confirmed in children. Fluoroquinolones are historically contraindicated in children because they cause arthrotoxicity in juvenile animals and are associated with reversible musculoskeletal events in both children and adults; therefore, they are not recommended in the absence of convincing evidence.

Choreito is a formula stemming from Japanese traditional (Kampo) medicine, originally developed from traditional Chinese medicine; it was the orthodox medicine in Japan until the 19th century, when modern Western medicine took over [14]. Nevertheless, some Kampo formulae are still officially registered in the Japanese Pharmacopoeia. Although Kampo extracts are crude drugs derived from plants, animals, and minerals, their quality is strictly controlled in accordance with the Japanese Pharmacopoeia by quantitative analysis of marker components using high-performance liquid chromatography. Kampo formulae are classified as dietary supplements outside Japan and are approved for marketing by the Food and Drug Administration in the United States.

Choreito is a crude product from *Polyporus umbellatus* sclerotium, *Wolfiporia extensa* sclerotium, *Alisma orientale* rhizome, aluminum silicate hydrate with silicon dioxide, and glue. Ergone isolated from *P. umbellatus* prevented early renal injury in a rat model of nephropathy [23] and may play a central role in the effect exerted by choreito. Pollakisuria was ameliorated in 93% of patients who received choreito for lower urinary tract symptoms in an open-label, single-arm study of 30 patients [24]. Choreito was also administered to patients with urolithiasis for enhancing the evacuation of stones after extracorporeal shock wave lithotripsy [25]. In these studies, no severe adverse effects were observed, suggesting high safety of choreito.

Considering the wide range of indications in genitourinary disorders, choreito may protect epithelial cells irrespective of the type of pathogens and thereby be an effective treatment option for the hemostasis of HC. Although the precise pathogenesis of BKV-HC remains unclear, urothelial cells infected with BKV in vitro detached without causing local cell lysis, which may be associated with the denudation of the damaged mucosa in patients with BKV-HC [26]. Choreito may protect urothelial cells from detaching, which may result in a significant reduction of the BKV load in urine, although the whole blood BKV load appears unchanged and the BKV burden itself is not reduced. Notably, unlike other antiviral agents or surgical interventions, no adverse effects were observed during choreito administration, although the mechanism of action of choreito remains unclear; hence, its safety cannot be easily predicted.

Our study has some limitations. The small number of study subjects in this single-center retrospective analysis may result in bias. Five of 8 subjects in the nonchoreito group had grade II to III GVHD, whereas 1 out of 6 subjects in the choreito group had grade IV GVHD. This difference in GVHD frequency could have been a contributing factor for the difference in HC severity and BKV clearance, although it was not statistically different ($P = .14$) among the 2 groups, possibly because of the small sample size. Children with concomitant AdV viruria were included only in the nonchoreito group, which may explain the longer time before CR in the nonchoreito group. In the present study, HC was significantly more severe in the choreito group than the nonchoreito group. This difference may represent the difference in preconditioning and donor sources: the choreito group included more cases of haplo-identical HSCT, which may have resulted in intensified immunosuppression. More severe HC correlates with a longer duration of HC [2]. Nevertheless, the duration of HC was significantly shorter in the choreito group, which exemplifies its effectiveness. Although the urine BKV load had significantly decreased 1 month after choreito treatment examined by the paired samples, this decrease could not be compared with that of the nonchoreito group because of a lack of paired samples in most of the patients in the nonchoreito group. Thus, the impact of choreito treatment on the urine BV virus load should be investigated in a prospective study where the BKV load is sequentially followed for every study subject.

In conclusion, choreito may be a safe and effective therapy for the hemostasis of late-onset BKV-HC following HSCT, although it may not decrease the BKV burden. Although its precise mechanism of hemostasis remains unclear, choreito may be administered as the first-line treatment for post-HSCT HC. Prospective, randomized studies are warranted to confirm the efficacy of choreito in the treatment of BKV-HC. Fundamental research aiming to identify the active ingredients and mechanisms of action is also essential.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.10.018.

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Bloodstream Infection after Stem Cell Transplantation in Children with Idiopathic Aplastic Anemia

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A B S T R A C T

Bloodstream infection (BSI) is the most common infectious complication of hematopoietic stem cell transplantation (HSCT) and can cause substantial morbidity and mortality. Identification of risk factors for BSI might be helpful in efforts to reduce transplantation-related death. This study analyzed the incidence of BSI and risk factors for BSI after HSCT in pediatric patients with aplastic anemia (AA). BSI occurred in 39 of the 351 patients with AA (11.1%). Onset of BSI occurred at a median of 8 days after HSCT (range, 0 to 92 days). The 5-year overall survival rate was lower in patients with BSI than in patients without BSI (63.32% ± 7.90% versus 93.35% ± 1.44%; $P < .0001$). Univariate analysis identified the following variables as associated with BSI: history of immunosuppressive therapy with antithymocyte globulin (ATG), transplantation from an unrelated donor, frequent blood transfusion before transplantation, major or major plus minor ABO type mismatch, graft-versus-host disease prophylaxis with tacrolimus and without cyclosporine, and long interval from diagnosis to transplantation. Among these factors, long interval from diagnosis to transplantation was the sole statistically significant risk factor for BSI on multivariate analysis. In patients who underwent HSCT from a related donor, age ≥14 years at transplantation was risk factor for BSI. In contrast, history of immunosuppressive therapy with ATG, frequent blood transfusion before HSCT, graft failure, and major or major plus minor ABO type mismatch were risk factors for BSI in patients who underwent HSCT from an unrelated donor. Because the overall 5-year survival rate without BSI was >90%, even in patients who were received a transplant from an unrelated donor, control of BSI is very important for successful HSCT in pediatric patients with AA.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is first-line therapy for severe aplastic anemia (AA). HSCT from an HLA-matched sibling donor is an established standard therapy for children with severe AA and is associated with high survival rates [1]. Outcomes of HSCT from an unrelated donor have gradually improved [2,3].

Bloodstream infection (BSI) is the most common infectious complication of HSCT and causes substantial morbidity and mortality [4,5]. Identification of risk factors for BSI may aid efforts to reduce transplantation-related deaths. We previously identified AA as a common risk factor for BSI in a retrospective multicenter study [6]. In the present study, we analyzed the incidence of BSI and risk factors for BSI after

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HSCT in pediatric patients with AA using the Transplant Registry Unified Management Program (TRUMP) system of the Japanese Society of Stem Cell Transplantation.

PATIENTS AND METHODS

Between 1980 and 2011, 1098 patients age ≤19 years who underwent HSCT for AA (excluding hereditary bone marrow failure, paroxysmal nocturnal hemoglobinemia, and secondary AA) were registered with the TRUMP system of the Japanese Society of Stem Cell Transplantation. Of these 1098 patients, 516 who underwent HSCT before 2000 were excluded from this analysis, owing to the drastic changes in infection control practices promulgated by the Japanese Society of Stem Cell Transplantation in 2000, including antibiotics and antifungal drugs and guidelines for infection management in the early post-transplantation period. Of the remaining 582 patients, 231 were excluded due to insufficient data; thus, our study group comprised 351 pediatric patients with AA who underwent HSCT, including 193 males and 158 females, with a median age of 11 years (range, 0 to 19 years).

Diagnosis and assessment of severity of disease were established according to published criteria [7]. Severity of AA at initial diagnosis was as follows: very severe, n = 84; severe, n = 137; nonsevere, n = 130. Severity of AA at HSCT was as follows: very severe, n = 122; severe, n = 166; nonsevere, n = 63. The median interval from diagnosis to transplantation was 337 days (range, 9 to 5261 days). Two hundred and seventy-eight patients had received some specific treatment for AA before transplantation, including steroids (n = 171), antithymocyte globulin (ATG; n = 210), cyclosporine (CsA; n = 244), and granulocyte colony-stimulating factor (n = 141). Stem cell source was bone marrow in 315 patients, peripheral blood in 12 patients, bone marrow plus peripheral blood in 1 patient, and cord blood in 23 patients. One hundred seventy-three patients had a related donor, 1 patient had a syngeneic donor, and 177 patients had an unrelated donor.

The conditioning regimen included ATG for 240 patients, cyclophosphamide for 317, fludarabine for 244, melphalan for 39, total body irradiation for 145, thoracoabdominal irradiation for 49, and total lymphoid irradiation for 70 patients. Graft-versus-host disease (GVHD) prophylaxis, defined as planed administration of immunosuppressive drugs before evidence of acute GVHD, included steroids in 17 patients, CsA in 160, tacrolimus in 191, and methotrexate in 319.

Twenty-four patients underwent a second HSCT, 3 patients underwent a third HSCT, and 1 patient underwent a fourth HSCT. Twenty-one patients had a bacterial or fungal infection at the time of transplantation. In patients with multiple HSCTs, each transplantation was analyzed separately.

BSI was defined as isolation of 1 or more recognized bacterial or fungal pathogens from 1 or more blood cultures and at least 1 of the following signs and symptoms within 24 hours of collection of a positive blood culture: fever (>38°C), chills or rigors, or hypotension. We classified ABO compatibility as minor (eg, from an type O donor to a type A, B, or AB recipient), major (eg, from a type A, AB, or B donor to an type O recipient), and major and minor (eg, type A donor to type B recipient). We defined an HLA match donor as a 6/6 HLA-A, -B, and -DR antigen match between recipient and donor, using low-resolution typing. The median duration of follow-up was 39 months. Data collected as of October 2012 were analyzed.

In univariate analysis, the chi square test and Fisher's exact test were used to assess risk factors for BSI. Multivariate stepwise regression was performed to explore the independent effects of variables that demonstrated a significant influence in univariate analysis ($P < .10$). Overall survival was analyzed using the Kaplan-Meier method, with differences compared using the log-rank test. Statistical analyses were performed using SPSS 11.0 for Windows release 11.0.1J (SPSS Japan, Tokyo, Japan).

