Table I. Characteristics of patients with relapsed or refractory ALCL according to the receipt of autologous or allogeneic HSCT.

	Autologous	Allogeneic	*P*
Patients (*n*)	23	24	
Age at HSCT (years)			
Median	15	13·5	0·27
Range	7–18	3–18	
Sex			
Male	17	21	0·24
Female	6	3	
Stage at diagnosis			
I	1	0	0·36
II	3	4	
III	11	6	
IV	4	8	
Unknown	4	6	
Disease status at HSCT			
CR2/CR≥3	14/2	5/3	0·01
Non-CR	7	16	
Conditioning			
TBI/TLI based	7/1	17/1	0·06
Non-TBI based	15	6	
Stem cell source			
BM	3	13	
PB	20	5	
CB	0	6	
Donor			
MRD	–	7	
MUD	–	2	
MMRD	–	6	
MMUD	–	7	
Unknown	–	2	

HSCT, haematopoietic stem cell transplantation; CR, complete remission; BM, bone marrow; CB, cord blood; PB, peripheral blood; MRD, matched related donor; MUD, matched unrelated donor; MMRD, mismatched related donor; MMUD, mismatched unrelated donor; TBI, total body irradiation; TLI, total lymphoid irradiation.

six patients and peripheral blood in five patients. Seven patients had human leucocyte antigen (HLA)-matched related donors, and two patients received stem cells from HLA-matched unrelated donors. Thirteen patients had HLA-mismatched donors. Engraftment was observed in 21 (88%) cases, occurring at a median of 17 d. Two patients died of infection and one died of disease progression before engraftment. The 5-year cumulative incidence of relapse was 28% ± 10% (Fig 1A). Treatment-related death occurred in five patients; four patients died of infectious complications and one patient died of acute graft-*versus*-host disease (GVHD). The 5-year cumulative incidence of TRM was 25% ± 10% (Fig 1B). Acute GVHD of any grade occurred in 13 patients, nine of whom had grade II–IV GVHD. The 5-year OS and EFS rates were 54% ± 10% and 50% ± 10%, respectively (Fig 2A, B). Seven of 24 patients had multiple relapses before their HSCT; the 5-year EFS rates among patients with and without multiple relapses were 43% ± 19% and 53% ± 12%, respectively ($P = 0·67$). We observed 5-year EFS rates of 63% ± 17% and 44% ± 12% among patients with CR and those with non-CR respectively, at allogeneic HSCT (Fig 3B), which did not constitute a significant difference ($P = 0·13$).

At HSCT, CR was less common among allogeneic HSCT recipients than it was among autologous HSCT recipients ($P = 0·01$). However, there were no significant differences between the autologous and allogeneic HSCT patients in terms of cumulative incidence of relapse ($P = 0·25$), cumulative incidence of TRM ($P = 0·40$), 5-year OS ($P = 0·95$) or 5-year EFS ($P = 0·63$).

RIC regimens

Of the 24 patients in the allogeneic group, four underwent allogeneic HSCT using RIC. Their outcomes are shown in Table II. One of the four patients died of bacterial infection and the other three patients survived in CR without relapse after allogeneic HSCT. Interestingly, none of these three patients were in CR at HSCT.

Discussion

Currently, the efficacy and toxicity of HSCT are poorly defined for childhood cases of relapsed or refractory ALCL. Evidence is especially lacking in regards to the efficacy and toxicity of allogeneic HSCT. The present study included 23 patients who underwent autologous HSCT and 24 patients who underwent allogeneic HSCT. Each of the patients was a child or adolescent who had relapsed or refractory ALCL and underwent HSCT in Japan. This report comprises the largest cohort concerning allogeneic HSCT for relapsed or refractory ALCL in childhood.

The Berlin-Frankfürt-Münster (BFM) cohort had efficacies of autologous HSCT (77% OS and 59% EFS among 39 children with relapsed ALCL) that lie at or above the upper range of previously reported series (Woessmann *et al*, 2011). In national case series from the United Kingdom and France, one of six and nine of 15 patients stayed in continuous CR (Brugières *et al*, 2000; Williams *et al*, 2002; Woessmann *et al*, 2011). The Center for International Blood and Marrow Transplant Research (CIBMTR) has reported another large series of autologous HSCTs that were performed for ALCL, noting an EFS of 35% in 24 patients (Gross *et al*, 2010). Previously, we have reported a retrospective analysis of relapsed ALCL, which included 26 patients in Japan (Mori *et al*, 2006). Three of the eight patients who underwent autologous HSCT survived in continuous CR. In the current study, the 5-year OS rate, EFS rate and cumulative incidence of relapse among the 23 patients who underwent autologous HSCT were 51%, 38% and 49%, respectively. These results are similar to the findings of a previous CIBMTR report (Gross *et al*, 2010). In a study of 64 adult and paediatric cases of autologous HSCT for ALCL, Fanin *et al* (1999) reported that disease status at HSCT

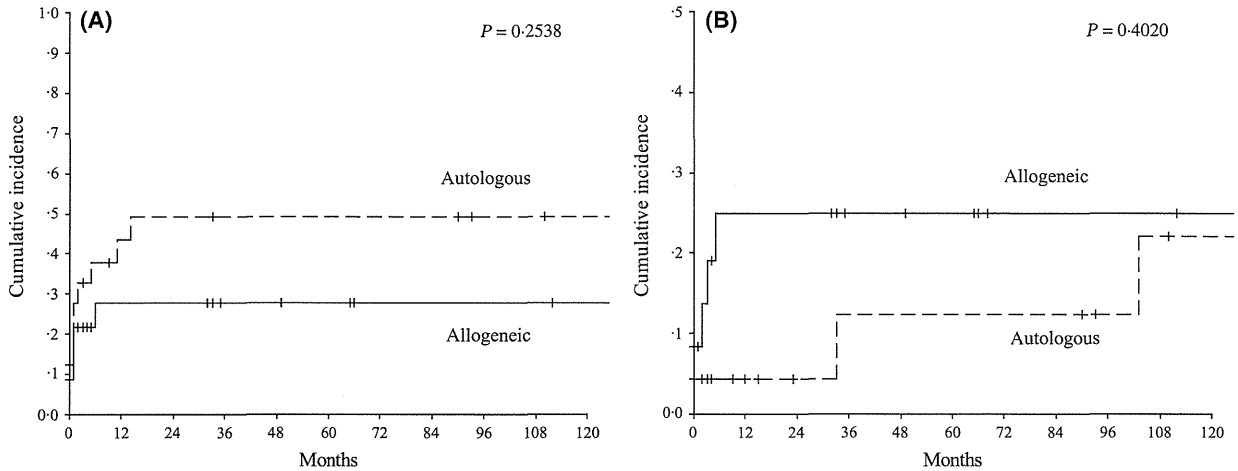

Fig 1. The cumulative incidence of relapse (A) and treatment-related mortality (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

