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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

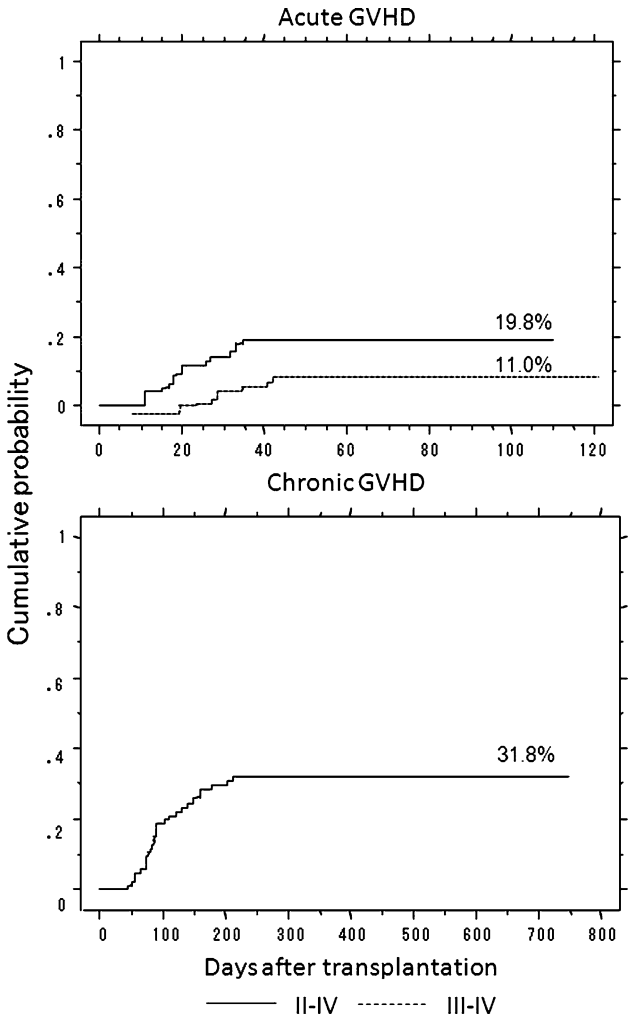

Fig. 1 Cumulative incidence of acute and chronic GVHD. *Upper* and *lower panels* show the cumulative incidence of acute and chronic GVHD, respectively

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

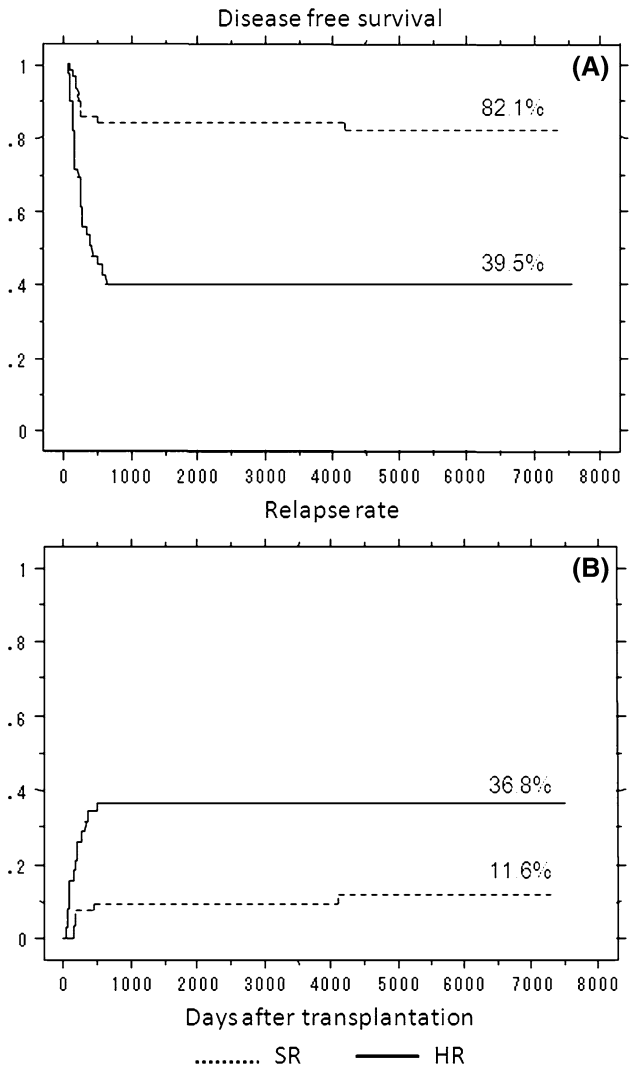

Fig. 2 **a** Disease-free survival. **b** Cumulative incidence of relapse. Standard-risk (*SR*) patients (*discontinuous line*), high-risk (*HR*) patients (*continuous line*)

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

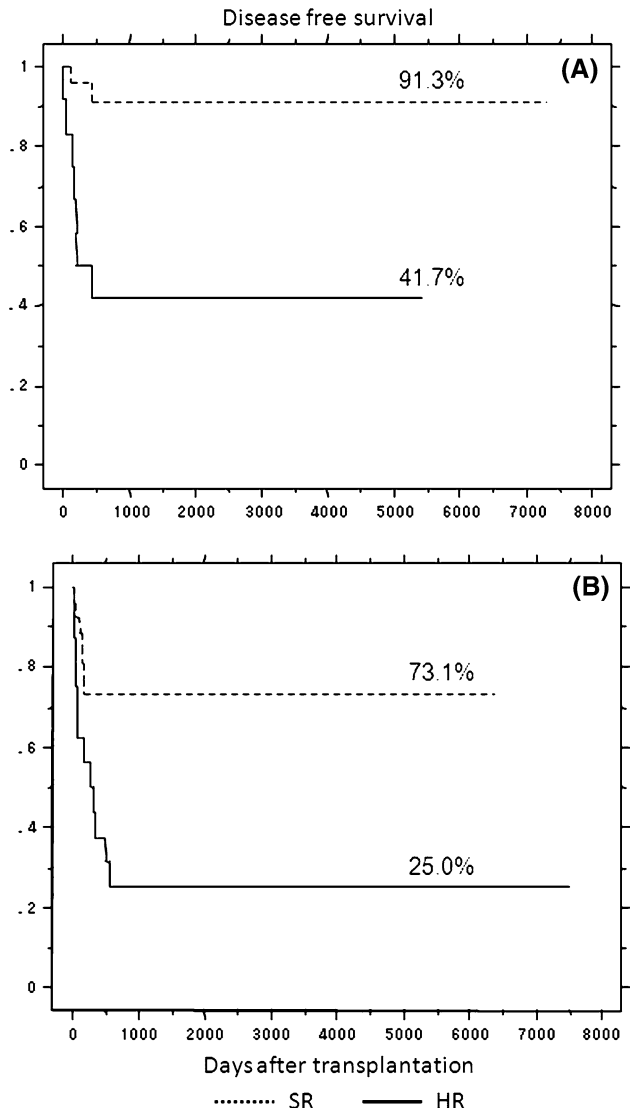

Fig. 3 Disease-free survival in patients with (**a**) acute myelogenous leukemia (*AML*) and (**b**) acute lymphoblastic leukemia (*ALL*). Standard-risk (*SR*) patients (*discontinuous line*), high-risk (HR) patients (*continuous line*)

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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