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ORIGINAL ARTICLE

Risk factors affecting cardiac left-ventricular hypertrophy and systolic and diastolic function in the chronic phase of allogeneic hematopoietic cell transplantation

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Chronic impairment of cardiac function can be an important health risk and impair the quality of life, and may even be life-threatening for long-term survivors of allogeneic hematopoietic cell transplantation (HCT). However, risk factors for and/or the underlying mechanism of cardiac dysfunction in the chronic phase of HCT are still not fully understood. We retrospectively investigated factors affecting cardiac function and left-ventricular hypertrophy (LVH) in the chronic phase of HCT. Sixty-three recipients who survived for >1 year after receiving HCT were evaluated using echocardiography. Based on simple linear regression models, high-dose TBI-based conditioning was significantly associated with a decrease in left-ventricular ejection fraction and the early peak flow velocity/atrial peak flow velocity ratio, following HCT (coefficient = −5.550, $P=0.02$ and coefficient = −0.268, $P=0.02$, respectively). These associations remained significant with the use of multiple linear regression models. Additionally, the serum ferritin (s-ferritin) level before HCT was found to be a significant risk factor for LVH on multivariable logistic analysis ($P=0.03$). In conclusion, our study demonstrated that a myeloablative regimen, especially one that involved high-dose TBI, impaired cardiac function, and that a high s-ferritin level might be associated with the development of LVH in the chronic phase of HCT.

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INTRODUCTION

The long-term survival rate after allogeneic hematopoietic cell transplantation (HCT) has significantly improved over recent decades because of improvements in the HCT procedure. However, chronic complications, such as cardiovascular disease, have become increasingly evident and constitute a serious problem in long-term survivors of HCT.[1–3] It is well known that the high-dose CY used for conditioning is a major cause of acute cardiotoxicity after HCT.[4,5] However, it is still unclear as to which factors contribute to chronic cardiotoxicity after HCT. It is possible that conditioning regimens consisting of high-dose CY or high-dose TBI influence cardiac function even in the chronic phase,[6,7] a contention that has not been evaluated in adult patients. Cytokines, such as IL-2 and TNF-alpha, have the potential to affect myocardial tissue adversely,[8] while TNF-alpha is also thought to be involved in the pathogenesis of chronic GVHD.[9] Chronic GVHD may therefore contribute to cardiac tissue damage via cytokines. However, it has not yet been determined whether chronic GVHD has a detrimental effect on cardiac function after HCT.

Left-ventricular hypertrophy (LVH) is often seen in patients with hypertension (HT). One report indicates that inflammatory cytokines, including IL-2 and TNF-alpha, have a role in the development of LVH.[10] It is still not clear which factors affect LVH in HCT recipients. Here we comprehensively evaluate the factors that affect chronic LVH and cardiac systolic and diastolic function after HCT, in a retrospective cohort study that used echocardiographic assessment.

MATERIALS AND METHODS

Study design

We examined left-ventricular systolic and diastolic function and LVH in patients who were eligible for the study as they had received HCT between April 2000 and June 2010 at our institution, had undergone an echocardiographic examination within the 2 months before HCT and had survived for >1 year after HCT. All the evaluated patients visited our hospital regularly. Patients who received more than two allogeneic HCT were excluded from analysis. We also statistically investigated the factors that influenced cardiac function after HCT. Written informed consent was obtained from all the enrolled patients and this study protocol was approved by the Institutional Review Board of our institution.

Cardiac evaluation

Echocardiographic examinations were performed using a Power Vision 6000 (Toshiba, Tokyo, Japan). An electrocardiographic tracing was recorded in the left-lateral decubitus position simultaneously with the echocardiogram. Transducer frequency was 2.5 or 3.5 MHz. We recorded left-ventricular end-diastolic dimension and left-ventricular end-systolic dimension from the left-parasternal long axis view at the high papillary muscle level and calculated left-ventricular ejection fraction (LVEF) by the Teichholtz method. Trans-mitral flow-velocity patterns were measured on an apical, four-chamber view according to the standard method. We assessed LVEF as a surrogate marker of LV systolic function and the early

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peak flow velocity/atrial peak flow velocity (E/A ratio) as a surrogate marker of LV diastolic function. 'LVH after HCT' was identified when the interventricular septal wall and/or the posterior wall became >12-mm thick after HCT.