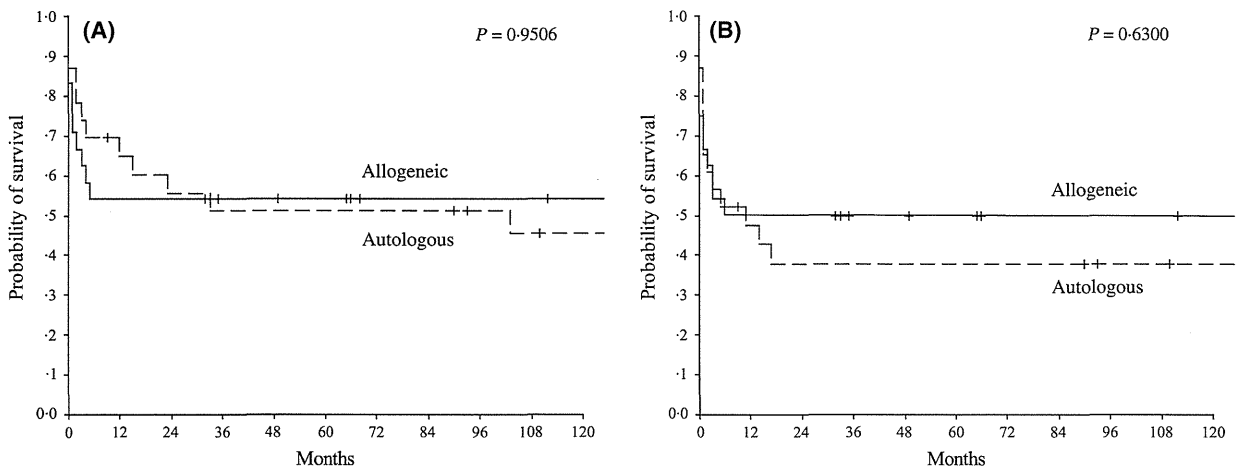

Fig 2. Overall survival (A) and event-free survival (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

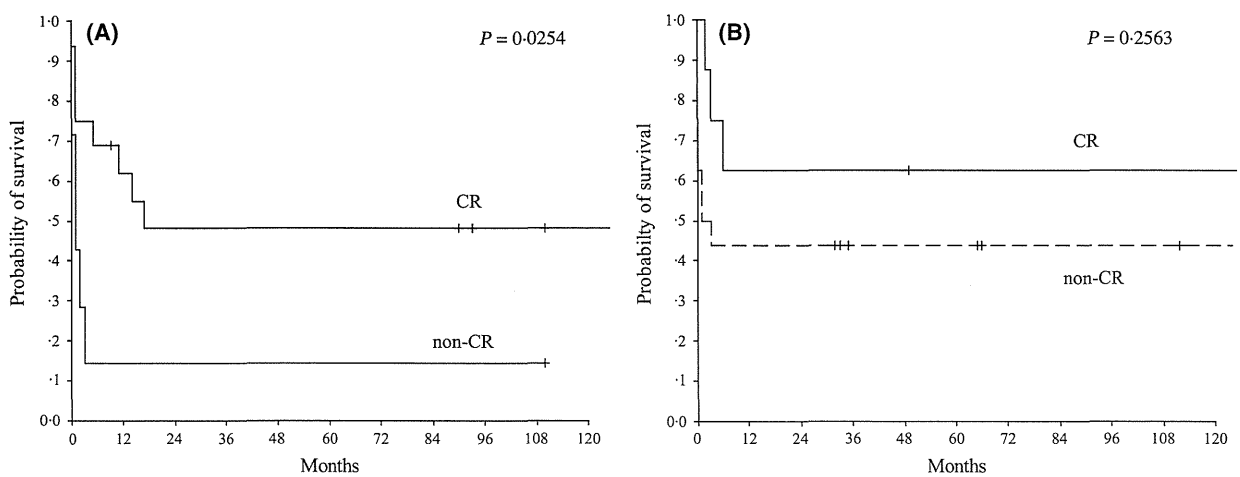

Fig 3. Event-free survival according to disease status at HSCT. (A) Autologous HSCT, (B) allogeneic HSCT. HSCT, haematopoietic stem cell transplantation; CR complete remission.

Table II. Details and outcomes of patients treated with reduced intensity conditioning and allogeneic HSCT.

Patients	Status at HSCT	Age at HSCT (years)	Donor	Stem cell source	Conditioning regimen	GVHD prophylaxis	aGVHD (Grade)	Extensive cGVHD	Outcome	Follow-up (months)
1	PR	3	UD	CB	TLI 2 Gy, Flu, Mel	Tac, MTX	III	–	CR	32
2	PR	9	UD	CB	Flu, Mel	Tac, MTX	II	–	CR	65
3	CR	18	UD	BM	Flu, Mel, ATG	Tac, MTX	0	NA	TRM	5
4	PR	16	UD	BM	Bu, Flu	Tac, MTX	III	+	CR	33

HSCT, haematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; UD, unrelated donor; BM, bone marrow; CB, cord blood; TLI, total lymphoid irradiation; Bu, busulfan; Flu, fludarabine; Mel, melphalan; ATG, antithymocyte globulin; GVHD, graft-*versus*-host disease; Tac, tacrolimus; MTX, methotrexate; aGVHD, acute GVHD; cGVHD, chronic GVHD; TRM, treatment-related mortality; NA, not applicable.

had predictive value for OS and EFS. In the current study, the EFS of the patients with CR at autologous HSCT was significantly higher than that of the patients with non-CR at autologous HSCT. Brugières *et al* (2000) reported that an interval of <12 months between diagnosis and relapse was associated with a higher risk of failure for the treatment of relapsed ALCL, including autologous HSCT. However, our cohort did not provide sufficient data to compare the risk of failure with the interval between diagnosis and relapse.

