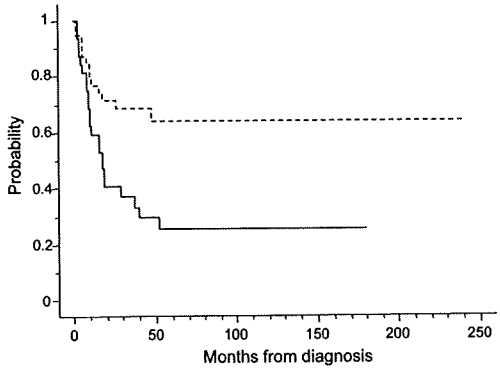

Figure 1. Kaplan-Meier estimate of overall survival in all patients according to *PTPN11* mutation status. *PTPN11*-mutation group (n = 32): *solid lines* *PTPN11* wild-type group (n = 39): *broken line*. The survival of *PTPN11*-mutation group was significantly inferior to survival of *PTPN11* wild-type group: 25% (95% CI: 17–33) *vs* 64% (95% CI: 56–72); p = 0.0029.

Table 4. *Probability of 5-y overall survival (OS) in 71 patients with JMML*

Variable	No. of patients	Probability (%)	95% CI	p
Mutational status				
PTPN11 mutation	32	25	17–33	0.0029
PTPN11 wild type	39	64	56–72	
RAS mutation	13	61	45–78	
No aberration	23	65	55–75	
Age at diagnosis				
Older than 24 mo	35	33	25–42	0.0030
24 mo or younger	36	58	48–67	
Cytogenetics				
Abnormal karyotype	16	22	11–34	0.0125
Normal karyotype	55	53	46–61	
Platelets count				
Less than 40×10^9/L	34	43	34–52	NS
40×10^9/L or more	37	49	40–58	
HbF level				
More than 10%	47	41	33–49	NS
10% or less	24	57	45–69	
Gender				
Male	43	43	35–51	NS
Female	28	49	38–60	

NS, not significant.

values for the *RAS* mutation group and the no aberration group were 61 and 65%, respectively. The three patients with NF1 all received HSCT. One patient died because of transplantation-related toxicity and the others survived without the disease. The prognostic significance of the initial clinical and laboratory parameters, together with mutational status, is shown in Table 4. In the univariate analysis, age greater than 24 mo (p = 0.0030) and presence of cytogenetic abnormality (p = 0.0125) were associated with poor prognosis, as was the presence of *PTPN11* mutation. Of particular interest cytogenetically is the fact that patients with monosomy 7 had a comparable outcome to that of children with a normal karyotype. However, all seven patients with an abnormal karyotype other than monosomy 7 died, and all had a *PTPN11* mutation. Multivariate analysis showed that none of the variables influenced survival (Table 6).

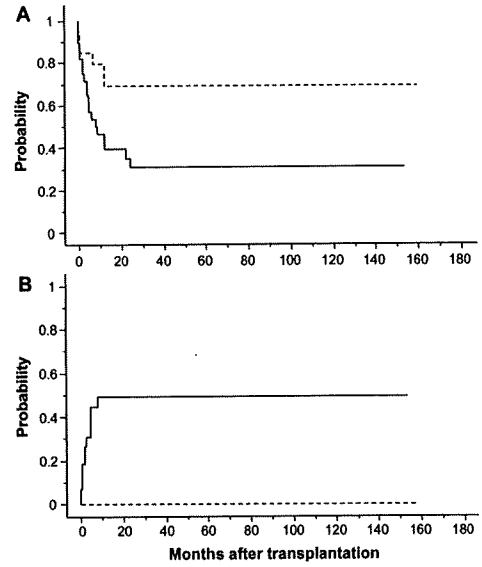

Figure 2. Kaplan-Meier estimate of overall survival and probability of relapse after HSCT in 48 patients according to *PTPN11* mutation status. *PTPN11*-mutation group (n = 28): *solid lines*. *PTPN11* wild-type group (n = 20): *broken lines*. (*A*) Overall survival. *PTPN11*-mutation group had significantly lower survival than *PTPN11* wild-type group: 30% (95% CI: 21–39) *vs* 69% (95% CI: 59–80); p = 0.018. (*B*) Relapse incidence. Whereas no relapse was observed in *PTPN11* wild-type group, the relapse incidence of *PTPN11*-mutation group was 49% (95% CI: 39–60); p = 0.001.

We then analyzed the prognostic value of *PTPN11* mutations in the 48 of 71 patients who received HSCT. *PTPN11* mutations were found in 28 of 48 (58%) patients, and of the 48 patients, 25 patients died after HSCT. As shown in Figure 2*A*, patients with a *PTPN11* mutation had significantly lower survival than patients without also in this cohort. (30 *versus* 69%; p = 0.018). We found that the presence of a *PTPN11* mutation was the most significantly associated factor with OS after HSCT, and followed by age greater than 24 mo and presence of cytogenetic abnormality (Fig. 3*A* and Table 5). No variables significantly associated with inferior survival after HSCT in a multivariate model (Table 6). In addition, we compared the probability of relapse after HSCT between patients with and without *PTPN11* mutations and found that the patients with a *PTPN11* mutation had significantly higher risk for relapse (p = 0.001) (Fig. 2*B*). No other variables including older age and cytogenetic abnormality arose statistically significant difference with the probability of relapse after HSCT (Fig. 3B and Table 5). Twelve patients died of relapse after transplantation and 13 died of transplantation-related toxicity. Notably, all 12 patients who died after relapse had a *PTPN11* mutation.

All four patients with a *PTPN11* mutation who did not receive HSCT died (at 3, 4, 19, and 29 mo after diagnosis), whereas 12 of 19 patients without a *PTPN11* mutation who did not receive HSCT remain alive, with a median follow-up of 80 mo (range, 21–240 mo) from diagnosis.

