Although preemptive therapy with CMV monitoring has been successful in preventing early CMV disease [8], the efficacy of such an approach beyond day 100 remains to be evaluated. We have been routinely continuing CMV monitoring beyond day 100 and administered GCV preemptively. In this study, we retrospectively analyzed the incidence, risk factors, and outcome of late CMV reactivation and disease in allogeneic HSCT patients.

2 Patients and methods

2.1 Study population

During a 7-year period (from January, 1998 to September, 2005), 205 adult patients (≥16 years old) underwent allogeneic HSCT for the first time at the University of Tokyo Hospital. Twenty-nine patients died within the first 100 days after HSCT, two of whom developed CMV disease. Among the remaining 176 patients who survived more than day 100 after HSCT, the day of the first negative antigenemia test without any antiviral agents beyond day 100 was defined as the starting point of late CMV antigenemia monitoring. The median period from HSCT to the starting point was 100 days (100–207 days). Eleven patients who developed CMV disease before the starting point and 64 who did not undergo CMV antigenemia assay at least five times after the starting point were excluded. Finally, 101 patients were included in the study.

The median follow-up was 12.6 months (range 4.8–74 months) after HSCT. The patient characteristics are shown in Table 1. Thirty-five, twenty, and forty-six patients received grafts from a HLA-matched related donor, a mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 40 out of 55 related donors provided a peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in the first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered high-risk diseases. Donors other than HLA-matched related donors were defined as alternative donors.

Table 1 Patients' characteristics

Characteristic	Total patients
Sex (male/female)	65/36
Age, median (range)	41.0 (16–66)
Serostatus before transplant	
Recipient CMV-positive/negative	90/11
Donor CMV-positive/negative	80/21
Underlying disease	
Acute leukemia	46
CML	21
MDS	12
NHL/ATL	11
SAA	5
Other	6
Graft source	
PBSC	40
BM	61
Donor type	
Matched related	35
Mismatched related	20
Unrelated	46
Preparative regimen	
Cy/TBI-based regimen	66
Bu/Cy-based regimen	11
ATG-including regimen	3
Flu-based reduced-intensity regimen	21
GVHD prophylaxis	
CsA + MTX	78
Tacrolimus + MTX	10
Alemtuzumab + CsA + MTX	13
Chronic GVHD	
Extensive	53
Limited	21
None	27

CMV cytomegalovirus, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin lymphoma, *ATL* adult T-cell leukemia/lymphoma, *SAA* severe aplastic anemia, *PBSC* peripheral blood stem cell, *BM* bone marrow, *Cy* cyclophosphamide, *TBI* total body irradiation, *Bu* busulfan, *ATG* anti-thymocyte globulin, *Flu* fludarabine, *GVHD* graft-versus-host disease, *CsA* cyclosporine, *MTX* methotrexate

2.2 Transplantation procedure

The conventional preparative regimen for leukemia/lymphoma was mainly performed with either total body irradiation (TBI) regimen [cyclophosphamide (Cy) at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days] or non-TBI regimen [Cy at the same dose combined with busulfan (Bu) at 4 mg/kg/day for 4 days]. In the TBI regimen, the dose of Cy was decreased to 40 mg/kg for 1 day and etoposide at 20 mg/kg for 2 days was added instead, in patients with impaired cardiac function. Fludarabine (Flu)-based regimens, including FB regimen (Flu at 30 mg/m^2/day for 6 days and Bu at 4 mg/kg/day for 2 days) with or without TBI at 4 Gy, FB16 regimen (Flu at the same dose with Bu at 4 mg/kg/day for 4 days), FM regimen (Flu 30 mg/m^2/day for 5 days and melphalan at 140 mg/m^2/day for 1 day), and FC regimen (Flu at

25 mg/m^2/day for 5 days and Cy at 60 mg/kg/day for 2 days), were used as reduced-intensity regimens for elderly or clinically infirm patients [9]. Gemcitabine at 1,000 mg/m^2/day for 3 days was added to the FB regimen for patients with pancreatic cancer [10]. The conditioning regimen for aplastic anemia was either a rabbit antithymocyte globulin (ATG) regimen (Cy at 50 mg/kg/day for 4 days and ATG at 5 mg/kg/day for 5 days with or without TBI at 4 Gy) or an alemtuzumab regimen (Cy at 25 mg/kg/day for 4 days and Flu at 30 mg/kg/day for 4 days combined with alemtuzumab at 0.2 mg/kg/day for 6 days, with or without TBI at 2 Gy).