The role of allogeneic HSCT has not been defined for cases of childhood ALCL. The currently available evidence is limited to a few reports. The BFM group reported a series of 20 paediatric patients who underwent allogeneic HSCT for relapsed or refractory ALCL, finding a 75% 3-year EFS (Woessmann *et al*, 2006). Twelve of the patients in this study were in CR at HSCT. The CIBMTR has reported another large series of allogeneic HSCTs that were performed for ALCL, observing an EFS of 46% for 12 relapsed or refractory patients (Gross *et al*, 2010). Giulino-Roth *et al* (2013) also reported the cases of 13 paediatric patients with ALCL, eight of whom underwent autologous HSCT and five of whom underwent allogeneic HSCT. The OS and disease-free survival rates were 83% and 77%, respectively. Although our previous study noted that all six patients who underwent allogeneic HSCT during their second CR survived without further relapse (Mori *et al*, 2006), 5-year OS and EFS rates were limited to 54% and 50% in the present study. Patients who underwent allogeneic HSCT while in CR accounted for only eight of the 24 cases. Indeed, the rate of CR at HSCT was lower in the current study than in previous reports of allogeneic HSCT. In the present study, we found no significant difference in EFS according to disease status (CR or non-CR) at allogeneic HSCT. However, the low CR rate at allogeneic HSCT might be associated with the survival rate in the current study, which was lower than the rates noted in previous reports.

In the present study, we observed a 25% TRM rate among patients who underwent allogeneic HSCT for relapsed and refractory disease. Although the cumulative incidence of TRM for allogeneic HSCT was higher than that for autologous HSCT, the difference was not significant ($P = 0{\cdot}40$) (Fig 1B). Several investigations have shown that RIC followed by allogeneic HSCT has the potential to reduce TRM and long-term toxicity in cases of malignant and non-malignant diseases (Carella *et al*, 2000; Dreger *et al*, 2003; Jacobsohn *et al*, 2004; Bradley *et al*, 2007). The BFM cohort of allogeneic HSCTs included one case in which an RIC regimen was administered to a patient with ALCL. The RIC regimen comprised total lymphoid irradiation (2 Gy), fludarabine and melphalan (Brugières *et al*, 2000). Another case in which an RIC regimen [thraco-abdominal irradiation (2 Gy), fludarabine and melphalan] was used has also been reported (Ohta *et al*, 2010). Both of these patients survived in continuous CR following allogeneic HSCT. In the present study, four patients received an RIC regimen followed by allogeneic HSCT. Of these four patients, three were in non-CR at allogeneic HSCT, yet survived in CR for 32–65 months without relapse after HSCT. These results suggest that RIC for relapsed or refractory ALCL may be useful in cases involving allogeneic HSCT, regardless of disease status. However, there are only a few reports of allogeneic HSCT using an RIC regimen for paediatric ALCL. Further evaluations of the efficacy of RIC are necessary and should include larger numbers of patients and a prospective design.

The treatment of relapsed or refractory ALCL remains a matter of debate. Recent studies have reported the efficacies of second-line treatments for relapsed or refractory ALCL, including vinblastine monotherapy, brentuximab vedotin and crizotinib. Brugières *et al* (2009b) studied 36 paediatric patients treated with weekly vinblastine for relapsed or refractory ALCL, finding that this treatment was highly efficacious, with a CR rate of 83%. Furthermore, the 5-year EFS rate was 30%, at which time all but two of the patients had stopped vinblastine for more than 2 years. In adults, a phase II trial of brentuximab vedotin was conducted in patients with relapsed or refractory systemic ALCL. Fifty of 58 patients (86%) achieved an objective response, including 33 patients (57%) in CR (Pro *et al*, 2012). The Children's Oncology Group reported a phase I study of crizotinib for paediatric patients with refractory ALCL, finding that seven of nine children acheived CR following crizotinib monotherapy (Mossé *et al*, 2013). Autologous and allogeneic HSCTs are associated with high rates of toxicities and TRM. Consequently, it will be necessary to speculate about the selection of second-line treatments for relapsed or refractory ALCL in children and adolescents.

In conclusion, both autologous and allogeneic HSCT can offer the prospect of durable disease-free survival for relapsed and refractory ALCL in childhood and adolescence. Patients with CR at the time of autologous HSCT had significantly greater EFS than patients with non-CR at the time of autologous HSCT. Our results suggest that allogeneic HSCT might provide a better outcome for patients who are resistant to chemotherapy after relapse, and those with non-CR at the time of HSCT. Furthermore, an RIC regimen followed by allogeneic HSCT might even be useful for these patients. However, the small number of patients in our cohort prevented us from investigating the efficacy of allogeneic HSCT with an RIC regimen. In the new era of molecular target drugs, the best candidates for autologous and allogeneic HSCT remain to be clarified by further analyses and prospective studies of relapsed or refractory ALCL in childhood and adolescence.

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Author contributions

R Kobayashi, T Mori and R Fukano designed the research study; M Chin, H Goto, Y Takahashi, J Hara, YD Park, M Inoue, Y Koga, J Inagaki, H Sakamaki, S Adachi, K Kawa, K Kato and R Suzuki collected the data; R Fukano analysed the data and wrote the paper. All authors reviewed the manuscript.

Conflict of interest

There are no conflicts of interest to declare.

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Choreito Formula for BK Virus–associated Hemorrhagic Cystitis after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Therapy for BK virus (BKV)–associated hemorrhagic cystitis (BKV-HC) is limited after hematopoietic stem cell transplantation (HSCT). We examined whether choreito, a formula from Japanese traditional Kampo medicine, is effective for treating BKV-HC. Among children who underwent allogeneic HSCT between October 2006 and March 2014, 14 were diagnosed with BKV-HC (median, 36 days; range, 14 to 330 days) after HSCT, and 6 consecutive children received pharmaceutical-grade choreito extract granules. The hematuria grade before treatment was significantly higher in the choreito group than in the nonchoreito group ($P = .018$). The duration from therapy to complete resolution was significantly shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$). In 11 children with macroscopic hematuria, the duration from treatment to resolution of macroscopic hematuria was significantly shorter in the choreito group than in the nonchoreito group (median, 2 days versus 11 days; $P = .0043$). The BKV load in urine was significantly decreased 1 month after choreito administration. No adverse effects related to choreito administration were observed. Choreito may be a safe and considerably promising therapy for the hemostasis of BKV-HC after HSCT.