DISCUSSION

Since the discovery of *PTPN11* mutations in JMML (9), biomedical and molecular research on this disease has pro-

gressed rapidly, and data on molecular aberrations are now of great importance in the diagnosis of JMML. In the current study, we confirmed that *PTPN11* mutations are the most frequent molecular aberrations (45%) in Japanese children with JMML. If a *PTPN11* mutation is present, it is important to rule out the possibility of NS, especially in infants, because the JMML-like disorder in these patients may spontaneously disappear without therapy, so it is considered distinct from common JMML (31). All mutations detected in our cohort were located in exons 3 and 13 of the *PTPN11* gene, which accords with previous findings that mutations associated with JMML exist only in these two exons (9,16). In contrast, mutations in NS are located in a much broader range of locations, in exons 2, 3, 4, 7, 8, and 13 (29). Kratz *et al.* (32) clearly demonstrated that a different spectrum of *PTPN11* mutations between JMML and JMML-like disorder with NS. According to Kratz *et al.*, all mutations in the present study were those associated with JMML, not a JMML-like disorder. These findings suggest that our study population included only patients with common JMML and that the observed mutations are somatic changes.

The prevalence of *PTPN11* mutations in our cohort was slightly higher than that reported previously (9–11), and the prevalence of *RAS* mutations was comparable with that found in previous studies (2,6–8). In other studies, the proportion of patients with clinically diagnosed NF1 has been found to be 9 and 14% (1,33), but in our cohort, the proportion was smaller, only 3 of 71 (4%) patients. A similar NF1 prevalence (4 of 83 patients; 5%) was observed in an ongoing prospective study conducted by the MDS Committee of the Japanese Society of Pediatric Hematology, so NF1 might be less prevalent in the Japanese population. Another possibility is that NF1 was under diagnosed because of the paucity of signs and symp-

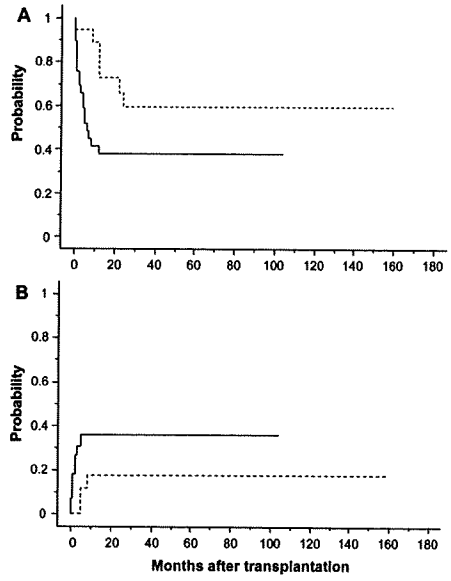

Figure 3. Kaplan-Meier estimate of overall survival and probability of relapse after HSCT in 48 patients according to age at diagnosis. Age >24 mo (n = 29): *solid lines*. Age ≦24 mo (n = 19): *broken lines*. (*A*) Overall survival. Age ≦24 mo: 59% (95% CI: 47–71) *vs* Age >24 mo: 38% (95% CI: 29–47); p = 0.033. (*B*) Relapse incidence. Age ≦24 mo: 19% (95% CI: 9–29) *vs* Age >24 mo: 35% (95% CI: 26–45); p = NS.

Table 6. *Multivariate analysis of survival for all 71 patients and 48 patients who received HSCT*

	Relative risk	95% CI	p
All patients (n = 71)			
PTPN11 mutation	1.854	0.852–5.139	NS
Older than 24 mo	2.011	0.925–4.790	NS
Abnormal karyotype	1.793	0.800–5.401	NS
Patients received HSCT (n = 48)			
PTPN11 mutation	2.226	0.852–5.814	NS
Older than 24 mo	1.707	0.742–3.925	NS
Abnormal karyotype	1.863	0.764–4.542	NS

NS, not significant.

Table 5. *Univariate analysis of 5-y overall survival (OS) and relapse incidence (RI) after HSCT in 48 patients with JMML*

		OS			RI		
Variable	No. of patients	Probability (%)	95% CI	p	Probability (%)	95% CI	p
Mutational status							
PTPN11 mutation	28	30	21–39	0.0181	49	39–60	0.0012
PTPN11 wild type	20	69	59–80		0	0–0	
RAS mutation	6	63	41–83		0	0–0	
No aberration	11	72	59–86		0	0–0	
Age at diagnosis							
Older than 24 mo	29	38	29–47	0.0331	35	26–45	NS
24 mo or younger	19	59	47–71		19	9–29	
Cytogenetics							
Abnormal karyotype	14	17	4–30	0.0474	48	32–64	NS
Normal karyotype	34	56	47–64		23	15–30	
Platelets count							
Less than 40×10^9/L	27	50	41–60	NS	22	13–30	NS
40×10^9/L or more	21	40	29–51		41	29–53	
HbF level							
More than 10%	37	45	37–54	NS	33	24–42	NS
10% or less	11	56	40–72		26	10–42	
Gender							
Male	29	40	30–49	NS	33	23–42	NS
Female	19	56	44–68		24	13–34	

NS, not significant.

toms in young children. Niemeyer *et al.* (1) found that patients with NF1 were more likely to have higher platelet counts and normal karyotypes. In our patients with NF1, no clinical parameters except for age seemed to differ from the other groups, although the number of patients was too small to draw any conclusions. The outcome of patients with NF1 remains unclear; therefore, further accumulations of prognostic data in this condition are needed.

Our analysis showed a striking correlation between mutational status and clinical and laboratory findings of known prognostic factors. Compared with the *RAS* mutation group and the no aberration group, age and HbF level at diagnosis were significantly higher in the *PTPN11* mutation group. Given that in previous reports older age at diagnosis and elevated HbF level have been repeatedly described as risk factors for survival (1,19–21,23–26), these results suggest that JMML with *PTPN11* mutation is a distinct subgroup and that the outcome for patients with this condition might be poorer. In this study, both *PTPN11* mutation and age were the strongest predictors of the probability of survival in univariate analyses. The poor survival of the *PTPN11* mutation group was also observed when only the patients who had been treated with HSCT were included in the analysis. Because multivariate analysis did not discriminate between age and *PTPN11* mutation, it remains unclear whether mutation in *PTPN11* is an independent predictor for poor survival. However, this could possibly be ascribed to the strong relationship between the *PTPN11* mutation group and older age. Poor outcome in patients with a *PTPN11* mutation may be due to the presence of several unfavorable factors, suggesting that previously recognized prognostic factors might reflect the genetic status.

