the first-line treatment in children with SAA. To obtain solid evidence on which to base treatment decisions, ideally, a randomized controlled trial is required. However, because of the rarity of the disease, no randomized controlled trials comparing IST with BMT from an MFD as first-line treatment for SAA exist, and only retrospective studies using data from registries or relatively small cohorts of patients are available. Following the previous report of 304 children treated from 1970 to 1988,[6] the EBMT SAA Working Party (SAAWP) reported a consecutive study of 911 children younger than 16 years initially treated with IST (n=304) or BMT (n=607) between 1991 and 2002, which indicated that first-line IST gave an overall survival rate comparable to that of first-line BMT (81% *versus* 79%).[10] Unfortunately, the analyses had several limitations, because the drugs used for IST varied (e.g., antithymocyte globulin only, cyclosporine A only, or a combination of antithymocyte globulin and cyclosporine A) and the donor types used for BMT were not consistent (15% of the donors were mismatched family donors or matched/mismatched unrelated donors, although the majority of those were MFD). In addition, neither EBMT study provided results on failure-free survival,[6,10] which seems to be much more important than survival alone. Recent advances in supportive care and salvage therapies have effectively rescued non-responders to IST.[24] On the other hand, relapse, clonal evolution in the IST group and secondary graft failure and late malignancy in the BMT group are serious problems in long-term survivors. That is the reason why overall survival is no longer the only endpoint to determine optimal first-line treatment in children with SAA. In Japan, we have conducted consecutive prospective trials with a unified IST regimen consisting of antithymocyte globulin and cyclosporine A since 1992, enrolling 386 SAA patients younger than 17 years. During the same period, 213 SAA patients younger than 17 years underwent BMT from an MFD and were registered into the TRUMP, which provided a unique opportunity to investigate updated evidence for treatment decisions in pediatric SAA, although this study also had limitations due to its retrospective nature.

This study confirmed the excellent outcomes obtained in Japanese children with SAA treated with BMT from an MFD or IST. Consistent with the EBMT studies,[10,22] the survival of children with SAA initially treated with IST has improved markedly since the 1980s, when first-line IST gave greatly inferior survival (with overall survival rates of around 40-50%) when compared with first-line BMT[1,5,6,21]; in the current analyses, the probability of overall survival at 10 years in the patients treated first-line with IST reached 88%, which was comparable to that of the group treated first-line with BMT. Recent significant advances in second-line SCT, especially with a matched unrelated donor, may contribute to this marked improvement in survival after first-line IST.[25-27] In our series, a certain number of patients underwent SCT from an alternative donor after failed IST as a second- or third-line treatment. When patients were subdivided into three groups (first-line BMT from an MFD, IST only, and SCT after failed IST groups), the 10-year overall survival rates in these groups were 91%, 93% and 79%, respectively (P<0.0001), confirming that, in the case of failure of IST, SCT from an alternative donor is a very good salvage option, whereas MFD BMT and IST are excellent first-line treatments for children with SAA.

Regarding survival with response after first-line treatment, we found that the failure-free survival rate in patients treated with IST plateaued over the past two decades after having slightly improved since the 1980s (from 40% in the 1980s to 56% currently).[1] Thus, unlike the overall survival results, failure-free survival in the IST group was significantly inferior to that in the MFD BMT group. Consistent with our observations, the EBMT group also demonstrated no significant improvement in outcomes in response to IST since the 1990s.[10] This may suggest that the IST regimen has not improved over time. Over the past decade, with the hypothesis that more intense IST might produce better outcomes, the addition of newer immunosuppressive agents, such as mycophenolate mofetil and sirolimus to antithymocyte globulin and cyclosporine A, has been tested, but has failed to improve responses.[28-31] The combination of antithymocyte globulin and cyclosporine A is, therefore, still regarded as the standard IST regimen. Another possibility is that we have reached a ceiling in the percentage of patients with the capacity to respond to IST.[18] In patients refractory to IST, the pathophysiology of the disease may be different from that in patients responsive to IST, which is thought to involve autoimmune processes, although there are no good markers to routinely or reliably distinguish non-responders from responders.[15,32-34] Further studies are needed to identify patients refractory to IST, because these patients might benefit from prompt alternative donor SCT.