INTRODUCTION

Hemorrhagic cystitis (HC) is a severe complication in patients undergoing hematopoietic stem cell transplantation (HSCT), resulting in significant morbidity, such as nephropathy and renal failure, prolonged hospitalization, and prolonged blood transfusion requirement [1,2]. Effects on mortality have also been reported in children undergoing HSCT [3]. Early-onset HC occurs within 1 week after HSCT and is mostly a symptom of regimen-related toxicity. Late-onset HC usually occurs after engraftment and is associated with viral infections, including those caused by the human polyomavirus BK (BKV), polyomavirus JC, adenovirus (AdV), and cytomegalovirus (CMV) [4]. BKV is the most frequent cause of late-onset HC and affects 5.3% to 21.2% of children undergoing HSCT [5-9]. BKV viruria is detected by real-time quantitative PCR (RT-PCR) in all patients with BKV-HC. A BKV load of more than 10^6 copies/mL in urine may be associated with a high risk of developing HC after HSCT [5]. However, asymptomatic BK viruria is detected in 50% to 100% of patients after HSCT [5,7,10], implicating that the presence of BKV viruria alone does not explain the pathogenesis of HC. High BKV viremia ($\geq 10^3$ copies/mL) is a better predictor of BKV-HC after HSCT, with a reported specificity of 93% [8]. Children with high BKV viremia ($\geq 10^4$ copies/mL) are at a higher risk of developing severe HC [6].

The standard treatment for BKV-HC has not been established [2]. Supportive therapy is provided to patients with mild BKV-HC, including intravenous hydration, bladder irrigation, and symptomatic relief treatment, such as the use of analgesics. Patients with severe BKV-HC require additional therapy. The current first line BKV-oriented therapy is intravenous cidofovir; however, its efficacy remains controversial [2]. Alternative strategies include intravesical instillation of cidofovir [2,7], hyperbaric oxygen therapy [11], leflunomide, and fluoroquinolone [12]; however, their effect is limited [13]. Invasive intervention such as vascular embolization or cystectomy may be necessary in uncontrollable HC.

Choreito is a formula derived from Japanese traditional Kampo medicine. The indication for choreito in the context of

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Kampo medicine is "dampness-heat" in the lower abdomen, the characteristic symptoms of which include dysuria, heat in the lower abdomen, and thirst. All these symptoms may be caused by inflammation and blood clots in the bladder. Based on this indication, choreito has been administered to patients with acute simple cystitis and urolithiasis, and its effectiveness has been confirmed [14]. Recently, choreito was successfully used to treat massive gross hematuria with clot retention in the bladder in a child with refractory acute lymphoblastic leukemia [14]. At present, choreito is covered by the national health insurance and is widely used for genitourinary symptoms in Japan.

Symptoms leading to the traditional use of choreito appear to overlap with symptoms associated with BKV-HC; indeed, some children receive choreito for HC. In this study, we retrospectively analyzed BKV-HC in children undergoing HSCT and evaluated the efficacy of choreito treatment.

PATIENTS AND METHODS

Definition

HC was defined as microscopic (blood in urine graded 1+ or more) or macroscopic hematuria combined with dysuria, pollakisuria, urinary urgency, and/or the sensation of residual urine in the absence of bacteria in urine as observed by culture [9]. *BKV-HC* was defined as the association of HC with BKV viruria and/or viremia. HC was graded according to the widely used criteria [15]. Grade I is defined as microscopic hematuria, grade II as macrohematuria, grade III as macroscopic hematuria with clots, and grade IV as macroscopic hematuria with renal or bladder dysfunction. The onset of BKV-HC was defined as the first day when patients presented with urinary symptoms, and *complete resolution* (CR) of HC was defined as blood in urine (− or + for hemoglobin) and disappearance of dysuria, pollakisuria, urinary urgency, and the sensation of residual urine related to HC.

Patient Inclusion Criteria of BKV-HC and Choreito Administration

Among the children (≤18 years old) who received allogeneic HSCT between October 2006 and March 2014 in Nagoya University Hospital, 14 were diagnosed with BKV-HC and included in the study. Their medical records were retrospectively analyzed. Patient characteristics are listed in Table 1. Intravenous fluids corresponding to 2.5 to 3.0 L/m^2/day with forced alkalinized diuresis were administered during conditioning, and patients treated with cyclophosphamide received prophylactic mesna for the prevention of HC. All the patients received acyclovir for herpes prophylaxis and weekly intravenous immunoglobulin for viral prophylaxis. Tacrolimus was intravenously administered for graft-versus-host disease (GVHD) prophylaxis in patients receiving HSCT from an unrelated donor. Cases of engraftment syndrome and GVHD were treated by methylprednisolone, followed by salvage therapies in nonresponding patients. Six children with BKV-HC diagnosed after March 2013 received a pharmaceutical-grade medicine, choreito extract granules (Tsumura & Co., Tokyo, Japan) with a dose of .2 g/kg per os daily in 3 divided doses (maximum, 7.5 g/day). Cidofovir and choreito were administered at the onset of macroscopic hematuria. Because it is not currently approved for clinical use in Japan, cidofovir was administered only to those who provided written informed consent.

Quantification of BKV DNA

Children undergoing HSCT were weekly monitored for plasma CMV, human herpesvirus 6, and Epstein-Barr virus, and those who met the criteria for HC underwent additional viral workup, including analysis for BKV, polyomavirus JC, and AdV. For 2 patients with BKV diagnosed before December 2009, BKV had been detected in urine by qualitative PCR. This qualitative PCR could not detect BKV in patients without HC. After January 2010, viruses were monitored by multiplex RT-PCR for quantification of DNA from BKV, polyomavirus JC, and AdV, as described previously [16]. In April 2010, BKV RT-PCR was used to screen all 30 hospitalized children with various hemato-oncological diseases who had neither HC-related symptoms nor abnormal urinalysis. All patients provided informed consent for viral PCR workup in accordance with the Declaration of Helsinki. This retrospective analysis was approved by the ethics committee of Nagoya University Graduate School of Medicine.

Statistical Analysis

Statistical analysis was performed using the Fisher's exact test for categorical variables and the Mann-Whitney's U test for continuous variables. The Wilcoxon signed-rank test was used for paired samples. Odds ratios with confidence intervals were estimated by the logistic regression. A probability (P) value <.05 was considered to indicate statistical significance. All statistical analyses were conducted using JMP Pro 11.0.0 (SAS Institute Inc., Cary, NC).

RESULTS

BKV Screening in Hemato-oncological Patients without Genitourinary Symptoms

All children with hemato-oncological disorders hospitalized in the same ward were screened for BKV viruria for the purpose of surveillance. BKV viruria was detected in 5 (17%) of 30 hospitalized children with various hemato-oncological diseases who had neither HC-related symptoms nor abnormal urinalysis. The median urine BKV load in children with asymptomatic viruria was 1.3×10^6 copies/mL (range, 3.5×10^3 to 2.0×10^9 copies/mL), which was significantly lower than that in children with BKV-HC (median, 5.4×10^{10} copies/mL; range, 8.3×10^7 to 1.5×10^{11} copies/mL; $P = .0021$).