For prophylaxis against GVHD, cyclosporine A (CsA) at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was administered combined with short-term methotrexate (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and optionally on day 11). For patients who received a graft from a haploidentical HLA-mismatched donor, alemtuzumab was added to the TBI regimen or the FB regimen at 0.2 mg/kg for 6 days [11]. Methylprednisolone (mPSL) or prednisolone (PSL) at 1 or 2 mg/kg was added for patients who developed grade 2–4 acute GVHD, whereas PSL at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, fungal, herpes simplex virus, and pneumocystis jirovecii infections consisted of fluconazole, tosufloxacin, acyclovir, and sulfamethoxazole/trimethoprim.

2.3 CMV antigenemia assay

CMV antigenemia assay was performed as described previously [12]. In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide, using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with monoclonal antibody C10/11 (Clonab CMV; Biotest, Dreieich, Germany), which targets CMV pp65 antigen, and reacted with goat alkaline phosphatase-labeled anti-mouse immunoglobulin (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

2.4 Preemptive therapy for CMV disease

Preemptive therapy against CMV disease was performed by weekly monitoring of CMV antigenemia after engraftment, as described previously [13]. Until June 2001, intravenous GCV was started at an induction dose of 10 mg/kg/day when ten or more CMV-positive cells were detected in patients who underwent HSCT from a HLA-matched related donor and when positive cells were detected at any level in patients who underwent HSCT from an alternative donor. From July 2001, the induction dose was decreased to 5 mg/kg/day and the threshold of antigenemia to start GCV was changed to twenty and three positive cells for patients who underwent HSCT from a HLA-matched related donor and an alternative donor, respectively [14]. The dose of GCV was increased to 10 mg/kg/day when rising antigenemia was observed. The dose of GCV was adjusted according to renal function [15]. GCV was continued until negative antigenemia was observed. Beyond day 100 after HSCT, CMV monitoring was continued at least every other week in 90 patients and at longer intervals in the remaining 11 patients. GCV was administered in a similar manner as before day 100, at the discretion of attending physicians.

2.5 Definition of late positive CMV antigenemia and CMV disease

Late positive CMV antigenemia was defined as the detection of CMV-positive cells at any level after the starting point. Recurrent CMV antigenemia was defined as the detection of CMV-positive cells after the negative conversion of late positive CMV antigenemia. All patients with symptoms compatible with CMV disease, such as interstitial pneumonia, colitis, or gastritis underwent extensive pathological and microbiological examination of biopsy specimens. Biopsy was performed in all cases of interstitial pneumonia, colitis, and gastritis. The diagnosis of these CMV diseases was made by histopathological examination and immunochemical staining of biopsy specimens, which demonstrated typical CMV inclusion bodies. To establish a definite diagnosis of CMV retinitis, patients received PCR to detect CMV-DNA using aqueous humor and/or ophthalmoscopy, which demonstrated typical findings of CMV retinitis, including a white fluffy retinal infiltrate with several areas of hemorrhage or a granular white area without hemorrhage.

2.6 Statistical analysis

The cumulative incidences of late positive CMV antigenemia and CMV disease, and the impact of possible confounding factors on these events were evaluated using Gray's method, considering death without each event as a competing risk [16]. The cumulative incidence of nonrelapse mortality (NRM), and the impact of possible confounding factors on NRM were evaluated, considering relapse as a competing risk. The development of chronic GVHD and the use of systemic corticosteroids at $\geq$0.5 mg/kg/day were treated as time-dependent covariates. Factors associated with at least borderline significance ($P < 0.10$) in univariate analyses were subjected to multivariate

analysis using backward stepwise proportional-hazard modeling. *P*-values <0.05 were considered significant.

3 Results

3.1 Incidence and risk factors for late positive CMV antigenemia

Overall, 51 of 101 patients developed late positive antigenemia at a median of 29 days (range 1–483 days) after the starting point, with a cumulative incidence of 53% (Fig. 1a). In univariate analyses, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were significantly associated with the development of late positive antigenemia (Table 2). In multivariate analysis, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were identified as independent risk factors for late positive antigenemia.