Presently, HSCT is the only curative treatment for JMML; however, disease recurrence remains the major cause of treatment failure. Notably, mutation in *PTPN11* was the only risk factor for relapse after HSCT in our study. Previously published studies have found that older age, elevated HbF level, and abnormal karyotype are patient-specific risk factors for relapse after HSCT (20,21). The finding that our patients with a *PTPN11* mutation had an association with all these factors and our results on risk factors for relapse also support the idea that the genetic status may be an explanation of previous prognostic factors.

In our study, all 12 patients who relapsed after HSCT had a *PTPN11* mutation, suggesting that patients with *PTPN11* mutation may experience an aggressive clinical course. In addition, patients with *PTPN11* mutation were more likely to receive HSCT, also suggesting that there was a bias attributable to the aggressive clinical course in these patients. Indeed, all patients in the *PTPN11* mutation group who did not receive HSCT died, whereas five of seven patients in the *RAS* mutation group and seven of 12 patients in the no aberration group were alive without HSCT. Moreover, all patients with an abnormal karyotype other than monosomy 7 had a *PTPN11* mutation, and all died, suggesting that clones with a *PTPN11* mutation might be more likely to acquire additional chromosomal alterations.

To the best of our knowledge, this is the first report to investigate the prognostic relevance of the GM-CSF signaling pathway-related genes in patients with JMML and demonstrate the correlation between mutational status and recognized prognostic factors. The finding that mutations in *PTPN11* or *RAS* and a clinical diagnosis of NF1 were mutually exclusive is consistent with the idea that these molecules act in the same pathway. Nonetheless, the clinical features were quite different in these groups. This difference might be caused by distinct gain-of-function effects of each gene on the GM-CSF pathway and unknown additional genetic alterations may cooperate with these mutations. Furthermore, considering the present and previous findings together, the previously recognized prognostic factors might reflect the genetic status of this pathway. Further biologic studies are necessary to clarify what kind of genetic alterations cooperate with altered GM-CSF pathway-related genes during the development of JMML.

In conclusion, JMML with mutation in *PTPN11* seems to be a distinct subgroup with specific clinical characteristics and poor outcome. Consideration should be given to early HSCT therapy in this group of patients and better strategies to lower the risk of relapse in these patients are warranted.

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ORIGINAL ARTICLE

Outcome of bone marrow transplantation from HLA-identical sibling donor in children with hematological malignancies using methotrexate alone as prophylaxis for graft-versus-host disease

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Abstract Most previous studies of graft-versus-host disease (GVHD) prophylaxis with methotrexate (MTX) alone in patients undergoing HLA-identical sibling donor bone marrow transplantation were performed in adults. With this background, we attempted to analyze the incidence and risk factors of GVHD in bone marrow transplantation (BMT) from an HLA-identical sibling donor in children with hematological malignancies using MTX alone as a prophylaxis for GVHD. Ninety-four patients received MTX by intravenous bolus injection, with a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60. The probability of developing grade II–IV acute GVHD and chronic GVHD was 19.1 and 31.8%, respectively. Age at transplantation and a female donor to male recipient were identified as risk factors for chronic GVHD in multivariate analysis, but no factors were identified for acute GVHD. The cumulative incidence of transplant-related mortality during the first 100 days was 9.6%. Disease-free survival at 5 years for standard- and high-risk patients was 82.1 and 39.5%, respectively. These results suggest that GVHD prophylaxis with MTX alone is safe and effective in young children under 10 years old at transplantation and in a setting other than female donor to male recipient.

Keywords HLA-identical sibling donor · GVHD prophylaxis · Methotrexate alone

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1 Introduction

Allogeneic bone marrow transplantation (BMT) is an effective treatment for patients with hematologic malignancies, bone marrow failure syndromes, and congenital disorders of the lymphohematopoietic system. The transplant outcome depends on the severity of complications such as graft failure, infection, graft-versus-host disease (GVHD), organ damage, and the disease stage GVHD is a major complication of allogeneic BMT that results in significant morbidity and mortality. It occurs, despite prophylaxis, in 30–50% of patients undergoing transplantation from HLA-identical sibling donors [1] and in 50–80% of patients with transplants from HLA-matched unrelated donors [2]. Previous studies have shown that the combination of cyclosporine-A (CyA) and four doses of methotrexate (MTX) is more effective than either agent alone in the prevention of GVHD [1]. Thus, a regimen including CyA or FK506 plus short-term MTX (sMTX) was established in adults, even for unrelated donors [3, 4]. Although several investigators have reported data from multicenter randomized clinical trials to evaluate the effectiveness of GVHD prophylaxis regimens in adults,

few data are available for pediatric patients, who usually show a lower incidence and less severe GVHD than adult patients. Ringden et al. [1] reported that the probability of developing acute GVHD did not differ between single or combined prophylaxis regimens in a pediatric population, and Locatelli et al. [5] reported that the incidence of GVHD in childhood was low compared to that in adults. Furthermore, Bacigalupo et al. [6] demonstrated in a randomized trial involving adults that GVHD prophylaxis with low-dose CyA (1 mg/kg per day) decreases the risk of relapse more than a higher dose (5 mg/kg per day), possibly because of a graft-versus-leukemia (GVL) effect. However, there is still a lack of data on pediatric patients, who usually show a different incidence and severity of GVHD than adult patients. Locatelli et al. confirmed that the use of low-dose CyA (1 mg/kg per day) led to a more favorable survival rate than regular-dose CyA (3 mg/kg per day) as a single prophylactic agent in pediatric patients [7]. However, in their report, almost all patients showed standard features, including acute leukemia in first or second complete remission (CR).

Herein, we report the effectiveness of MTX as a single agent for GVHD prophylaxis in 94 pediatric patients with hematological malignancies who underwent BMT from HLA-identical sibling donors including high-risk features. We also retrospectively analyzed the risk factors and incidence of GVHD.

2 Patients and methods

2.1 Patient characteristics

Ninety-four patients, aged 1–15 (median: 8 years old) received transplantations from HLA-identical sibling donors at the Japanese Red Cross Nagoya First Hospital between 1984 and 2000. The clinical characteristics of the patients are shown in Table 1.