Patient Characteristics of Cases with BKV-HC after HSCT

Table 1 summarizes the patient characteristics of 14 children who underwent HSCT and later developed BKV-HC. In patients 1 and 2, BKV was detected in urine by qualitative

Table 1
Patient Demographics of BKV-HC after HSCT

UPN	Choreito Treatment	Age, yr	Sex	Diagnosis	Clinical Status	Preconditioning Regimen	Stem Cell Source	GVHD Prophylaxis
1	No	15.3	M	AA	Non CR	CY + ATG + TBI 5 Gy	UR-BM	FK + sMTX
2	No	16.0	M	AA	Non CR	FLU + CY + Campath + TBI 3 Gy	UR-BM	FK + sMTX
3	No	12.3	M	B-ALL	CR1	MEL + TBI 12 Gy	UR-BM	FK + sMTX
4	No	11.8	M	CML	CyCR	FLU + MEL + TBI 3 Gy	UR-BM	FK + sMTX
5	No	7.1	F	T-ALL	CR2	FLU + MEL + ATG + TBI 12 Gy	Haplo	FK + sMTX
6	No	5.7	M	NB	CR1	FLU + MEL + TBI 2 Gy	UR-CB	FK + sMTX
7	No	15.4	M	CMML	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
8	No	7.8	M	B-ALL	CR2	MEL + ATG + TBI 12 Gy	UR-BM	FK + sMTX
9	Yes	14.3	M	AA	Non CR	FLU + MEL + ATG + TBI 3 Gy	Haplo	FK + sMTX
10	Yes	5.4	M	MDS	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
11	Yes	10.1	F	AA	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
12	Yes	12.2	F	CMML	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX
13	Yes	6.8	M	B-ALL	CR2	MEL + TBI 12 Gy	UR-BM	FK + sMTX
14	Yes	7.5	M	MDS	Non CR	FLU + MEL + ATG + TBI 5 Gy	Haplo	FK + sMTX

UPN indicates unique patient number; M, male; AA, aplastic anemia; Cy, cyclophosphamide; ATG, antithymocyte globulin; TBI, total body irradiation; UR, unrelated; BM, bone marrow; FK, tacrolimus; sMTX, short course of methotrexate; FLU, fludarabine; Campath, alemtuzumab; ALL, acute lymphoblastic leukemia; MEL, melphalan; CML, chronic myelogenous leukemia; CyCR, cytological complete remission; F, female; Haplo, haploidentical transplant; NB, neuroblastoma; CB, cord blood; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome.

PCR; therefore, other agents including preconditioning could have contributed to HC. Six of the 14 children received choreito because of BKV-HC. All patients were older than 5 years (median, 11 years; range, 5.4 to 16 years). Antithymoglobulin or alemtuzumab was administered to 10 of 14 children (71%) as a preconditioning. Notably, all the children received total body irradiation with various doses.

Children were diagnosed with BKV-HC at a median 36 days (range, 14 to 330 days) (Table 2) after HSCT. Six of 14 patients (43%) had grade II to IV acute GVHD, and 11 of 14 (79%) received steroids for treatment of engraftment syndrome and/or acute GVHD before being diagnosed with BKV-HC. Three children with acute GVHD grade III or IV received intensified immunosuppressive treatment for steroid-resistant GVHD; 1 received infliximab and the other 2 received infliximab, basiliximab, and mesenchymal stem cells. All 3 responded well to additional therapy for acute GVHD. Concomitant AdV viruria was detected in 2 of 14 children (14%), and 12 of 14 children (86%) developed CMV and/or Epstein-Barr virus infection after HSCT. AdV titers in the urine were 2.6×10^8 copies/mL in patient 3 and 1.8×10^8 copies/mL in patient 7 at the time of diagnosis. CMV viruria was not detected in any of these 14 children when BKV-HC was diagnosed. Six children were receiving gancyclovir and/or foscarnet for CMV reactivation at the time of BKV-HC diagnosis.

Treatment for BKV Cystitis with Choreito

Six of 14 children with BKV-HC diagnosed after October 2013 received choreito (Tables 1 to 3). All 6 fulfilled the Kampo indication for receiving choreito ("lower energizer dampness-heat" in patients 9, 11, 12, 13, and 14, and "heat binding in the lower energizer" in patient 10). Patient characteristics, including age at HSCT, sex, underlying disease, engraftment syndrome, acute GVHD frequency and grade, immunosuppressive treatment, absolute lymphocyte count, antiviral therapy, duration of steroid use before the diagnosis of BKV-HC, and duration from HSCT to the onset of BKV-HC, did not differ significantly between the choreito group and the nonchoreito group (Tables 1 and 2). However, the hematuria grade at the time of diagnosis of BKV-HC was significantly higher in the choreito group than in the nonchoreito group ($P = .018$) (Table 2). Choreito was administered over a median of 5 days after the onset of symptoms related to BKV-HC (range, 2 to 16 days), and this interval was not statistically different from that of other treatments (median, 4 days; range, 1 to 23 days; $P = .43$) (Table 3). The urine BKV load before treatment amounted to a median of 2.6×10^{10} copies/mL (range, 1.3×10^9 to 6.3×10^{10} copies/mL) in children receiving choreito, which was not statistically different from that in those not receiving choreito (median, 3.4×10^{10} copies/mL; range, 8.3×10^7 to 1.3×10^{11} copies/mL; $P = .67$) (Table 3). Similarly, the BKV load in whole blood before treatment was not statistically different between the choreito and nonchoreito groups ($P = .24$, Table 3).

In all 14 children with BKV-HC, the duration from the start of therapy to CR as defined by disappearance of dysuria, pollakisuria, urinary urgency, and the sensation of residual urine was significantly shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$) (Table 3, Figure 1A): the odds ratio of choreito versus nonchoreito was .63 (95% confidence interval, .22 to .93; $P = .0031$). With regard to 11 children with HC graded ≥ II at the beginning of therapy, the administration of choreito

