection.

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Feasibility of Reduced-Intensity Cord Blood Transplantation as Salvage Therapy for Graft Failure: Results of a Nationwide Survey of Adult Patients

Fusako Waki,[1] Kazuhiro Masuoka,[2] Takahiro Fukuda,[1] Yoshinobu Kanda,[3] Mika Nakamae,[4] Kimikazu Yakushijin,[5] Katsuhiro Togami,[6] Kaichi Nishiwaki,[7] Yasunori Ueda,[8] Fumio Kawano,[9] Masaharu Kasai,[10] Koji Nagafuji,[11] Maki Hagihara,[12] Kazuo Hatanaka,[13] Masafumi Taniwaki,[14] Yoshinobu Maeda,[15] Naoki Shirafuji,[16] Takehiko Mori,[17] Atae Utsunomiya,[18] Tetsuya Eto,[19] Hitoshi Nakagawa,[20] Makoto Murata,[21] Toshiki Uchida,[22] Hiroatsu Iida,[23] Kazuaki Yakushiji,[24] Takuya Yamashita,[25] Atsushi Wake,[2] Satoshi Takahashi,[26] Yoichi Takaue,[1] Shuichi Taniguchi[2]

To evaluate whether rescue with cord blood transplantation (CBT) could improve the poor survival after graft failure (GF), we surveyed the data of 80 adult patients (median age, 51 years) who received CBT within 3 months of GF (primary 64, secondary 16), with fludarabine-based reduced-intensity regimens with or without melphalan, busulfan, cyclophosphamide, and/or 2-4 Gy total-body irradiation (TBI). A median number of 2.4×10^7/kg total nucleated cells (TNC) were infused, and among the 61 evaluable patients who survived for more than 28 days, 45 (74%) engrafted. The median follow-up of surviving patients was 325 days, and the 1-year overall survival rate was 33% despite poor performance status (2-4, 60%), carryover organ toxicities (grade 3/4, 14%), and infections (82%) prior to CBT. Day 100 transplantation-related mortality was 45%, with 60% related to infectious complications. Multivariate analysis showed that the infusion of TNC $\geq 2.5 \times 10^7$/kg and an alkylating agent–containing regimen were associated with a higher probability of engraftment, and that high risk-status at the preceding transplantation and grade 3/4 organ toxicities before CBT were associated with an increased risk of mortality. In conclusion, in an older population of patients, our data support the feasibility of CBT with a reduced-intensity conditioning regimen for GF.

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From the [1]Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [2]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [3]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [4]Hematology, Graduate School of Medicine, Osaka City University, Osaka, Japan; [5]Hematology/Oncology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [6]Department of Hematology and Clinical Immunology, Kobe City General Hospital, Kobe, Japan; [7]Division of Hematology and Oncology, Department of Internal Medicine, Jikei University Kashiwa Hospital, Kashiwa, Japan; [8]Department of Hematology/Oncology, Kurashiki Central Hospital, Kurashiki, Japan; [9]Department of Hematology, National Hospital Organization Kumamoto Medical Center, Kumamoto, Japan; [10]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [11]Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Science, Fukuoka, Japan; [12]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [13]Department of Internal Medicine, Rinku General Medical Center, Izumisano, Japan; [14]Division of Hematology and Oncology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [15]Department of Hematology, Oncology, and Respiratory Medicine, Okayama University Graduate School of Medicine, Okayama, Japan; [16]Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan; [17]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan; [18]Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan; [19]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [20]Department of Hematology, Kyoto First Red Cross Hospital, Kyoto, Japan; [21]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [22]Department of Hematology & Oncology, Japanese Red Cross Society Nagoya Daini Hospital, Nagoya, Japan; [23]Department of Hematology, Meitetsu Hospital, Nagoya, Japan; [24]Division of Hematology and Oncology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan; [25]Hematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; and [26]Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

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Correspondence and reprint requests: Takahiro Fukuda, MD, Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan (e-mail: tafukuda@ncc.go.jp).

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INTRODUCTION

Graft failure or rejection (GF) is a serious problem early after allogeneic stem cell transplantation (SCT) using cord blood (CB) [1-6], an HLA-mismatched donor [7], and nonmyeloablative or reduced-intensity conditioning (RIC) regimens [8-13]. The incidence of GF was low after SCT from an HLA-matched related (2%) [14] or unrelated donor (0.7%-1.7%) [15,16]. In contrast, the incidence of GF was 14%-22% for SCT from an HLA-mismatched unrelated donor [15], 8%-20% for cord blood transplantation (CBT) [17,18], and 5%-21% for SCT from an unrelated donor using RIC [12,13]. The outcome of GF becomes generally poor because of an increased risk of infectious complications, which occur during prolonged severe neutropenia with associated organ toxicities. Whereas the survival rate after GF was 8% when no rescue transplantation was performed [19], the survival rate improved to 25%-40% when a second transplantation was performed [19-22].