RESULTS

Assessment of BSI in All 351 Patients Who Underwent HSCT

BSI occurred in 39 of the 351 patients with AA (11.1%). Onset of BSI occurred at a median of 8 days after transplantation (range, 0 to 92 days). The bacteria that were isolated are summarized in Table 1. *Staphylococcus* spp were detected in 11 patients, and *Streptococcus* spp were detected in 7 patients. Gram-positive cocci were detected in 20 patients (51.3%); gram-positive bacilli, in 5 patients (12.8%); gram-negative bacilli, in 11 patients (28.2%); and *Candida* spp, in 3 patients (7.7%). The 5-year overall survival rate was lower in patients with BSI compared with patients without BSI (65.32% ± 7.90% versus 93.35% ± 1.44%; $P < .0001$) (Figure 1). The cause of death was directly associated with BSI in 5 of the 13 patients with BSI who died.

Table 1
Organisms Isolated from Blood Cultures of Patients with AA Who Underwent HSCT

Organism	n
Staphylococcus	11
Staphylococcus epidermidis	8
Staphylococcus haemoliticus	1
Coagulase-negative staphylococci	1
Staphylococcus sp	1
Streptococcus	7
Streptococcus mitis	4
Streptococcus viridans	1
α-streptococci	1
Streptococcus sp	1
Micrococcus	1
Enterococcus	1
Bacillus	4
Gram-positive rods	1
Escherichia coli	1
Enterobacter cloacae	2
Acinetobacter	1
Pseudomonas aeruginosa	4
Stenotrophomonas maltophilia	3
Candida	3

We performed univariate and multivariate analyses to identify risk factors for BSI in the patients with AA (Table 2). Variables associated with BSI on univariate analysis included (1) history of immunosuppressive therapy with ATG, (2) transplantation from an unrelated donor, (3) frequent blood transfusions before HSCT, (4) major or major plus minor ABO mismatch, (5) tacrolimus as acute GVHD prophylaxis with use of CsA, and (6) extended interval from diagnosis of AA to HSCT. Infectious complications at the time of HSCT were not associated with BSI after transplantation. Multivariate analysis identified extended interval from diagnosis to HSCT (>300 days) as the sole statistically significant risk factor for BSI (Table 3).

Assessment of BSI in 158 Patients Who Underwent First HSCT from a Related Donor

BSI occurred in 11 of 158 patients with AA who underwent first HSCT from a related donor (7.0%). The 5-year overall survival rate was lower in patients with complicated BSI compared with patients without BSI (81.82% ±

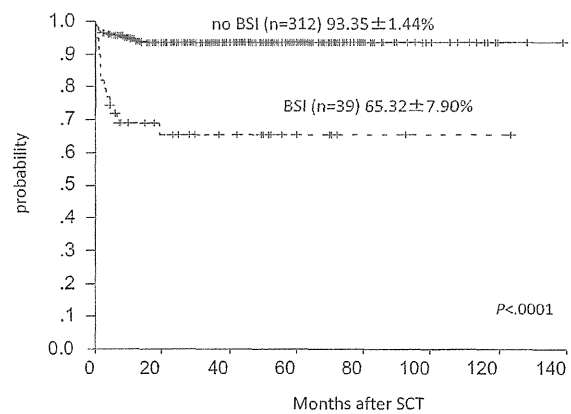

Figure 1. Kaplan-Meier estimate of overall survival for patients with BSI (n = 40; 63.68% ± 7.87%) and patients without BSI (n = 311; 93.65% ± 1.41%); $P < .0001$.

Table 2
Clinical Characteristics of 351 Patients Who Underwent HSCT

	BSI (n = 39)	No BSI (n = 312)	*P*
Sex, male/female, n	21/18	172/140	1.000
Age, yr, median	11	11	.568
Previous treatment, n (%)			
ATG	33 (85)	177 (57)	**.001**
CsA	34 (87)	210 (67)	**.010**
Both ATG and CsA	33 (85)	171 (55)	**<.001**
Bacterial/fungal infection at HSCT, n (%)	3 (8)	18 (6)	.716
First HSCT, n (%)	37 (95)	286 (92)	.754
Stem cell source, n (%)			.959
BM	35 (90)	280 (90)	
PB	1 (3)	11 (4)	
BM + PB	0 (0)	1 (0)	
CB	3 (8)	20 (6)	
Donor, n (%)			**.044**
Syngeneic	0 (0)	1 (0)	
Related	12 (31)	161 (52)	
Unrelated	27 (69)	150 (48)	
Receipt of ≥20 transfusions before HSCT, n (%)			
RBCs	20 (51)	84 (27)	**.003**
Platelets	21 (54)	102 (33)	**.012**
ABO type match, n (%)			**.014**
Match	16 (41)	172 (55)	
Major mismatch	14 (36)	47 (15)	
Minor mismatch	6 (15)	54 (17)	
Major plus minor mismatch	3 (8)	39 (13)	
HLA compatibility (low-resolution typing), n (%)			.846
Matched	30 (77)	231 (74)	
Mismatched	9 (23)	81 (26)	
Conditioning regimen, n (%)			
ATG	30 (77)	210 (67)	.275
Fludarabine	27 (69)	217 (70)	1.000
Cyclophosphamide	36 (92)	281 (90)	1.000
Irradiation	33 (85)	231 (74)	.173
GVHD prophylaxis, n (%)			
Steroid	0 (0)	17 (5)	.235
CsA	11 (28)	149 (48)	**.040**
Tacrolimus	28 (72)	163 (52)	**.026**
Methotrexate	37 (95)	282 (90)	.555
Acute GVHD grade II-IV, n (%)	5 (13)	55 (18)	.651
Graft failure, n (%)	5 (13)	16 (5)	.070
Time from diagnosis to HSCT, d, median	447	305.5	**.020**
Time from diagnosis to HSCT >300 d, n (%)	30 (77)	157 (50)	**.002**
Severity at diagnosis, n (%)			.641
Very severe	7 (18)	77 (25)	
Severe	16 (41)	121 (39)	
Nonsevere	16 (41)	114 (37)	
Severity at HSCT, n (%)			.426
Very severe	17 (44)	105 (34)	
Severe	15 (38)	151 (48)	
Nonsevere	7 (18)	56 (18)	

BM indicates bone marrow; PB, peripheral blood; CB, cord blood.
*Irradiation included total body irradiation, thoracoabdominal irradiation, and total lymphoid irradiation.

11.63% versus 95.84% ± 1.66%; $P = .0379$). Univariate analysis of variables associated with BSI identified age ≥14 years at HSCT as the sole risk factor (Table 4).

Assessment of BSI in 165 Patients Who Underwent First HSCT from an Unrelated Donor

BSI occurred in 26 of 165 patients with AA who underwent a first HSCT from an unrelated donor (15.8%). The 5-year overall survival rate was lower in patients with complicated BSI compared with those without BSI (55.75% ± 10.14% versus 90.77% ± 2.25%; $P < .0001$). In univariate analysis, variables associated with BSI included a history of immunosuppressive therapy with ATG, frequent blood transfusion before transplantation, graft failure, and major or major plus minor ABO type mismatch (Table 4).

Table 3
Multivariate Analysis of 351 Patients with AA

	Hazard Ratio	*P*	95% Confidence Interval
Interval from diagnosis to HSCT >300 d	2.430	**.041**	1.036-5.702
≥20 RBC or platelet transfusions before HSCT	1.843	.109	0.873-3.891
Major or major plus minor ABO type mismatch	1.595	.199	0.783-3.250
Unrelated donor	1.233	.673	0.466-3.262
Use of tacrolimus	1.167	.755	0.442-3082
Previous treatment with ATG or CsA	1.115	.839	0.392-3.170

DISCUSSION

In the literature, the incidence of BSI after the early phase of HSCT in children has ranged from 25% to 30% [4-8]. In our previous study, the incidence of BSI after HSCT (including patients with malignant and nonmalignant diseases) was 8.7% [6]. In the study of Sarashina et al. [6], nonmalignant disease, especially AA and Wiskott-Aldrich syndrome, were identified as risk factors for BSI after HSCT (17.2%).

The present study is the first to analyze BSI after HSCT in pediatric patients with AA. Our data show a lower incidence of BSI in these patients (11.1%) compared with our previous study, but a higher incidence than that seen in patients with other diseases in that study. In our previous study, BSI was not associated with survival, and the survival rate was nearly identical in patients with BSI and those without BSI; however, in the present study, the survival rate was lower in patients with BSI. Patients with malignant diseases were included in the previous study, whereas only patients with AA were analyzed in the present study. In patients with malignant disease, the relapse rate of the original disease was lower in patients with BSI; this difference might account for the discrepant results between the previous and present studies.

In the present study, univariate analysis identified a history of immunosuppressive therapy with ATG, receipt of a transplant from an unrelated donor, frequent blood transfusions before HSCT, major or major plus minor ABO type mismatch, GVHD prophylaxis with tacrolimus but without CsA, and extended interval from diagnosis to HSCT as risk factors for BSI. Poutsiaka et al. [9] previously reported an association between BSI after HSCT and acute GVHD; however, our data do not corroborate this finding. Interestingly, the risk factors for BSI identified in the present study are associated with one another. Generally, patients without a related HLA-matched donor are treated with immunosuppressive therapy. If this therapy is not effective, then HSCT with an unrelated donor is performed. These patients often receive numerous blood transfusions and have a extended interval between diagnosis and transplantation. Furthermore, tacrolimus (rather than CsA) may be selected for GVHD prophylaxis. Among these factors, an extended interval between diagnosis and HSCT was the sole statistically significant risk factor for BSI identified by multivariate analysis.