Table 2
Clinical Characteristics of Patients with BKV Cystitis

UPN	Engraftment Syndrome	Acute GVHD		ALC at the Diagnosis of BKV-HC ($\times 10^9$/L)	Steroid Use (d before BKV-HC)	Other Immunosuppressants	Onset of BKV-HC (d from SCT)	Hematuria (Grade)	Viruria (Urine log copy/mL)	CMV (Whole Blood log copy/mL)	Viral Infections	Antiviral Therapy at BKV-HC
		Stage	Grade									
1	+	-	-	4.7	14	-	35	II	BKV	0.0	CMV, EBV	GCV
2	-	skin 3	II	.3	24	-	65	II	BKV	3.1	CMV	PFA
3	+	-	-	.8	10	-	36	III	BKV (9.2), AdV (8.4)	0.0	CMV	PFA
4	+	skin 3	II	1	90	-	330	II	BKV (7.9)	0.0	CMV, EBV	PFA
5	+	skin 2, gut 1	II	.2	-	-	14	II	BKV (10.8)	2.6	CMV	-
6	-	skin 2, gut 3	III	1	10	INX	45	I	BKV (10.9)	0.0	-	-
7	-	skin 3, gut 2	III	.6	67	INX, BSX, MSC	86	I	BKV (11.1), AdV (8.3)	3.0	CMV, EBV	-
8	+	-	-	2	2	-	27	II	BKV (10.0)	3.2	CMV	-
9	-	-	-	.2	-	-	16	III	BKV (9.1)	2.9	EBV	-
10	+	skin 2, liver 4, gut 2	IV	.8	12	INX, BSX, MSC	25	III	BKV (9.2)	0.0	-	-
11	+	-	-	1.8	30	-	48	III	BKV (9.5)	0.0	CMV, EBV	GCV + PFA
12	+	-	-	.2	45	-	67	III	BKV (10.8)	2.7	CMV	GCV
13	-	-	-	1.3	-	-	21	III	BKV (10.7)	0.0	CMV	-
14	+	-	-	.5	6	-	26	I	BKV (10.7)	0.0	EBV	-

ALC indicates absolute lymphocyte count; SCT, stem cell transplanation; EBV, Epstein-Barr virus; GCV, gancyclovir; PFA, foscarnet; INX, infliximab; BSX, basiliximab; MSC, mesenchymal stem cell transplantation.

Table 3
Summary of Treatment for Patients with BKV Cystitis

UPN	Duration from Onset to Tx, d	Primary Tx for BKV	Hematuria Grade at Tx	Hematuria Grade ≤I (d from Tx)	CR (d from Tx)	Urine BKV Load before Tx (log copy/mL)	Plasma BKV Load before Tx (log copy/mL)	Urine BKV Load 1 mo after Tx (log copy/mL)	Plasma BKV Load 1 mo after Tx (log copy/mL)	Possible Complications
1	7	Cidofovir (5 mg/kg qwk ×2), hydration	II	11	17	N/A	N/A	N/A	N/A	None
2	4	Bladder irrigation, hydration	II	16	55	N/A	N/A	N/A	N/A	None
3	14	Cidofovir (1 mg/kg qwk ×2), hydration	III	28	66	9.2	0.0	6.5	3.8	Renal failure
4	4	Hydration	II	10	15	7.9	0.0	N/A	N/A	None
5	2	Hydration	II	5	16	10.8	0.0	N/A	N/A	None
6	1	Hydration	I	N/A	15	10.9	3.0	10.5	3.6	None
7	1	Hydration	I	N/A	15	11.1	0.0	N/A	N/A	None
8	23	Hydration	II	8	23	10.0	0.0	N/A	N/A	None
9	16	Choreito, cidofovir (1 mg/kg qwk ×11), hydration	III	4	6	9.1	4.0	8.7	4.6	None
10	5	Choreito	III	2	4	9.2	3.1	8.3	4.0	None
11	2	Choreito	III	2	16	9.5	0.0	7.8	0.0	None
12	4	Choreito	III	3	17	10.8	0.0	8.2	5.8	None
13	5	Choreito	III	2	7	10.7	5.0	4.4	0.0	None
14	16	Choreito	I	N/A	11	10.7	2.1	10.5	3.2	None

Tx indicates treatment; qwk, every week; N/A, not applicable or available.

significantly shortened the duration from the onset to BKV-HC grade ≤ I (median, 2 days; range, 2 to 4 days) in comparison with that in the nonchoreito group (median, 11 days; range, 5 to 28 days; $P = .0043$) (Table 3, Figure 1B). The duration from start of therapy to CR was also significantly shorter in the choreito group (median, 7 days; range, 4 to 17 days) than in the nonchoreito group (median, 20 days; range, 15 to 66 days; $P = .048$) (Table 3, Figure 1C): here, the odds ratio of choreito versus nonchoreito was .66 (95% confidence interval, .14 to .95; $P = .0058$).

Sequential Analysis of BKV Load after Choreito Treatment

BKV-HC–related symptoms improved significantly earlier in children receiving choreito, and we studied whether these earlier improvements were related to the clearance of BKV. The BKV load in urine and whole blood was monitored after the diagnosis of BKV-HC in children receiving choreito. The urine BKV load generally decreased over time. The median urine BKV load was 1.7×10^8 copies/mL (range, 2.6×10^4 to 3.1×10^{10} copies/mL) 1 month after BKV-HC diagnosis when all children had achieved CR, and they experienced a statistically significant decrease in BKV load since the time of diagnosis ($P = .031$; Wilcoxon signed-rank test for paired samples) (Table 3, Figure 2A). At the time of CR, only 1 of 6 children had a urine BKV load lower than 1.3×10^6 copies/mL, which was the median urine BKV load in children with asymptomatic viruria. The BKV load in whole blood appeared stable during the course of BKV-HC, and no significant decrease was observed a month after diagnosis ($P = .44$) (Table 3, Figure 2B).

All 6 children eventually finished taking choreito, and relapse of HC was not observed, except for in 1 patient who experienced relapse twice (patient 9). This patient was diagnosed with idiopathic aplastic anemia and received a bone marrow transplant from an unrelated donor; however, the graft was rejected and he underwent haplo-identical HSCT as the second HSCT. Because he developed chronic GVHD, he was administered prednisolone, which was increased during the exacerbation of chronic GVHD and which may have contributed to the prolonged elevation of the BKV load. Every time the patient had a relapse of BKV-HC, he was administered choreito, and his genitourinary symptoms resolved within a few days (Supplemental Figure 1).

Safety and Tolerability of Treatment

All children were able to take choreito per os. Notably, there were no adverse effects due to choreito intake, and renal function impairment was not observed in children receiving choreito (Table 3). The reported adverse effects of choreito include drug allergy and mild gastric discomfort [14], which were not observed in any of the children. In the nonchoreito group, 1 patient (patient 3) who received cidofovir for BKV infection developed impaired renal function, possibly resulting from renal toxicity of cidofovir and post-renal acute kidney injury due to clot retention.

