INTRODUCTION

Reactivation of varicella zoster virus (VZV) is a common event in patients undergoing hematopoietic cell transplantation (HCT) [1-5]. In HCT recipients, VZV reactivation frequently occurs as localized zoster and sometimes as disseminated cutaneous lesions resembling varicella with or without visceral involvement, which results in a high mortality rate. The most common complication associated with zoster in healthy individuals is chronic and often debilitating pain called postherpetic neuralgia (PHN), which can last for several yeas and may reduce quality of life. Although many previous studies have shown a high incidence of VZV reactivation after HCT, the incidence and risk of PHN in HCT recipients have not yet been clarified.

PATIENTS AND METHODS

Patients

To assess the incidence and risk factors associated with PHN after post-HCT VZV infection, we conducted a retrospective chart review of 418 consecutive patients who underwent HCT in Hokkaido Hematology Study Group (HHSG) between April 2005 and March 2007. HHSG is a multicentric clinical study group that includes "all" hematology departments in Hokkaido prefecture, consisting of 26 clinical groups of 19 institutes. VZV infection was defined by the appearance of typical cutaneous vesicular lesions or the detection of the VZV antigen. Information on pretransplant therapeutic exposures, HCT procedures, and posttransplant health complications was obtained via evaluation form. A total of 418 patients were included in this study. Patients characteristics are summarized in Table 1. Male/female ratio was 221/197, median age at HCT was 47 years (range: 0-69 years), autologous HCT/allogeneic HCT/syngeneic HCT ratio was 154/263/1, and median length of follow-up was 344 days (range: 3-1165 days). Short-term (up to 6 weeks) administration of acyclovir (ACV) or valacyclovir (VACV) has been widely used as prophylaxis against herpes simplex virus (HSV) in Japan. Duration of prophylactic ACV or VACV differed in each institution. The current Japanese medical insurance system only covers oral ACV at 1000 mg/day from HCT day −7 to day 35 in allogeneic HCT. In an autologous transplantation setting, duration of prophylactic antiviral drug administration varied from 0 to 239 days (median of 7 days), and in an allogeneic transplantation setting, duration varied from 10 to 189 days (median of 43 days). Duration of prophylactic antiviral drug administration was longer in an allogeneic HCT setting than in an autologous HCT setting ($P < .001$).

Table 1. Patients' Characteristics.

Male/Female:	221/197
Age (Median):	0-69/(47)
VZV Infection (+)/(-):	78/340
Hemotologica Disease:	
Acute Myelogenous leukemia	93
Acute Lymphoblastic leukemia	48
Myelodysplastic syndrome	30
Chronic myelogenous leukemia	10
Non-Hodgkin lymphoma	115
Severe aplastic anemia	14
Hodgkin lymphoma	10
Multiple myeloma	46
Plasma cell dyscrasia	8
Congenital disease	7
Solid tumor	17
Adult T cell leukemia	5
Secreloderma	5
Myeloproliferative disease	5
Juvenile myelomonocytic leukemia	3
Chronic lymphocytic leukemia	1
Chronic neutrophilic leukemia	1
Stem cell source:	
auto PBSCT	154
allogeneic	263
related BMT	38
related PBSCT	55
related CBT	2
unrelated BMT	95
unrelated CBT	73
syngeneic	1
Preparative regimen in allo- HCT	
CST/RIST	146/117

PBSCT indicates peripheral blood stem cell transplantation; CST, conventional stem cell transplantation; RIST, reduced intensity stem cell transplantation; CBT cord blood transplantation; BMT bone marrow transplantation; HCT, hematopoietic cell transplantation; VZV, varicella zoster virus

Diagnosis of Clinical VZV Infection

VZV infection was defined by the appearance of typical cutaneous vesicular lesions or the detection of VZV antigen. Localized zoster was defined as the presence of vesicular lesions in a dermatomal distribution. Disseminated zoster was defined as a generalized vesicular eruption that is identical to that of varicella. Visceral dissemination was defined as clinical evidence of internal organ involvement in the absence of other identified pathogens that might have accounted for the clinical syndrome. PHN was defined as dermatomal pain that persisted beyond rash healing.

Statistical Analysis

The incidence of VZV reactivation was calculated by the Kaplan-Meier method, and differences between groups were compared using the log-rank test. We performed univariate analysis for comparisons between different groups of patients or clinical data using the chi-square test and *t*-test, as appropriate. We performed multivariable logistic regression modeling with the forward stepwise method to assess which predictors independently contribute to prediction of PHN and to what extent using odds ratios with 95%

CI. All *P*-values were 2-sided, and a value of $P = .05$ was used as a cutoff for statistical significance. Patients who remained free of VZV infection after transplantation were censored at the time of their last follow-up or death from unrelated causes. A case with syngeneic transplantation was dealed with as an autologous transplantation. Analyses were done with Dr. SPSS for Windows (version 8.0.1J).

RESULTS

VZV Reactivation

Seventy-eight patients developed VZV infection after HCT (M/F = 36/42; median age, 48 [range: 3-68] years; auto/allo/syngeneic = 29/48/1). Sixty-two patients had localized zoster (single dermatome in 53, double dermatomes in 9), 12 patients had disseminated zoster (rash like chicken pox), and 4 patients had visceral zoster (involvement of the gastrointestinal [GI] tract). No VZV infection occurred during the period of prophylactic antiviral drug administration. All cases were treated with ACV or VACV, and there was no VZV infection-related death. The incidence of VZV infection in females (21.3%) was slightly higher than that in males (16.7%), but the difference was not statistically significant ($P = .22$). The incidences of VZV infection were not different between age groups. After resolution of VZV infection, VZV infection re-occurred in 5 cases (localized zoster in 4 cases, and visceral zoster in 1 case). Cumulative incidences of VZV infecion in allo-HCT and auto-HCT recipients were estimated to be 34% and 22%, respectively, at 2 years after HCT (log-rank $P = .23$) (Figure 1). In autologous HCT, 96.6% of the cases of VZV infection occurred during the first year after HCT, but in allogeneic HCT, only 75.5% of the cases of VZV infection occurred during the first year after HCT. The cumulative incidences of VZV infection in auto-HCT and allo-HCT recipients were not statistically different because the incidence curves crossed at 1 year after HCT. However, in an autologous setting, prophylactic usage of ACV was shorter (0-239 days, median of 7 days) than that in an allogeneic HCT setting (10-189 days, median of 43 days) ($P < .001$). Because no VZV infection occurred during the period of prophylactic antiviral drug administration, earlier onset of VZV infection in auto-HCT recipients may result from the shorter period of prophylactic antiviral drug usage. In an allogeneic setting, the rate of VZV infection in patients who underwent related donor bone marrow transplantation (rBMT) was lower than that in patients who underwent transplantations of stem cells of other sources (Figure 2).

Incidence of PHN

Twenty-seven (35%) of the 78 patients with VZV infection suffered PHN after resolution of VZV infection (M/F = 15/12; median age, 56 [range: 21-64] years; auto/allo = 13/14). Although incidences of VZV infection were not different between age groups, the incidence of PHN increased with advancement of age (Figure 3). Univariate analysis showed advanced age to be a risk factor in both allogeneic HCT and autologous HCT (Table 2). In allogeneic HCT, tacrolimus usage and graft-versus-host disease (GVHD) at onset of VZV infection were shown to be risk factors. There was no significant difference between the incidence of PHN in patients in whom antiviral therapy was initiated within 24 hours of clinical onset (no Tx delay) and that in patients in whom antiviral therapy was initiated after 24 hours from onset (Tx delay). Multivariate analysis showed that advanced age is the only risk factor in autologous HCT ($P = .0075$; OR = 1.14; 95% CI, 0.97-1.33). On the other hand, advanced age ($P = .0097$; OR = 1.06; 95% CI, 1.01-1.12), male sex ($P = .0055$; OR = 12.7; 95% CI, 1.61-100.1), and GVHD prophylaxis with a tacrolimus-based regimen ($P = .0092$; OR = 9.56; 95% CI, 1.44-63.3) were associated with increased risk of PHN in allogeneic HCT. Results of statistical analysis between autologous versus allogeneic HCT (data not shown), onset of VZV infection after HCT, gammaglobulin usage,

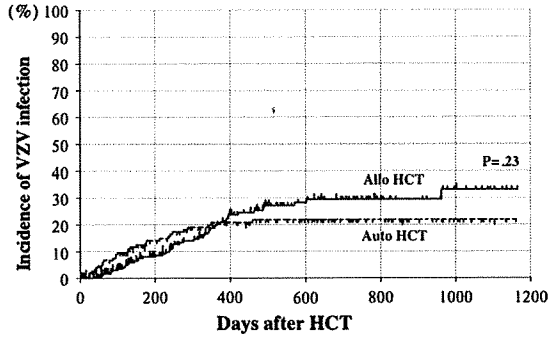

Figure 1. Incidence of VZV infection after HCT (auto versus allo).

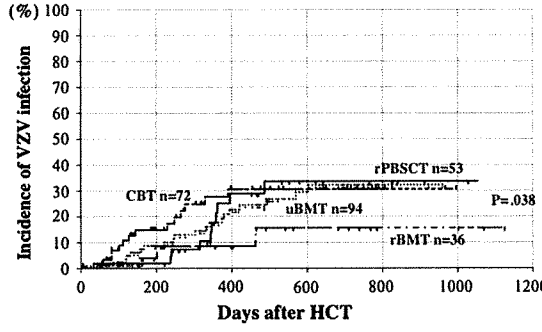

Figure 2. Incidence of VZV infection after HCT (stem cell source).