Regarding numerous blood transfusions before HSCT, some recent studies have investigated the relationship

Table 4
Clinical characteristics of 158 patients who underwent HSCT from related donor as first HSCT and 165 patients who underwent HSCT from unrelated donor as first HSCT

	Related Donor (n = 158)			Unrelated Donor (n = 165)		
	BSI (n = 11)	No BSI (n = 147)	*P*	BSI (n = 26)	No BSI (n = 139)	*P*
Sex, male/female, n	6/5	84/63	1.000	10/16	77/62	.136
Age, yr, median	15	11	**.050**	11	11	.675
Age ≥14 yr, n (%)	8 (73)	45 (31)	**.007**			
Previous treatment, n (%)						
ATG	5 (45)	49 (33)	.512	25 (96)	110 (79)	**.050**
CsA	6 (56)	68 (46)	.756	25 (96)	124 (89)	.472
Both ATG and CsA	5 (45)	48 (33)	.509	25 (96)	107 (77)	.030
Bacterial/fungal infection at HSCT, n (%)	2 (18)	10 (7)	.198	1 (4)	7 (5)	1.000
Stem cell source, n (%)			.863			.734
BM	10 (91)	139 (95)		23 (88)	125 (90)	
PB	1 (9)	6 (4)		0 (0)	0 (0)	
BM + PB	0 (0)	1 (1)		0 (0)	0 (0)	
CB	0 (0)	1 (1)		3 (12)	14 (10)	
Donor, n (%)			1.000			
Syngeneic	0 (0)	1 (1)		-	-	
Related	11 (100)	146 (99)		-	-	
Receipt of ≥20 transfusions before HSCT, n (%)						
RBCs	2 (18)	10 (7)	.198	17 (65)	57 (41)	**.031**
Platelets	3 (27)	26 (18)	.425	17 (65)	58 (42)	**.032**
ABO type match, n (%)			.718			**.024**
Match	7 (64)	97 (66)		8 (31)	62 (45)	
Major mismatch	2 (18)	18 (12)		12 (46)	26 (19)	
Minor mismatch	2 (18)	20 (14)		3 (12)	29 (21)	
Major and minor mismatch	0 (0)	12 (8)		3 (12)	22 (16)	
HLA compatibility (low-resolution typing), n (%)			1.000			.248
Matched	9 (82)	122 (83)		21 (78)	95 (68)	
Mismatched	2 (18)	25 (17)		5 (22)	44 (32)	
Conditioning regimen, n (%)						
ATG	7 (64)	91 (62)	1.000	22 (81)	108 (78)	.602
Fludarabine	5 (45)	87 (59)	.528	20 (77)	109 (78)	.333
Cyclosphosphamide	10 (91)	138 (94)	.525	24 (92)	130(94)	.685
Irradiation	6 (56)	85 (58)	1.000	25 (96)	128 (92)	.693
GVHD prophylaxis, n (%)						
Steroid	0 (0)	4 (3)	1.000	0 (0)	10 (7)	.365
CsA	10 (91)	119 (81)	.690	1 (4)	20 (14)	.203
Tacrolimus	1 (9)	28 (19)	.690	25 (96)	119 (86)	.203
Methotrexate	10 (91)	131 (89)	1.000	25 (96)	132 (95)	1.000
Acute GVHD grade II-IV, n (%)	0 (0)	13 (9)	.601	5 (19)	37 (27)	.624
Graft failure, n (%)	0 (0)	5 (3)	1.000	5 (19)	7 (5)	**.024**
Mean time from diagnosis to HSCT, d	91	80	.426	474.5	455	.183
Severity at diagnosis, n (%)			.735			.664
Very severe	2 (18)	41 (28)		5 (19)	35 (25)	
Severe	5 (45)	65 (44)		10 (38)	42 (30)	
Nonsevere	4 (36)	41 (28)		11 (42)	62 (45)	
Severity at HSCT, n (%)			.490			.094
Very severe	5 (45)	59 (40)		12 (46)	35 (25)	
Severe	3 (27)	64 (44)		10 (38)	75 (54)	
Nonsevere	3 (27)	24 (16)		4 (15)	29 (21)	

*Irradiation included total body irradiation, thoracoabdominal irradiation, and total lymphoid irradiation.

between pretransplantation hyperferritinemia and post-transplantation outcomes [10,11]. In many of these reports, hyperferritinemia was associated with adverse outcomes after allogeneic HSCT. Moreover, iron overload is associated with proliferation of bacteria and fungus [12]. These observations suggest that iron chelating agents should be administered before HSCT in patients who have received frequent blood transfusions.

We analyzed BSI after HSCT in patients undergoing first transplantation from related and unrelated donors to clarify the risk factors for BSI. In both groups, survival rates were significantly lower in patients with BSI than in those without BSI. Surprisingly, the survival rate of patients undergoing HSCT from an unrelated donor without BSI exceeded 90%, not significantly different from that seen in patients undergoing HSCT from a related donor. This finding suggests that prevention of BSI is important to improving outcomes after HSCT. In patients who underwent HSCT from a related donor, age ≥14 years at transplantation was identified as a risk factor for BSI, although the incidence of BSI was evidently lower than that in patients undergoing HSCT from an unrelated donor. Older patients tend to have more severe oral mucositis. Furthermore, we previously identified age >10 years as a risk factor for fungal infection in patients with hematologic malignancies [13]. In contrast, in patients who underwent HSCT from an unrelated donor, variables associated with BSI included a history of immunosuppressive therapy with ATG, frequent transfusions before transplantation, graft failure, and major or major plus minor ABO type mismatch.

The impact of ABO incompatibility on clinical outcomes remains controversial [14,15]. ABO incompatibility in

allogeneic HSCT is associated with an increased risk of delayed erythroid reconstitution, pure RBC aplasia, and acute and delayed hemolysis; however, ABO incompatibility has not been identified as a risk factor for BSI. The ABO blood group antigens consist of oligosaccharide glycoproteins and are expressed in erythrocytes as well as in neutrophils, platelets, and vascular endothelial and epithelial cells. The ABO antigens could be immunologic targets for ABO-incompatible donor or recipient lymphocytes, thereby affecting GVHD and engraftment [16]. These phenomena may contribute to the development of BSI.

In conclusion, because the 5-year overall survival rate without BSI exceeded 90%, even in patients who underwent HSCT from an unrelated donor, controlling BSI is very important for a successful outcome of HSCT in patients with pediatric AA.

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Comparison of Continuous and Twice-Daily Infusions of Cyclosporine A for Graft-Versus-Host-Disease Prophylaxis in Pediatric Hematopoietic Stem Cell Transplantation

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Background. Cyclosporine A (CsA) is used widely for graft-versus-host disease (GVHD) prophylaxis in hematopoietic stem cell transplantation (HSCT); however, the optimal schedule of its administration has not been established. Although comparative studies of adult patients undergoing HSCT have demonstrated enhanced efficacy and safety of twice-daily infusion (TD) compared with continuous infusion (CIF) of CsA, to our knowledge, similar studies have not yet been performed in pediatric groups. ***Procedure.*** A self-administered questionnaire was used to retrospectively compare the clinical outcome and incidence of CsA-associated adverse events of 70 pediatric acute myelogenous leukemia patients who were receiving CsA by TD (n = 36) or CIF (n = 34) as GVHD prophylaxis for their first allogeneic HSCT. ***Results.*** The cumulative incidences of grade II–IV acute GVHD and chronic GVHD, as well as the overall survival and event-free survival rates, did not differ significantly between the TD and CIF groups; however, the incidence of severe hypertension was significantly higher in the CIF group than the TD group. ***Conclusions.*** The analysis presented here indicates that TD and CIF administration of CsA have similar prophylactic effect on pediatric GVHD and suggest that TD is associated with a lower rate of toxicity than CIF in pediatric patients undergoing HSCT. Pediatr Blood Cancer 2015;62:291–298. © 2014 Wiley Periodicals, Inc.

Key words: cyclosporine; graft-versus-host disease; hematopoietic stem cell transplantation; pediatric

INTRODUCTION

The immunosuppressive drug cyclosporine A (CsA), which is usually combined with short-term treatment with methotrexate (MTX), is used widely for the prophylaxis of graft-versus-host disease (GVHD). Traditionally, CsA is typically administrated intravenously in the early period after allogeneic hematopoietic stem cell transplantation (HSCT), after which the treatment is converted to oral administration [1].

Target CsA concentrations of 250–450 ng/ml are widely accepted for continuous infusion (CIF) of CsA [2]: however, these concentrations are not sufficient to prevent GVHD in adult patients undergoing HSCT. Although CIF of CsA at higher target concentrations (450–550 ng/ml) is more effective at preventing GVHD, these concentrations are associated with adverse effects, including hypertension and acute nephrotoxicity [3,4]. The immunosuppressive effect of CsA, which occurs via calcineurin inhibition, is concentration-dependent rather than time-dependent and its greatest pharmacodynamic effect occurs within the first 2 or 3 hr after exposure [5,6]. Hence, twice-daily infusion (TD) of CsA is used during renal, liver, and heart transplantation to reduce the occurrence of graft rejections [7].

TD administration of CsA with peak concentration monitoring has also been employed as an optimized GVHD prophylaxis regimen for adult patients undergoing HSCT [8–10]. However, the dose, target blood level, and mode of intravenous infusion vary

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among transplant institutions, and the optimal schedule of CsA administration has not yet been established. Furthermore, the comparative studies of the efficacy of various modes of CsA treatment have not yet been performed in pediatric HSCT despite of the wide use of both TD and CID modes. Therefore, the aim of this study was to evaluate the efficacy and safety of the TD and CIF modes of CsA administration for the treatment of pediatric HSCT. For this aim, we analyzed the data of pediatric patients with acute myelogenous leukemia (AML) as a single disease entity, which is one of the most popular pediatric hematological malignancies.

MATERIALS AND METHODS

Study Design and Data Collection

Using data for patients with AML provided by the Transplant Registry Unified Management Program (TRUMP) [11], which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the following criteria were used to select candidates for the self-administered questionnaire: (i) children with a diagnosis of AML who were younger than 18 years old; (ii) children in which allogeneic transplantation was performed for the first time during January 2006 and December 2009; (iii) children administered CsA for GVHD prophylaxis; and (iv) children administered CsA for more than 28 days after the first transplant. The data were extracted from the database in the Japan Society for Stem Cell Transplantation Registry and 99 cases from 58 institutions were selected as candidates. The questionnaire was distributed to gather additional information about the mode of CsA administration, the daily dose; the blood concentration of CsA; and CsA-associated adverse effects, including hypertension, renal toxicity, hyperglycemia, hyperbilirubinemia, thrombotic microangiopathy (TMA), hepatic veno-occlusive disease of liver (VOD), and encephalopathy. Of the 58 transplant institutions surveyed, 44 (75.9%) responded and data for 70 patients with AML were included in the study. This study was approved by the Data Management Committee of the Nationwide Survey of the JSHCT, and the institutional ethics committees of Kyoto University Hospital and Nagoya University Hospital.