All patients received MTX alone for GVHD prophylaxis. Patients were classified as having standard- or high-risk disease based on previously described criteria [8, 9]. Briefly, patients were categorized as standard-risk cases if they had acute lymphoblastic leukemia (ALL) in first or second complete remission (CR), acute myelogeneous leukemia (AML) in first CR, chronic myelogenous leukemia (CML) in the first chronic phase (CP), or malignant lymphoma in first CR. The other 38 patients, including those who received a second transplantation (five cases), were categorized as high-risk cases. Chromosomal abnormalities classified as standard risk included ALL with translocations of 9;22 (three cases) and 11q23 (three cases), as well as AML with translocations 8;21 (six cases) and 15;17 (two cases). ALL patients with 9;22 (four cases) and 11q23 (two

Table 1 Patient and donor characteristics

Patients	$n = 94$		%
Sex	Female	43	45.7
	Male	51	54.3
Age, median (range)		8 (1–15)	
	<10	56	59.6
	≥10	38	40.4
Disease	ALL	42	44.7
	CR1–2	27	
	CR3–5	3	
	Relapse	12	
	AML	35	37.2
	CR1	23	
	CR2	5	
	Relapse	7	
	AUL	4	4.3
	CR1	2	
	Relapse	2	
	CML	3	3.2
	CP1	2	
	BP	1	
	ML	5	5.3
	CR1	3	
	Relapse	2	
	MDS	5	5.3
Risk[a]	Standard risk	56	59.6
	High risk	38	40.4
Time at SCT	First	89	94.5
	Second	5	5.5
Conditioning	TBI	30	32
	Non-TBI	64	68
Post-BMT growth factor	None	43	45.7
	G-CSF	51	54.3
Donors	Age	9 (1–21)	
Donor sex	Female	45	47.9
	Male	49	52.1
Donor/patient sex	F to F	24	25.5
	F to M	21	22.3
	M to F	19	20.2
	M to M	30	31.9
ABO blood group	Compatible	63	67
	Minor mismatch	10	10.6
	Major mismatch	12	12.8
	Major and minor mismatch	9	9.6

ALL acute lymphoblastic leukemia, *CR* complete remission, *AML* acute myelogeneous leukemia, *AUL* acute unclassified leukemia, *CML* chronic myelocytic leukemia, *CP* chronic phase, *BP* blastic phase, *ML* malignant lymphoma, *MDS* myelodysplastic syndrome, *SCT* stem cell transplantation, *TBI* total body irradiation*standard risk; ALL CR1 or −2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

[a] Standard risk; ALL CR1 or −2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

cases) were included as high-risk patients because they received BMT at relapse. As of December 2005, the median follow-up duration was 161 (66–249) months. HLA typing of the donors and recipients was performed by serology. Previous chemotherapy regimens varied because the patients were treated at their referring institutions.

2.2 Pretransplant preparative regimens

The conditioning regimens are described in Table 1. Thirty-two patients received a preparative regimen consisting of busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m^2), and 32 patients received busulfan (4 mg/kg per day × 2 days) in addition to LPAM + TBI (12–13.2 Gy). Thirty patients received other TBI-based regimens, such as cytarabine (CA) (4–6 g/m^2 per day × 2 days)/cyclophosphamide (CY) (60 mg/kg per day × 2 days)/TBI, CY/TBI, thiotepa (TEPA) (800 mg/m^2)/TBI, TEPA/CY/TBI, LPAM/TBI, and VP-16 (60 mg/kg per day)/LPAM/TBI.

2.3 Prophylaxis and treatment of GVHD

All patients received MTX alone as GVHD prophylaxis. MTX was scheduled to be given intravenously as a bolus injection at a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60, shorter than the Seattle protocol [10]. Folinic acid was given at 3 mg orally in divided doses on the next day of MTX injection to prevent mucositis caused by MTX. When patients developed acute GVHD of grade II or more, and extensive-type chronic GVHD, steroid therapy was started. If patients showed no improvement, CyA was added, according to the physician's assessment.

Acute GVHD was evaluated on an individual basis according to the standard criteria by Glucksberg [10]. Chronic GVHD was assessed as either limited or extensive, based on clinical and/or histological findings, as described by Glucksberg and Shulman, respectively [10, 11]. Mucositis and liver dysfunction were graded using the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Interstitial pneumonia was diagnosed based on the clinical condition and computed tomography. If patients developed mucosal toxicity, liver/renal dysfunction, and interstitial pneumonia, the dose of MTX was withheld, at the physician's discretion.

2.4 Engraftment

Engraftment of neutrophils and platelets was defined as the first of three consecutive days with an absolute neutrophil count (ANC) >0.5 × 10^9/l and unsupported platelet count >50 × 10^9/l.

2.5 Statistical analysis

Acute and chronic GVHD, overall survival, disease-free survival (DFS), rate of relapse of malignant diseases, and transplant-related mortality (TRM) were assessed using the cumulative incidence and Kaplan–Meier product limit estimates. Significance between patient populations was tested using the log-rank test. In DFS analysis, both relapse and death in remission due to any cause were considered events, whereas, in relapse rate analysis, only disease relapse was considered as failure. In TRM analysis, all deaths not due to disease relapse were considered events. Risk factors of acute and chronic GVHD were analyzed using Cox proportional hazard analysis. Children showing sustained donor engraftment and surviving for more than 21 days and more than 100 days after the transplant were assessable for the occurrence and severity of acute and chronic GVHD, respectively. Factors that appeared to be predictive of developing grade II–IV acute GVHD and chronic GVHD in univariate analysis ($P < 0.10$) were considered for inclusion in multivariate Cox regression models. The likelihood ratio test was used to determine whether variables should be added or dropped from the multivariate model. The STATA package (STATACORP LP, College Station, TX, USA) was used for data analysis.

3 Results

3.1 Engraftment

The median amount of infused marrow-nucleated cell dose was 4.0 × 10^8/kg (range: 0.98–7.2 × 10^8/kg), and 92 patients (98%) showed neutrophil engraftment at a median of 17 days (range: 10–40), and 67 patients (71%) exhibited platelet engraftment at a median of 35 days. In patients receiving granulocyte colony-stimulating factor (G-CSF) after BMT, neutrophil engraftment was confirmed at 15 days, and that without G-CSF was confirmed at 20 days ($P < 0.01$). Three patients died before neutrophil engraftment of hepatic veno-occlusive disease (VOD) or invasive fungal infection with bacterial pneumonia, and 28 patients died prior to platelet engraftment.