Importantly, all patients in the current analyses were treated with horse antithymocyte globulin (Lymphoglobulin), which has recently been withdrawn from Asian and European markets and replaced by rabbit antithymocyte globulin. To date, there are only limited studies using rabbit antithymocyte globulin as first-line IST for pediatric aplastic anemia, and thus, the effectiveness of this form of antithymocyte globulin for pediatric patients remains controversial.[35-38] The change of product might result in different outcomes in response to IST for children with SAA.

Survival after BMT from an MFD in children with SAA has exceeded 90% for the past two decades, and this has remained unchanged when compared with our previous observation in the 1980s. In this study, the major causes of treatment failure were primary and secondary graft failure, but notably, most patients with secondary graft failure were rescued by second transplantation or careful observation. In addition to short-term complications, long-term sequelae, such as chronic GVHD and late malignancy, should be taken into consideration to make optimal treatment decisions, especially in children. Our results showed that acute and chronic GVHD were relatively uncommon

Table 4. Multivariate analysis of favorable factors for survival in all 599 patients with SAA.

Overall survival	Hazard ratio	95% CI	P
First-line treatment: BMT	1.619	0.881-2.977	NS
Treatment period: 2000-2009	1.536	0.556-2.753	NS
Age: <10 years	2.207	1.240-3.927	0.007
Failure-free survival	**Hazard ratio**	**95% CI**	**P**
First-line treatment: BMT	4.497	2.935-6.891	<0.0001
Treatment period: 2000-2009	1.090	0.812-1.464	NS
Age: <10 years	1.113	0.833-1.488	NS

BMT: bone marrow transplantation; NS: not significant.

in the setting of BMT from an MFD for pediatric SAA, which is consistent with recently reported results from the EBMT SAAWP, with 11% of grade II to IV acute GVHD and 4% of extensive chronic GVHD after BMT from an MFD for SAA in all age groups.[39] Regarding late malignancy, Kikuchi *et al.* recently published data from 329 Japanese children with SAA from the nationwide registry, confirming a low incidence of late malignancy after BMT from an MFD; the cumulative incidence of late malignancy was 0.8% at 10 years and 2.5% at 20 years, respectively, which was much lower than the cumulative incidences in reports from western countries.[4] In the present series, only one patient developed a late malignancy (myelodysplastic syndrome), and was saved by second BMT. These observations suggest that this approach has been already established as first-line treatment for children with SAA.

In conclusion, our updated data clearly demonstrate that children receiving BMT from an MFD as first-line treatment have a significant advantage over children managed with first-line IST, given the dramatically better failure-free survival and the lower incidence of associated long-term sequelae in the BMT group, which supports the current algorithm for treatment decisions that recommends BMT for pediatric SAA when an MFD is available. On the other hand, IST using the combination of antithymocyte globulin and cyclosporine A is the treatment of choice for children with SAA without an MFD considering the comparable overall survival with BMT from an MFD, which could possibly be ascribed to recent improvements in outcomes after SCT from an alternative donor. In other words, patients have an excellent chance of survival, even after failed first-line IST, when they undergo second-line SCT from an alternative donor.