DISCUSSION

Unlike its effect in immunocompetent patients, HC is life threatening in immunocompromised patients with hemato-oncological disease, particularly among patients undergoing HSCT [17]. To our knowledge, prospective studies of the treatment for BKV-HC are not available, and there are no standard treatment guidelines for post-HSCT HC. Treatment modalities are limited, particularly in children, partly owing to few reports on children receiving pharmaceutical and

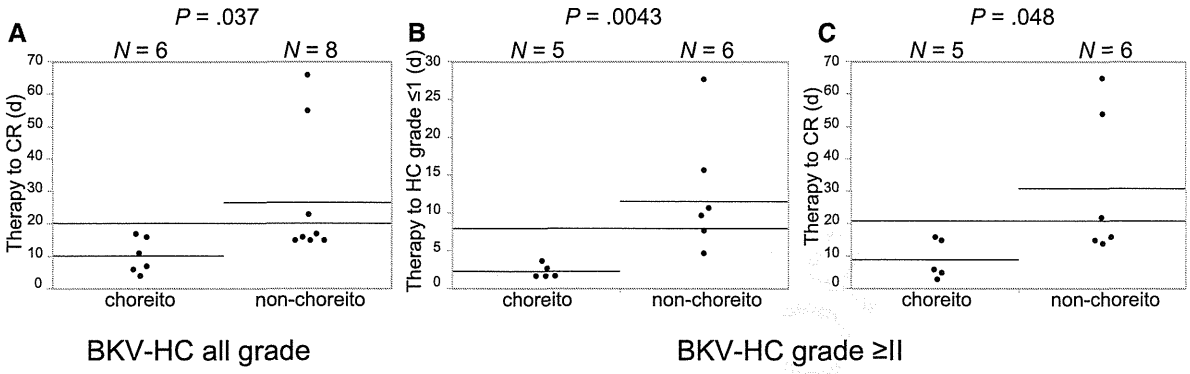

Figure 1. Comparison of choreito and nonchoreito treatment for BK virus-associated hemorrhagic cystitis (BKV-HC). The duration from the beginning of therapy to complete resolution (CR), as defined by the absence of dysuria, pollakisuria, urinary urgency, or the sensation of residual urine, was shorter in the choreito group (median, 9 days; range, 4 to 17 days) than in the nonchoreito group (median, 17 days; range, 15 to 66 days; $P = .037$) (A). When comparing children with HC graded ≥ II, the administration of choreito significantly shortened the duration from the onset to BKV-HC grade ≤ I (median, 2 days; range, 2 to 4 days) in comparison with that in the nonchoreito group (median, 11 days; range, 5 to 28 days) (B). The duration from start of therapy to CR was also significantly shorter in the choreito group (median, 7 days; range, 4 to 17 days) than in the nonchoreito group (median, 20 days; range, 15 to 66 days; $P = .048$) (C).

surgical treatments [4,18-20]. Intravenous hydration with forced diuresis is conducted; however, this is supportive treatment only without reliable efficacy.

At present, cidofovir is the only commercially available antiviral agent against BKV, and its efficacy for BKV-HC has been investigated only in retrospective studies [19-21]. In the report from the European Group for Blood and Marrow Transplantation, intravenous or intravesical cidofovir was administered to 62 patients with BKV-HC [21]. Of the 62 patients, 41 (66%) achieved CR and 8 (13%) had partial response after cidofovir treatment; however, no improvement or deterioration was observed in 12 patients (19%). CR is related to clearance of BK viremia in patients with BK viremia detected at the beginning of treatment, and the median time to clearance is 37 days (range, 7 to 102 days). Of 57 patients receiving intravenous cidofovir, 17 (30%) experienced renal toxicity. In a pediatric cohort, 19 children received cidofovir for BKV-HC grade ≥ II [19]. Macroscopic hematuria resolved in 15 (79%) after a median of 22 days (range, 9 to 63 days). In 1 patient, HC progressed to grade IV during cidofovir treatment. Notably, the baseline creatinine level appeared to be elevated after treatment. Another pediatric cohort included 12 children with BKV-HC treated by intravenous and/or intravesical cidofovir [20]. The median duration of symptoms was 25 days (range, 9 to 73 days) and no persistent nephrotoxicity was observed. Compared with cidofovir treatment, children treated with choreito treatment in our study experienced no impairment of renal function; all patients with BKV-HC achieved CR and BKV-HC resolved earlier.

Hyperbaric oxygen therapy is another alternative treatment for BKV-HC [11,22]. A retrospective study included 16 patients with BKV-HC grade ≥ II (5 patients under 19 years of age), 15 (94%) of whom achieved CR after a median of 17 days (range, 4 to 116 days) [11]. In a pediatric cohort of 10 children with BKV-HC grade ≥ II, 9 (90%) achieved CR after a median of 15 days (range, 10 to 37 days), including spontaneous resolution [22]. Hyperbaric oxygen is generally well tolerated; however, it requires a high-cost facility and adverse effects have been reported, including ruptured tympanum.

Other alternative therapies include leflunomide and fluoroquinolone antibiotics [12]; however, experience is limited, even in adults [13]. Few reports of leflunomide use in the setting of HSCT are available and its safety has not been

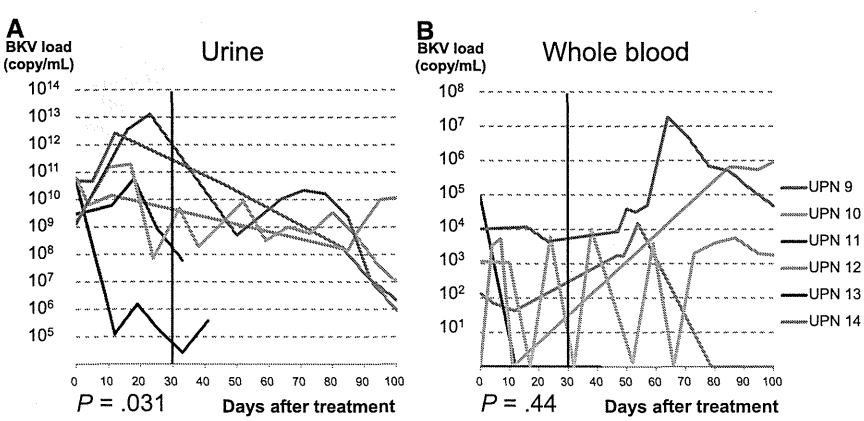

Figure 2. BK virus (BKV) load after choreito treatment. The BKV load before treatment amounted to a median of 2.6×10^{10} copies/mL in urine (range, 1.3×10^9 to 6.3×10^{10} copies/mL) and a median of 6.5×10^2 copies/mL in whole blood (range, 0 to 9.0×10^4 copies/mL). The median urine BKV load was 1.7×10^8 copies/mL (range, 2.6×10^4 to 3.1×10^{10} copies/mL) 1 month after BKV-HC diagnosis, and the BKV load had significantly decreased since the time of diagnosis (Wilcoxon signed-rank test, $P = .031$) (A). The BKV load in whole blood appeared stable during the course of BKV-HC, and no significant decrease was observed a month after diagnosis (Wilcoxon signed-rank test, $P = .44$) (B).