3.2 Acute GVHD

In 91 evaluable patients, 30 (33%) developed grade I–IV acute GVHD. The cumulative incidence of grades II–IV and III–IV acute GVHD was 19.8 and 11%, respectively (Fig. 1). For 18 patients who developed acute GVHD (grade ≥II), MTX was replaced with prednisolone for the treatment of acute GVHD. CyA was added in 11 patients to treat GVHD, and ATG (anti-thymocyte globulin) was

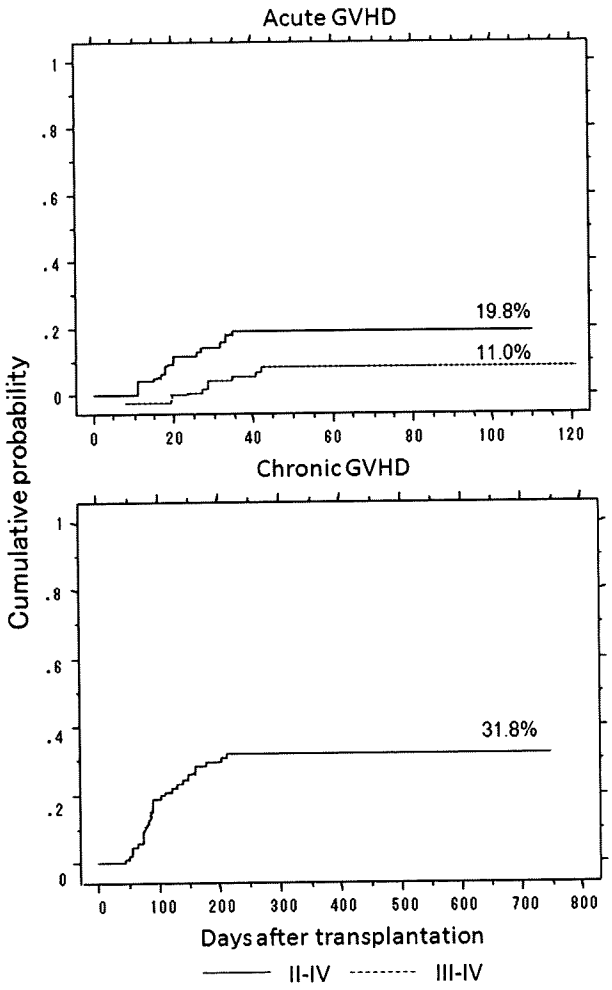

Fig. 1 Cumulative incidence of acute and chronic GVHD. *Upper* and *lower panels* show the cumulative incidence of acute and chronic GVHD, respectively

Table 2 Univariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	*P* value
Sex			
Male	1.00		
Female	1.12	0.53–2.38	0.77
Patient age (years)			
<10	1.00		
≥10	2.45	1.13–5.24	0.02
Risk			
Standard risk	1.00		
High risk	1.71	0.80–3.66	0.17
Conditioning			
TBI +	1.00		
TBI −	0.94	0.43–2.05	0.88
Busulfan +	1.00		
Busulfan −	0.86	0.38–1.97	0.73
Donors age			
<10	1.00		
≥10	1.14	0.54–2.43	0.73
Donor sex			
Male	1.00		0.26
Female	1.55	0.73–3.32	0.02
Donor/patient sex			
M to M	1.00		
F to F	1.80	0.48–6.70	0.38
M to F	2.99	0.90–9.93	0.07
F to M	3.87	1.21–12.34	0.02
ABO blood group			
Compatible	1.00		
Mismatch	1.12	0.51–2.45	0.77

RR indicates relative risk, *CI* confidence interval

given to four patients. Although the data are not shown, no risk factors for the development of grade II–IV acute GVHD were identified in univariate analysis.

3.3 Chronic GVHD

Although GVHD at 100 days or later after transplantation is defined as chronic GVHD by the classical criteria [10], typical clinical and histological features of chronic GVHD could occur as early as 40 days post-transplantation. In our study, 27 of 85 assessable patients (31.8%) developed limited (seven patients) or extensive (20 patients) chronic GVHD (Fig. 1), and ten of 27 patients with cGVHD stopped receiving MTX. Sixteen of 27 patients developed cGVHD before 100 days after transplantation, including 11 patients diagnosed by histological examination and five patients with diagnostic signs based on the National Institutes of Health (NIH) consensus criteria [12]. On univariate analysis, an older patient age (>10 years old) and female donor to male recipient were significantly associated with the risk of developing chronic GVHD (Table 2). Even in multivariate analysis, these two factors were identified as significant risk factors for chronic GVHD, and female donor to male recipient was the most significant predictive factor in different pairs of sex combinations (Table 3).

3.4 Compliance and toxicity of MTX administration

Twenty-three patients stopped receiving MTX by day +60, with a median of day +25 (range: 1–46), and a median of six doses (range: 1–9). The reasons for MTX discontinuation were the treatment of acute GVHD (nine patients), liver dysfunction (ten patients, including six patients with VOD and four patients with abnormal liver function test (grade 3 NCI-CTC)), two with respiratory failure, and two early deaths with severe infection. No patients stopped

Table 3 Multivariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	*P* value
Patient age (years)			<0.001
<10	1.00		
≥10	3.09	1.40–6.84	
Donor/patient sex			<0.001
M to M	1.00		
F to F	1.55	0.42–5.80	0.51
M to F	3.32	0.99–11.08	0.05
F to M	4.80	1.48–15.57	<0.001

receiving MTX because of grade IV mucositis of NCI-CTC. For these patients who stopped receiving MTX before day +60, prednisolone was started. The risk factors for MTX discontinuation were acute GVHD (≥grade 2) and second stem cell transplantation (SCT) (data not shown). Eighteen of 23 patients who stopped receiving MTX survived for more than 100 days after transplantation, and ten of 18 patients developed chronic GVHD. Thirteen patients (14.8%) developed interstitial pneumonia, and five of 13 patients died of respiratory failure (two cases) or other reasons (three cases).