Fifty (98%) of the 51 patients with late positive antigenemia showed negative conversion of antigenemia after a median of 21 days (range 2–430 days). The median peak antigenemia level was only two positive cells per two slides (range 1–268). Twenty-eight patients developed late CMV antigenemia with consistently less than three positive cells (low-level antigenemia), 25 of whom showed negative conversion without GCV administration (Table 3A). Of the remaining 23 patients who developed high-level antigenemia with three or more positive cells, all but one who died of invasive aspergillosis (IA) achieved negative conversion, with ($n = 17$) or without ($n = 5$) GCV administration. Twenty-nine of the 50 patients (58%) had recurrent antigenemia at a median of 14 days (range 3–714 days) after the first negative conversion (Table 3B). The second recurrence was observed in 17 of 26 patients, after a median of 21 days (range 4–323 days) after the second negative conversion.

3.2 Incidence and risk factors for late CMV disease

Eight patients developed late CMV disease at a median of 54 days (range 14–248 days) after the starting point, with a cumulative incidence of 8%, (Fig. 1b). Female sex, the use of alemtuzumab in the conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, and the development of chronic GVHD were associated with a higher incidence of late CMV disease with at least borderline significance ($P < 0.10$) (Table 4). Among these, the use of alemtuzumab in the conditioning regimen was the only independent risk factor for late CMV disease.

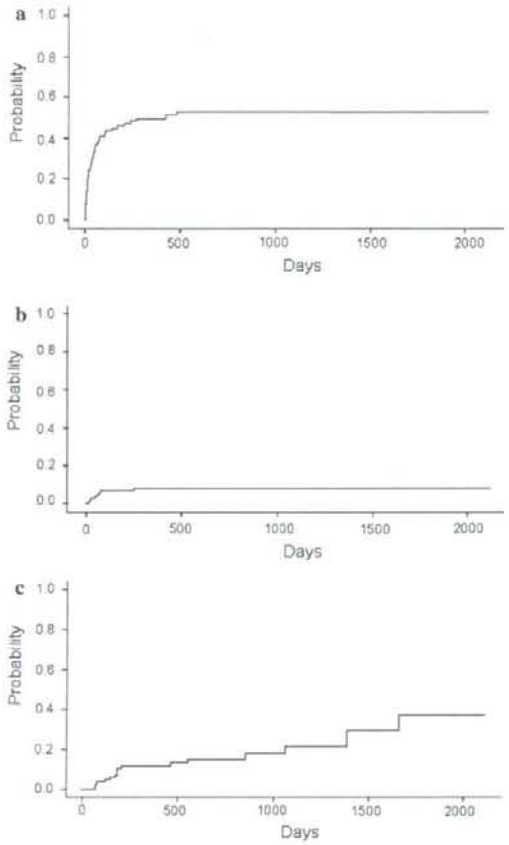

Fig. 1 Cumulative incidences of **a** late positive CMV antigenemia, **b** late CMV disease, and **c** non-relapse mortality. The definition of the starting point is described in the text

Late CMV disease involved retinitis in four, colitis in three, and gastritis in one (Table 5). Three of the four patients with retinitis were asymptomatic. Although all of these CMV diseases were successfully treated with GCV, two patients died of bronchiolitis obliterans and IA, after the resolution of CMV disease. Among the eight patients with late CMV disease, seven developed CMV disease after the development of late positive antigenemia. Five developed CMV disease after the first episode, and the other two after recurrent antigenemia. The median peak antigenemia level was ten positive cells per two slides (range 4–186). The remaining patient developed late CMV disease before the development of late positive antigenemia. All patients achieved negative conversion by the administration of intravenous GCV.

Table 2 Risk factors for late positive CMV antigenemia

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	63	0.30
≤40 years old	45	44	
Sex			
Male	65	52	0.40
Female	36	54	
Disease risk			
Standard risk	40	51	0.80
High risk	61	54	
Graft source			
Bone marrow	61	46	0.18
Peripheral blood	40	64	
Donor type			
Matched related donor	35	47	0.51
Alternative donor	66	56	
Regimen			
TBI regimen	72	54	0.81
Non-TBI regimen	29	50	
Regimen			
With alemtuzumab	13	69	0.041
Without alemtuzumab	88	47	
Donor CMV			
Seropositive	80	57	0.18
Seronegative	21	33	
Recipient CMV			
Seropositive	90	58	0.0069
Seronegative	11	9	
Prior CMV antigenemia			
Yes	74	61	0.0053
No	27	32	
Prior use of GCV			
Yes	59	62	0.012
No	42	41	
Year of transplant			
Before June 2001	30	50	0.77
After July 2001	71	54	
Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	3.29 (1.81–5.97)	<0.0001
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	2.77 (1.16–4.45)	0.017
No	45		