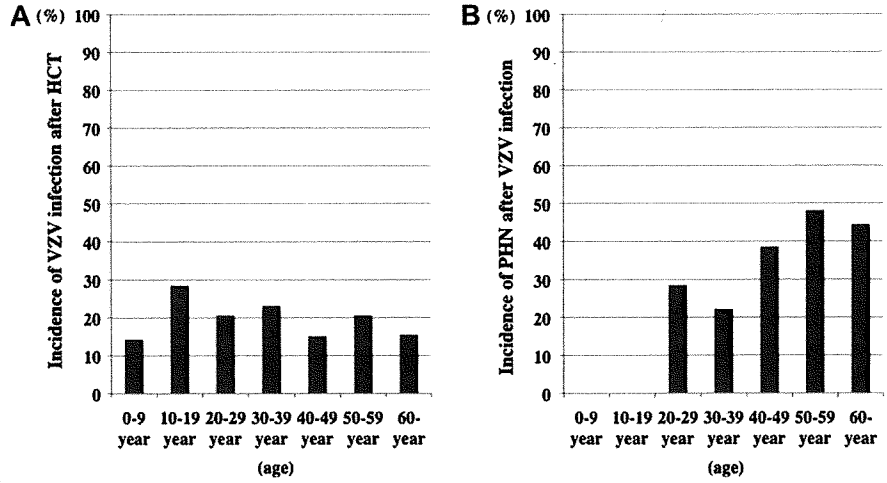

Figure 3. (A) Incidence of VZV infection after HCT. (B) Incidence of PHN after VZV infection.

localization of rash, conventional stem cell transplantation (SCT) versus reduced-intensity stem cell transplantation (RIST), stem cell source, and GVHD at onset of VZV infection were not significant.

DISCUSSION

Herpes Zoster after HCT

Reactivation of latent VZV, presenting as localized zoster or as disseminated infection, is a common and potentially serious complication in HCT recipients. Previous studies revealed that 23% to 60% of patients could be expected to develop VZV infection after HCT [1-4]. Analyses of risk factors such as allogeneic versus autologous transplant, GVHD, underlying disease, and pre-BMT irradiation have not revealed definitive associations [1,2]. In our study, age, sex, SCT versus RIST, and total body irradiation (TBI) did not show a definitive association with VZV infection after HCT. Tomonari et al. [6] reported a high risk of VZV infection after cord blood transplantation (CBT). In our series, CBT has a similar risk of VZV infection similar to that of other stem cell sources except related bone marrow, which showed a lower risk.

Cutaneous and Visceral Dissemination

VZV infection after HCT sometimes progressed to systemic infection. In previous series, 2% to 20% of the cases of VZV infection were disseminated zoster, and 3% to 5% of cases were visceral dissemination such as acute abdomen, pneumonitis, and central nervous system (CNS) involvement [1]. Risk of disseminated disease has not been studied in detail. Previously, we showed an association of preexisting (before onset of VZV infection) anti-VZV IgG titer and disseminated VZV infection [5]. In our previous observations, herpes zoster occurred despite prolonged existence of anti-VZV IgG after HCT. Recipients with lower preexisting VZV-IgG titer had higher viral copies in their serum at onset of VZV infection and tended to present as a disseminated disease. It is well known that cell-mediated immunity to VZV is a major determinant of the risk and severity of herpes zoster; however, decreasing humoral immunity after HCT might contribute to disseminated VZV infection.

Second Episodes of Herpes Zoster

Five patients (one autologous HCT recipient, 4 allogeneic HCT recipients) developed 2 episodes of VZV infection after HCT. The average interval between episodes was 9 months (range: 2-31 months). Four recipients had recurrence as localized zoster and 1 recipient had recurrence as visceral zoster (esophageal involvement). Two of the 5 patients with multiple VZV infections suffered PHN. In immunocompetent individuals, a second episode after resolution of zoster is quite unusual, but HCT recipients sometimes have plural episodes of VZV infection, indicating the failure to reconstitute VZV specific immunity because of prolonged insufficient immunity [1].

PHN

PHN is the most common complication of herpes zoster in immunocompetent as well as immunocompromised patients. Many patients develop severe physical and social disabilities as a consequence of their unceasing pain. Because the effect of treatment is disappointing once the syndrome has occurred, the importance of PHN-preventive strategies is widely recognized. HCT recipients apparently

Table 2. Risk Factors of Postherpetic Neuralgia

	Autologous HCT (n = 30)					Allogeneic HCT (n = 48)				
			Univariate	Multivariate				Univariate	Multivariate	
	no PHN (n = 17)	PHN (n = 13)	P value	Odds Ratio (95% CI)	P-value	no PHN (n = 35)	PHN (n = 13)	P-value	Odds ratio (95% CI)	P-value
Male/Female	9/8	7/6	.96			12/23	8/5	.089	12.7 (1.62-100.1)	.0055
Age (median)	8-61 (49)	44-64 (57)	.00023	1.14 (0.97-1.33)	.0075	3-68 (38)	25-60 (48)	.028	1.06 (1.01-1.12)	.0097
Onset of VZV infection after HCT <1year / >1year	16/1	13/0	0.37			27/8	9/4	.57		
Delay of ACV administration no Tx delay / Tx delay	7/9	7/5	0.45			14/21	7/6	.39		
Gammaglobulin usage (-) / (+)	8/5	8/4	0.79			18/10	9/4	.76		
Localization of rash Including face / no facial lesion	6/9	2/11	0.15			8/26	4/9	.61		
CST / RIST	—	—	—	—	—	18/17	6/7	.75		
Allogeneic stem cell source										
uBM/rBM/rPB/CB*	—	—	—	—	—	14/3/6/12	7/0/4/2	.32		
GVHD prophylaxis CsA base / FK base*	—	—	—	—	—	22/13	4/9	.047		
GVHD at onset of VZV infection (-) / (+)	—	—	—	—	—	18/17	3/10	.078	9.56 (1.44-63.3)	.0092

*uBM, unrelated bone marrow; rBM, related bone marrow; rPB, related peripheral blood; CB, cord blood; CsA, Cyclosporine A; FK, tacrolimus; CST, conventional stem cell transplantation ; RIST, reduced-intensity stem cell transplantation; HCT, hemotopoietic cell transplantation ; VZV varicella zoster virus; ACV, acyclovir; GVHD, graft-versus-host disease; PHN, postherpetic neuralgia; CI confidence interval.

seem to be at higher risk of PHN. Locksley et al. [7] observed PHN in 25% of HCT recipients, a much higher incidence than the expected incidence of about 9% in healthy individuals. Koc et al. [8] reported that PHN and peripheral neuropathy occurred in 43% of HCT recipients. There has been no report in which prevalence and precise risk of PHN after VZV infection in HCT recipients was described. Previous studies in immunocompetent individuals showed that advanced age, acute pain severity, presence of severe rash, rash duration before consultation, greater degree of sensory impairment, ophthalmic location, and psychologic distress were potential predictors of PHN [9,10]. Advanced age is the strongest risk factor in immunocompetent adults [11]. The incidence of PHN was reported to be much higher in immunocompetent patients over 60 years old [11]. In HCT recipients, the incidence of PHN was higher at a younger age than that in healthy individuals. This study for the first time clarified the risk of PHN in HCT recipients. The incidence of PHN in HCT patients of a younger age (age > 20 years) was higher than that in healthy individuals. Previous observations support the belief that a decline in cell-mediated immunity to VZV can lead to a higher incidence and greater severity of herpes zoster and PHN [12]. Because of severe cellular immunoincompetence, the risk of PHN is considered to be high in HCT recipients. In our series, the incidences of VZV infection were not significantly different in males and females, but the incidence of PHN was higher in males only in allogeneic HCT recipients. The effect of sex on risk of herpes zoster or PHN is controversial [11].

Prophylaxis

In our series, immediate antiviral drug administration did not reduce the incidence of PHN. A previous study in immunocompetent individuals showed that antiviral therapy reduced the severity and duration of herpes zoster, but did not prevent the development of PHN [13]. One factor potentially limiting the effect of antiviral agents on chronic pain is the fact that VZV replication occurs for several days or weeks before a rash appears and a diagnosis can be made. Therefore, to prevent PHN, we should prevent VZV reactivation itself. Some studies have shown successful long-term usage of ACV for prophylaxis of VZV infection after HCT. Administration of ACV for a period as long as that immunosuppressant usage and at least 1 year after HCT even at a low dose (ACV 200-400 mg daily) successfully reduced the incidence of VZV reactivation at 1 year after HCT, but VZV reactivation after cessation of ACV administration was still high (29%-32.1%) [14,15]. Another study showed that subclinical reactivation was important to reconstitute donor-derived

VZV-specific immunity [16]. It has been shown that in vivo reexposure to VZV antigens without clinical symptoms may boost immunity and thereby prevent subsequent symptomatic VZV reactivation [16]. Therefore, administration of ACV suppresses VZV reactivation, but at the same time might also suppress recovery of VZV-specific immunity by preventing contact of immune cells with the VZV antigen. Active immunization by a vaccine is theoretically reasonable; however, the current VZV vaccine used worldwide is a live attenuated vaccine, and, therefore, cannot be used in HCT recipients for a period within 2 years after transplantation [17]. Inactivated varicella vaccine may be useful for the early reconstitution of adaptive immunity to VZV after HCT [1,18]. A live attenuated vaccine does not work during antiviral drug usage, but inactivated vaccine can be administered during prophylactic antiviral drug usage. Therefore, 1 possible approach is to administer an inactivated VZV vaccine before the discontinuation of prophylactic antiviral drug administration. Appropriate duration of administration and dose of a prophylactic antiviral drug and appropriate timing of inactivated VZV vaccine administration must be studied prospectively.