Based on the recommendation outlined in a previous report [12], myeloablative conditioning (MAC) was classified as a regimen including at least 5 Gy of total body irradiation (TBI) as a single fraction, at least 8 Gy or TBI in fractionated doses, or oral or intravenous administration of busulfan at doses greater than 8 mg/kg. All other conditioning regimens were classified as nonmyeloablative reduced intensity conditioning (RIC). For transplantation using related bone marrow (BM) or peripheral blood (PB), or unrelated cord blood (CB), HLA matching was assessed using serological data for the HLA-A, HLA-B, and HLA-DR loci. For transplantation using unrelated BM, HLA matching was assessed using allelic data for HLA-A, HLA-B, and HLA-DRB1.

Endpoints

The primary endpoint of this study was to compare the cumulative incidences of grade II-IV and grade III-IV acute GVHD, and CsA-associated adverse events between the TD and CIF groups. Other endpoints were to compare the overall survival (OS) and event-free survival (EFS) rates, and the cumulative incidences of chronic GVHD, non-relapse mortality (NRM), and relapse between the TD and CIF groups. Acute and chronic GVHD was diagnosed and graded by the attending physicians of each hospital according to the consensus criteria [13,14]. Hypertension, renal toxicity, hyperglycemia, and hyperbilirubinemia were evaluated using the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4.0), and severe adverse events were defined as grade 2 and higher. Diagnosis of VOD, TMA, and encephalopathy were made based on characteristic clinical findings, positive laboratory data, or positive radiological findings by the attending physicians at each hospital.

Statistical Analysis

The characteristics of patients in the TD and CIF groups were compared using Fisher's exact test for categorical variables and two-sample Wilcoxon's test for continuous variables. OS and EFS rates were estimated using the Kaplan–Meier method [15], and the groups were compared using the log-rank test. The cumulative incidences of grade II–IV acute GVHD, chronic GVHD, NRM, and relapse were estimated, and the groups were compared using the log-rank test. Competing events were engraftment failure, relapse, or NRM without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. To determine prognostic factors associated with the development of grade II–IV acute GVHD and chronic GVHD, log-rank test and a Cox regression test were used. The following variables were examined in the univariate analysis: mode of CsA administration, patient age, sex match, stage of AML, HSCT type, ABO match, conditioning regimen, and CMV serostatus. Factors with $P < 0.2$ in log-rank tests were included in the Cox regression model. To determine prognostic factors associated with the development of severe hypertension, Fisher's exact test and a logistic regression test were used. The following variables were examined in the univariate analysis: mode of CsA administration, patient age, occurrence of grade I hypertension before HSCT, use of melphalan (Mel), use of $\geq$8 Gy of TBI, conditioning regimen, use of prednisolone or methylprednisolone for GVHD prophylaxis and/or treatment, and HSCT type. Factors with $P < 0.2$ in Fisher's exact tests were included in the logistic regression model. All statistical analyses were performed using Stata software (version 12; StataCorp, TX). The authors had full access to the data and assume responsibility for their integrity. The P values were two-sided and $P < 0.05$ was considered significant for all analyses.

RESULTS

Characteristics of the Patients

Of 70 pediatric patients with AML, 36 (51.4%) and 34 (48.6%) received TD and CIF of CsA, respectively. The characteristics of the patients and the associated clinical data are listed in Table I. Most of the patients received MAC (58 of 70 patients; 82.9%), and most underwent short-term treatment with MTX in combination with CsA (63 of 70 patients; 90.0%). Prednisolone was administrated to only two patients (2.9%). There were no significant differences between any of the baseline characteristics of the TD and CIF groups (Table I). The median time to switch to oral administration of CsA in the TD and CIF groups were 41 days (range, 20–73 days) and 36 days (range, 21–84 days), respectively. In the TD group, CsA was administrated over two (n = 15), three (n = 19), four (n = 1), or

TABLE I. Characteristics of the 70 Patients Included in the Study

Variable	TD (n = 36)	%	CIF(n = 34)	%	*P*-value
Recipient age (years), median (range)	9 (0–17)		10 (1–17)		0.120
Patient sex					
Male	20	55.6	20	58.8	0.813
Female	16	44.4	14	41.2	
Sex match					
Match	15	41.7	13	38.2	0.323
Male to female	10	27.8	5	14.7	
Female to male	7	19.4	13	38.2	
Missing	4	11.1	3	8.8	
Diagnosis					
M0	0	0	2	5.9	0.663
M1	5	13.9	7	20.6	
M2	8	22.2	8	23.5	
M3	0	0	1	2.9	
M4	5	13.9	3	8.8	
M5a	7	19.4	2	5.9	
M5b	0	0	1	2.9	
M6	1	2.8	1	2.9	
M7	7	19.4	6	17.6	
With MD	1	2.8	2	5.9	
Others	2	5.6	1	2.9	
De novo					
De novo	33	91.7	31	91.2	1.000
Secondary	3	8.3	3	8.8	
WBC at diagnosis (/μl), median (range)	19,700 (1,300–405,900)		9,750 (610–290,000)		0.428
Stage					
1CR	21	58.3	17	50.0	0.562
2CR	3	8.3	6	17.6	
NCR	12	33.3	11	32.3	
HSCT type					
MR-BM/CB	15	41.7	13	38.2	0.641
MR-PB	3	8.3	7	20.6	
MMR-BM/PB	4	11.1	4	11.8	
MU-BM	2	5.6	2	5.9	
U-CB	12	33.3	8	23.5	
ABO match					
Matched	23	63.9	18	52.9	0.811
Minor mismatched	4	11.1	6	17.6	
Major mismatched	4	11.1	4	11.8	
Major-minor mismatched	5	13.9	6	17.6	
Conditioning regimen					
MAC	31	86.1	28	82.4	0.750
RIC	5	13.9	6	17.6	
GVHD prophylaxis					
+MTX	34	94.4	29	85.3	0.153
+PSL	0	0	1	2.9	
+MTX, PSL	1	2.8	0	0	
CsA alone	1	2.8	4	11.8	
CMV serostatus					
Negative donor to negative patient	5	13.9	4	11.8	0.924
Positive donor to negative patient	2	5.6	2	5.9	
Negative donor to positive patient	6	16.7	4	11.8	
Positive donor to positive patient	12	33.3	15	44.1	
Unknown	11	30.6	9	26.5	
Follow-up (days), median (range)	700.5 (56–1,599)		567.5 (69–1,409)		0.282

MD, myelodysplasia; WBC, white blood cell; 1CR, first complete remission; 2CR, second complete remission; NCR, no complete remission; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; MTX, methotrexate; PSL, prednisolone.

five hours (n = 1). None of the patients underwent *in vivo* or *ex vivo* T cell depletion.

Treatment Outcome

The median follow-up duration was 590.5 days (range, 56–1599 days). The OS (Fig. 1A) and EFS (Fig. 1B) rates did not differ significantly between the TD and CIF groups. Furthermore, there were no significant differences in the cumulative incidences of grade II–IV acute GVHD (Fig. 1C) and chronic GVHD (Fig. 1D) between the TD and CIF groups. The differences in the cumulative incidences of grade II–IV acute GVHD or chronic GVHD were also not significant when the dataset was limited to patients treated with CsA and MTX (data not shown). There were no significant differences in the cumulative incidence of grade III–IV acute GVHD between the TD and CIF groups (grade III–IV acute GVHD at day 100: TD group, 0%; CIF group, 3.0 ± 3.0%; $P = 0.303$). The cumulative incidences of relapse and NRM did not differ