Table 2 continued

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	2.13	1.04–4.38	0.040
Chronic GVHD	2.87	1.54–5.35	0.00095
With alemtuzumab	2.54	1.20–5.37	0.015
Recipient CMV seropositive	13.0	1.77–95.6	0.012

3.3 Incidence and risk factors for non-relapse mortality

Seventeen patients died of non-relapse causes more than 100 days after HSCT, with a 3-year cumulative incidence of 22% (Fig. 1c). Male sex and the use of systemic corticosteroids at ≥0.5 mg/kg were associated with a higher NRM with at least borderline significance (Table 6). The use of systemic corticosteroids at ≥0.5 mg/kg was identified as independently significant for NRM in multivariate analysis. The direct causes of death included non-infectious pulmonary complications (NIPC) in seven patients, infections other than CMV in five, gastrointestinal bleeding in two, multiple organ failure in two, and acute myocardial infarction in one. Fifteen of the seventeen patients received systemic corticosteroids at ≥0.5 mg/kg after the starting point, for severe chronic GVHD in eight, NIPC in four, respiratory failure caused by infections in two, and hemophagocytic syndrome in one.

4 Discussion

This study demonstrated that the cumulative incidence of late CMV disease was successfully decreased and CMV-related mortality was completely avoided by preemptive therapy with extended CMV antigenemia monitoring beyond day 100, in spite of a high frequency of late CMV reactivation. The use of alemtuzumab was the only significant independent risk factor for late CMV disease, while recipient CMV seropositivity, the use of alemtuzumab, chronic GVHD and high-dose steroids were important determinants for late positive antigenemia. A significant correlation between the development of late positive antigenemia and these risk factors is consistent with the clinical observation that the development of chronic GVHD and the use of alemtuzumab or high-dose steroid resulted in delayed recovery of CMV-specific immune response, leading to an increased incidence of late CMV reactivation [17, 18]. Extended CMV-antigenemia monitoring is strongly recommended in such patients with profound immunosuppression.

Table 3 Time course of late positive CMV antigenemia

A. Time course of CMV-Ag in 51 patients who developed late positive antigenemia

Peak level after late positive Ag	Number of patients (*n*)	Use of GCV	Number of patients (*n*)	Peak value of CMV-Ag	Patients with negative conversion
CMV-Ag < 3	28	(−)	25	1 (1–2)	25
		(+)	3	2 (1–2)	3
CMV-Ag ≥ 3	23	(−)	5	4 (3–8)	5
		(+)	18	12 (3–268)	17

B. Recurrence of late positive CMV antigenemia

Late positive CMV antigenemia	Number of patients (*n*)	Patients with late positive Ag (*n*)	Value of CMV-Ag at late positive Ag	Peak value of CMV-Ag	Use of GCV	Patients with negative conversion
First late positive Ag	101	51	1 (1–268)	2 (1–268)	21	50
Second late positive Ag	50	29	1 (1–18)	1 (1–86)	14	26
Third late positive Ag	26	17	2 (1–126)	3 (1–126)	8	16

Table 4 Risk factors for late CMV disease

Univariate analysis

Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	9	0.66
≤40 years old	45	7	
Sex			
Male	65	5	0.094
Female	36	15	
Disease risk			
Standard risk	40	8	0.90
High risk	61	9	
Graft source			
Bone marrow	61	5	0.18
Peripheral blood	40	13	
Donor type			
Matched related donor	35	3	0.17
Alternative donor	66	11	
Regimen			
TBI regimen	72	10	0.29
Non-TBI regimen	29	3	
Regimen			
With alemtuzumab	13	31	0.00097
Without alemtuzumab	88	5	
Donor CMV			
Seropositive	80	9	0.58
Seronegative	21	5	
Recipient CMV			
Seropositive	90	9	0.31
Seronegative	11	0	
Prior CMV antigenemia			
Yes	74	11	0.075
No	27	0	
Prior use of GCV			
Yes	59	12	0.080
No	42	2	
Year of transplant			
Before June 2001	30	3	0.26
After July 2001	71	10	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	9.27 (1.11–77.5)	0.040
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	1.12 (0.21–5.90)	0.90
No	45		