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Increased number of $CD16^{+}CD56^{dim}$ NK cells in peripheral blood mononuclear cells after allogeneic cord blood transplantation

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ABSTRACT

In the present study, we investigated subpopulations of natural killer (NK) cells and the expression of stimulatory and inhibitory NK receptors after adult blood and bone marrow transplantation (BBMT) and cord blood transplantation (CBT). There were significant increases in $CD16^{+}CD56^{dim}$ cell proportion and in absolute number in peripheral blood mononuclear cells (PBMC) during a period of 4–9 months after CBT compared with these in normal PBMC, cord blood (CB), and in PBMC after BBMT. Also, increased numbers of $CD16^{+}CD56^{dim}$ NK cells were sustained in some patients until 4 years after CBT. This $CD16^{+}CD56^{dim}$ cell subset after CBT exhibited decreased expression of NKG2A compared with that in CB and increased expression of NKG2C. Purified $CD16^{+}CD56^{dim}$ cells from patients 8–9 months after CBT exhibited significantly higher levels of cytolytic activity against K562 than did purified $CD16^{+}CD56^{bright}$ cells and also whole PBMC. The $CD16^{+}CD56^{dim}$ cell subset with a high level of cytolytic activity significantly increased after CBT, and these cells may be responsible for NK cell–mediated immunity after CBT.

1. Introduction

Human natural killer (NK) cells are large granular lymphoid cells defined as being membrane $CD3^{-}$, $CD16^{+}$, and/or $CD56^{+}$ and account for approximately 10–15% of cells in lymphocytes [1–4]. The majority of adult peripheral blood NK cells are $CD16^{+}$ $CD56^{+}$, with a minor population of cells that are $CD16^{-}$ $CD56^{+}$. NK cells can attack target cells such as tumor cells and pathogen-infected cells without prior sensitization and without major histocompatibility restriction. It has been proposed that there are five stages of development of human NK cells from BM-derived human stem cells [4]. From stage 1 to 3, NK cells become committed to the NK cell lineage; stage 4 $CD56^{bright}$ NK cells may preferentially produce interferon-γ, and then stage 5 $CD56^{dim}$ NK cells may preferentially mediate cellular cytotoxicity.

Neonatal cord blood (CB) cells have been demonstrated to contain a higher percentage of NK cells [5], albeit immature with low level of cytolytic activity [6]. It has also been reported that cord blood contains $CD16^{+}56^{-}$ cells with low levels of cytolytic activity and that these cells are possible precursors of mature NK cells [7]. However, expression levels of perforin and granzyme B have been reported be higher in CB NK cells, and it has been suggested that CB NK cells are phenotypically and functionally mature [8].

Cord blood transplantation (CBT) has been increasingly used for the treatment of hematologic malignancies in adults [9–11]. It has been suggested that cord blood is a source of stem cells that is as safe and effective as bone marrow or mobilized peripheral blood.

Overall results for CBT recipients have been shown to be better than those for BMT recipients in terms of graft-versus-host disease (GVHD) because of the immaturity of T cells in cord blood [12], and results for CBT recipients are also potentially better in terms of transplantation-related mortality (TRM) and disease-free survival (DFS). Therefore, it was suggested that immunocompetent cells other than T cells may mediate the graft-versus-leukemia (GVL) effect after CBT. NK cells have an important role in the GVL effect after HLA-mismatched stem cell transplantation [13,14]. CBT is often carried out from HLA-mismatched donors, and NK cells may therefore contribute to the development of GVL after CBT. However, there is little knowledge about subpopulations of peripheral blood NK cells after allogeneic CBT.

In this study, we analyzed subpopulations of NK cells after adult blood and bone marrow transplantation (BBMT) and allogeneic CBT to characterize the development of NK cells after transplantation.

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Table 1
Patient characteristics and outcome after allo-cord blood transplantation

Age/Gender	Diagnosis	Conditioning	GVHD prophylaxis	aGVHD	cGVHD	Months/16^+56^{dim} (%)	Months	Outcome
33/F	MDSovert nonCR	Ara-C+CY+TBI (12)	FK+sMTX	III	0	4M/23.5 9M/15.2	61	Alive
65/M	MM PR	Flu+Bu+TBI (2)	CsA+sMTX	II	Limited	4M/19.6	57	Alive
48/M	AML (M4) CR2	Flu+Bu+TBI (4)	CsA+sMTX	III	0	7M/12.3	55	Alive
28/F	AML (M3) CR3	Flu+Bu+TBI (2)	CsA+sMTX	NE	NE	NE	2	Rejection
	Second CBT	CY+TBI (6)	FK	I	0	5M/4.0	55	Alive
52/M	AML(M5b) CR1	Ara-C+CY+TBI (12)	FK+sMTX	0	Limited	5M/29.4	42	Alive
51/F	MDSovert nonCR	Flu+Bu+TBI (8)	CsA+sMTX	0	Extensive	4M/39.3 8M/36.4	11	Dead (relapse)
26/F	PhALL CR1	Ara-C+CY+TBI (12)	FK+sMTX	0	Limited	5M/33.9 8M/44.5	23	Alive
29/F	AML(M4) CR2	Ara-C+CY+TBI (12)	FK+sMTX	I	Limited	6M/38.5 9M/26.6	21	Alive
40/F	ALL(L2) CR1	Ara-C+CY+TBI (12)	FK+sMTX	II	Extensive	4M/10.8	16	Alive

GVHD, graft-versus-host disease; NE, not evaluated.

2. Subjects and methods

2.1. Patients and blood samples

Fourteen healthy peripheral blood samples and 14 cord blood samples were obtained from Hokkaido Red Cross Blood Bank (Sapporo, Japan). Thirty peripheral blood samples from 11 patients after peripheral blood stem cell transplantation (PBSCT) or bone marrow transplantation (BMT) from serologic HLA-matched donors and 21 samples from 9 patients after CBT (Table 1) were obtained. Eight CBT patients underwent transplantation of serologic HLA four of six loci matching CB, and one patient underwent transplantion five of six loci matching CB. We obtained HLA-C serologic typing as possible. There were no group 1 HLA-C among cord blood units and no GVHD direction mismatch concerning about HLA-C group between cord blood units and recipients. One patient had relapsed with leukemia 8 months after CBT among our nine patients with hematologic malignancy who underwent CBT (relapse-free survival period, 8 to >61 months; mean, >38 months). Among these nine patients who underwent CBT, acute GVHD of grade I, grade II, and grade III developed in two, two, and two patients, respectively, and chronic GVHD of limited type and of extensive type developed in four and two patients, respectively. All patients achieved complete donor-type chimerism (>97% of donor type) within 3 months after CBT. Informed consent for the analysis of blood cells was obtained from all patients.

2.2. Immunofluorescent staining for flow-cytometric analysis and monoclonal antibodies

The following antibodies were used in this study: the phycoerythrin (PE)–conjugated monoclonal antibody (mAb) HP-3D9 (anti-CD94), obtained from Ancell (Bayport, MN); Z199 (anti-NKG2A), ON72 (anti-NKG2D), EB6 (anti-CD158a), GL183 (anti-CD158b), Z27.3.7 (anti-CD158e1, NKB1), Z25 (anti NKp30), Z231 (anti-NKp44), and BAB281 (anti-NKp46), obtained from Immunotec (Marseilles, France); 134591 (anti-NKG2C), obtained from R\&D Systems (Minneapolis, MN); and FITC-conjugated anti-CD16 and Cy5-conjugated anti-CD56, obtained from Becton Dickinson (San Jose, CA). Lymphocytes were gated using FSC and SSC. The three-color fluorescence intensity of the cells was analyzed using a FACS Calibur. $CD16^+56^{bright}$ cells and $CD16^+CD56^{dim}$ cells were sorted using JSAN (Japan-made sort analyzer, Bay bioscience, Kobe, Japan). Statistical analysis was performed using Student's *t*-test.

2.3. Evaluation of cytolytic activity of $CD16^+56^{bright}$ cells and $CD16^+CD56^{dim}$ cells

The cytolytic activities of unfractionated PBMC, purified $CD16^+56^{bright}$ cells, and $CD16^+CD56^{dim}$ cells were tested against ^{51}Cr-labeled human erythroleukemic K562 cells (5×10^3) using a 4-hour standard ^{51}Cr release assay (effector-to-target ratio, 10:1).

2.4. Long-term observation of $CD16^+CD56^{dim}$ NK cells after CBT

An additional 13 PBMC samples from six patients were analyzed during 12–50 months after CBT. All these patients were alive 20–50 months after CBT.

3. Results

3.1. Subpopulations and absolute number of NK cells after stem cell transplantation at engraftment phase

PBMC within 2 months after CBT contained higher proportion of $CD16^{dim}CD56^{bright}$ cells than those after BBMT and contained more $CD16^+CD56^{bright}$ cells than those in normal adult blood and cord blood and more $CD16^+CD56^{dim}$ cells than those in other blood (data

Table 2
Absolute number of natural killer cells after stem cell transplantation

n	$CD16^{dim}CD56^{bright}$	$CD16^+CD56^{bright}$	$CD16^+CD56^{dim}$
Within 2 months			
BBMT 11	88 ± 135	86 ± 151	14 ± 17
CBT 8	101 ± 65	81 ± 44	29 ± 23
At 4–9 months			
BBMT 19	55 ± 31	118 ± 62	59 ± 47
CBT 11	130 ± 128[b]	345 ± 404[b]	771 ± 455[a]

BBMT, blood and marrow stem cell transplantation; CBT, cord blood transplantation.
Values indicate absolute numbers of indicated marker-expressing cells (/μl). Significant differences were found in values 4–9 months after CBT compared with values after BBMT (blood and marrow stem cell transplantation) and CBT.
[a] $p < 0.01$.
[b] $p < 0.05$.