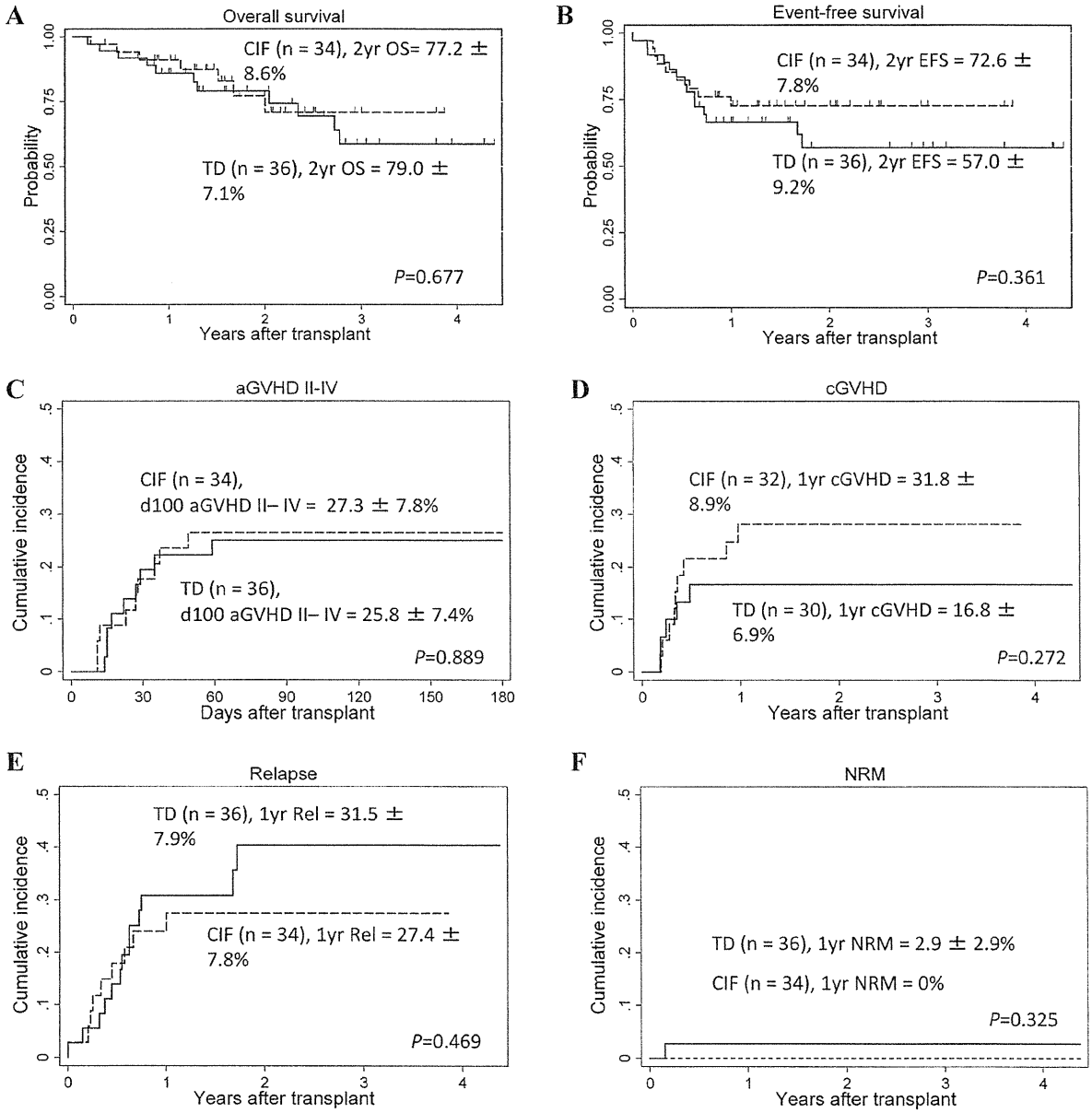

Fig. 1. The overall survival (**A**) and event-free survival (**B**) rates, and the cumulative incidences of grade II–IV acute GVHD (**C**) and chronic GVHD (**D**) among patients grouped by the mode of CsA administration. The cumulative incidences of relapse (**E**) and non-relapse mortality (**F**) among patients grouped by the mode of CsA administration. The solid and dashed lines indicate the TD and CIF groups, respectively.

significantly between the TD and CIF groups (Fig. 1E and F). Next, a univariate analysis was performed to evaluate the impact of potential confounding factors on the development of grade II–IV acute GVHD. Stage of AML, HSCT type, and CMV serostatus were identified as risk factors for grade II–IV acute GVHD; however, a multivariate analysis using a Cox regression test demonstrated that no independent risk factors were identified (Table II). For chronic GVHD, stage of AML, HSCT type, and conditioning regimen were identified as risk factors; however, a multivariate analysis demonstrated that no independent risk factors were identified.

Incidence of CsA-associated Adverse Events

The incidences of CsA-associated adverse events in the TD and CIF groups during the first 28 days after transplantation were compared. For each adverse event, patients who had grade 2 or higher toxicity before transplantation were excluded from the analysis. The incidence of severe hypertension was significantly higher in the CIF group than the TD group; however, the incidences of severe renal toxicity, hyperglycemia, and hyperbilirubinemia, TMA, VOD, and encephalopathy did not differ significantly between the two groups (Table III).

Univariate and Multivariate Analyses of Factors Related to the Development of Severe Hypertension

Univariate analysis was performed to evaluate the impact of potential confounding factors on the development of severe hypertension. As shown in Table IV, CIF administration of CsA, grade 1 hypertension before HSCT, the use of melphalan, and conditioning regimen were identified as risk factors for severe hypertension. A multivariate analysis using a logistic regression test was then performed to identify independent risk factors for the development of severe hypertension. CIF administration of CsA was identified as the sole independent significant risk factor.

Daily Doses and Trough Blood Concentration of CsA

In a previous study of adult patients receiving CsA, the incidence of grade II–IV acute GVHD was significantly higher and renal toxicity was significantly less frequent in the CIF group than the TD group [8]. In the adult study, patients in the TD group received a higher dose of CsA than those in the CIF group and the trough blood concentrations in these two groups were maintained at 150–300 ng/ml and 250–400 ng/ml, respectively [8]. To enable a direct comparison of the results, the daily doses and trough blood

TABLE II. Univariate and Multivariate Analyses of the Effects of Pre-transplantation Factors on the Incidence of Grade II-IV Acute GVHD in the 70 Patients Included in the Study

				Multivariate analysis	
Characteristics	Factors (n)	Grade II-IV acute GVHD	Univariate analysis *P*-value	Odds ratio (95% CI)	*P*-value
CsA mode	TD (36)	27.3 ± 7.8	0.889	1.02 (0.40–2.58)	0.973
	CIF (34)	25.8 ± 7.4			
Age group	0–9 (34)	21.2 ± 7.1	0.284	N.E.	N.E.
	10–17 (36)	31.4 ± 7.9			
Sex match	Match (28)	25.0 ± 8.2	0.294	N.E.	N.E.
	Male to female (15)	40.7 ± 12.9			
	Female to male (20)	27.8 ± 10.6			
	Missing (7)	0			
Stage	1CR (38)	15.8 ± 5.9	0.015	1.58 (0.95–2.63)	0.075
	2CR (9)	55.6 ± 16.6			
	NCR (23)	33.3 ± 10.3			
HSCT type	MR-BM/CB (28)	25.0 ± 8.2	0.011	1.00 (0.75–1.34)	0.987
	MR-PB (10)	0			
	MMR-BM/PB (8)	62.5 ± 17.1			
	MU-BM (4)	66.7 ± 27.2			
	U-CB (20)	21.0 ± 9.4			
ABO match	Matched (41)	20.6 ± 6.5	0.455	N.E.	N.E.
	Minor mismatched (10)	40.0 ± 15.5 %			
	Major mismatched (8)	37.5 ± 17.1			
	Major-minor mismatched (11)	27.3 ± 13.4			
Conditioning regimen	MAC (59)	28.1 ± 6.0	0.536	N.E.	N.E.
	RIC (11)	18.2 ± 11.6			
CMV serostatus	Negative donor to negative patient (9)	11.1 ± 10.5	0.159	1.19 (0.80–1.70)	0.390
	Positive donor to negative patient (4)	0			
	Negative donor to positive patient (10)	30.0 ± 14.5			
	Positive donor to positive patient (27)	42.3 ± 9.7			
	Unknown (20)	15.8 ± 8.4			

N.E., not evaluated; 1CR, first complete remission; 2CR, second complete remission; NCR, no complete remission; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood; MAC, myeloablative conditioning; RIC, reduced intensity conditioning.

TABLE III. The Incidences of Complications (≥grade 2 and ≥grade 3) in Patients Grouped by the Mode of CsA Administration

Complication	CsA mode	Cases	≥grade2	*P*-value	≥grade3	*P*-value
Hypertension	TD	36	2 (5.5%)	0.021	0 (0%)	0.010
	CIF	34	9 (26.5%)		6 (17.6%)	
Hyperglycemia	TD	36	3 (8.3%)	0.466	0 (0%)	0.225
	CIF	33	5 (15.2%)		2 (6.1%)	
Renal toxicity	TD	36	6 (16.7%)	0.261	1 (2.8%)	1
	CIF	34	2 (5.9%)		1 (2.9%)	
Hyperbilirubinemia	TD	36	1 (2.8%)	0.608	0 (0%)	0.478
	CIF	33	2 (6.1%)		1 (3.0%)	

concentrations of CsA were evaluated in the 70 patients included in this study during the first 28 days after transplantation.

No significant differences in the daily doses of CsA were observed between the TD and CIF groups at days 7, 14, 21, and 28 (Fig. 2A). The trough blood concentrations of CsA in the TD group at days 7, 14, 21, and 28 were 122.9 ± 68.1 ng/ml, 158.7 ± 71.5 ng/ml, 187.2 ± 102.5 ng/ml, and 190.6 ± 93.0 ng/ml, respectively. The corresponding concentrations in the CIF group were 294.8 ± 83.3 ng/ml, 350.9 ± 138.3 ng/ml, 335.8 ± 132.3 ng/ml, and 310.5 ± 119.0 ng/ml, respectively. At days 7, 14, 21, and 28, trough concentrations of CsA below 150 ng/ml occurred in 58.3%, 52.9%, 38.2% and 31.0% of patients in the TD group, respectively. Trough concentrations below 250 ng/ml occurred in 17.6%, 14.7%, 23.5% and 30.0% of patients in the CIF group at days 7, 14, 21, and 28, respectively. These data indicate that, compared with the CIF group, a significantly higher percentage of patients in the TD group were treated with a lower dose of CsA during the first two weeks after transplantation than that reported in a previous study of CsA administration to adults undergoing HSCT[8] ($P = 0.009$ and $P = 0.002$ at days 7 and 14, respectively) (Fig. 2B).