The treatment of GF generally depends on 2 major basic mechanisms, that is, (1) poor graft function and (2) immunologically mediated graft rejection. Although the boost infusion of $CD34^+$ stem cells, selected or unmanipulated, has been reported to be effective in the former case [23,24], in the latter case, retransplantation with immunosuppressive conditioning is required for effective reconstitution of hematopoiesis [21,25-27]. Nevertheless, transplantation-related mortality (TRM) is still high because at the second SCT, most patients have poor performance status (PS), organ toxicities, carryover infection because of prolonged cytopenia, and difficulties in finding a suitable donor on an emergency basis. An additional problem is overlapping regimen-related toxicity (RRT) because of the conditioning regimen for the second SCT.

CB is a readily available stem cell source and, with the current development of efficient banking systems, most patients can readily find a suitable CB unit [28]. Many reports have shown the feasibility of reduced-intensity cord blood transplantation (RICBT) in older patients and patients with comorbidities [29,30]. Additionally, small case series of patients who were successfully rescued with retransplantation using CB after GF have also been reported [31-36]. Hence, CBT is a potential target of clinical research for GF. Nevertheless, the inevitable risks associated with CBT, that is, slower neutrophil engraftment and resultant higher risk of GF [17,18], may become critical barriers. To investigate whether salvage therapy with RICBT is a feasible therapeutic option for adult patients suffering from GF, we conducted a nationwide survey of RICBT that was performed as salvage therapy for GF.

PATIENTS AND METHODS

Data Sources and Patient Selection

Questionnaires were sent to 131 transplant centers in Japan, and 42 centers agreed to enroll consecutive cases in this study. This study was approved by the institutional review board of the National Cancer Center. The inclusion criteria for this study were as follows: (1) patients with hematologic disorders above age 16 years who received allogeneic SCT between January 2000 and April 2006, which resulted in primary or secondary GF, and (2) those who subsequently received fludarabine-based RICBT as salvage therapy within 3 months of the diagnosis of GF. The definition of a RIC regimen was according to the previous report by Giralt [37]. Patients who had relapse or disease progression before rescue RICBT were not included.

The total number of allogeneic SCT performed during this study period in 42 centers was 5622 including related donors (n = 2556), unrelated donors (n = 1907) and cord blood donors (n = 1159). Among 240 patients who experienced GF, 146 underwent salvage SCT and 94 did not. The stem cell source was CB (n = 102) or non-CB (n = 44). Among the 102 CBT recipients, 80 patients fulfilled the criteria for this study after excluding 12 patients who received myeloablative conditioning and 10 patients who received no toxic drug as conditioning regimen (antithymocyte globulin [ATG] only, n = 5; steroid only, n = 3; total lymphoid irridiation [TLI] only, n = 1; no conditioning, n = 1).

Definitions

Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count (ANC) exceeded 500/mm^3 of peripheral blood. Primary GF was defined according to a previous report [15] as (1) failure of ANC to surpass 500/mm^3 or (2) absence of donor T cells (<5%) before relapse, disease progression, second SCT, or death. Secondary GF was defined as (1) decrease in ANC <100/mm^3 at 3 determinations or (2) absence of donor T cells (<5%) after the initial engraftment without recovery before relapse, disease progression, second SCT, or death. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction (PCR) for short tandem repeats or variable numbers of

tandem repeats was used to detect donor cells at a sensitivity of 1% to 5% [38]. Whole blood, $CD3^+$ selected, or marrow cells were assessed for chimerism at the time of neutrophil engraftment depending on the decision at each transplant center. HLA matching was reported using serological typing of HLA-A and HLA-B and allele typing of HLA-DRB1 of donor-recipient pairs except for 5 patients. Standard risk was defined as all complete remission of hematologic malignancy, chronic phase of chronic myeloid leukemia, or aplastic anemia. High risk was defined as other status of hematologic malignancy and all myelodysplastic syndrome refractory anemia with excess blasts (MDS-RAEB), including nonremission atypical CML. PS was defined according to the ECOG criteria [39]. RRT was evaluated by the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) [40]. The diagnosis and clinical grading of acute graft-versus-host disease (aGVHD) were based on the established criteria [41]. Relapse was defined as an increase of blast more than 5% in bone marrow with hematologic malignancy.