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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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.

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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Conflict of interest: Nothing to declare.

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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 1 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

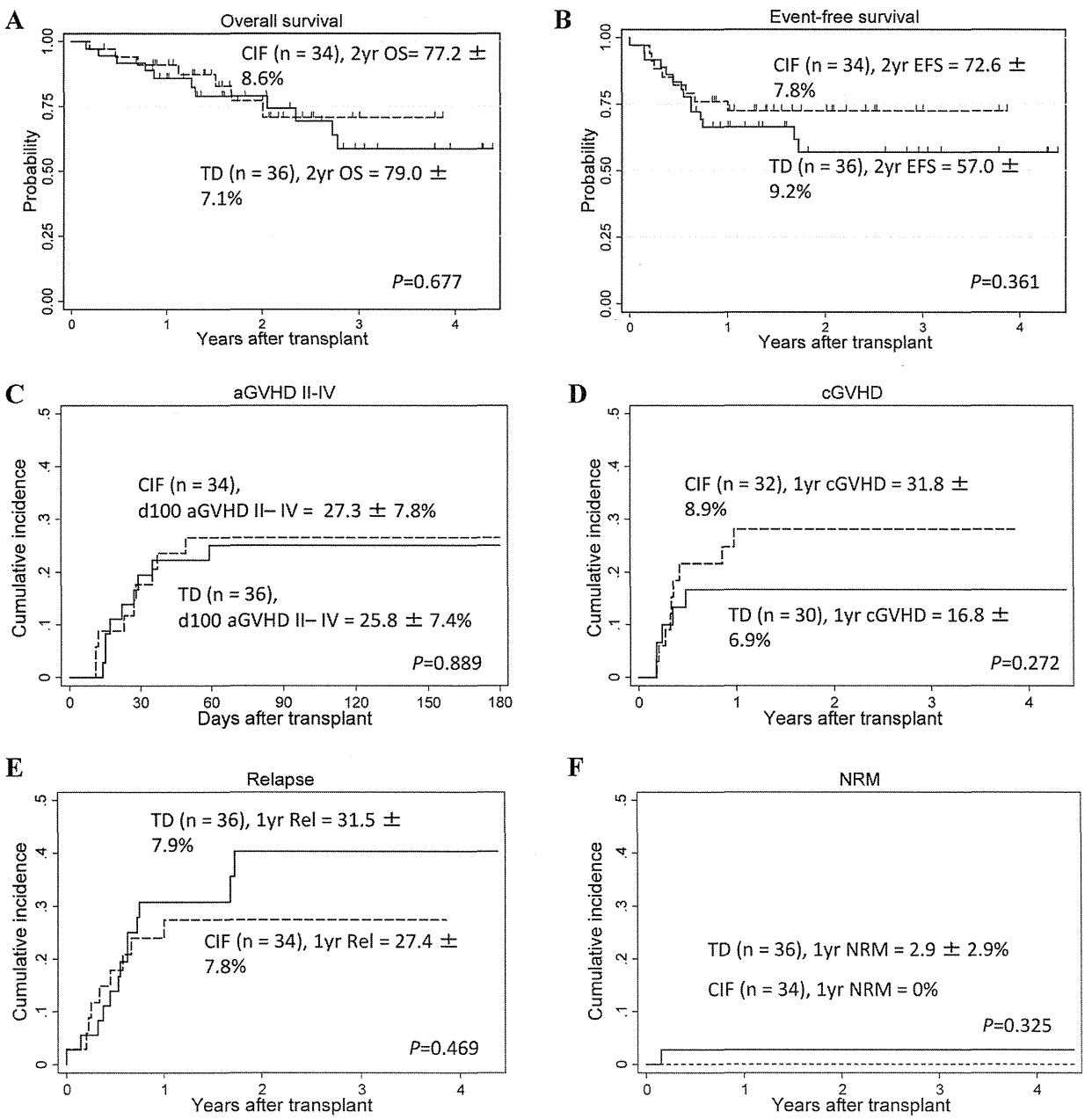

Fig. 1. The overall survival (**A**) and event-free survival (**B**) rates, and the cumulative incidences of grade II–IV acute GVHD (**C**) and chronic GVHD (**D**) among patients grouped by the mode of CsA administration. The cumulative incidences of relapse (**E**) and non-relapse mortality (**F**)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

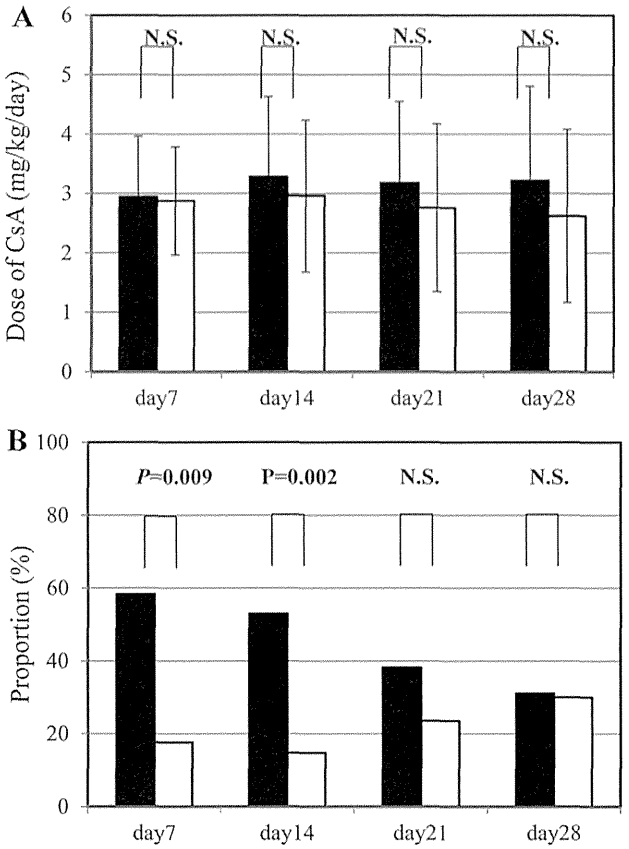

Fig. 2. (**A**) The daily doses of CsA administrated to patients in the TD (solid bars) and CIF (open bars) during the first 28 days after transplantation. (**B**) The percentages of patients in the TD (solid bars) and CIF (open bars) groups with trough concentration of CsA below 150 ng/ml (TD group) 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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