DISCUSSION

Because uncontrolled variables, such as patient age and underlying disease, may influence the incidence or severity of acute GVHD, it is necessary to evaluate the efficacy and safety of different types of GVHD prophylaxis within homogenous groups of patients. To achieve this aim, a nationwide survey was performed to select pediatric AML cases who had recently received their first allogeneic transplantation and had been treated with CsA for GVHD prophylaxis. Historically, CsA was administered to most pediatric patients via CIF; however, the mode of CsA administration in Japan has gradually shifted to TD over the last few years. Consequently, the 70 patients selected for inclusion in this study were divided approximately equally between the TD and CIF groups, which enabled a reliable comparison of the effect of CIF and TD

TABLE IV. Univariate and Multivariate Analyses of the Effects of Pre-transplantation Factors on the Incidence of Severe Hypertension (HT) in the 70 Patients Included in the Study

				Multivariate analysis	
Characteristics	Factors (n)	≥grade2	Univariate analysis *P*-value	Odds ratio (95% CI)	*P*-value
CsA mode	TD (36)	2 (5.6%)	0.022	7.99 (1.37–46.4)	0.021
	CIF (34)	9 (26.5%)			
Age group	0–9 (34)	6 (17.6%)	0.750	N.E.	N.E.
	10–17 (36)	5 (13.9%)			
Grade 1 hypertension before HSCT	Yes (65)	9 (13.8%)	0.173	6.26 (0.69–57.2)	0.173
	No (5)	2 (40.0%)			
Mel	Yes (31)	7 (22.6%)	0.196	0.35 (0.06–1.86)	0.217
	No (39)	4 (10.3%)			
TBI ≥8 Gy	Yes (43)	6 (14.0%)	0.739	N.E.	N.E.
	No (27)	5 (18.5%)			
PSL/mPSL for GVHD prophylaxis and/or treatment	Yes (14)	4 (28.6%)	0.212	N.E.	N.E.
	No (56)	7 (12.5%)			
Conditioning regimen	MAC (59)	7 (11.9%)	0.063	3.45 (0.56–21.4)	0.182
	RIC (11)	4 (36.4%)			
SCT type	MR-BM/CB (28)	2 (7.1%)	0.325	N.E.	N.E.
	MR-PB (10)	2 (20.0%)			
	MMR-BM/PB (8)	2 (25.0%)			
	MU-BM (4)	0 (0%)			
	U-CB (20)	5 (25.0%)			

N.E., not evaluated; Mel, melphalan; TBI, total body irradiation; PSL, prednisolone; mPSL, methylprednislone; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood.

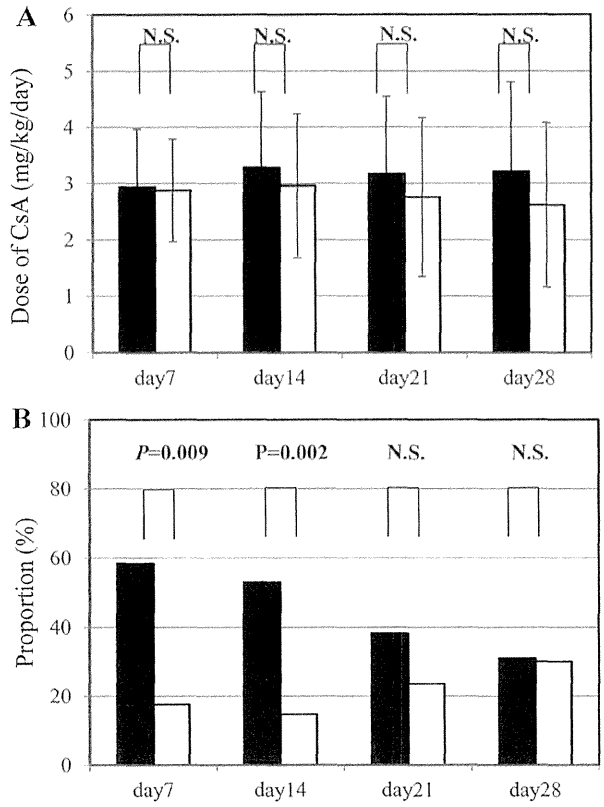

Fig. 2. (**A**) The daily doses of CsA administrated to patients in the TD (solid bars) and CIF (open bars) during the first 28 days after transplantation. (**B**) The percentages of patients in the TD (solid bars) and CIF (open bars) groups with trough concentration of CsA below 150 ng/ml (TD group) or 250 ng/ml (CIF group) during the first 28 days after transplantation. N.S., not significant. The data are presented as the mean ± SD.

administration of CsA among relatively homogenous pediatric populations.

In a previous study of adults undergoing HSCT, renal dysfunction was significantly less frequent in the CsA CIF group than the TD groups [8]. By contrast, in the current study, the incidences of CsA-associated adverse events, including renal dysfunction, were comparable in the TD and CIF groups. A possible explanation for the lack of increased renal dysfunction in the TD group observed here is that a large proportion of the pediatric TD patients (>50% in the first 14 days after transplantation) had trough concentration of CsA less than those reported in the adult study. By contrast, a significantly smaller proportion of pediatric patients in the CIF group had trough concentrations lower than those reported in the adult study. Alternatively, the pharmacokinetics and adverse effects of CsA may differ between pediatric and adult patients. Notably, CIF of CsA was identified as the sole independent risk factor for the development of severe hypertension, although TMA and encephalopathy, both of which are closely related to CsA-associated hypertension, occurred rarely in both the TD and CIF groups. Clinicopathological findings, as well as animal model studies, have indicated that CsA-induced acute reversible nephrotoxicity, caused by vasoconstriction of the afferent arterioles, might trigger the development of chronic irreversible damage to renal vessels, interstitial tubules, and glomeruli [16]. Furthermore, hypertension can persist long-term in some HSCT survivors [17], and the presence of multiple cardiovascular risk factors, including hypertension, is associated with an increased risk of late cerebrovascular disease and coronary artery disease after HSCT [18]. TD administration of CsA to pediatric patients undergoing HSCT may reduce the risk of late-occurring sequelae in long-term survivors.

Unlike a comparative previous study in adults [8], the analysis presented here fails to demonstrate the superiority of TD over CIF of CsA for the prevention of acute GVHD in pediatric patients undergoing HSCT. The lower incidence of acute GVHD in pediatric patients undergoing HSCT than adult patients undergoing HSCT, reported previously [19], may be related to similar efficiencies of different types of GVHD prophylaxis in children. Alternatively, it is possible that the peak concentrations of CsA did not reach levels sufficient to induce beneficial effects in a considerable proportion of the pediatric patients in the TD group. The limitations of this study include a retrospective analysis of small numbers of patients within the groups. Therefore, prospective randomized controlled studies are required to evaluate the efficiency and safety of TD administration alongside measurements of the peak concentration of CsA in pediatric patients undergoing HSCT.

In summary, this study demonstrates that TD is a potentially promising mode of CsA administration to pediatric HSCT patients, since the incidence of severe hypertension was lower in the TD group than the CIF group. Additional prospective studies of larger pediatric populations, including long-term follow-ups, are required to validate the efficacy and safety of TD administration of CsA.

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First-line treatment for severe aplastic anemia in children: bone marrow transplantation from a matched family donor *versus* immunosuppressive therapy

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ABSTRACT

The current treatment approach for severe aplastic anemia in children is based on studies performed in the 1980s, and updated evidence is required. We retrospectively compared the outcomes of children with acquired severe aplastic anemia who received immunosuppressive therapy within prospective trials conducted by the Japanese Childhood Aplastic Anemia Study Group or who underwent bone marrow transplantation from an HLA-matched family donor registered in the Japanese Society for Hematopoietic Cell Transplantation Registry. Between 1992 and 2009, 599 children (younger than 17 years) with severe aplastic anemia received a bone marrow transplant from an HLA-matched family donor (n=213) or immunosuppressive therapy (n=386) as first-line treatment. While the overall survival did not differ between patients treated with immunosuppressive therapy or bone marrow transplantation [88% (95% confidence interval: 86-90) *versus* 92% (90-94)], failure-free survival was significantly inferior in patients receiving immunosuppressive therapy than in those undergoing bone marrow transplantation [56% (54-59) *versus* 87% (85-90); $P<0.0001$]. There was no significant improvement in outcomes over the two time periods (1992-1999 *versus* 2000-2009). In multivariate analysis, age <10 years was identified as a favorable factor for overall survival ($P=0.007$), and choice of first-line immunosuppressive therapy was the only unfavorable factor for failure-free survival ($P<0.0001$). These support the current algorithm for treatment decisions, which recommends bone marrow transplantation when an HLA-matched family donor is available in pediatric severe aplastic anemia.

Introduction

Aplastic anemia is defined as peripheral blood pancytopenia caused by bone marrow failure; the pathogenesis of this disease is thought to involve autoimmune processes.[1-3] The principal interventions responsible for improved survival in aplastic anemia are bone marrow transplantation (BMT) and immunosuppressive therapy (IST). In children, BMT from an HLA-matched family donor (MFD) is the treatment of choice for severe aplastic anemia (SAA).[1,4-6] For children lacking an MFD, IST with a combination of antithymocyte globulin and cyclosporine has been used as a therapeutic option.[6-10] However, this treatment approach is based on the results of comparative studies between these therapies that were conducted mainly in the 1980s, and there have been few recent studies that compare the outcome of BMT recipients with comparable patients receiving IST.

The largest pediatric series in previous studies was reported by the European Group for Blood and Marrow Transplantation (EBMT) and included 304 children treated from 1970 to 1988; that study indicated survival was better following first-line BMT than after first-line IST (63% *versus* 48%; $P=0.002$) but did not compare failure-free survival after the two therapies.[6] Our previous analysis showed a significant advantage for patients receiving BMT from an MFD as first-line treatment in a study of 100 children with SAA who were treated between 1984 and 1998.[1] In patients who received first-line IST, 10-year overall and failure-free survival rates were 55% and 40%, respectively, both of which were markedly inferior to the rates in patients who initially underwent BMT, which was associated with 10-year overall survival and failure-free survival rates greater than 90%. Since the 1980s, the outcomes of both BMT and IST have improved, likely due to better supportive care and advanced treatment and transplantation protocols. A recently published Cochrane review regarding BMT from an MFD and IST as first-line treatment also pointed out that all studies included in the analysis

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had a high risk of bias due to their study design and were conducted more than 10 years ago and may not be applicable to the standard of care of today.[11] Updated evidence to aid treatment decisions in pediatric SAA is, therefore, required.

In children, the choice of an appropriate treatment is particularly influenced by the long-term sequelae of the disease and its therapy. Thus, failure-free survival is much more important than survival alone when analyzing the long-term outcomes of children with aplastic anemia. Lack of response, relapse, and clonal evolution are problematic in the IST setting, whereas graft failure, acute and chronic graft-*versus*-host disease (GVHD), and infectious complications limit the success of BMT. In the present study, we compared the outcomes of children with SAA who received IST or BMT from an MFD as first-line treatment using data from nationwide IST and BMT registries.