3.5 Relapse and survival

The relapse rate for all patients was 22%, with a median of 5.73 months (range: 0.87–137). The relapse rates of standard-risk (SR) and high-risk (HR) patients were 11.6 and 36.8%, respectively, which were significant ($P = 0.002$) (Fig. 2).

The rate of transplant-related mortality (TRM) was 7.1 and 27.5% in SR and HR patients, respectively ($P = 0.01$).

Causes of death are listed in Table 4. Relapse was the most frequent cause of death. After relapse, respiratory failure (e.g., interstitial pneumonia, bronchiolitis obliterans) was the major cause of death. The probability of transplant-related mortality was 14.4% for all patients, and that of early (<100 days) TRM was 9.6%. The risk of transplant-related mortality was significantly greater in HR patients (TRM: 27.5%, early TRM: 18.4%) compared to SR patients (all TRM: 7.1%, early TRM: 3.6%) ($P = 0.01$). Disease-free survival (DFS) for all patients was 64.9% at 5 years, and was significantly higher in SR (82.1%) compared to HR (39.5%) patients ($P = 0.001$) (Fig. 2).

Stratifying the risk of disease, we analyzed the GVL effect with or without cGVHD. In fact, the relapse rate for SR patients with cGVHD was 6.7% compared with the 14.1% observed in patients without cGVHD ($P = 0.52$). For HR patients, the development of cGVHD was associated with a lower relapse rate (25%) than that of patients without cGVHD (47.4%), even though this was not significant ($P = 0.15$). In the same way, no significant

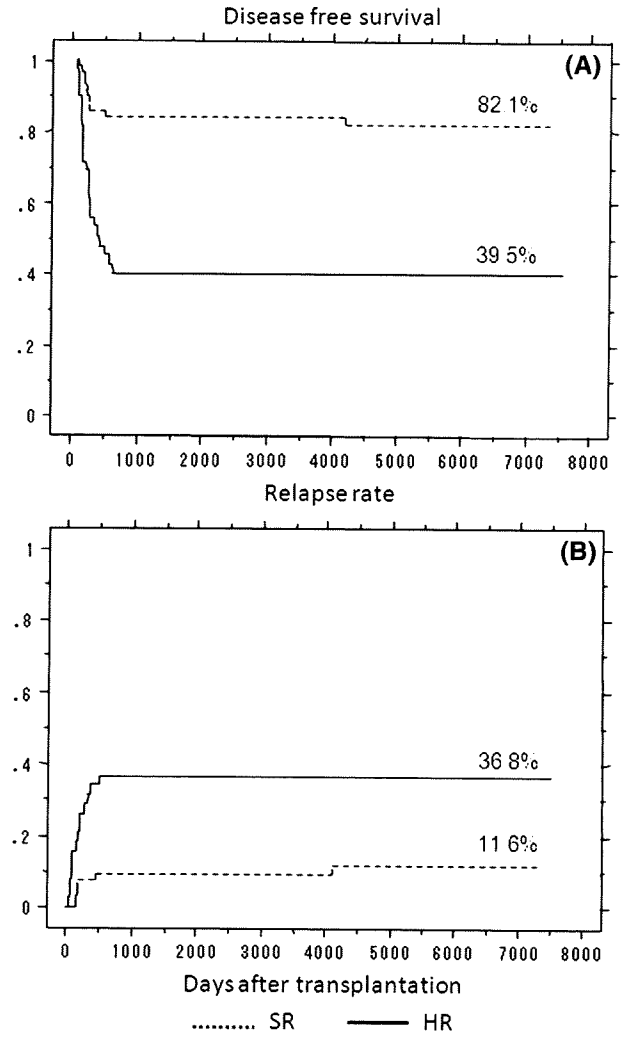

Fig. 2 **a** Disease-free survival. **b** Cumulative incidence of relapse. Standard-risk (*SR*) patients (*discontinuous line*), high-risk (*HR*) patients (*continuous line*)

Table 4 Cause of death ($n = 32$)

Cause	
Relapse	18
Rejection	1
Interstitial pneumonitis	2
Obstructive bronchiolitis	4
Infection	1
Acute GVHD	1
Veno occlusive disease	3
CNS toxicity	2

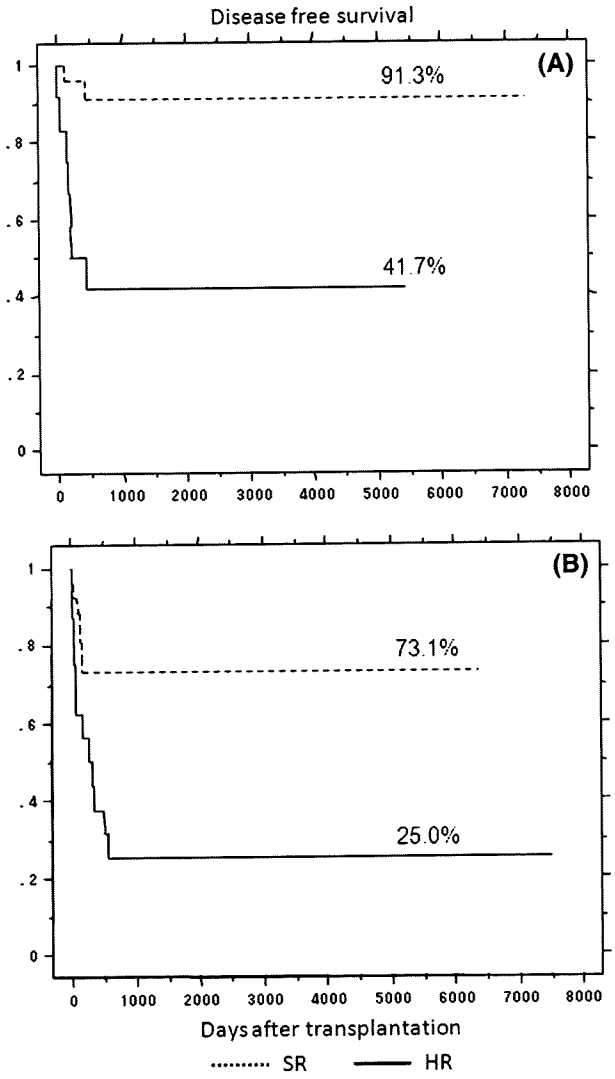

Fig. 3 Disease-free survival in patients with (**a**) acute myelogenous leukemia (*AML*) and (**b**) acute lymphoblastic leukemia (*ALL*). Standard-risk (*SR*) patients (*discontinuous line*), high-risk (HR) patients (*continuous line*)

difference was observed for DFS between patients with or without cGVHD (64.2 vs. 66.7%, respectively, P = NS). Meanwhile, stratifying the type of disease, DFS in AML patients was 91.3% in SR and 41.7% in HR patients, and the relapse rate was 4.3 and 41.7%, respectively. In ALL patients, DFS was 73.1% in SR and 25% in HR patients, and the relapse rate was 16 and 50%, respectively (Fig. 3).