Statistical analysis

We assessed the possible factors that influence cardiac function after HCT, including intensity of the conditioning regimen (high-dose, TBI-based conditioning regimens vs high-dose busulfan (BU)-based conditioning regimens vs all others), sex, age, a history of HT, hemoglobin concentration, serum ferritin (s-ferritin) levels, chronic GVHD and cumulative dose of anthracyclines. All the high-dose TBI-based conditioning regimens included TBI 1200 cGy and CY 120 mg/kg. High-dose BU-based conditioning regimens used oral BU 16 mg or i.v. BU 12.8 mg and CY 120 mg/kg; all other regimens were defined as reduced intensity conditioning (RIC). HT was defined as a history of HT within 1 year after HCT. We also analyzed data on hemoglobin concentration determined 1 year after HCT, and s-ferritin levels taken before HCT. We defined a history of chronic GVHD as the presence of chronic GVHD within 1 year of HCT. We calculated the relative cumulative dose of anthracyclines with the use of a multiplier of 1 for doxorubicin, 0.5 for DNR, 1.6 for idarubicin, 3.4 for mitoxantrone and 0.1 for aclarubicin.[11]

All statistical tests were interpreted at the 5% significance level. All *P*-values and 95% confidence intervals were two-sided. Simple or multiple linear regression analysis was used to assess the associations of the pre-transplant or the post-transplant variables with the differences in LVEF or E/A measured before HCT and at the follow-up visit. To assess whether the correlation between the type of pre-transplant conditioning regimen and measures, including change in LVEF or E/A, varied significantly with covariates that included age, sex or chronic GVHD, first-order interactions between the variable and the covariates were inserted into a regression model that contained the interaction term and both covariates as main effects. Analysis of residuals was performed to examine model fit and adherence to regression assumptions. Multi-colinearity was assessed using a variance inflation factor. A variance inflation factor exceeding 10 is thought to indicate serious multi-colinearity, and values >4.0 may be a cause for concern.[12] Changes in LVEF or E/A before and after HCT were tested for significance with a paired *t*-test. Repeated measures analysis of variance was used to assess the differences in the degree of change in LVEF or E/A before and after HCT among the groups divided by the type of pre-transplant conditioning regimen. Univariable or multivariable logistic regression analysis was used to estimate the odds ratio for incidence of LVH. Nonlinear effects of continuous independent variables were evaluated using quadratic and log transformations. The presence of effect modification was tested by the insertion of first-order interaction terms into appropriate regression models. We calculated the 95% confidence interval for each odds ratio. All statistical analyses were performed using PASW Statistics 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Study cohort

One hundred and twenty-three patients underwent allogeneic HCT at our institution between April 2000 and June 2010. In all, 25 patients died and 20 patients had moved to another hospital, leaving 78 outpatients at our institution. Of these, seven were missing pre-HCT echocardiographic data and five patients had received more than two allogeneic HCT. Sixty-six patients thus met the study criteria. However, consent for the study could not be obtained from three patients. Ultimately, a total of 63 patients were eligible for participation in the study; of these 63 patients, 56 without LVH at baseline were eligible for analysis of the incidence of LVH.

Patient characteristics

Characteristics of study subjects at baseline are shown in Table 1. The diagnoses in the 63 patients included *de novo* AML in 22 patients, ALL in 16 patients, myelodysplastic syndrome (MDS)/AML in nine patients, non-Hodgkin's lymphoma in five patients, CML in three patients, adult T-cell leukemia in three patients, aplastic anemia in three patients and one each of biphenotypic acute leukemia and chronic active EB virus infection. In most of the patients, CsA was used for prophylaxis against GVHD. Just one patient received mediastinal radiation therapy prior to HCT.