Methods

Patients

Between 1992 and 2009, a total of 599 consecutive children (younger than 17 years) with acquired SAA underwent BMT from an MFD or received IST as first-line treatment in Japan; 213 patients with an MFD underwent BMT and were registered in the Transplant Registry Unified Management Program (TRUMP) conducted by the Japanese Society for Hematopoietic Cell Transplantation, and 386 patients without an MFD were enrolled in two consecutive prospective multicenter trials (AA-92/97) conducted by the Japanese Childhood Aplastic Anemia Study Group and were initially treated with IST (Table 1). The disease severities were defined as previously reported.[12,13] Underlying inherited marrow failure disorders were excluded clinically and by chromosome fragility testing. Marrow cytogenetic studies were performed for all patients, and patients with clonal cytogenetic abnormalities were excluded from this study. Patients with paroxysmal nocturnal hemoglobinuria with clinical symptoms and positive findings on the Ham test/sucrose test were also excluded from this analysis. All treatments were performed after obtaining written informed consent from patients or their parents in accordance with the Declaration of Helsinki.

Immunosuppressive therapy and bone marrow transplantation procedures

The characteristics of the treatment procedures are detailed in Table 2. Three hundred and eighty-six patients were enrolled in the AA-92 (n=84) and AA-97 (n=302) trials, and all the patients were initially treated with a combination of antithymocyte globulin and cyclosporine A. Response to IST and disease relapse were evaluated as previously reported.[12] Transplantation data were collected with the use of standardized forms provided by the TRUMP. A total of 213 patients underwent BMT from an MFD as first-line treatment following the local protocols for conditioning regimens and GVHD prophylaxis. Patients who did not reach neutrophil counts >0.5×10^9/L for 3 consecutive days after transplantation were considered to have had primary graft failure. Patients with initial engraftment in whom absolute neutrophil counts subsequently declined to <0.5×10^9/L were considered to have had secondary graft failure. Acute and chronic GVHD were evaluated according to standard criteria.[14-16] More details on methods are provided in the *Online Supplementary Methods section*.

Statistical analyses

The date of analysis was July 30, 2012. Survival probabilities were estimated by the Kaplan-Meier method and compared between different groups of patients using the log-rank test. The influence of potential risk factors on overall survival and failure-free survival was assessed according to first-line treatment (BMT or IST), time period of treatment (1992-1999 or 2000-2009), age and other variables related to each treatment. Overall survival was defined as the time from diagnosis to death or last follow-up. Failure-free survival was defined as survival with treatment response. Death, primary or secondary graft failure, and secondary malignancy in the BMT group, and death, relapse, disease progression requiring stem cell transplantation (SCT) from an alternative donor or second IST, clonal evolution and evolution to paroxysmal nocturnal hemoglobinuria in the IST group were consid-

Table 1. Patients' characteristics.

	First-line treatment		
	BMT n=213	IST n=386	P
Age at diagnosis, year, median (range)	10 (0-16)	9 (0-16)	NS
Age at treatment, year, median (range)	11 (0-16)	9 (0-16)	NS
Gender			
Male / female	119/94	217/169	NS
Etiology, n. of patients (%)			
Idiopathic	204 (96)	312 (81)	<0.0001
Hepatitis	7 (3)	67 (17)	
Others	2 (1)	7 (2)	
Severity, n. of patients (%)			
Very severe aplastic anemia	–	227 (59)	–
Severe aplastic anemia	–	159 (41)	
Interval diagnosis-treatment, days, median (range)	84 (14-4605)	15 (1-180)	<0.0001
Time periods of treatment, n. of patients (%)			
1992-1999	121 (57)	155 (40)	0.0001
2000-2009	92 (43)	231 (60)	

BMT: bone marrow transplantation; IST: immunosuppressive therapy; NS: not significant.

ered treatment failures. For multivariate analyses, the Cox proportional hazard regression model was used. *P* values less than 0.05 were considered statistically significant. This study was approved by the institutional ethics committee of the Japanese Red Cross Nagoya First Hospital.

Results

Patients' characteristics

The characteristics of the 599 children are detailed in Table 1. The groups treated first-line with BMT (n=213) or IST (n=386) were similar with regards to age at diagnosis, age at treatment and male/female ratio. The majority of patients in both groups had a diagnosis of idiopathic disease, although the proportion of patients with non-idiopathic disease was higher in the IST group. Seven patients (3%) in the BMT group and 67 patients (17%) in the IST group suffered from hepatitis-associated disease. Nine patients had drug-induced or virus-associated disease. Information on the proportion of very severe disease was not available for 141 patients who underwent BMT because the severity of the SAA was not a required item for the registry. The clinical features of these patients were similar to those of the remaining patients. In the IST group, details regarding the severity of disease were provided for all patients: 227 (59%) had very severe disease and 159 (41%) suffered from severe disease. As expected, the time to treatment was significantly longer in the BMT group; the median interval between diagnosis and treatment was 15 days (range, 1-180 days) and 84 days (range, 14-4605 days) for those treated with IST and BMT, respectively. In accordance with decisions taken by the patients and the parents, ten patients underwent BMT more than 5 years after diagnosis. None of the patients who received IST before BMT from an MFD were included in the BMT group.

Table 2. Treatment characteristics.

Bone marrow transplantation	**213**
Conditioning regimen, n.	
High-dose CY (200 mg/kg) -based	158
CY ± low-dose irradiation	86
CY + ATG ± low-dose irradiation	72
FLU + CY (100-120 mg/kg) -based	44
FLU + CY ± low-dose irradiation	29
FLU + CY + ATG ± low-dose irradiation	15
Myeloablative	11
CY + TBI (10-12 Gy)	7
BU + CY	4
GVHD prophylaxis, n.	
CyA + MTX	174
CyA alone	23
Tacrolimus + MTX	6
Others	10
Immunosuppressive therapy	**386**
IST trial, n.	
AA-92	84
AA-97	302
IST regimen, n.	
CyA + ATG	140
CyA + ATG + G-CSF	246

CY: cyclophosphamide; ATG: antithymocyte globulin; FLU: fludarabine; TBI: total body irradiation; BU: busulfan; CyA: cyclosporine; MTX: methotrexate; G-CSF: granulocyte colony-stimulating factor.

Immunosuppressive therapy

Response to IST at 6 months was not evaluable in 11 patients for the following reasons: early death (n=7) or BMT from an alternative donor within 6 months of IST (n=4). The causes of the early deaths were sepsis (n=3), interstitial pneumonia (n=2), hemolysis of unknown cause (n=1) and accidental ingestion (n=1). Of the patients who underwent BMT from an alternative donor within 6 months, two patients died of graft failure or cardiac toxicity related to the preconditioning regimen. Overall, 238 of the 375 evaluable patients (63%) improved with first-line IST and achieved a partial response (n=151) or complete response (n=87) at 6 months. All of these patients achieved transfusion independence.

For all 386 patients who received IST initially, the 10-year overall survival rate was 88% [95% confidence interval (CI): 86-90], as shown in Figure 1A, and the median follow-up time for living patients was 106 months (range, 22-224 months). In contrast to the high rate of overall survival, the result regarding survival with response was unsatisfactory, the 10-year failure-free survival rate being 56% (95%

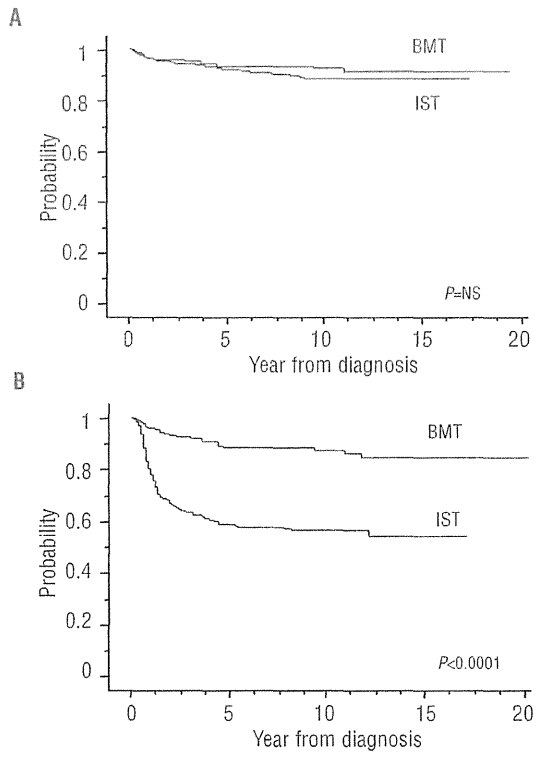

Figure 1. Survival of 599 children with severe aplastic anemia according to first-line treatments with immunosuppressive therapy (IST) (n=386) or bone marrow transplantation (BMT) (n=213). (A) Overall survival. The 10-year overall survival was 88% (95% CI: 86-90) in the IST group and 92% (95% CI: 90-94) in MFD BMT recipients (*P*=NS). (B) Failure-free survival. The 10-year failure-free survival was 56% (95% CI: 54-59) in the IST group and 87% (95% CI: 85-90) in the BMT group (*P*<0.0001).

CI: 54-59) (Figure 1B). The cause of treatment failure included death in 12 patients [due to intracranial hemorrhage (n=2), pneumonia (n=1), traffic accident (n=1) and sudden death (n=1) in addition to the seven early deaths], relapse in 23 patients, disease progression requiring second-line treatment in 109 patients, evolution to myelodysplastic syndrome in 15 patients, and appearance of paroxysmal nocturnal hemoglobinuria in two patients. After failed IST, a total of 113 patients underwent SCT from an alternative donor as second- or third-line treatment. The 10-year overall survival of these patients who received a transplant after failed IST was 79% (95% CI: 75-83) with a median of 435 days from diagnosis and SCT. We then analyzed the influence of potential risk factors for survival in the IST group. The prognostic significance of the clinical parameters is shown in Table 3. In the univariate analysis, age younger than 10 years at diagnosis was associated with a favorable overall survival rate [93% (95% CI: 91-95) *versus* 82% (95% CI: 78-86); *P*=0.012], and this was confirmed in a multivariate model. However, the rate of failure-free survival did not differ between patients in the two age groups. No other variables were significantly associated with survival after IST in either univariate or multivariate analyses.