4 Discussion

In this study, we analyzed the probability and risk factors of GVHD using MTX monotherapy as a prophylaxis in HLA-matched sibling bone marrow transplantation for patients with hematological malignancies. In previous studies, the incidence of GVHD using MTX as a prophylaxis was 48–53% for grade II–IV acute GVHD and 9–36% for chronic GVHD [1, 13]. In a randomized study of patients with leukemia, the incidence and severity of acute GVHD was lower in patients receiving CyA + MTX than in those with CyA monotherapy [14]. Furthermore, compared with MTX alone, CyA was associated with lower rates of interstitial pneumonia, treatment-related mortality, and treatment failure [1]. However, these studies were exclusively performed in adult populations, and few reports have described the incidence and severity of GVHD using MTX monotherapy as a prophylaxis in a pediatric population. Aschan et al. [15], demonstrated that MTX combined with CyA increases leukemic relapse compared to monotherapy, even though it decreases GVHD, and the GVL effect is supported by studies that improved leukemia-free survival in adults with AML who had acute or chronic GVHD [16]. Based on previous experience, the risk of GVHD in a pediatric population has been considered to be lower than that in adults, and an older patient age is a risk factor for the development of GVHD [17]. For the above reasons, a single agent could be sufficient for the prevention of GVHD in pediatric patients. Koga et al. [8] reported no significant difference in the incidence of acute GVHD (grades II–IV) or any type of chronic GVHD between patients who received MTX or CyA (28.3 vs. 44% for acute GVHD and 19 vs. 20% for chronic GVHD, respectively).

In this study, we reported the feasibility of GVHD prophylaxis with MTX alone in 94 pediatric patients with hematological malignancies. Although the incidence of chronic GVHD was comparable with previous studies, the incidence of acute GVHD using MTX alone as a prophylaxis was lower in our study. This reason could be due to the genetic homogeneity of Japanese [9]. The relapse rate was 11.6% in standard-risk and 36.8% in high-risk patients. In the standard-risk setting, this result was superior to other reports [6, 7, 13]. The survival rate of all patients was 64.9%, which is also comparable to previous reports [7, 18, 19]. Especially, in standard-risk patients with AML, the DFS rate was higher than in previous reports [19, 20]. Neudorf et al. [19] reported the results of allogeneic bone marrow transplantation for children with AML in first CR using MTX alone as GVHD prophylaxis. The patients received 4 × 4 mg/kg of busulfan and 50 mg/kg × 4 of cyclophosphamide as a conditioning regimen and MTX alone as GVHD prophylaxis until day 100. In their study, the incidence of chronic GVHD, overall survival, and DFS rates were 21, 67, and 57%, respectively. In our study, AML patients received MTX until day 60 as GVHD prophylaxis, and the incidence of chronic GVHD in our patients was relatively higher (31%), but the 91% DFS rate

and 4.3% relapse rate in SR patients were superior to those of previous reports. Similarly to what Matsuyama et al. reported previously, almost all of our patients received busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m^2) as a conditioning regimen [21]. Probably, our results are dependent on the graft-versus-leukemia effect and eradication of leukemic cells by melphalan.

Based on karyotypic analysis at diagnosis, AML patients with translocations 8;21 and 15;17 are classified as having a favorable risk. Slovak et al. [22] observed superior overall survival after transplantation compared to chemotherapy among AML patients showing favorable chromosomal abnormalities. Conversely, Schlenk et al. [23] observed no difference between allogeneic stem cell transplantation (SCT) and intensive chemotherapy for this group of AML patients. Indeed, our current practice does not suggest that AML with an abnormal karyotype of t(8;21) and t(15;17) is an indication for sibling donor SCT in the first remission. However, in our study, among AML patients without these favorable abnormal karyotypes, DFS was 93% in standard-risk and 41.7% in high-risk patients (data not shown).

Although Horeowitz et al. [24] reported the direct antileukemic effect of MTX on relapse after transplantation for ALL, in our study, DFS for standard-risk ALL patients was not superior to that of AML patients. The reasons may be that, in our study, more AML patients received transplantation at first CR and the graft-versus-leukemia effect might occur more preferentially in AML patients [25].

Although one of the major toxicities of MTX is mucositis, it was not a reason for MTX cessation in this study. The major reason for its cessation was liver dysfunction because of GVHD or VOD, and predictive factors of MTX cessation were the development of acute GVHD (≥ grade 2) and second transplantation. Ringden et al. [1] reported that MTX was associated with increased rate of interstitial pneumonia, treatment-related mortality, and treatment failure, compared with CyA in adult patients. However, in our study, the incidence of interstitial pneumonia was 14.8%, being lower than in previous reports [1, 24].

In the search for predictive factors of GVHD development, patient age and female donor to male recipient were found to be significant for the development of chronic GVHD, but no risk factors for acute GVHD were identified. Neudorf et al. [19] demonstrated that children older than 10 years are at a higher risk for developing severe acute GVHD, and others reported that age at transplantation and female donor to male recipient were risk factors for chronic GVHD in adult and pediatric populations [26]. Although Kollman et al. [27] demonstrated that donor age was a significant risk factor for GVHD, we did not document donor age as a risk factor of GVHD. Although the data are not shown, patient age and female donor to male recipient were also significant risk factors for extensive chronic GVHD. In this study, the association of acute and chronic GVHD with a reduced risk of relapse was not documented, along with the association with overall survival, for patients with each high- or standard-risk malignancy. In the future, in addition to MTX, calcineurin inhibitors should be considered for patients undergoing bone marrow transplantation from an HLA-identical sibling in the setting of patients aged over 10 years old and a female donor to male recipient.