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Authorship and Disclosures

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Hematopoietic Stem Cell Transplantation for Patients With Acute Lymphoblastic Leukemia and Down Syndrome

Hiroaki Goto, MD, PhD,[1]* Takashi Kaneko, MD, PhD,[2] Yoko Shioda, MD, PhD,[3] Michiko Kajiwara, MD, PhD,[4] Kazuo Sakashita, MD, PhD,[5] Toshiyuki Kitoh, MD, PhD,[6] Akira Hayakawa, MD, PhD,[7] Mizuka Miki, MD, PhD,[8] Keisuke Kato, MD,[9] Atsushi Ogawa, MD,[10] Yoshiko Hashii, MD, PhD,[11] Takeshi Inukai, MD, PhD,[12] Chiaki Kato, MD, PhD,[13] Hisashi Sakamaki, MD, PhD,[14] Hiromasa Yabe, MD, PhD,[15] Ritsuro Suzuki, MD, PhD,[16] and Koji Kato, MD, PhD[17]

Background. Hematopoietic stem cell transplantation (HSCT) is one curable option for high-risk acute lymphoblastic leukemia (ALL); however, transplant-related toxicities might be severe in patients with Down syndrome and ALL (DS-ALL). ***Procedure.*** HSCTs performed in patients with DS-ALL were identified in the Japan Society for Hematopoietic Cell Transplantation registry. ***Results.*** In the registry data, 11 patients with DS-ALL were identified. The median age at HSCT was 9 years (range: 6–22 years). Six patients underwent HSCT at non-remission status. Allogeneic grafts were utilized in all patients, including eight patients who received HSCT from unrelated donors. Reduced intensity conditioning regimens were used in three patients. All patients achieved neutrophil engraftment by a median of day 18 (range: day 11–61). Ten patients experienced grade 3 or more infectious episodes. Six patients experienced complications of the respiratory system. The incidences of II–IV or III–IV acute GVHD were nine (81.8%) or seven patients (63.6%), respectively. Chronic GVHD was observed in five (55.6%) out of nine evaluable patients. Seven patients died at a median of 6 months (range: 0–24 months) after HSCT. Two-year relapse-free and overall survival were 33.3% (95% CI: 2.5–64.1%) or 37.5% (95% CI: 5.9–69.1%), respectively. The causes of death were relapse (n = 2), infection (n = 2), bleeding (n = 1), thrombotic microangiopathy (n = 1), and chronic GVHD (n = 1). ***Conclusions.*** Therapy-related mortality accounted for five out of seven deceased patients in this case series. Attempts to reduce toxicities should be considered in HSCT for patients with DS-ALL. Pediatr Blood Cancer

Key words: acute lymphoblastic leukemia; Down syndrome; GVHD; relapse; transplantation

INTRODUCTION

Patients with Down syndrome (DS) are known to be at high risk of developing acute leukemia [1,2]. Different from acute myeloid leukemia (AML) associated with DS which is known to have excellent prognosis [3,4], treatment results of patients with DS and acute lymphoblastic leukemia (ALL) have been reported to be worse compared with those in patients without DS [5–7]. The poor prognosis of patients with DS-ALL has been suggested to be attributed to the biology of ALL cells [8,9], higher toxicity of chemotherapy [10,11], and less intensification of treatment [12,13].

Hematopoietic stem cell transplantation (HSCT) is an option for cure of high-risk or relapsed ALL. In patients with DS-ALL, however, the role of HSCT has not been established. Earlier studies reported high therapy-related mortality (TRM) after HSCT [14,15]. In contrast, more recent studies identified ALL relapse rather than TRM as the main cause of treatment failure [16,17].

In this study, we accessed the national HSCT registry data to obtain further information to assess the risks and benefits of HSCT for patients with DS-ALL.

PATIENTS AND METHODS

In the Japanese Society of Hematopoietic Cell Transplantation (JSHCT) registry from April 1977 to December 2011, 13 patients with DS were identified to have received HSCT against ALL. Two patients were excluded from the study because of the guardians' refusal to allow participation in the clinical study (n = 1) and insufficient data to confirm that the patient had DS (n = 1). In total, the clinical courses of 11 patients with DS-ALL who underwent HSCT were studied. The missing data in the JSHCT registry and additional clinical information such as doses of methotrexate (MTX) in GVHD prophylaxis, grades of transplant-related toxicities (TRTs), and comorbidities at the time of HSCT were obtained by direct contact with transplant institutions. Grading of TRT was scored according to Common Terminology Criteria for Adverse Events version 4.0. Survival after HSCT was calculated using the Kaplan–Meier method. This study was approved by the