Table 1. Characteristics of study subjects at baseline

No of patients	n = *63*
Median age at HCT (range)	42 years (range, 17–69)
Gender (male/female)	30/33
Diagnosis	
De novo AML	22 (35%)
ALL	16 (25%)
MDS/AML	9 (14%)
NHL	5 (8%)
All others	11 (17%)
Conditioning regimen	
High-dose TBI-based	20 (32%)
High-dose BU-based	18 (29%)
Reduced intensity	25 (40%)
Stem cell source	
Related BM	3 (5%)
Related peripheral blood	21 (33%)
Unrelated BM	29 (46%)
Unrelated cord blood	10 (16%)
History of hypertension within 1 year of HCT	4 (6%)
Chronic GVHD within 1 year of HCT	33 (52%)
LVEF <55% before HCT	2 (3%)
Median hemoglobin level 1 year after HCT	12.7 g/dL (range, 5.6–15.7)
Median serum ferritin level before HCT	566 ng/mL (range, 30–7377)
Median cumulative anthracycline dose	90 mg/m^2 (range, 0–458)
Median follow-up time	1394 days (range, 462–3932)

Abbreviations: HCT = allogeneic hematopoietic cell transplantation; LVEF = left-ventricular ejection fraction; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma. The follow-up time indicates the time between HCT and the post-transplant echocardiographic examination.

HCT conditioning regimen

The high-dose TBI-based conditioning regimen included CY with TBI in eleven patients, CY with TBI and etoposide (60 mg/m^2) in one patient and CY with TBI and cytosine arabinoside (8 g/m^2) in eight patients. The high-dose BU-based conditioning regimen included BU and CY in 14 patients or BU and CY with TLI (750 cGy) in four patients. RIC consisted of BU (oral or i.v. 8 mg/kg) and fludarabine (180 mg/m^2) with/without TBI (400 cGy) in 22 patients, melphalan (140 mg/m^2) and fludarabine (150 mg/m^2) in one patient, oral BU (8 mg/kg) and cladribine (0.66 mg/kg) in one patient and CY (200 mg/kg) with TLI (750 cGy) in one patient.

Factors affecting LV systolic and diastolic function and LVH

Mean LVEF and E/A values pre- and post HCT in each conditioning group are shown in Table 2. The LVEF and E/A before HCT in the two myeloablative conditioning groups were higher than those in cases who underwent RIC. In the interval from pre- to post-HCT, the LVEF decreased significantly in the high-dose, TBI-based conditioning group as did the E/A with both high-dose TBI-based and high-dose BU-based conditioning (Table 2, Figures 1a and b). In the simple linear regression analysis that assessed the relationship between LVEF or E/A and other factors, high-dose TBI-based conditioning was significantly associated with a decrease in LVEF

Table 2. Characteristics of patients divided according to conditioning regimens: mean LVEF and E/A values with ranges before and after HCT

	n	*Mean age (range)*	*Pre-HCT mean LVEF (%) (range)*	*Post-HCT mean LVEF (%) (range)*	P-*value*	*Pre-HCT mean E/A (range)*	*Post-HCT mean E/A (range)*	P-*value*
High-dose TBI-based	20	34.9 (19–59)	67.0 (64–72)	59.0 (28–69)	0.001	1.51 (0.9–2.5)	1.17 (0.6–2.0)	<0.001
High-dose BU-based	18	37.6 (19–60)	64.4 (55–76)	64.7 (55–76)	0.86	1.61 (0.7–2.2)	1.24 (0.5–1.9)	0.004
RIC	25	49.8 (19–69)	62.7 (46–79)	60.3 (41–72)	0.08	1.11 (0.7–1.9)	1.04 (0.5–1.7)	0.31

Abbreviations: A = mitral late diastolic velocity; E = peak early mitral inflow velocity; E/A = the ratio of peak mitral E to A filling velocities; HCT = allogeneic hematopoietic cell transplantation; LVEF = left-ventricular ejection fraction; RIC = reduced intensity conditioning. *P*-values represent the difference between pre- and post-HCT values; $P<0.05$ is statistically significant.