Table 3
Inhibitory natural killer cell receptors on $CD16^+CD56^{dim}$ cells 4–9 months after transplantation

n	CD94	NKG2A	CD158a	CD158b	CD158e1
Normal PB 14	64.5 ± 12.8[b]	23.2 ± 15.4	9.8 ± 8.5	22.4 ± 15.3[b]	12.3 ± 8.0
CB 14	73.0 ± 11.0	49.4 ± 10.4[a]	16.5 ± 10.0[b]	23.6 ± 9.2[a]	7.8 ± 6.1[a]
BBMT 19	74.3 ± 19.1	29.2 ± 18.1[b]	10.1 ± 8.3	25.0 ± 12.1[b]	20.2 ± 14.3
CBT 13	76.3 ± 9.3	16.1 ± 15.3	9.2 ± 5.8	34.6 ± 8.2	19.5 ± 10.7

BBMT, blood and marrow stem cell transplantation; CB, cord blood; CBT, cord blood transplantation; PB, peripheral blood.
Values indicate percentage of indicated marker-expressing cells. Significant differences were found in values after CBT compared with normal peripheral blood mononuclear cells and CB cells and with values after BBMT and CBT.
[a] $p < 0.01$.
[b] $p < 0.05$.

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Table 4
Stimulatory natural killer cell receptors on CD16$^+$CD56^{dim} cells 4–9 months after transplantation

n	NKG2C	NKG2D	NKp30	NKp44	NKp46
Normal PB 14	22.1 ± 19.2	25.1 ± 20.3[a]	4.6 ± 4.3	0.6 ± 0.6	15.7 ± 10.9
Cord blood 14	8.3 ± 6.1[a]	66.4 ± 17.1[a]	17.3 ± 13.8[a]	0.7 ± 0.6	38.0 ± 22.8
BBMT 19	18.5 ± 14.9[a]	69.1 ± 14.3[a]	4.0 ± 3.4	0.6 ± 0.6	26.1 ± 16.1
CBT 13	32.5 ± 12.2	50.8 ± 9.1	1.8 ± 3.3	0.3 ± 0.7	27.2 ± 17.9

BBMT, blood and marrow stem cell transplantation; CBT, cord blood transplantation; PB, peripheral blood.
Values indicate percentage of indicated marker-expressing cells. Significant differences were found in values after CBT compared with normal PB and cord blood and with values after BBMT and CBT.
[a] $p < 0.01$.

not shown). However, absolute numbers of theses cells are almost same between after BBMT and CBT (Table 2).

3.2. Subpopulations and absolute number of NK cells 4–9 months after stem cell transplantation

PBMC 4–9 months after CBT contained higher proportion of CD16$^+$CD56^{dim} cells than those in normal adult blood and cord blood and also after BBMT (mean ± standard deviation, 25.7% ± 12.7% vs 2.2% ± 0.9%, 2.4% ± 2.3%, and 4.0% ± 2.5%, $p < 0.01$). Also, PBMC after CBT at the chronic phase contained significantly more absolute number of CD16^{low}CD56^{bright}, CD16$^+$CD56^{bright}, and CD16$^+$CD56^{dim} cells than those after BBMT (Table 2).

3.3. Inhibitory and stimulatory NK cell receptors on CD16$^+$CD56^{dim} cells after 4–9 months after stem cell transplantation

Expression level of inhibitory and stimulatory NK cell receptors on CD16$^+$CD56^{dim} cells in normal PBMC, CB and also PBMC 4–9 months after BBMT vary according to each blood cell source (Tables 3, 4). However, the CD16$^+$CD56^{dim} cell subset after CBT exhibited decreased expression of NKG2A compared with CB and after BBMT (16.1% ± 15.3% vs 49.4% ± 10.4% and 29.2% ± 18.1%, $p < 0.01$, 0.05, respectively) and increased expression of NKG2C compared with CB and after BBMT (32.5% ± 12.2% vs 8.3% ± 6.1% and 18.5% ± 14.9%, $p < 0.01$, respectively).

3.4. Cytolytic activity of subpopulations of NK cells after CBT

Purified CD16$^+$CD56^{dim} cells (Fig. 1) from PBMC obtained from an AML (M4) patient 9 months after CBT (Case 1) exhibited a significantly higher level of cytolytic activity against K562 than the activity of purified CD16$^+$CD56^{bright} cells and whole PBMC (Table 5). Purified CD16$^+$CD56^{dim} cells obtained from a PhALL patient 8 months after CBT (Case 2) also exhibited a significantly higher level of cytolytic activity than that of whole PBMC. These two patients were alive more than 34 months after CBT.

3.5. Long-term observation of CD16$^+$CD56^{dim} NK cells after CBT

Increased proportion and absolute number of CD16$^+$CD56^{dim} NK cells (~10–20% and >200/μl) after CBT sustained during 2–4 years. At the time of writing, all of these CBT patients were alive without relapse (Fig. 2).

4. Discussion

Immune recovery early after transplantation is thought to be thymus independent [15]; after early recovery, naïve lymphocytes derived from the differentiation of donor hematopoietic stem cells colonize lymphoid tissues and sustain late immune recovery. The second recovery involves selection of donor-derived precursor cells in the thymus and peripheral selection sites [16].

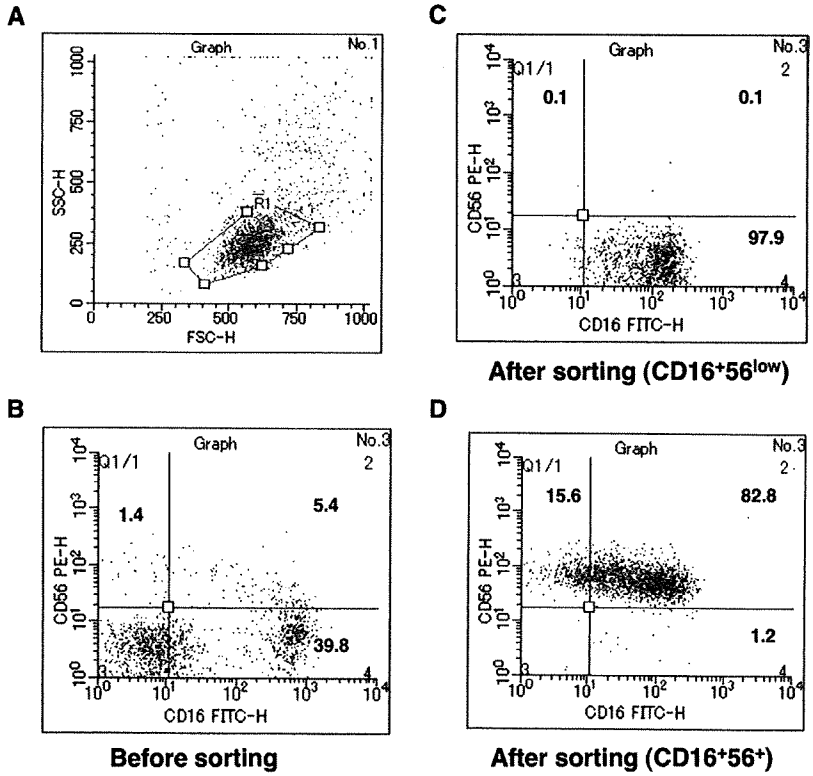

Fig. 1. Surface expression of CD16 and CD56 on PBMC from a patient after CBT and sorting of CD16$^+$CD56^{dim} and CD16$^+$CD56^{bright} cells. (A) Lymphocytes were gated using FSC and SSC. (B) PBMC were stained with anti-CD56 (PE) and anti-CD16 (FITC). (C) After sorting of CD16$^+$CD56^{dim} cells. (D) After sorting of CD16$^+$CD56^{bright} cells. The percentage of cells is shown in each quadrant.

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Although a much smaller number of lymphocytes is transferred with CBT, recovery of lymphocyte number and function has been reported to be rapid and comparable to that after BMT [17]. On the other hand, Hamza *et al.* reported that donor-derived lymphocyte recovery was slower in CBT patients at early phase, however, surpassed from day 60 to 365 [18]. Concerning lymphocyte subset reconstitution after CBT, it has been reported that recovery of NK cells, $CD19^+$ cells, $CD8^+$ cells, and $CD4^+$ cells was achieved at a median of 2–3, 6, 8–9, and 12 months, respectively [19,20]. This prompt immune recovery may be favored by the reduction of incidence and severity of GVHD after CBT. Although innate immunity reconstitute quickly, T-cell lymphopoeisis may be compromised for years because of the decrease of thymopoiesis following transplantation. Early arising and persisting NK cells would suggest the possibility of an alternative and safer GVL effect after CBT than after conventional transplantation from adult donors, where T-cells are predominant [21–23].