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[1]Division of Hemato-Oncology and Regenerative Medicine, Kanagawa Children's Medical Center, Yokohama, Japan; [2]Division of Hematology and Oncology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [3]Division of Hematology/Oncology, National Center for Child Health and Development, Tokyo, Japan; [4]Department of Pediatrics, Tokyo Medical and Dental University Hospital Faculty of Medicine, Tokyo, Japan; [5]Department of Pediatrics, Shinshu University School of Medicine, Nagano, Japan; [6]Department of Hematology/Oncology, Shiga Medical Center for Children, Shiga, Japan; [7]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; [8]Department of Pediatrics, Hiroshima University Hospital, Hiroshima, Japan; [9]Division of Pediatric Hematology and Oncology, Ibaraki Children's Hospital, Ibaraki, Japan; [10]Department of Pediatrics, Niigata Cancer Center Hospital, Niigata, Japan; [11]Department of Pediatrics, Osaka University Hospital, Osaka, Japan; [12]Department of Pediatrics, University of Yamanashi, Faculty of Medicine, Yamanashi, Japan; [13]Department of Hematology, Meitetsu hospital, Meitetsu, Japan; [14]Division of Hematology, Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, Tokyo, Japan; [15]Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Tokai, Japan; [16]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [17]Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

Conflict of interest: Nothing to declare.

*Correspondence to: Hiroaki Goto, 2-138-4 Mutsukawa Minami-ku, Yokohama, Japan. E-mail: hgoto39@aol.com

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ethics committee of Kanagawa Children's Medical Center and the ethics committee of the JSHCT.

RESULTS

Characteristics of Patients and HSCT

The characteristics of the patients with DS-ALL are shown in Table I. Five were female and six were male. The median age at diagnosis of ALL was 7 years (range: 4–21 years). Median age at HSCT was 9 years (range: 6–22 years). The immunophenotype of ALL was B cell precursor in all patients, including the case of secondary leukemia, who developed ALL after the treatment for AML (case 2). Data on cytogenetics were available in 10 out of the 11 patients, and two had additional chromosomal aberrations (der[16]t[1;16][q2;q2.1]; case 2, del [9]; case 5) besides trisomy 21. Disease status at the time of HSCT was first complete remission (CR) in three patients (case 5 and 11 failed to achieve CR after the first-line induction therapy), second or later CR in two, relapse in five, and induction failure in one. Comorbidities included three patients with diabetes mellitus, two with thyroid dysfunction, and one with mild aortic regurgitation.

These patients underwent HSCT between April 2001 and April 2011. All patients received allogeneic grafts: five unrelated bone marrow (BM), two related BM, one related peripheral blood, and three unrelated cord blood. Total body irradiation at a dose of 8–12 Gy was used as part of the conditioning regimen in six patients. Two patients received high-dose busulfan regimens, and three received fludarabine and melphalan-based reduced intensity regimens. GVHD prophylaxis regimens are summarized in Table II.

Outcome

Neutrophil engraftment was reported in all patients at median day 18 (range: day 11–61). Platelet recovery over $2 \times 10^4/\mu l$ was achieved in all but one patient (case 9) at median day 37 (range: day 21–58; the recovery date was unavailable in case 6).

Among six patients who received HSCT at non-remission status, one patient did not achieve complete remission even after HSCT (case 1). Among 10 patients who achieved complete remission before or after HSCT, ALL recurrence was observed in four patients 4–11 months after HSCT (case 3, 5, 6, and 10). At the time of this study, four patients are alive 16–77 months after HSCT (case 2, 3, 7, and 11). One patient who received HSCT at ALL relapse has survived over 2 years after HSCT (case 7). In total, the 2-year overall survival or leukemia-free survival rate was 37.5% (95% CI: 5.9–69.1%) or 33.3% (95% CI: 2.5–64.1%), respectively. Main causes of death were reported as ALL (n = 2), infection (n = 2), chronic GVHD (n = 1), thrombotic microangiopathy associated with chronic GVHD (n = 1), or bleeding (n = 1). Thus, in this case series, TRT was considered as the major cause of death rather than ALL relapse. Three patients died of TRT without ALL recurrence.