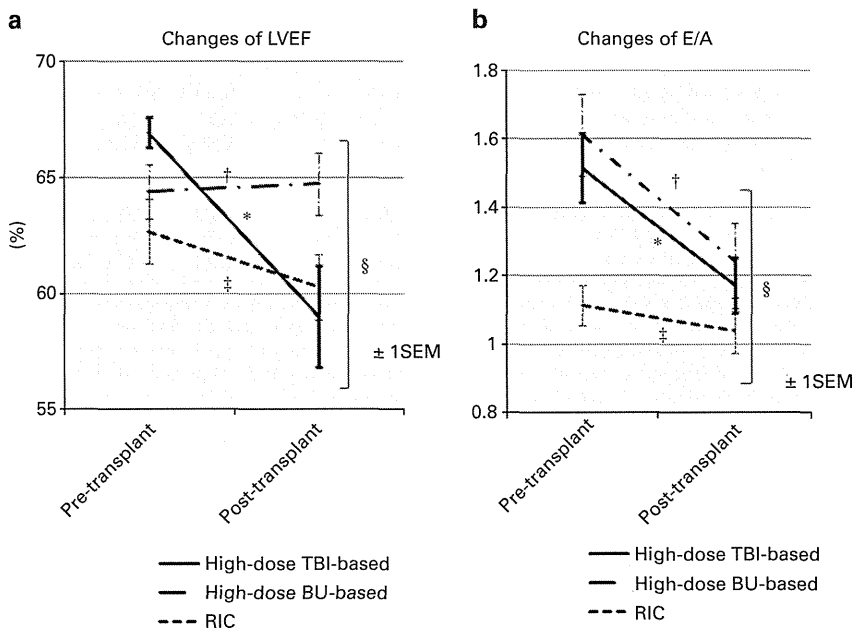

Figure 1. (**a**) Changes in LVEF from pre-transplant to post-transplant. *$P=0.001$; †, ‡: not statistically significant; and §: there were statistically significant differences among the three groups ($P=0.005$). (**b**) Changes of E/A from pre-transplant to post-transplant. *$P<0.001$; †$P=0.004$; ‡: not statistically significant; and §: there were statistically significant differences among the three groups ($P=0.02$).

Table 3. Simple linear regression analysis of changes in LVEF and E/A before and after HCT ($n=63$)

	Changes of LVEF		*Changes of E/A*	
	β (95% CI)	P-*value*	*β (95% CI)*	P-*value*
High-dose TBI-based (vs RIC)	**−5.55 (−10.16 to −0.94)**	**0.02**	**−0.27 (−0.49 to −0.04)**	**0.02**
High-dose BU-based (vs RIC)	2.73 (−2.02 to 7.49)	0.25	**−0.29 (−0.53 to −0.06)**	**0.01**
Sex (female/male)	1.43 (−2.75 to 5.62)	0.50	0.07 (−0.13 to 0.27)	0.47
Age at HCT (year)	0.05 (−0.10 to 0.21)	0.50	0.00 (−0.01 to 0.01)	0.52
HT (yes/no)	2.54 (−6.04 to 11.12)	0.56	−0.22 (−0.63 to 0.19)	0.28
Hgb level (g/dL)	0.33 (−0.75 to 1.42)	0.54	0.04 (−0.02 to 0.09)	0.16
s-ferritin level (ng/mL)	0.00 (−0.00 to 0.00)	0.57	−0.00 (−0.00 to 0.00)	0.44
Chronic GVHD (yes/no)	1.18 (−3.01 to 5.37)	0.58	−0.05 (−0.25 to 0.15)	0.62
Anthracyclines (mg/m^2)	0.01 (−0.01 to 0.02)	0.36	0.00 (−0.00 to 0.00)	0.87

Abbreviations: A = mitral late diastolic velocity; anthracyclines = cumulative dose of anthracyclines; β = regression coefficient; Chronic GVHD = the presence of chronic GVHD within 1 year of HCT; CI = confidence interval; E = peak early mitral inflow velocity; E/A = the ratio of peak mitral E to A filling velocities; HCT = allogeneic hematopoietic cell transplantation; Hgb level = hemoglobin level 1 year after HCT; HT = a history of hypertension within 1 year of HCT; LVEF = left-ventricular ejection fraction; RIC = reduced intensity conditioning; s-ferritin = serum ferritin level before HCT. $P<0.05$ is statistically significant. Bold type indicates data that achieved statistical significance.

after HCT (coefficient = −5.550, $P=0.02$). Patients who received a high-dose TBI-based or high-dose BU-based conditioning regimen had a significant decrease in E/A after HCT (coefficient = −0.268, $P=0.02$ and coefficient = −0.293, $P=0.01$, respectively) (Table 3).