Cord blood cells themselves before transplantation have been demonstrated to contain a higher percentage of NK cells [5] and $CD16^+CD56^{dim}$ cells with low level of cytolytic activity, and these cells are possible precursors of mature NK cells [7]. The results of our previous preliminary study showed a significant increase in the $CD16^+CD56^{dim}$ cell subset during a period of 4–9 months after CBT [24]. Lu *et al.* reported that an increase in the $CD16^+CD56^{dim}$ NK cell count in PB was observed in seven (64%) of 11 CBT patients [25]. However, dynamic changes in subpopulations of NK cells after CBT have not been clarified. In this study, we demonstrated a marked increase of proportion and absolute number of $CD16^+CD56^{dim}$ cells 4–9 months after CBT. Also, increased proportion and absolute number of $CD16^+CD56^{dim}$ NK cells (~10–20% and >200/μl) after CBT sustained for more than 2 years. On the other hand, $CD16^+CD56^{dim}$ cell number was 138 ± 33 ($n = 6$; mean, 40 months) after adult stem cell transplantation. Although $CD16^+CD56^{dim}$ cells comprise monocytes, the $CD16^+CD56^{dim}$ cells had a high level of cytolytic activity against HLA class I–negative NK cell target K562 cells. The $CD16^+CD56^{dim}$ cells expressed inhibitory and stimulatory NK cell receptors, and exhibited decreased expression of inhibitory NKG2A and increased expression of stimulatory NKG2C, compared with cord blood and also PBMC after BBMT. Therefore, stage 4 $CD16^-CD56^{bright}$ NK cells may be differentiated into stage 5 $CD16^+CD56^{dim}$ NK cells with cytotoxicity [4] after CBT at the chronic phase. $CD16^+CD56^{dim}$ cells have been reported to exist in CB with low levels of cytolytic activity as possible precursors of mature NK cells [7]. However, $CD16^+CD56^{dim}$ cells 8–9 months after CBT had a higher level of cytolytic activity against K562 cells than did $CD16^+CD56^{bright}$ cells in this study. Therefore, $CD16^+CD56^{dim}$ cells after CBT may comprise a mature NK cell subset.

In our nine patients who underwent CBT, only one patient with MDS overt leukemia in the non-CR state had relapsed with leukemia 8 months after CBT during a mean observation period of 38 months (range, 11–61 months). There seemed to be no definitive correlation between GVHD and $CD16^+CD56^{dim}$ cells.

The present results suggest that NK cell subsets and expression of NK cell receptors after SCT may vary depending on stem cell source. Also, the increase in $CD16^+CD56^{dim}$ NK cells with strong cytolytic activity after CBT may have an important role in the GVL effect after CBT. However, we need confirmation for short- and long-term reconstitution of NK cells after CBT with a greater number of patients.

Table 5
Cytolytic activity of $CD16^+CD56^{dim}$ cells against K562 cells

	Whole	$CD16^+CD56^{bright}$	$CD16^+CD56^{dim}$
Case 1	20.3 ± 0.4[a]	53.9 ± 3.7[a]	74.1 ± 2.0
Case 2	38.0 ± 1.6[a]	ND	78.0 ± 4.6

ND, not determined.
Values indicate the percentage ^{51}Cr release against K562 cells using 4-hour standard ^{51}Cr release assay. Significant differences were found in values of $CD16^+CD56^{dim}$ compared with whole cells and $CD16^+CD56^{bright}$ cells.
[a] $p < 0.01$.

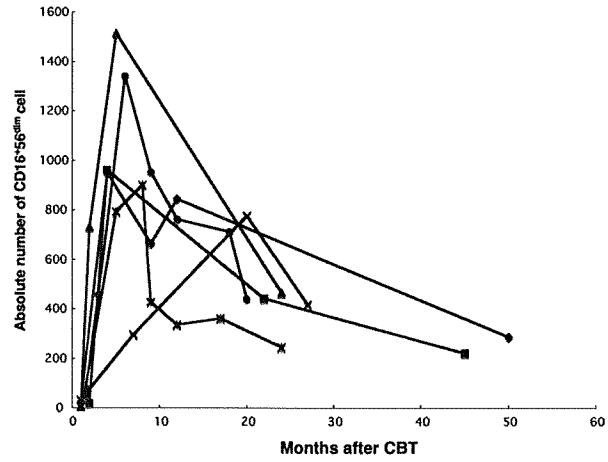

Fig. 2. Long-term observation of absolute number of $CD16^+CD56^{dim}$ NK cells after CBT. Increased absolute number of $CD16^+CD56^{dim}$ NK cells (~200–400/μl) after CBT sustained during 2–4 years.

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ORIGINAL ARTICLE

Cardiac and autonomic nerve function after reduced-intensity stem cell transplantation for hematologic malignancy in patients with pre-transplant cardiac dysfunction

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Abstract Recent reports have shown that cardiomyopathy caused by hemochromatosis in severe aplastic anemia is reversible after reduced-intensity allogeneic stem-cell transplantation (RIST). We comprehensively evaluated cardiac and autonomic nerve function to determine whether cardiac dysfunction due to causes other than hemochromatosis is attenuated after RIST. In five patients with cardiac dysfunction before transplant, we analyzed the changes in cardiac and autonomic nerve function after transplant, using electrocardiography (ECG), echocardiography, radionuclide angiography (RNA), serum markers, and heart rate variability (HRV), before and up to 100 days after transplant. There was no significant improvement in cardiac function in any patient and no significant alteration in ECG, echocardiogram, RNA, or serum markers. However, on time-domain analysis of HRV, the SD of normal-to-normal RR intervals (SDNN) and the coefficient of variation of the RR interval (CVRR) decreased significantly 30 and 60 days after transplant (P=0.04 and 0.01, respectively). Similarly, on frequency-domain analysis of HRV, low and high frequency power (LF and HF) significantly and temporarily decreased (P=0.003 and 0.03, respectively). Notably, in one patient who had acute heart failure after transplantation, the values of SDNN, CVRR, r-MSSD, LF, and HF at 30 and 60 days after transplantation were the lowest of all the patients. In conclusion, this study suggests that (a) RIST is well-tolerated in patients with cardiac dysfunction, but we cannot expect improvement in cardiac dysfunction due to causes other than hemochromatosis; and (b) monitoring HRV may be useful in predicting cardiac events after RIST.

Keywords Cardiac dysfunction · Heart rate variability · Reduced intensity allogeneic stem-cell transplantation · Acute heart failure

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is recognized as a curative treatment for hemato-

logic malignancy. However, myeloablative regimens are difficult to administer in elderly or ill patients because of increased transplantation-related toxicity. Therefore, reduced-intensity conditioning regimens for allo-HSCT have been developed for these vulnerable patients, who are ineligible for myeloablative conditioning. There are, however, no reports that describe whether reduced-intensity allogeneic stem-cell transplantation (RIST) is sufficiently well tolerated in patients with single-organ comorbidity, including cardiac, pulmonary, renal, or hepatic dysfunction. Therefore, the safety of RIST for patients with impaired organ function has not been sufficiently verified. Indeed, the existence of pretransplant organ dysfunction can worsen the prognosis after transplantation whether nonmyeloablative conditioning or myeloablative conditioning is used [1, 2]. Furthermore, in RIST utilizing melphalan and fludarabine, a high incidence of severe left ventricular failure (three of 21 patients; 14%) was reported [3]. This report casts doubt on whether RIST is safe in patients with cardiac dysfunction.

In contrast, three recent reports have shown marked improvement in cardiac function after transplant in patients with severe aplastic anemia (AA) and cardiomyopathy in secondary hemochromatosis, despite the underlying mechanism being unknown [4–6]. Conversely, the reversibility of cardiac dysfunction due to causes other than hemochromatosis has not been investigated. Hence, the primary objective of this study was to evaluate how cardiac dysfunction due to causes other than hemochromatosis changes after RIST.

Heart rate variability (HRV), which is generally recognized as an index of sympathovagal balance and autonomic cardiovascular control, has been investigated in various diseases that precipitate sudden death. Recently accumulated evidence has shown that a decrease in RR interval variability is strongly associated with sudden death and/or cardiac events after a myocardial infarction [7–10]. Furthermore, the usefulness of HRV as a clinical tool has been explored in numerous other conditions such as congestive heart failure, vasovagal syncope, hypertrophic cardiomyopathy, obstructive sleep apnea, diabetic neuropathy, and various neurological conditions [11–16]. In this study, we comprehensively analyzed the changes in cardiac function after RIST, assessed by various methods including electrocardiography (ECG), echocardiography, radionuclide angiography (RNA), natriuretic peptides and troponin T, and autonomic nerve function on 24-h Holter ECG for HRV.

Materials and methods

Patients

We prospectively enrolled five consecutive patients with pretransplant cardiac dysfunction from a group of 116 patients who had undergone allo-HSCT between September 2003 and August 2007 at our institute. In this study, the patients who were eligible for this study were those who had a left ventricular ejection fraction (LVEF) of under 50% on pretransplant radionuclide angiography, had ever been noted to have an LVEF of under 50% on echocardiography, or had a history of heart failure prior to transplant. The median age at transplantation was 42 years old (range 37–55). Pretransplant LVEFs were less than 50% in two patients, two patients had previously had an LVEF of under 50%, and the remaining patient had a history of heart failure prior to transplantation and an LVEF of under 50% at pretransplant assessment. No patient received mediastinal radiation therapy before transplantation nor did any patient have a history of hypertension, diabetes mellitus, and/or ischemic heart disease. The median level of serum ferritin prior to transplant was 189.6 ng/ml (range 58.3–1837.9), and there were no patients who were diagnosed as having cardiac hemochromatosis on echocardiography. We therefore strongly suspected anthracycline treatment as the cause of cardiac dysfunction in all five patients. As in a previous report [17], we calculated the cumulative anthracycline dose using the following ratios: daunorubicin 0.5, pirarubicin 0.8, mitoxantrone 3.4, idarubicin 1.6, and epirubicin 0.6, with cardiotoxicity of doxorubicin considered to be 1.0. These patients included three patients with non-Hodgkin's lymphoma (intravascular lymphoma, mantle cell lymphoma, and follicular lymphoma) who where refractory to chemotherapy, one patient with acute myeloid leukemia in her second complete remission (CR), and one patient with acute lymphoblastic leukemia in his first CR (Table 1). This study was approved by the Institutional Review Board. The concept, procedure, and potential risks of the study were explained, and written informed consent was obtained from all enrolled patients.