Multivariate analysis

Factors	Relative risk	95% CI	*P*-value
With alemtuzumab	8.20	2.02–33.3	0.0032

The incidence of late CMV disease in our series was lower than that in previous studies, where antiviral agents were not used preemptively beyond day 100 [1–3]. In a recent study by Boeckh et al. [2], 17.8% of the patients

Table 5 Characteristics of patients who developed late CMV disease

UPN	Sex/age	Diagnosis	Preparative regimen	cGVHD grade	Steroid at CMV disease	Starting point to late positive Ag / disease (days)	Treatment	CMV disease	Lymphocyte counts at CMV disease	Peak value of late CMV antigenemia	Negative conversion	Survival months (months)	Cause of death
1	M/38	ALL	Cam/Cy/TBI	Limited	0	18/60	Intravenous GCV	Retinitis (asymptomatic)	1,505	10	+	29.8+	Alive
2	F/43	NHL	Cy/TBI	Extensive	0	1/14	Intravenous GCV / vitrectomy	Retinitis (asymptomatic)	840	6	+	21.4+	Alive
3	M/42	AA	Cam/Flu/Cy/TBI	Extensive	PSL 30 mg	2/48	Intravenous GCV	Colitis	1,125	12	+	12.1+	Alive
4	F/57	AML	Cam/Flu/Bu/TBI	None	0	4/27	Intravenous/ intreavetreal GCV	Retinitis (asymptomatic)	<400	28	+	6.2	IA
5	F/41	ALL	Cam/Cy/TBI	Limited	0	4/77	Intravenous GCV	Retinitis(symptomatic)	1,890	4	+	9.6+	Alive
6	F/25	CML	Cy/TBI	Extensive	mPSL 20 mg	42/17	Intravenous GCV /foscarnet	Gastritis	748	0	+	33.5	BO
7	F/38	AML	Cy/TBI	Extensive	0	7/248	Intravenous GCV	Colitis	704	6	+	48.6+	Alive
8	M/56	NHL	Flu/BU	Extensive	PSL 10 mg	36/69	Intravenous GCV	Colitis	2,465	186	+	38.0+	Alive

UPN unique patient number, *cGVHD* chronic graft-versus-host-disease, *CMV* cytomegalovirus, *GCV* ganciclovir, *AA* aplastic anemia, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *CML* chronic myelogenous leukemia, *NHL* non-Hodgkin lymphoma, *TBI* total body irradiation, *Cam* alemtuzumab, *Cy* cyclophsphamide, *Flu* fludarabine, *Bu* Busulfan, *PSL* predonisolone, *mPSL* methylpredonisolone, *IA* invasive aspergillosis, *BO* bronchiolitis obliterans

Table 6 Risk factors for 3-year non-relapse mortality

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	21	0.55
≤40 years old	45	9	
Sex			
Male	65	22	0.092
Female	36	3	
Disease risk			
Standard risk	40	14	0.65
High risk	61	16	
Graft source			
Bone marrow	61	16	0.60
Peripheral blood	40	15	
Donor type			
Matched related donor	35	21	0.10
Alternative donor	66	12	
Regimen			
TBI regimen	72	14	0.26
Non-TBI regimen	29	18	
Regimen			
With alemtuzumab	13	8	0.56
Without alemtuzumab	88	16	
Donor CMV			
Seropositive	80	15	0.60
Seronegative	21	18	
Recipient CMV			
Seropositive	90	15	0.14
Seronegative	11	22	
Prior CMV antigenemia			
Yes	74	15	0.30
No	27	18	
Prior use of GCV			
Yes	59	18	0.65
No	42	12	
Year of transplant			
Before June 2001	30	14	0.78
After July 2001	71	16	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	1.35 (0.35–5.21)	0.67
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	10.5 (2.34–47.0)	0.0021
No	45		

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	1.05	2.34–47.0	0.0021

who did not receive antiviral agents preemptively more than 3 months after HSCT, developed late CMV disease, including pneumonia and gastrointestinal disease predominantly, with a mortality rate of 46%. Other studies have also confirmed that CMV pneumonia was the leading manifestation of late CMV disease with an associated mortality rate ranging from 60 to 80%, in the absence of preemptive therapy [1, 4]. In contrast, we exclusively observed late CMV retinitis and/or gastrointestinal disease, none of which was directly related to death. These findings suggest that preemptive therapy with extended CMV antigenemia monitoring, reduced the incidence of late CMV disease and eradicated fatal CMV disease.