Bone marrow transplantation

In the BMT group, 209 patients (98%) achieved primary engraftment at a median of 16 days after transplantation. As shown in Figure 1A and 1B, the 10-year overall survival and failure-free survival rates for all 213 patients who were treated initially with BMT from an MFD were 92% (95% CI: 90-94) and 87% (95% CI: 85-90), respectively. When the analysis was applied to the patients who underwent BMT within 180 days from diagnosis, similar results were observed; the 10-year overall survival and failure-free survival rates were 94% (95% CI: 92-96) and 89% (95% CI: 86-92), respectively. The median follow-up time for living patients was 101 months (range, 18-213 months). The cause of treatment failure included primary graft failure in two patients, secondary graft failure in ten patients, second malignancy in one patient, and death due to other complications in 12 patients. Although both patients without primary engraftment died, nine of the ten patients with secondary graft failure remain alive; eight were saved by a second transplant, and one recovered spontaneously. Twenty-five of 209 patients (12%) who had achieved primary engraftment developed grade II to IV acute GVHD, and extensive chronic GVHD was observed in 13 of 209 patients (6%) alive 100 days after BMT.

The prognostic significance of the clinical parameters, including variables related to transplantation, was then assessed. We found no association between age, gender, etiology, interval between diagnosis and BMT, or time period of treatment and treatment outcome (Table 3). Of particular interest with regards to conditioning regimens is the fact that the addition of antithymocyte globulin produced an improvement of overall survival [96% (95% CI: 92-99) *versus* 87% (95% CI: 84-91); *P*=0.021], whereas the rate of failure-free survival was comparable. A fludarabine-based regimen did not affect outcome after BMT from an

Table 3. Univariate analysis of 10-year overall survival (OS) and failure-free survival (FFS), according to first-line treatment.

	IST					BMT				
		OS		FFS			OS		FFS	
Variable	N. of patients	% (95% CI)	*P*	% (95% CI)	*P*	N. of patients	% (95% CI)	*P*	% (95% CI)	*P*
Age at diagnosis										
Younger than 10 years	219	93 (91-95)	0.012	57 (54-61)	0.754	89	95 (93-98)	0.163	92 (89-95)	0.200
10 years or older	167	82 (78-86)		55 (51-59)		124	90 (87-93)		84 (81-88)	
Gender										
Male	217	87 (84-90)	0.628	60 (56-64)	0.089	119	91 (87-94)	0.383	87 (83-90)	0.679
Female	169	90 (87-92)		52 (48-56)		94	94 (91-97)		88 (84-91)	
Etiology										
Idiopathic	312	88 (86-91)	0.661	54 (51-57)	0.185	204	92 (90-95)	0.568	87 (85-90)	0.934
Other	74	87 (83-92)		66 (60-71)		9	88 (76-99)		88 (76-99)	
Severity										
Very severe	227	90 (88-92)	0.600	57 (53-60)	0.965		–		–	
Severe	159	85 (82-89)		56 (52-60)						
Interval diagnosis-treatment										
Less than median days	187	91 (88-93)	0.537	60 (57-64)	0.170	105	95 (92-97)	0.322	91 (88-94)	0.362
Median days or more	199	86 (83-89)		53 (49-56)		108	90 (87-94)		85 (82-89)	
Time periods of treatment										
1992-1999	155	85 (82-88)	0.119	54 (50-58)	0.545	121	91 (89-94)	0.510	87 (84-90)	0.801
2000-2009	231	92 (90-94)		59 (56-63)		92	95 (93-98)		89 (85-93)	
Conditioning regimen										
With ATG		–		–		87	96 (92-99)	0.021	86 (83-90)	0.648
Without ATG						126	87 (84-91)		85 (82-89)	
GVHD prophylaxis										
CyA + MTX		–		–		174	93 (90-95)	0.924	88 (85-91)	0.809
Others						39	93 (88-98)		86 (80-93)	

ATG: antithymocyte globulin; CyA, cyclosporine; MTX, methotrexate.

MFD, although the number of patients treated with such regimens was too small to draw any conclusions. Multivariate analysis showed that none of the variables significantly influenced survival.

Survival and prognostic factors

The overall outcomes of the 599 children with SAA, stratified according to their first-line treatment, are shown in Figure 1A and 1B. Our data clearly showed a significant advantage for children receiving BMT from an MFD as first-line treatment; the failure-free survival was significantly superior in patients treated with BMT than in those in whom IST was used ($P<0.0001$), whereas the overall survival of patients in these two treatment groups did not differ. Figure 2A and 2B show survival curves in all patients treated in the two sequential time periods, 1992-1999 and 2000-2009: results were comparable over time [10-year overall survival: 88% (95% CI: 86-90) *versus* 93% (95% CI: 91-95); 10-year failure-free survival: 67% (95% CI: 65-70) *versus* 68% (95% CI: 66-71)], indicating no significant improvement in the last two decades. When age groups were considered, overall survival at 10 years in the younger group (<10 years old) was significantly better than that in the other age groups [93% (95% CI: 92-95) *versus* 85% (95% CI: 83-88); $P=0.007$], although no difference in failure-free survival was observed (Figure 3A and 3B). The favorable overall survival in the younger group may be mostly due to that observed in the first-line IST group. In multivariate analysis, age younger than 10 years at diagnosis was identified as a favorable factor for overall survival ($P=0.007$), and choice of first-line BMT from an MFD was confirmed as an independent favorable factor for failure-free survival ($P<0.0001$), as shown in Table 4.

Discussion

For children with SAA, BMT and IST have been accepted as standard treatments during the past three decades. The current guideline recommends BMT from an MFD as the treatment of choice for pediatric SAA[17-19] based on the results of comparative studies performed in the 1980s.[1,5,6,20,21] On the other hand, recent prospective studies with intensified IST for pediatric SAA have resulted in dramatic improvements in survival.[22,23] For example, a study from the EBMT showed a 100% overall survival rate at 6 years after first-line IST in 31 SAA patients younger than 20 years treated from 2002 to 2008.[22] These excellent overall survival results after IST have led to discussion about

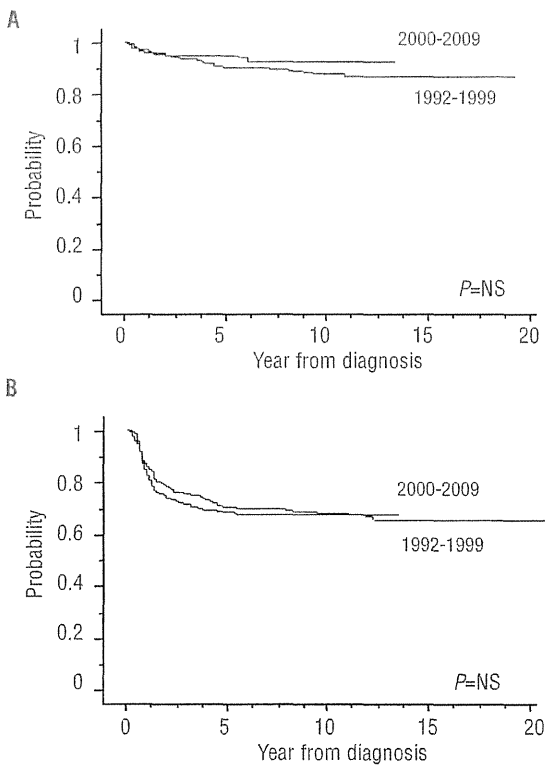

Figure 2. Survival of patients according to time periods of treatment: 1992-1999 (n=276) or 2000-2009 (n=323). (A) Overall survival. The 10-year overall survival was 88% (95% CI: 86-90) in 1992-1999 *vs.* 93% (95% CI: 91-95) in 2000-2009. (B) Failure-free survival. The 10-year failure-free survival was 67% (95% CI: 65-70) in 1992-1999 *vs.* 68% (95% CI: 66-71) in 2000-2009.

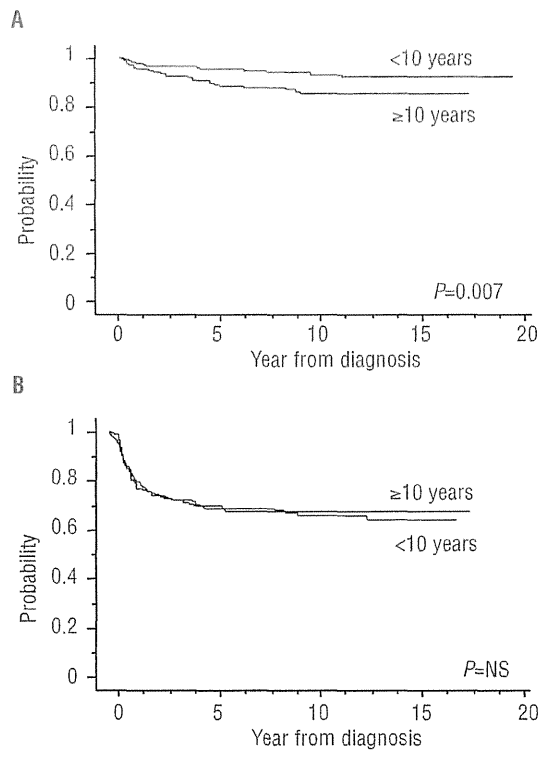

Figure 3. Survival of patients according to age at diagnosis: <10 years (n=308) or ≥10 years (n=291). (A) Overall survival. The 10-year overall survival in the younger group (<10 years) was significantly better than that in the other group [93% (95% CI: 92-95) *vs.* 85% (95% CI: 83-88); $P=0.007$]. (B) Failure-free survival. No difference in failure-free survival at 10 years was observed [67% (95% CI: 65-70) *vs.* 63% (95% CI: 59-67)].