Allogeneic HSCT

Three patients received allo-HSCT from HLA-identical unrelated donors, and two patients from HLA-mismatched cord blood (Table 1). HLA matching (HLA-A, -B, and -DR) was determined by DNA genotyping in three unrelated BMTs, and only serologic typing was performed for the two cord blood transplants (CBT). Three patients received transplants from donors with matched blood types, one patient received a transplant with a major blood type mismatch, and one patient received a transplant with a minor blood type mismatch.

Reduced-intensity conditioning was employed for all five patients. Of the three patients from HLA-identical unrelated donors, two patients received reduced-intensity conditioning including fludarabine/oral busulfan (fludarabine 30 mg/m^2/day, on days −7 to −2 and oral busulfan 4 mg/kg/day, on days −4 and −3), and the remaining patient

Table 1 Patients characteristics

No.	Sex	Age	Disease	Disease status	Source	HLA Mismatch (A, B, DR)	aGVHD prophylaxis	Eligibility	Cumulative dose of anthracycline (mg/m^2)
1	F	39	AML	CR2	uBM	Allele match	CsA + sMTX	LVEF 37%	250
2	F	37	IVL	Ref	uBM	Allele match	CsA + sMTX	LVEF 46%, a history of AHF	380
3	F	55	FL	Ref	CB	2 Ag	CsA alone	a history of decreased LVEF in 45%	391.8
4	M	42	ALL	CR1	uBM	Allele match	CsA + sMTX	LVEF 48%	240
5	F	46	MCL	Ref	CB	2 Ag	CsA + sMTX	a history of decreased LVEF in 39%	150

We calculated the cumulative dose of anthracycline as daunorubicin 0.5, pirarubicin 0.8, mitoxantrone 3.4, idarubicin 1.6, epirubicin 0.6, when the intensity of the cardiotoxicity of doxorubicin was considered to be 1.0
IVL intravascular B cell lymphoma, *FL* follicular lymphoma, *MCL* mantle cell lymphoma, *Ref* chemo-refractory, *uBM* unrelated bone marrow, *Ag* antigen, *CB* cord blood, *CsA* cyclosporine, *sMTX* short-term methotrexate, *LVEF* left ventricular ejection fraction

received fludarabine/intravenous busulfan (fludarabine 30 mg/m^2/day, on days −9 to −4 and intravenous busulfan 1.6 mg/kg/day, on days −9 and −5). In contrast, the two patients who underwent CBT had fludarabine/intravenous busulfan/total body irradiation (fludarabine 30 mg/m^2/day, on days −9 to −4, intravenous busulfan 1.6 mg/kg/day, on days −9 and −5, and total body irradiation 4 Gy divided into two fractions/day, on days −3, −2, or −1).

As prophylaxis for acute graft-versus-host disease (aGVHD), four patients received both cyclosporine A (CsA) and short-term methotrexate (MTX), and one patient who received cord blood was given CsA alone. Intravenous MTX was administered on day 1 (10 mg/m^2), and on days 3 and 5 (7 mg/m^2). The doses of CsA were adjusted to a target trough level between 150 and 250 ng/ml until day 100, except where disease progression or drug toxicity occurred, and then tapered unless GVHD occurred. aGVHD was diagnosed clinically, graded according to standard criteria and confirmed by appropriate biopsies. Chronic GVHD (cGVHD) was also defined according to the standard criteria. In CBT, pre-engraftment immune reactions (PIR) were defined according to the report of Kishi et al. [18].

The evaluation of cardiac and autonomic nerve function

We evaluated cardiac function with 12-lead electrocardiograms (ECG), echocardiography, radionuclide angiography (RNA), serum markers, and autonomic nerve function with heart rate variability assessed on 24-h ECG monitoring (within the 100 days before transplant) and posttransplantation [on days 30 (±4), 60 (±6), and 100 (±12)].

The 12-lead ECG

Twelve-lead ECG was performed at rest, and the QTc interval was measured automatically using a novel recording system (FDX-6521, Fukuda Denshi, Tokyo, Japan). In brief, QT intervals were measured for 15 s at 25 mm/s. QTc intervals were then calculated by correcting the QT interval using the Bazett formula [19].

Echocardiography

Two-dimensional echocardiographic examinations were performed using a Power Vision 6000 (Toshiba, Tokyo, Japan). We assessed the following variables: (1) posterior wall (PW) and interventricular septal wall (IVS) for evaluation of left ventricular hypertrophy, (2) left ventricular end-diastolic dimension (LVDd) and end-systolic dimension (LVDs) for evaluation of left ventricular dilatation, (3) early peak flow velocity/atrial peak flow velocity (E/A) and deceleration time (DcT) for evaluation of left ventricular diastolic function. During the examination, the gain setting was optimized to a level just below background noise, and the transducer frequency was set to 2.5 or 3.5 MHz.

RNA

Radionuclide angiography was performed with the gated-equilibrium technique. Blood cells were targeted with 99 mTc-pertechnetate, facilitated by pyrophosphate and injected intravenously. ECG-gated gamma camera scanning was performed using a VertexPlus (ADAC /Philips, CA, USA) from two directions; the left anterior oblique and anterior views were obtained in order to image the left ventricle separately from the right ventricle, and the left atrium and 64×64 matrix was used. Acquisition time was 180 s, and ECG-gated images were acquired with 20 frames per cardiac cycle. LVEF and peak-filling rate (PFR) were calculated as parameters of left ventricular systolic and diastolic function.

Serum markers (natriuretic peptide measurement)

We employed human atrial natriuretic peptide (hANP) and brain natriuretic peptide (BNP) as parameters of cardiac function. Blood samples for natriuretic peptide measurement were drawn from the patients into chilled tubes containing aprotinin and 1 mg of ethylenediamine tetraacetic acid (EDTA)/ml. The tubes were immediately placed on ice and centrifuged at 4°C, and the separated plasma was stored at −80°C before assaying. According to the manufacturer's recommended protocol (SRL, Tokyo, Japan), hANP and BNP were measured with a commercially available immunoradiometric assay. The normal values of hANP and BNP are less than or equal to 40 and 20 pg/ml, respectively.

Heart rate variability

Data obtained from the 24-h ambulatory ECG recordings were analyzed with RR data analysis software (MemCalc/CHIRAM version 1, Suwa Trust, Tokyo, Japan). We employed markers including the coefficient of variance of the RR interval (CVRR), the SD of the NN interval (SDNN), and the squares of the differences between adjacent normal-to-normal RR intervals (r-MSSD) in time domain analysis and the low-frequency (LF) area, high-frequency (HF) area, and LF/HF ratio in frequency domain analysis.

Other parameters

In addition, we evaluated parameters that possibly influence HRV, such as systolic and diastolic blood pressure, heart rate, body temperature, CsA trough, and hemoglobin to assess which parameters affected HRV in this study. For analysis, we used the averaged values of each parameter on the three preceding days that were closest to the day when the HRV evaluations were conducted.

The diagnosis of acute heart failure

Clinical criteria for acute heart failure were established by referring to those used in the Framingham study [20]. We diagnosed acute heart failure when a minimum of two major criteria or when one major and two minor criteria were present concurrently. Major criteria included orthopnea, pulmonary congestion, pulmonary rales, a gallop rhythm, and jugular venous distention. Minor criteria included tachycardia (rate>120/min), shortness of breath, ankle edema, and hepatomegaly.

Statistical analysis

The Friedman test was used for detecting changes pre- and posttransplantation (30, 60, and 100 days after transplant) in all serum markers, parameters on echocardiography, RNA, ECG and HRV, systolic and diastolic blood pressure, heart rate, body temperature, CsA trough level, hemoglobin, and C-reactive protein. All P values were two-sided, and a significance level of 0.05 was used.

Results

Table 1 shows the five patients' characteristics. The median follow-up time was 324 days (range 127–1517). Engraftment was achieved in all five patients (neutrophil engraftment occurred in the three patients with unrelated BMT 13, 17, and 21 days after transplantation; in the two patients with CBT, it occurred 27 and 31 days after transplant, respectively). No patient died within 100 days of the transplant, although in one patient (no. 2), the disease relapsed within 100 days, as she had disease recurrence in the central nervous system at day 42. In this case, therefore, CsA was tapered and stopped by day 60. Although salvage chemotherapy and donor lymphocyte infusion were performed on days 65 and 76, respectively, she died from disease progression on day 127 (Table 2). An angiotensin-receptor antagonist was administered from before transplantation in one patient (no. 5), but angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and diuretics were not administered before transplantation in any patient.