The use of alemtuzumab was the only independent significant risk factor for late CMV disease, although it was not associated with increased non-relapse mortality. This finding is inconsistent with the conclusion of previous studies that the use of alemtuzumab did not result in an increased incidence of CMV disease in two previous studies despite a strong association with the high frequency of CMV reactivation [11, 17]. In this study, four of the 13 patients who received alemtuzumab developed late CMV disease, three of whom had retinitis. Two cases of retinitis were asymptomatic and diagnosed by ophthalmologic screening. We performed routine ophthalmologic screening as a standard practice only in patients who received alemtuzumab, based on the association between a high incidence of CMV reactivation and delayed posttransplant immune reconstitution by alemtuzumab [11, 17]; therefore, asymptomatic retinitis might have been overlooked in patients who did not receive alemtuzumab in this study or in patients who received alemtuzumab in other studies.

More than half of the patients with late positive antigenemia developed low-level antigenemia with consistently less than three positive cells, and 90% showed negative conversion without GCV administration. In all high-level antigenemia patients, except for one who died of IA while on preemptive GCV, negative conversion without progression to fatal disease was obtained by preemptive therapy; therefore, three positive cells per two slides might be an appropriate threshold to start GCV beyond day 100 after transplantation.

In conclusion, late positive antigenemia was frequently observed beyond day 100 after transplantation, especially in profoundly immunosuppressed patients who received alemtuzumab, high-dose steroids, or who developed chronic GVHD. Preemptive therapy with extended CMV antigenemia monitoring beyond day 100, not only reduced the incidence of late CMV disease, but also completely prevented fatal CMV disease; therefore, extended CMV monitoring is recommended at least for patients with such risk factors.

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References

1. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996;88:4063–71.
2. Boeckh M, Leisenring W, Riddell SR, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. Blood. 2003;101:407–14.
3. Einsele H, Hebart H, Kauffmann-Schneider C, et al. Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. Bone Marrow Transplant. 2000;25:757–63.
4. Nguyen Q, Champlin R, Giralt S, et al. Late cytomegalovirus pneumonia in adult allogeneic blood and marrow transplant recipients. Clin Infect Dis. 1999;8:618–23.
5. Krause H, Hebart H, Jahn G, Müller CA, Einsele H. Screening for CMV-specific T cell proliferation to identify patients at risk of developing late onset CMV disease. Bone Marrow Transplant. 1997;19:1111–6.
6. Wolf DG, Lurain NS, Zuckerman T, et al. Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients. Blood. 2003;101:463–5.
7. Crippa F, Corey L, Chuang EL, Sale G, Boeckh M. Virological, clinical, and ophthalmologic features of cytomegalovirus retinitis after hematopoietic stem cell transplantation. Clin Infect Dis. 2001;32:214–9.
8. Kanda Y, Mineishi S, Saito T, et al. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. Bone Marrow Transplant 2001;27:437–44.
9. Niiya H, Kanda Y, Saito T, et al. Early full donor myeloid chimerism after reduced-intensity stem cell transplantation using a combination of fludarabine and busulfan. Hematologica 2001;86:1071–4.
10. Kanda Y, Komatsu Y, Akahane M, et al. Graft-versus-tumor effect against advanced pancreatic cancer after allogeneic reduced-intensity stem cell transplantation. Transplantation. 2005;79:821–7.
11. Kanda Y, Oshima K, Asano-Mori Y, et al. In vivo alemtuzumab enables haploidentical HLA-mismatched hematopoietic stem cell transplantation without ex vivo graft manipulation. Transplantation. 2005;79:1351–7.
12. Kurihara T, Hayashi J, Matsuoka T, Ito A. HCMV pp65 antigenemia assay using indirect alkaline phosphatase staining method. Bio Med Res. 1995;16:125–9.
13. Asano-Mori Y, Oshima K, Sakata-Yanagimoto M, et al. High-grade cytomegalovirus antigenemia after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2005;36:813–9.
14. Kanda Y, Mineishi S, Saito T, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: a prospective evaluation. Transplantation. 2002;73:568–72.
15. Asano-Mori Y, Kanda Y, Oshima K, et al. Pharmacokinetics of ganciclovir in haematopoietic stem cell transplantation recipients with or without renal impairment. J Antimicrob Chemother. 2006;57:1004–7.

16. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695–706.
17. Chakrabarti S, Mackinnon S, Chopra R, et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of alemtuzumab in delaying immune reconstitution. Blood. 2002;99:4357–63.
18. Hakki M, Riddell SR, Storek J, et al. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. Blood. 2003;102:3060–7.

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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.
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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,

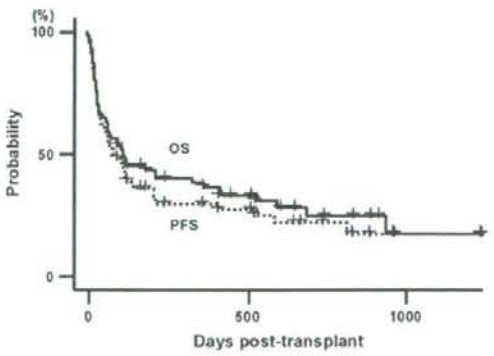

Figure 1. OS and PFS estimates for 70 patients with hematologic diseases treated with RI-UCBT.

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.

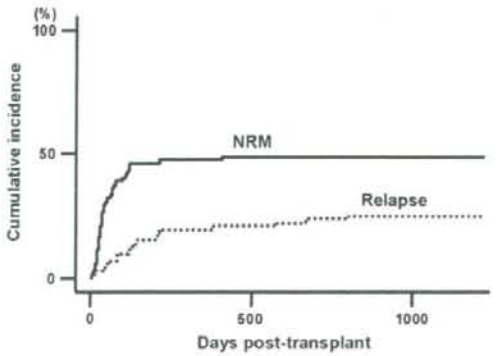

Figure 2. NRM and disease progression. Cumulative incidence estimates of NRM and disease progression for all 70 patients.

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].

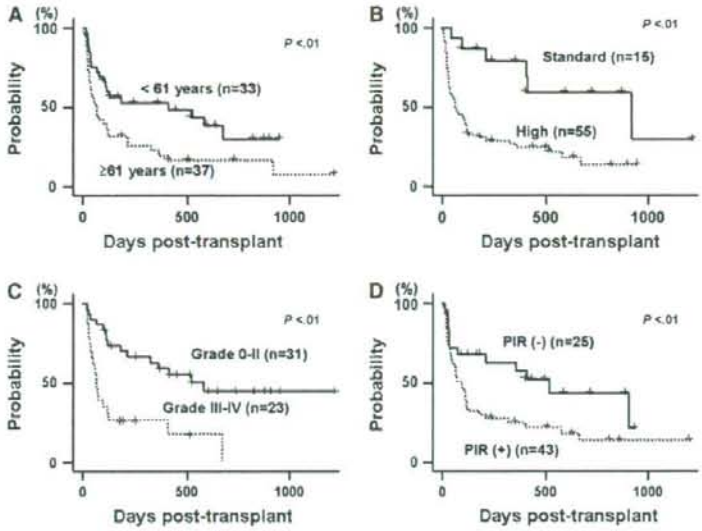

Figure 3. OS estimates after RI-UCBT (n = 70). (A) Effect of age. (B) Effect of disease status. (C) Effect of severity of aGVHD. (D) Effect of PIR.

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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REFERENCES

1. Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107:2099-2107.
2. Ringden O, Horowitz MM, Gale RP, et al. Outcome after allogeneic bone marrow transplant for leukemia in older adults. *JAMA*. 1993;270:57-60.
3. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*. 2001;97:631-637.
4. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. 2001;97:3390-3400.
5. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*. 1998;91:756-763.
6. Brunstein CG, Wagner JE. Cord blood transplantation for adults. *Vox Sang*. 2006;91:195-205.
7. Schoemans H, Theunissen K, Maertens J, Boogaerts M, Verfaillie C, Wagner J. Adult umbilical cord blood transplantation: a comprehensive review. *Bone Marrow Transplant*. 2006;38:83-93.
8. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med*. 2004;351:2265-2275.
9. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276-2285.
10. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation of adult patients with hematologic malignancies. *Blood*. 2004;104:3813-3820.
11. Barker JN, Weisdorf DJ, DeFor TE, et al. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood*. 2003;102:1915-1919.
12. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res*. 2004;10:3586-3592.
13. Koh LP, Chao NJ. Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol Blood Marrow Transplant*. 2004;10:1-22.
14. Komatsu T, Narimatsu H, Yoshimi A, et al. Successful engraftment of mismatched unrelated cord blood transplantation following reduced intensity preparative regimen using fludarabine and busulfan. *Ann Hematol*. 2006;86:49-54.
15. Ballen KK, Spitzer TR, Yeap BY, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol Blood Marrow Transplant*. 2007;13:82-89.
16. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplant outcomes in 110 adults with hematological disease. *Blood*. 2007;110:3064-3070.
17. Narimatsu H, Matsumura T, Kami M, et al. Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients. *Biol Blood Marrow Transplant*. 2005;11:429-436.
18. Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation*. 2005;80:34-40.
19. Narimatsu H, Terakura S, Matsuo K, et al. Short-term methotrexate could reduce early immune reactions and improve outcomes in umbilical cord blood transplantation for adults. *Bone Marrow Transplant*. 2007;39:31-39.
20. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity intex: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912-2919.
21. Sullivan KM. Graft-versus-host-disease. In: Thomas ED, Blume KG, Forman SJ, editors. *Hematopoietic Cell Transplantation*, 4th ed. Boston, MA: Blackwell Science; 1999. p. 515-536.

22. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis.* 2002;34:7-14.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
24. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999;18:695-706.
25. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Statist.* 1988;16:1141-1154.
26. Rocha V, Rio B, Brunstein C, et al. Unrelated cord blood transplantation after reduced intensity conditioning (RIC) in adults with hematological malignancy. An EBMT-Eurocord-Netcord, Société Française de Greffe de Moelle et de Therapie Cellulaire and University of Minnesota Collaborative study. *Blood.* 2007;110:603a.
27. Takami A, Takamatsu H, Yamazaki H, et al. Reduced-intensity unrelated cord blood transplantation for treatment of metastatic renal cell carcinoma: first evidence of cord-blood-versus-solid-tumor effect. *Bone Marrow Transplant.* 2006;38:729-732.
28. Howrey RP, Martin PL, Driscoll T, et al. Graft-versus-leukemia-induced complete remission following unrelated umbilical cord blood transplantation for acute leukemia. *Bone Marrow Transplant.* 2000;26:1251-1254.
29. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood.* 2001;97:2962-2971.
30. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood.* 2002;100:1611-1617.
31. Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood.* 2001;98:2332-2338.
32. Long GD, Laughlin M, Madan B, et al. Unrelated umbilical cord blood transplantation in adult patients. *Biol Blood Marrow Transplant.* 2003;9:772-780.
33. Cornetta K, Laughlin M, Carter S, et al. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). *Biol Blood Marrow Transplantation.* 2005;11:149-160.
34. Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med.* 1985;313:765-771.
35. Beatty PG, Hansen JA, Longton GM, et al. Marrow transplantation from HLA-matched unrelated donors for treatment of hematologic malignancies. *Transplantation.* 1991;51:443-447.
36. Kanda Y, Chiba S, Hirai H, et al. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991-2000). *Blood.* 2003;102:1541-1547.
37. Anasetti C, Beatty PB, Storb R, et al. Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol.* 1990;29:79-91.
38. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. *Semin Hematol.* 2006;43:3-10.
39. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med.* 2001;344:1815-1822.
40. Ballen KK. New trends in umbilical cord blood transplantation. *Blood.* 2005;105:3786-3792.
41. Sullivan KM, Deeg HJ, Sanders J, et al. Hyperacute graft-v-host disease in patients not given immunosuppression after allogeneic marrow transplantation. *Blood.* 1986;67:1172-1175.
42. Saliba RM, de Lima M, Giralt S, et al. Hyperacute GVHD: risk factors, ontcomes, and clinical implications. *Blood.* 2007;109:2751-2758.
43. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 2001;28:181-185.
44. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (Prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood.* 1998;92:2303-2314.
45. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood.* 2000;96:2062-2068.
46. Osunkwo I, Bessmertny O, Harrison L, et al. A pilot study of tacrolimus and mycophenolate mofetil graft-versus-host disease prophylaxis in childhood and adolescent allogeneic stem cell transplant recipients. *Biol Blood Marrow Transplant.* 2004;10:246-258.
47. Cutler C, Li S, Ho VT, et al. Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation. *Blood.* 2007;109:3108-3114.