In this study, we reported the results of BMT from HLA-identical sibling donors in 94 pediatric patients with hematological malignancies using MTX alone as GVHD prophylaxis, and the relapse rate, OS, and DFS were found to be favorable compared to previous reports. In conclusion, we consider that the use of MTX alone is feasible to prevent severe acute GVHD and may reduce the risk of leukemia recurrence, possibly because of an enhanced GVL effect in the pediatric population, although the incidence of chronic GVHD was comparable to previous reports. In the future, a randomized control study should be considered to document the availability of MTX alone as GVHD prophylaxis in pediatric patients with hematological malignancies.

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Unrelated Cord Blood Transplantation for Severe Aplastic Anemia

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ABSTRACT

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

KEY WORDS
Unrelated cord blood transplantation • Severe aplastic anemia

INTRODUCTION

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

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

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

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

PATIENTS AND METHODS

Patients

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

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

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

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

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

Recipient-Donor HLA Matching

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

Transplantation Procedure

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

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

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

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

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

Definitions and Statistical Analysis

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

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

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

RESULTS

Engraftment

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

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

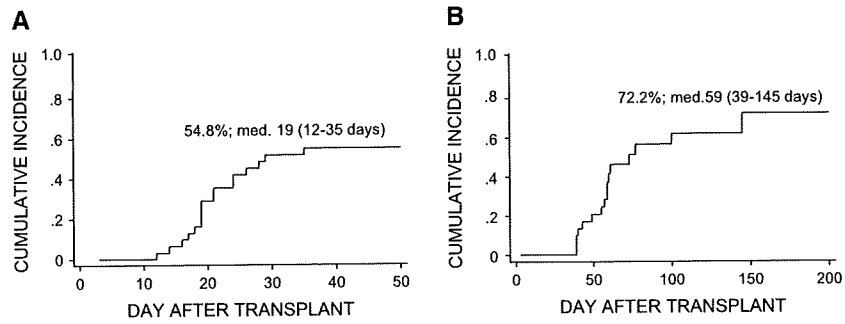

Figure 1. (A) Cumulative incidence of sustained donor neutrophil engraftment (>0.5 × 10^9/L) and (B) platelet engraftment (>50 × 10^9/L) after unrelated cord blood transplantation in patients with aplastic anemia.

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

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

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

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

GVHD and Viral Infections

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

Survival

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

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

DISCUSSION

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

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

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

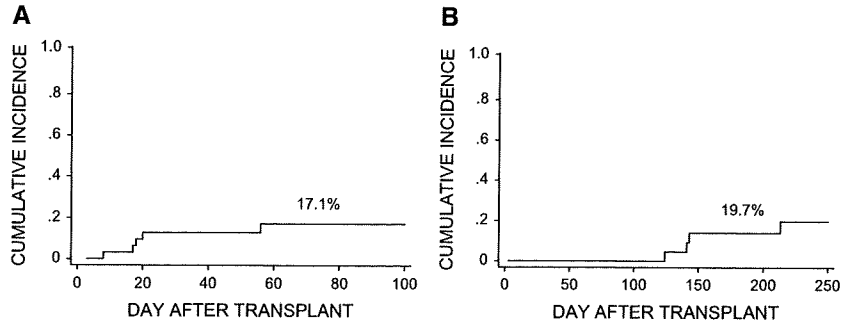

Figure 2. Cumulative incidence of ≥ grade II aGVHD (A) and cGVHD (B) in patients with aplastic anemia who received UCBT.

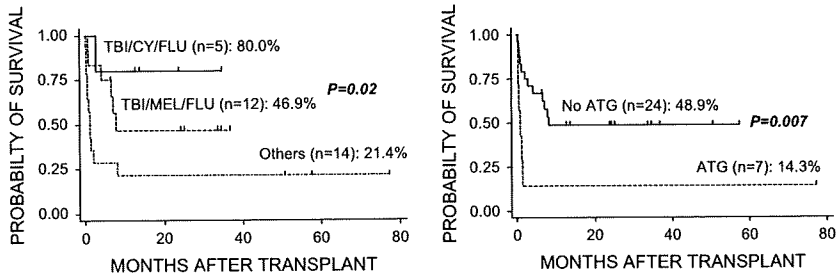

Figure 3. Probability of survival after conditioning regimens in patients with aplastic anemia, who received unrelated cord blood transplantation. TBI: total body irradiation, CY: cyclophosphamide, MEL: melphalan, FLU: fludarabine, ATG: antithymocyte globulin.

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

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

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

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

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

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

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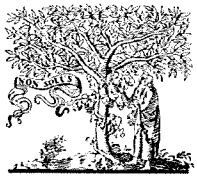

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Elevated serum cytokine levels are associated with human herpesvirus 6 reactivation in hematopoietic stem cell transplantation recipients

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Summary Although it has been demonstrated that human herpesvirus 6 (HHV-6) reactivation generally occurs approximately 2–3 weeks after transplantation in the hematopoietic stem cell transplantation (HSCT) recipients, the mechanism of viral reactivation remains unclear. To explore the relationship between HHV-6 reactivation and plasma cytokine levels, 24 HSCT recipients underwent measurements of plasma proinflammatory cytokine levels (IL-6, TNF-α, IL-1β, and IFN-γ), viral isolation, and serological assays. Of these patients, 14 developed an HHV-6 reactivation, and 9 developed HHV-6 viremia approximately 2–3 weeks after transplantation. IL-6 levels were significantly higher in the recipients with an HHV-6 reactivation than in the subjects without an HHV-6 reactivation at 1 week, 2 weeks, and 4 weeks after transplantation. In addition, the level of TNF-α was significantly higher in recipients with an HHV-6 reactivation than in those without an HHV-6 reactivation at 2 weeks post-transplantation. Low levels of IL-1β and IFN-γ were detected in a small number of the plasma samples, although there were no significant differences between the two groups in the levels of these cytokines.

Abbreviations: CMV, cytomegalovirus; GVHD, graft-versus-host disease; HHV-6, human herpesvirus 6; HSCT, hematopoietic stem cell transplantation; IE, immediate early; TNF, tumor necrosis factor.

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