Of the five patients, none developed acute heart failure early after the conditioning chemotherapy. Pre-engraftment immune reactions (PIR) occurred in the two patients who had CBT, on days 8 and 13, but neither developed acute heart failure during the period of evaluation (Table 2). Three patients had aGVHD (grades I, III, and IV, respectively). The two patients with grade III and IV aGVHD were given high-dose steroids. Diuretics were appropriately given to patients with body weight gain during PIR or aGVHD. Three patients had sepsis, two cases of which were severe. One patient (no. 3) experienced prolonged MRSA sepsis and another patient (no. 4) experienced septic shock due to catheter infection. Two patients died from disease progression. One patient (no. 3) developed acute heart failure that might partly have been caused by pneumonia and drug-induced acute renal failure 147 days after transplantation. In this patient, acute progressive dyspnea, bilateral pleural effusions, cardiomegaly, and leg edema appeared soon after pneumonia and drug-induced acute renal failure. We diagnosed her as having acute heart failure (New York Heart Association functional class IV) and performed endotracheal intubation, administered catecholamines and diuretics, and discontinued or reduced the dose of drugs thought to be nephrotoxic. Thereafter, she recovered rapidly, and she was extubated 156 days posttransplant.

Table 2 Outcomes

No.	Neutrophil engraftment	aGVHD	cGVHD	Severe infection	AHF	Outcome (Cause of death)
1	Day 21		Limited			Alive 1,517+
2	Day 17		NA			Dead 127 (relapse)
3	Day 31	IV (day 13, skin and gut)	Extensive	Prolonged MRSA sepsis >2 months from day 56	+Day 147	Alive 461+
4	Day 13	III (day 29, gut)	Limited	Septic shock at day 61		Alive 324+
5	Day 27	I (day 49, gut)	Limited			Dead 170 (relapse)

aGVHD acute graft-versus host disease, *cGVHD* chronic graft-versus-host disease, *AHF* acute heart failure after transplantation, *MRSA* methicillin-resistant *Staphylococcus aureus*

Table 3 shows the posttransplantation changes in cardiac parameters in all five patients. All parameters for RNA, echocardiography, natriuretic peptides, and ECG showed no significant changes at any of the evaluation points. On the other hand, some parameters in the analysis of HRV changed significantly (Fig. 1). SDNN and CVRR significantly decreased at 30 to 60 days after transplantation and then recovered (P=0.04 and 0.01, respectively). Moreover, LF and HF also temporarily decreased (P=0.003 and 0.03, respectively). In particular, the patients with acute heart failure had the lowest values of SDNN, CVRR, r-MSSD, LF, and HF at 30 to 60 days after transplant (Fig. 1, dashed line). LF/HF did not change significantly.

Table 4 shows changes in the parameters that might possibly have influenced HRV, including systolic and diastolic blood pressure, heart rate, body temperature, CsA trough levels, and hemoglobin. On analysis, diastolic blood pressure and body temperature showed a statistically significant change (P=0.03 and 0.04, respectively).

Discussion

In this study, we observed no significant improvement of impaired cardiac function, but in contrast, we did detect a temporary, marked decrease in HRV soon after SCT.

As mentioned earlier, there have been three Japanese reports of the use of RIST for severe AA in patients with impaired cardiac function [4–6]. Before HSCT, LVEFs in these three cases were 20–40%. In all three cases, cardiomyopathy was caused by secondary hemochromatosis attributable to heavy transfusions. Although the mechanism was not clarified, LVEFs in all three cases dramatically recovered to a normal level, a few months to years after transplant. In one case, prompt improvement in cardiac function was seen with persistent iron deposition and without normalization of hemoglobin level [4]. These reports demonstrate that cardiomyopathy is reversible in patients with secondary hemochromatosis and severe AA. It is therefore interesting to determine whether cardiac

Table 3 Posttransplant changes of cardiac parameters

	Baseline	Day 30	Day 60	Day 100	*P*
Radionuclide ventriculography					
LVEF (%)	48 (37–64)	51 (41–59)	45 (38–55)	47 (39–58)	0.39
PFR (EDV/s)	2.0 (1.3–3.1)	1.8 (1.6–3.2)	1.5 (1.2–3.2)	1.9 (1.3–2.7)	0.56
Echocardiography					
IVS (mm)	7.7 (6.8–9.3)	10.0 (8.0–11.0)	8.9 (7.7–12.8)	8.6 (6.8–10.7)	0.08
PW (mm)	9.6 (8.3–11.2)	10.7 (8.0–11.8)	10.7 (9.5–12.2)	10.8 (8.0–13.2)	0.42
LVDd (mm)	45 (37–58)	44 (40–56)	47 (32–53)	46 (37–54)	0.42
LVDs (mm)	34 (28–44)	34 (27–42)	35 (24–43)	33 (31–42)	0.90
E/A	0.91 (0.72–1.17)	1.24 (0.84–2.24)	1.10 (0.85–2.08)	0.87 (0.55–1.15)	0.83
DcT (ms)	185.5 (160–220)	196 (124–212)	234 (96–296)	186 (172–296)	0.62
Natriuretic peptides					
hANP (ng/ml)	15 (12–40)	25 (12–119)	35 (10–98)	29 (10–66)	0.80
BNP (ng/ml)	36 (5–100)	68 (5–183)	21 (9–180)	35 (2–107)	0.61
Electrocardiography					
QTc (ms)	435 (422–490)	427 (408–478)	422 (408–457)	415 (405–421)	0.14

LVEF left ventricular ejection fraction, *PFR* peak filling rate, *IVS* intraventricular septal wall, *PW* posterior wall, *LVDd* left ventricular end-diastolic dimension, *LVDs* left ventricular end-systolic dimension, *E/A* early peak flow velocity/atrial peak flow velocity, *DcT* deceleration time, *hANP* human atrial natriuretic peptide, *BNP* brain natriuretic peptide

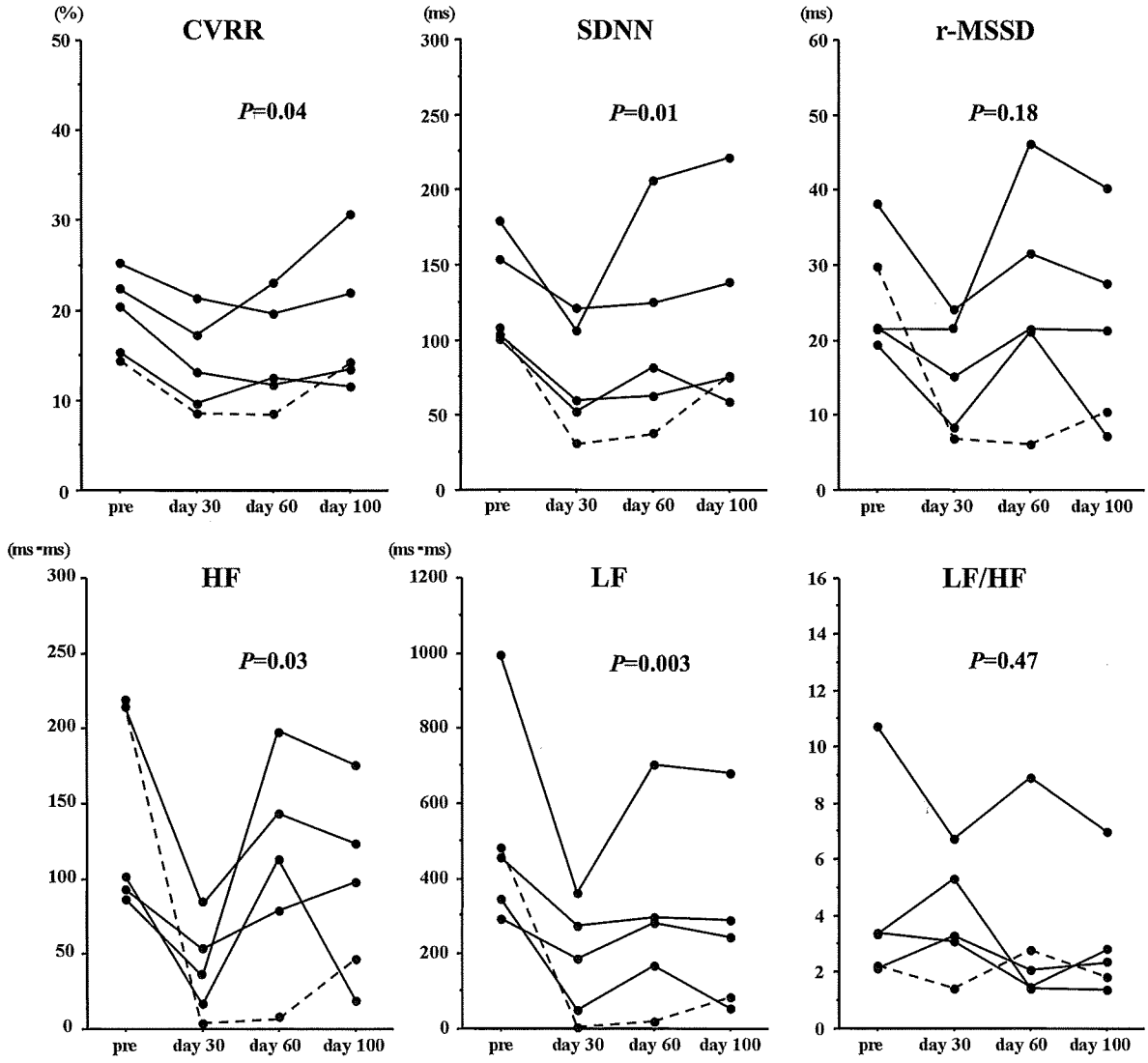

Fig. 1 Changes in HRV indicators during the first 100 days after allogeneic stem cell transplantation. The value of SDNN in the time-domain analysis and the values of HF and LF in the frequency-domain analysis significantly and temporarily decreased 30 days after transplantation. *SDNN* the standard deviation of all normal beats, *r-MSSD* the square root of the mean of the sum of squared differences between adjacent normal-to-normal intervals, *HF* high frequency power, *LF* low frequency power

dysfunction caused by other etiologies such as chemotherapy can similarly be restored. However, no significant improvement was observed in any patient in this study, suggesting that it is likely that the reversibility of cardiac function depends on the etiology of the cardiac dysfunction.