Furthermore, we tested several regression models to assess the relationship between LVEF or E/A and the conditioning regimen, using multiple linear regression analyses (Table 4). The high-dose, TBI-based conditioning regimen was significantly associated with

Table 4. Multiple linear regression models of changes in LVEF and E/A before and after HCT ($n = 63$)

	Changes of LVEF				*Changes of E/A*			
	β (95% CI)	*β′*	P-*value*	R^2	*β (95% CI)*	*β′*	P-*value*	R^2
Model 1								
High-dose TBI-based (vs RIC)	**−5.37 (−10.08 to −0.67)**	**−0.31**	**0.03**		**−0.29 (−0.52 to −0.06)**	**−0.34**	**0.01**	
High-dose BU-based (vs RIC)	2.92 (−1.92 to 7.77)	0.16	0.23	0.17	**−0.31 (−0.55 to −0.08)**	**−0.36**	**0.01**	0.14
Chronic GVHD	0.94 (−3.03 to 4.92)	0.06	0.64		−0.11 (−0.30 to 0.09)	−0.14	0.28	
Model 2								
High-dose TBI-based (vs RIC)	**−5.54 (−10.17 to −0.91)**	**−0.31**	**0.02**		**−0.27 (−0.50 to −0.04)**	**−0.32**	**0.02**	
High-dose BU-based (vs RIC)	2.86 (−1.92 to 7.64)	0.16	0.24	0.17	**−0.29 (−0.52 to −0.05)**	**−0.33**	**0.02**	0.13
s-ferritin level	−0.00 (−0.00 to 0.00)	−0.09	0.45		−0.00 (0.00 to 0.00)	−0.08	0.50	
Model 3								
High-dose TBI-based (vs RIC)	**−4.86 (−9.64 to −0.07)**	**−0.28**	**0.05**		**−0.30 (−0.53 to −0.06)**	**−0.35**	**0.01**	
High-dose BU-based (vs RIC)	3.68 (−1.39 to 8.74)	0.20	0.15	0.18	**−0.33 (−0.58 to −0.09)**	**−0.39**	**0.01**	0.14
Anthracyclines	0.01 (−0.00 to 0.03)	0.14	0.29		0.00 (−0.00 to 0.00)	−0.12	0.35	
Model 4								
High-dose TBI-based (vs RIC)	**−5.55 (−10.24 to −0.85)**	**−0.32**	**0.02**		**−0.29 (−0.52 to −0.06)**	**−0.34**	**0.01**	
High-dose BU-based (vs RIC)	2.73 (−2.07 to 7.53)	0.15	0.26	0.16	**−0.29 (−0.52 to −0.05)**	**−0.33**	**0.02**	0.15
HT	0.04 (−8.12 to 8.19)	0.00	0.99		−0.25 (−0.65 to 0.14)	−0.16	0.20	

Abbreviations: A = mitral late diastolic velocity; anthracyclines = cumulative dose of anthracyclines; β = regression coefficient; β′ = standardized regression; Chronic GVHD = the presence of chronic GVHD within 1 year of HCT; CI = confidence interval; E = peak early mitral inflow velocity; E/A = the ratio of peak mitral E to A filling velocities; HCT = allogeneic hematopoietic cell transplantation; HT = a history of hypertension within 1 year of HCT; LVEF = left-ventricular ejection fraction; RIC = reduced intensity conditioning; s-ferritin = serum ferritin level before HCT. High-dose TBI-based (vs RIC) and high-dose BU-based (vs RIC) regimens achieved significance in all models. $P < 0.05$ is statistically significant. Bold type indicates data that achieved statistical significance.

a decrease in LVEF and E/A after HCT, and the high-dose BU-based conditioning regimen showed a significant decrease in E/A after HCT, following adjustment for chronic GVHD, s-ferritin, cumulative dose of anthracyclines and a history of HT (Table 4). In addition, after adjustment for sex, age and hemoglobin concentration, the high-dose TBI-based conditioning regimen was significantly associated with a decrease in LVEF and E/A after HCT, and the high-dose BU-based conditioning regimen showed a significant decrease in E/A after HCT (Supplementary Table 1).