Transplant-Related Toxicities

More than grade 3 non-hematological toxicity was observed in all but one patient. The most frequent TRTs were infection (10/11 patients: 90.9%) and mucositis (7/11 patients: 63.6%). As shown in Table II, all seven patients with more than grade 3 mucositis developed infection such as febrile neutropenia (n = 3), sepsis (n = 3), or pneumonia (n = 1). TRT in the respiratory system was also common, including acute respiratory distress syndrome (ARDS, n = 2), pleural effusion (n = 2), pneumonia (n = 2), and airway obstruction (n = 1).

GVHD

The incidence of acute GVHD was relatively high in this case series (10/11 patients: 90.9%). Greater than grade II acute GVHD was observed in nine patients (81.8%), and grade III acute GVHD was seen in seven (63.6%). Five (55.6%) out of nine evaluable patients developed chronic GVHD, which was related to transplant-related mortality in two patients.

Patients with DS are known to be susceptible to MTX-toxicity [18] and the optimal dose of MTX as GVHD prophylaxis is not standardized in patients with DS. Therefore, besides the JSHCT registry data, doses of MTX used in GVHD prophylaxis were additionally investigated in this study. As shown in Table II, the doses were considered to be relatively low, especially in four patients, in whom the doses were as follows: 10 mg/m^2 followed by a single dose of 7 mg/m^2; 7.5 mg/m^2 followed by three doses of 5 mg/m^2; four doses of 5 mg/m^2; and three doses of 2.5 mg/m^2, respectively. These four patients developed grade II–III acute GVHD, although a clear association between MTX doses and the occurrence of GVHD was not observed in this case series.

DISCUSSION

In previous studies, survival after HSCT in patients with acute leukemia and DS was 19–48% [14,17,19,20], which is comparable with our results. In this study, six out of 11 patients with DS-ALL received HSCT at non-remission status. Uncontrolled disease status at the time of HSCT might have affected the treatment results. However, contrary to recent reports that indicated that leukemia relapse was the major cause of death in patients with DS-ALL after HSCT, three patients in this case series (27.3% of total and 42.9% of deceased patients) died of TRT without ALL recurrence. Because patients' backgrounds such as disease status, donor sources or transplant procedures were highly heterogeneous in this study, we could not specify the single factor associating with mortality after HSCT. However, our results suggest TRT associated with HSCT is still a major problem in patients with DS-ALL.

DS is frequently complicated by congenital and acquired diseases such as heart defects or metabolic disorders. The prevalence of congenital heart defects or thyroid dysfunction in patients with DS is about 44–58% or 28–40% [21], respectively. Considering these incidences, comorbidity at the time of HSCT in this case series was not significantly frequent so that it could explain the high rate of therapy-related mortality. The relatively low frequency of severe congenital disease in this study suggests that the indication of HSCT has been restricted in patients with DS-ALL because of their physical condition.

Infection and mucositis were frequent TRTs associated with HSCT in patients with DS-ALL. The mucocutaneous complication is prevalent even during conventional-dose chemotherapy in patients with DS and acute leukemia, and this is possibly related to the high incidence of infection [10]. Mucositis caused by the conditioning regimen may have resulted in the high incidence of infection after HSCT as well. In this study, all seven patients who had more than grade 3 mucositis developed severe infection. Complications in the respiratory system were also frequent,