In all five patients, there were no significant changes in cardiac parameters such as left ventricular systolic and diastolic function on echocardiography and RNA, serum troponin T (data not shown), BNP and hANP, and QTc on ECG, throughout the monitoring period. One patient had acute heart failure after the completion of all assessments, but she recovered with appropriate monitoring and therapy. These results suggest that RIST is relatively well-tolerated in patients with cardiac dysfunction, provided management is adequate.

To prevent the development of acute heart failure in patients with impaired left ventricular function present before transplantation, we need to pay attention to the following points: (1) careful control of fluid intake and output by monitoring body weight and urine output, or checking for cardiomegaly and dilatation of the vena cava on chest X-ray and echocardiography; (2) careful monitoring of

Table 4 Posttransplant changes of possible parameters[a] influencing HRV

	Baseline	Day 30	Day 60	Day 100	*P*
Systolic blood pressure (mmHg)	101 (97–117)	116 (92–137)	109 (91–136)	111 (97–118)	0.35
Diastolic blood pressure (mmHg)	60 (57–64)	76 (62–87)	68 (67–84)	69 (68–74)	0.03
Heart rate (/min)	79 (76–93)	89 (76–120)	85 (72–109)	86 (84–88)	0.08
Body temperature (°C)	36.5 (36.2–36.8)	37.2 (36.8–38.2)	36.8 (36.7–38.1)	37.2 (37–37.5)	0.04
CsA trough (ng/ml)	0	227 (193–277)	237 (0–328)	160 (0–378)	0.07
Hemoglobin (g/dl)	8.8 (8.3–14.3)	9.9 (7.7–14.2)	9.4 (8–12.1)	10.2 (7.9–10.9)	0.55
C-reactive protein (mg/dl)	0.1 (0.01–0.21)	0.2 (0.1–2.34)	0.12 (0.1–1.99)	0.25 (0.03–2.38)	0.23

CsA cyclosporine

[a] Each data is the five patients' median value of the 3 days average data on or about the day HRV went

decline in kidney function due to renal toxicity caused by calcineurin inhibitors, antibiotics, antifungal drugs, or antiviral drugs and prompt adjustment of the dose of these drugs; and (3) transfusing at a slower rate than usual.

One study reported that prophylactic treatment with the ACE inhibitor enalapril not only prevented deterioration in left ventricular dysfunction prior to transplant, but also brought about an increase in LVEF in all patients [21]. Another study also demonstrated that early treatment with enalapril might prevent late cardiotoxicity due to high-dose chemotherapy in a randomized trial in 114 high-risk patients, identified by an increased troponin I value [22]. In our study, instead of receiving an ACE inhibitor, one patient (no. 5) started to receive an angiotensin receptor blocker before transplantation; she had no cardiac dysfunction after transplantation. Although the effectiveness of ACE inhibitors or angiotensin receptor blockers has not been sufficiently validated for preventing cardiac events in patients with pretransplant cardiac dysfunction, it may be useful to consider prophylactic administration of an ACE inhibitor or angiotensin receptor blocker.

In this case series, almost all patients had a temporary decrease in HRV posttransplant, and the patient with acute heart failure had an especially marked decrease prior to the onset of acute heart failure (Fig. 1, dashed line). Previous studies have reported a correlation between HRV and proinflammatory cytokines [23, 24], CsA [25], blood pressure [26], heart rate [27], anemia [28], and C-reactive protein [29, 30] as possible factors that influence changes in HRV after transplantation. In fact, the significant elevation of body temperature and diastolic blood pressure might have influenced the decrease in HRV. On time-domain analysis of HRV, a number of authors have identified a decreased SDNN value as a univariate risk factor for all-cause mortality, expressed as a dichotomized variable in the patients with chronic heart failure [31]. This review notes that an SDNN value of less than 44 ms was determined statistically to be the optimum cutoff point [32], and the value of 44 ms is similar to the value of <50 ms that best predicts mortality in myocardial infarction patients [7]. The predictive value of SDNN can be explained by the speculation that a reduction in the SDNN reflects the summed influence of abnormalities in sympathetic, parasympathetic and rennin–angiotensin activities, abnormal chemoreceptor function, changes in respiratory pattern, and physical inactivity in congestive heart failure [33].

Conversely, among the indicators used in frequency-domain analysis of HRV, a reduced LF value is reportedly highly predictive of sudden cardiac death in chronic heart failure [31]. Despite high levels of sympathetic activation, decreases in the LF value were often observed in patients with chronic heart failure, which may be secondary to abnormalities in central autonomic regulation and impairment of beta adrenergic receptor sensitivity [33]. Furthermore, a reduced LF value was reported as a marker of sympathetic overactivity, and it was concluded that a reduced short-term LF value during controlled breathing (<13 ms^2) is a powerful predictor of sudden death in patients with chronic heart failure [34]. In our case with acute heat failure, the LF value as well as the SDNN value temporarily but dramatically decreased to less than 44 ms and 13 ms^2, respectively, before the occurrence of acute heat failure, indicating that decreases in SDNN and/or LF values might be associated with the development of acute heat failure after transplantation. HF and r-MSSD are generally considered to be predominantly determined by the parasympathetic nervous system. In a rat study, it was reported that CsA induced a decrease in SDNN and r-MSSD values [25]. Therefore, in our study, CsA treatment after transplantation might play a role in the significant decrease in the HF value and the mild decrease in r-MSSD, by its suppression of parasympathetic activity.

This study has the following limitations: (1) the population is small and from a single institution; (2) lack of clarity about the causes for the decrease in HRV; and (3) we do not know the lower limit of cardiac function in patients still eligible for RIST. However, to our knowledge, there has been no prior report of comprehensive analyses of cardiac and autonomic nervous system function in patients with impaired cardiac function who underwent allo-HSCT.

In conclusion, RIST is relatively well tolerated in patients with pretransplant cardiac dysfunction, but apparent improvement of cardiac function cannot be expected except in cardiomyopathy induced by secondary hemochromatosis. We could not determine whether changes in autonomic function are associated with the development of cardiac events after transplant. Additional studies of a larger cohort are therefore required to confirm the usefulness of HRV for prediction of cardiac events after transplantation.

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CNS Complications of Hematopoietic Stem Cell Transplantation

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OBJECTIVE. With the worldwide increase in the use of hematopoietic stem cell transplantation (HSCT), a high level of diligence is required for radiologists to understand HSCT-related complications in the CNS. This article describes the clinical background of HSCT and complications that occur in a time-dependent manner through the course of HSCT and addresses pivotal issues in diagnostic imaging.

CONCLUSION. Acknowledging the realm of imaging manifestations and the underlying mechanism of HSCT will enhance diagnostic accuracy and optimize treatment decisions.

Hematopoietic stem cell transplantation (HSCT) is an accepted treatment option for various hematopoietic disorders, genetic disorders, inborn errors of metabolism, and autoimmune disorders. More than 20,000 transplantations are performed yearly, for a total of more than 230,000 transplantations to date, according to the annual report of the Center for International Blood and Marrow Transplant Research [1]. Complications in the posttransplantation period are mostly related to hematopoietic and immune system aplasia and to the alloreactivity of donor cells. Because preparative regimens destroy the recipient's immune and hematopoietic systems, immunologic recovery depends on engraftment and proliferation of the infused stem cells. The most common and clinically significant complications are infection, vascular disorders, therapy-induced cytotoxicity, graft-versus-host disease (GVHD), and recurrence of preexisting diseases. Early diagnosis is crucial to successful management and good prognosis, because CNS complications are potentially devastating. CT and MRI play an important role in early diagnosis, which maximizes the chance of prompt therapy for HSCT-related complications. To accurately interpret imaging manifestations both before and after transplantation, it is imperative to understand the clinical course and immunologic status of patients in terms of the timeline of events associated with transplantation.

The first HSCT was performed with allogeneic bone marrow by Thomas et al. [2] in 1957 to treat a patient with hematologic malignant disease after high-dose chemoradiation therapy. Since then, various terms have been used to describe the procedure, depending on the pretransplantation conditioning regimen (full- or reduced-intensity myeloablation), donor type (human leukocyte antigen [HLA]-identical twins, autologous or allogeneic), and the source of stem cells (bone marrow, peripheral blood cells, or cord blood cells). Improved therapeutic designs in HSCT with highly detailed matching between donor and recipient have improved clinical outcome. At the same time, however, clinical studies have shown CNS abnormalities in 11–59% of recipients, and autopsy studies have revealed neuropathologic abnormalities in more than 90% of patients who died after HSCT [3]. The frequency and diversity of neurologic complications largely depend on the degree and duration of myelosuppression, immunosuppression, and GVHD, which affect the immune reconstitution process of recipients [4]. CNS complications are frequently high-risk factors for transplantation-related morbidity and mortality [5]. Thus early recognition of these complications with imaging and appropriate management will allow early intervention with immunotherapy or additional chemotherapy to improve the chance of survival. Although a brief review of imaging features of CNS complications in children has been published [6], there is still a need for understanding the pathophysiologic mechanism of CNS complications before and after transplantation. This review summarizes the

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