Twelve patients received a new diagnosis of LVH after HCT. Several logistic regression models were tested to assess the effects of the pre- and the post-transplant variables on the incidence of LVH (Table 5). With logistic regression analysis, insertion of a quadratic transformation of s-ferritin alone improved the fit compared with the linear model. Because both the linear and the quadratic transformation of s-ferritin were significant in all models at $P < 0.05$, both of these variables were retained in all models that included s-ferritin (Table 5). The s-ferritin level was identified as a significant risk factor for LVH after HCT in both the univariable and multivariable logistic analyses (Table 5). However, we could not identify a significant effect of chronic GVHD on cardiac function or LVH after HCT.

DISCUSSION

Our study shows that myeloablative conditioning, in particular regimens that included high-dose TBI, adversely affected cardiac function even in the chronic phase of HCT. Unexpectedly, we could not establish a significant correlation between chronic GVHD and cardiac function. Notably, the s-ferritin level was identified as an independent risk factor for LVH after HCT.

A long-term follow-up study of cardiac function after HCT in children showed similar results, with a report that TBI was a risk factor for cardiac dysfunction.[13] In contrast, Auner *et al.*[7] reported that high-dose TBI combined with high-dose CY was not associated with a significant decrease in cardiac function. However, these investigators evaluated cardiac function after a relatively short-term follow-up period, with a median of just 5 months. Radiotherapy-induced late cardiotoxicity has often been described in patients with Hodgkin's lymphoma who received mediastinal radiotherapy.[14–17] Pathological examination of radiotherapy-induced myocarditis showed diffuse interstitial fibrosis and microcirculatory damage, leading to capillary obstruction and extensive fibrosis,[14] with the potential to cause LV systolic and diastolic dysfunction.[16,17] In addition, CY-containing regimens without high-dose TBI significantly reduced LV diastolic function but not systolic function. Therefore, our results suggest that chronic LV systolic dysfunction after HCT might largely reflect a late, toxic effect of TBI.

There were differences in the pre-transplant LVEF and E/A in the three groups of conditioning regimens in our study—the LVEF and E/A before HCT in the two myeloablative conditioning groups were better than those in the RIC group. We thought that the results likely indicated that the patients receiving myeloablative conditioning regimens were younger and/or had fewer cardiac complications than the RIC patients.

Our data demonstrated that the s-ferritin level pre-HCT, rather than age or a history of HT, was significantly associated with development of LVH after HCT. A high s-ferritin level has been thought to reflect iron overload.[18] Although transfusion-related iron overload was reported to be causally associated with LVH,[19] the causal association between the s-ferritin level and LVH is still unclear. However, release of ferritin could be induced by IL-1 beta or TNF-alpha.[20] Thus, the relationship between the s-ferritin level and LVH might indicate that IL-1 beta or TNF-alpha was involved in the pathogenesis of myocyte hypertrophy. Further study will be required to elucidate the precise mechanism of development of LVH after HCT.

In our study, the presence of chronic GVHD did not appear to have any significant impact on cardiac function after HCT. There are some reports of cardiac dysfunction during GVHD but these are just case series.[21–24] However, a larger, retrospective cohort study showed no statistically significant difference in the incidence of congestive heart failure among patients with acute GVHD, those with a history of chronic GVHD and

Table 5. Logistic regression models of incidence of LVH in subjects without LVH at baseline ($n = 56$)

	Univariable logistic analysis		*Multivariable logistic analysis*	
	OR (95% CI)	*P-value*	*OR (95% CI)*	*P-value*
Conditioning regimen				
RIC	1.00 (reference)			
High-dose TBI-based	1.10 (0.25–4.88)	0.90		
High-dose BU-based	1.18 (0.24–5.89)	0.84		
Sex				
Male	1.00 (reference)			
Female	2.89 (0.76–11.06)	0.12		
Age at HCT	**1.08 (1.01–1.14)**	**0.02**	1.07 (1.00–1.15)	0.06
HT				
No	1.00 (reference)			
Yes	0.26 (0.02–4.42)	0.35		
Hgb level	**0.65 (0.45–0.92)**	**0.01**	0.81 (0.54–1.20)	0.29
s-ferritin level (ng/mL)				
$\leqslant 600$[a]	1.00 (reference)			
>600	3.95 (0.94–16.60)	0.06		
s-ferritin model[b]	**1.00 (1.00–1.01)**	**0.01**	**1.00 (1.00–1.01)**	**0.03**
Chronic GVHD				
No	1.00 (reference)			
Yes	1.20 (0.33–4.31)	0.78		
Anthracyclines	1.00 (1.00–1.01)	0.65		