TABLE I. Characteristics of DS-ALL Patients

Case	Age at Diag.*1 (yr)	Age at HSCT (yr)	Gender	Diag.	karyotype	ALL status at HSCT	Complications at HSCT	Donor type	HLA match*2	Conditioning	Relapse after HSCT	Outcomes (mo after HSCT)	Cause of death
1	4	6	Male	BCP	+21	Rel		uBM	6/8 allele	TBI 8 Gy(4)*3, Flu 150 mg/m^2, CY 120 mg/kg	no CR*4	Dead, 6 mo	cGVHD
2	8	8	Female	BCP, secondary	+21, der(16)t (1;16) (q2;q2.1)	1st CR		uBM	7/8 allele	CY 120 mg/kg, ETOP 60 mg/kg, Bu 16 mg/kg	no	Alive, 24 mo+	
3	7	12	Male	BCP	+21	2nd CR	DM (steroid induced)	rPB	8/8 allele	Flu 180 mg/m^2, Mel 180 mg/m^2, ATG 1.25 mg/kg	yes	Alive, 16 mo+	
4	7	12	Male	BCP	+21	Rel		uBM	5/6 allele	Flu 125 mg/m^2, Mel 210 mg/m^2, TBI 3 Gy(1)	no	Dead, 24 mo	TMA
5	8	9	Male	BCP	+21, del(9)	1st CR		uBM	6/6 allele	TBI 12 Gy (6), CY 2800 mg/m^2	yes	Dead, 4 mo	ALL
6	9	9	Male	BCP	+21	IF		uCB	5/6 antigen	TBI 8 Gy (4), CY, Tepa	yes	Dead, 11 mo	ALL
7	6	9	Male	BCP	+21	Rel	DM	uCB	5/6 allele	Flu 125 mg/m^2, Mel 140 mg/m^2, TBI 6 Gy (3)	no	Alive, 55 mo+	
8	21	22	Female	BCP	NE	Rel	epilepsy hypothyroidism	uBM	6/6 allele	TBI 12 Gy (6), Mel 180 mg/m^2, ETOP 50 mg/kg, Bu 6.4 mg/kg	no	Dead, 5 mo	Bleeding
9	5	12	Female	BCP	+21	>3rd CR		rBM	4/8 allele	TBI 12 Gy(6), CY 120 mg/m^2	—	Dead, 0 mo	Infection
10	6	7	Female	BCP	+21	Rel		rBM	7/8 antigen	Bu 16 mg/kg, Mel 180 mg/m^2, ETOP 50 mg/kg	yes	Dead, 8 mo	Infection
11	18	19	Female	BCP	+21	1st CR	aortic regurgitation hyperthyroidism DM	uCB	6/6 allele	TBI 10 Gy(6), CA 12 g/m^2, CY 120 mg/m^2	no	Alive, 77 mo+	

Diag., diagnosis; mo: months; BCP, B cell precursor; ALL: acute lymphoblastic leukemia; Rel, relapse; CR, complete remission; IF, induction failure; DM, diabetes melitus; uBM, unrelated bone marrow; rPB, related peripheral blood; uCB, unrelated cord blood; rBM, related bone marrow; TBI, total body irradiation; Flu, fludarabine; CY, cyclophosphamide; Bu, busulfan; Mel, melphalan; ETOP, etoposide; CA, cytarabine; NE, data not evaluable; TMA, thrombotic microangiopathy; *1: diagnosis of ALL; *2: 6 allele/antigen = HLA-A, B, DR loci, 8 allele/antigen = HLA-A, B, C, DR loci; *3: fractions of irradiation; *4: the patient did not achieve CR.