First Transplant Procedures

Patients and transplantation characteristics at the first SCT that resulted in subsequent GF are summarized in Table 1. The median age of the 80 patients was 51 years (range: 17-68). Disease risk before the first SCT was standard risk in 49 patients (61%) and high risk in 31 patients (39%). Donor source for the first SCT included unrelated CB in 74% and unrelated bone marrow (BM) in 20%. Because the Japan Marrow Donor Program does not permit the donation of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (PBSC) from unrelated donors, the stem cell source from unrelated donors was BM or CB. GVHD prophylaxis varied among the transplant centers.

After the first SCT, 64 patients experienced primary GF at a median of 28 days (range: 16-56 days), and 16 patients experienced secondary GF at a median of 36 days (range: 20-156). Data for chimerism analysis were available in 65 patients (primary GF, n = 49; secondary GF, n = 16). Among them, 45 patients had <5% donor cells (primary GF, n = 40, 82%; secondary GF, n = 5, 31%), which suggested immunologically mediated graft rejection, and 20 patients had donor cells ranging from 5% to 100% (primary GF, n = 9, 18%; secondary GF, n = 11, 69%), which suggested poor graft function.

Second Rescue Transplant Procedures

Patients and transplantation characteristics at the second SCT using RICBT as salvage therapy for GF are summarized in Table 2. The median intervals between the first SCT to the second SCT and the diagnosis of GF to the second SCT were 47 days and 15 days, respectively. Forty-eight patients (60%) had poor PS at the second SCT, and 11 patients (14%) had grade 3 or 4 carryover organ toxicities. Within 3 weeks of the start of conditioning for the second SCT, 66 patients (82%) had documented infection or febrile neutropenia that required intravenous antibiotics. More than half of the patients received a graft with serologic 2- or 3-locus HLA mismatches. We also examined the effect of HLA mismatch with serologic HLA-A, B and allele DRB1 except for 5 patients whose allele typing was not performed. The median body weight of the recipients was 55 kg (range: 33-110), and the median number of total nucleated cells (TNC) was 2.4×10^7/kg recipient body weight (range: 1.03-4.3) at cryopreservation. All patients received a fludrabine-containing reduced-intensity regimen with or without 2-4 Gy TBI. As there are no

Table 1. Patients and Tranaplantation Characteristics at the First SCT

Parameters	n = 80*
Median age at first SCT (range)	51 years (17-68)
Male/female	34/46
Underlying diagnosis†	
AML	43 (54%)
MDS	10 (13%)
ALL	13 (16%)
Other	14 (18%)
Disease risk‡	
Standard risk	49 (61%)
High risk	31 (39%)
Preceding chemotherapy	
Yes	66 (83%)
No§	14 (17%)
Conditioning⊥	
Myeloablative	37 (46%)
Reduced-intensity	43 (54%)
Donor and stem cell source	
Related BM or PB	5 (6%)
Unrelated BM	16 (20%)
Unrelated CB	59 (74%)
Type of GF	
Primary	64 (80%)
Secondary	16 (20%)

SCT indicates stem cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; BM, bone marrow; PB, peripheral blood; CB, cord blood; GF, graft failure; RAEB, refractory anemia with excess blasts; CML, chronic myelogeneous leukemia; CY, cyclophosphamide; TBI, total-body irradiation; BU, busulfan.

*Before undergoing the SCT that resulted in GF, 6 patients had received preceding transplantation.

†AML included overt AML evolved from MDS. MDS included RAEB-I or II (n = 9) and atypical CML (n = 1). Other diagnoses included non-Hodgkin lymphoma (n = 6), aplastic anemia (n = 5), and CML (n = 3).

‡Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

§Forteen patients included MDS (n = 7), AML (n = 2), or aplastic anemia (n = 5).

⊥Myeloablative conditionings included CY/TBI (n = 27), BU/CY (n = 6), and other TBI-based regimen (n = 4). Reduced-intensity conditionings included fludarabine-based (n = 37), cladribine-based (n = 2), and others (n = 4) with (n = 26) or without (n = 17) 2-4 Gy TBI.

established standard RIC regimens for CBT after GF, the different conditioning regimens were chosen at the discretion of the attending physicians. G-CSF was administered in all but 1 patient after CBT.

Statistical Analyses

The primary endpoint of this study was the engraftment rate in patients who survived for more than 28 days after salvage RICBT. The secondary endpoints were TRM, overall survival (OS), and progression-free survival (PFS) from the day of salvage RICBT. For calculation of PFS, 5 patients with aplastic anemia were excluded from the analysis. OS and PFS were estimated using the Kaplan-Meier method. The cumulative incidences of engraftment and TRM were evaluated using Gray's method, considering death without engraftment and relapse, respectively, as competing risks. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of OS, PFS, TRM, and relapse after the second transplantation over time across patient subgroups.

Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. Finally, P values of $<.05$ were considered statistically significant. Clinical factors that were assessed for their association with engraftment rate, TRM, and OS included sex, patient age at the time of the first SCT (<50 years versus ≥50 years), disease risk at the first SCT (standard risk versus high risk), conditioning for the first SCT (myeloablative versus reduced-intensity), PS at the second SCT (0-1 versus 2-4), carryover organ toxicities at the second SCT (grade 0-2 versus 3-4), carryover infection at the second SCT (documented versus febrile neutropenia/none), conditioning regimens for the second SCT (containing alkylating agents versus others), including TBI at the second SCT (non-TBI versus TBI 2-4 Gy), use of MTX (yes versus no), TNC (<2.5 versus ≥2.5 × 10^7/kg), and numbers of HLA mismatches in the graft-versus-host direction (0-1 versus 2-3) and host-versus-graft direction (0-1 versus 2-3). The statistical analysis was performed with SAS ver.8 (SAS Institute, Cary, NC).

Table 2. Patients and Transplantation Characteristics at the Second SCT (RICBT) for GF

Parameters	n = 80
Median time interval between	
The first and second SCT	47 days (range: 27-203)
Diagnosis of GF and the second SCT	15 days (range: 4-61)
PS at the second SCT	
0-1	32 (40%)
2-4	48 (60%)
Carryover organ toxicities at the second SCT*	
Grade 0-2	69 (86%)
Grade 3-4	11 (14%)
Carryover infection at the second SCT†	
Documented	40 (50%)
Febrile neutropenia	26 (32%)
None	14 (18%)
The median TNC of CB	2.4 × 10^7/kg (range: 1.03-4.3)
Numbers of serological HLA mismatch in GVH direction	
0-1	32 (40%)
2-3	48 (60%)
HVG direction	
0-1	33 (41%)
2-3	47 (59%)
Conditioning‡	
Flu alone	20 (25%)
Flu + Mel	22 (28%)
Flu + Bu	18 (22%)
Flu + CY	17 (21%)
Flu + others	3 (4%)
with 2-4 Gy TBI	35 (44%)
without TBI	45 (56%)
GVHD prophylaxis§	
CSP alone	17 (21%)
CSP + sMTX	6 (8%)
TAC alone	40 (50%)
TAC + sMTX	8 (10%)
Others	9 (11%)

SCT indicates stem cell transplantation; RICBT, reduced-intensity cord blood transplantation; GF, graft failure; PS, performance status; TNC, total nucleated cells; CB, cord blood; HLA, human leukocyte antigen; GVH, graft-versus-host; HVG, host-versus-graft; Flu, fludarabine; Mel, melphalan; Bu, busulfan; CY, cyclophosphamide: TBI, total-body irradiation; GVHD, graft-versus-host disease; CSP, cyclosporine; sMTX, short-term methotrexate; TAC, tacrolimus.

*Grade of organ toxicities was evaluated by the CTCAE v3.0 [40]. Grade 3 toxicities included liver (n = 5), lung (n = 3), renal/bladder (n = 2), heart (n = 1), stomatitis (n = 1), and central nervus system (n = 1). Grade 4 toxicity included lung only (n = 1).

†Documented infection included bacteremia (n = 27), pneumonia (n = 5), aspergillus infection (n = 3), subcutaneous abscess (n = 2), and others (n = 3).

‡The median total doses of each conditioning regimen were as follows: Flu (138 mg/m^2), Mel (80 mg/m^2), Bu (8 mg/kg), and CY (60 mg/kg). Antithymocyte globulin was also used in 8 patients (Flu alone [n = 5], Flu + Mel [n = 1], and Flu + Bu [n = 2]). Other conditioning regimens included Flu plus thiotepa (n = 2) or etoposide (n = 1). Twelve patients received 2 Gy TBI and 23 patients received 4 Gy TBI.

§Other prophylaxis included CSP/TAC plus mycophenolate mofetil (n = 7) or prednisolone (n = 2).

RESULTS

Neutrophil and Platelet Engraftment (Table 3)

The cumulative incidences of neutrophil engraftment and death without engraftment are shown in Figure 1A. Among 61 patients who survived for more than 28 days after the second SCT, 45 (74%) achieved neutrophil engraftment at a median of 21 days (range: 13-44) (Table 3). The other 33 patients failed to achieve engraftment because of early TRM within 28 days after RICBT (n = 17), early relapse (n = 3) at days 22-25, or primary GF (n = 13). The remaining 2 patients died of TRM within 28 days after obtaining neutrophil engraftment. Among 13 patients who experienced primary GF after second SCT, chimerism analyses were performed in 4 patients to confirm the diagnosis of GF at a median of 25 days (range: 21-28).