TABLE II. GVHD Prophylaxis Regimens and Transplant-Related Toxicities

Case	GVHD prophylaxis	MTX (mg/m^2)	acute GVHD	chronic GVHD	Transplant-related toxicities > grade 3
1	FK, MTX, mPSL	10 × 1, 7 × 2	grade III (skin 2, gut 4)	extensive	none
2	FK, MTX	10 × 1, 7 × 1	grade III (skin 3, gut 2)	limited	ARDS, FN, mucositis
3	CsA, MTX	10 × 1, 7 × 3	none	limited	candidemia, pleural effusion, edema, hypokalemia
4	FK, MTX	15 × 1, 10 × 2	grade III (skin 2, gut 3)	extensive	FN, airway obstruction (mucosal damage), mucositis
5	FK, MTX	7.5 × 1, 5 × 3	grade II (skin3)	none	FN, mucositis
6	CsA, MTX, PSL	NA	grade III	none	aspergillosis, bacterial pneumonia
7	FK, MTX	15 × 1, 10 × 3	grade I (skin 1)	none	FN
8	FK, MTX	15 × 1, 10 × 3	grade III (skin 2, gut 2)	NE	sepsis (pseudomonas aeruginosa), radiation dermatitis, pleural effusion, mucositis
9	CsA	none	grade III (skin 3, liver 2)	NE	ARDS, VOD, pneumonia, mucositis
10	CsA, MTX	5 × 4	grade III (skin 1, gut 2)	none	sepsis (pseudomonas aeruginosa), mucositis
11	FK, MTX	2.5 × 3	grade II (skin 3, liver 1)	extensive	sepsis (staphylococcus epidermidis), mucositis

FK, taclorimus; MTX, methotrexate; mPSL, methyl prednisolone; CsA, cyclosporine A; PSL, prednisolone; ARDS, acute respiratory distress syndrome; FN, febrile neutropenia; VOD, veno-occlusive disease; NA, data not available; NE, not evaluable.

consistent with the results in previous studies [14,15]. Patients with DS are vulnerable by nature to respiratory complications, and have been reported to develop ARDS and acute lung injury more frequently compared with children without DS after lung and airway distress such as mechanical ventilation [22]. Such inherited condition of patients with DS could be associated with the high incidence of lung complications after HSCT.

Reducing the intensity of the conditioning regimen is a possible consideration to improve survival after HSCT in patients with DS-ALL. In patients with DS-AML, survival after HSCT was better when a reduced intensity regimen was employed [20]. In patients with ALL, however, the efficacy of a reduced intensity conditioning regimen for HSCT is a matter of debate. Several retrospective studies reported that reduced intensity HSCT for ALL resulted in a higher relapse rate, but comparable survival with myeloablative HSCT [23,24]. Reduction of the conditioning intensity might be beneficial in patients who are vulnerable to toxicities associated with high-dose chemo-radiotherapy, such as children with DS, although further studies are required to evaluate if reduced intensity stem cell transplantation improves survival of patients with DS and high risk ALL.

In this study, the incidence of acute or chronic GVHD was relatively high, consistent with an earlier study [16]. Theoretically, severe mucositis or cutaneous damage after the conditioning regimen might increase the risk of developing GVHD [25]. Impaired thymic function in children with DS which is indicated by low TCR excision circle levels in blood might lead to the high incidence of GVHD [26,27]. GVHD prophylaxis is also a factor associated with the development of GVHD. Most patients in this study received the standard prophylaxis regimen consisting of a calcineurin inhibitor and MTX. However, doses of MTX were rather low in some patients compared to the standard doses [28,29]. Severe MTX-related toxicity in patients with DS has been well characterized. However, it is due to tissue sensitivity and the pharmacokinetics of MTX are not different between DS and patients without DS [18]. Low-dose MTX is possibly not sufficient to repress donor lymphocytes which are more tolerant to MTX than tissues of patients with DS.

Due to the high TRM rate, it is not acceptable to consider HSCT as the standard treatment option for patients with DS-ALL. Discovering appropriate GVHD prophylaxis is one of the solutions to improve survival, such as the use of folinic acid after administering the MTX dose which might allow the use of standard doses of MTX [30,31]. Reducing the intensity of the conditioning regimens is another conceivable option, although it might be associated with an increased risk of ALL relapse. To develop the optimal HSCT procedure for patients with DS-ALL, a study in a larger cohort, which could be achieved by international collaboration, is necessary.

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