Abbreviations: anthracyclines = cumulative dose of anthracyclines; Chronic GVHD = the presence of chronic GVHD within 1 year of HCT; CI = confidence interval; HCT = allogeneic hematopoietic cell transplantation; Hgb level = hemoglobin level 1 year after HCT; HT = a history of hypertension within 1 year of HCT; LVH = left-ventricular hypertrophy; OR = odds ratio; RIC = reduced intensity conditioning; s-ferritin = serum ferritin level before HCT. $P < 0.05$ is statistically significant. Bold type indicates data that achieved statistical significance. [a]Median value of s-ferritin level. [b]s-ferritin model = (s-ferritin) $- \beta 2/\beta 1 \times$ (s-ferritin)^2; $\beta 1$ and $\beta 2$ denote coefficients of s-ferritin and (s-ferritin)^2, respectively; $\beta 1 = 3.184\text{e-}3$ and $\beta 2 = -8.050\text{e-}7$, in the univariable model; and $\beta 1 = 3.591\text{e-}3$ and $\beta 2 = -9.958\text{e-}7$, in the multivariable model.

those with active GVHD.[25] Uderzo *et al.*[13] report similar findings in childhood HCT.

This study was limited by its retrospective, nonrandomized design and small study population. The incidence of cardiac dysfunction might be underestimated because of survivor bias. In addition, some patients were excluded from analysis because of a change in hospital or lack of echocardiographic data pre-HCT. A further limitation was related to the assessment of cardiac diastolic dysfunction. E/A, used as a surrogate marker of cardiac diastolic function in our study, can be influenced by multiple factors that include heart rate,[26,27] loading conditions[26,28] and/or valvular regurgitation.[29] However, none of our patients had features of heart failure, such as tachycardia, dyspnea or edema. In addition, no patient had severe mitral regurgitation or stenosis.

In conclusion, our results suggest that intensive conditioning, especially regimens that involved high-dose TBI for HCT, had a significant negative effect on chronic cardiac function; a high level of s-ferritin was also found to be a crucial risk factor for LVH in the chronic phase after HCT. Long-term survivors who received an intensive conditioning regimen should therefore have their cardiac function carefully monitored, as well as the function of other organs. In the future, a larger, adequately powered, prospective study of adult HCT patients will be required to confirm our observations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CASE REPORT

Detection of donor-derived CMV-specific T cells in cerebrospinal fluid in a case of CMV meningoencephalitis after cord blood stem cell transplantation

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Abstract Cytomegalovirus (CMV) meningoencephalitis is a rather rare complication after allogeneic stem cell transplantation. We describe here the case of a 59-year-old man with acute myeloid leukemia who developed CMV meningoencephalitis after cord blood transplantation. The patient presented with a sudden onset of neurological symptoms, such as convulsion, on day 37. The analysis of cerebrospinal fluid (CSF) sample revealed an increase in the number of cells, which were of donor (cord blood) origin, consisting mainly of T cells. No bacteria were detected in the CSF sample. Real-time PCR analysis revealed that the CSF sample was positive for CMV, but was negative for HHV-6, adenovirus, or BK virus. The patient was diagnosed with CMV meningoencephalitis and received cidofovir. His neurological symptoms were gradually improved and completely disappeared by day 60. CMV-specific dextramer-positive $CD8^+$ T cells were detected in the peripheral blood and CSF samples, with the frequency being much higher in the CSF. To our knowledge, this is the first report on the appearance of CMV-specific T cells in CSF samples from a patient with CMV meningoencephalitis. Cord blood-derived CMV-specific T cells may develop early after transplantation, enter the intrathecal compartment, and likely contribute to the regulation of CMV-meningoencephalitis.

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Keywords Cytomegalovirus · Viral encephalitis · Cord blood transplantation · Dextramer

Introduction

Cytomegarovirus (CMV) meningoencephalitis (CMV-ME) is a rather rare complication, occurring in 6 % of patients with viral encephalitis after allogeneic stem cell transplantation (SCT) [1]. Recently, we had a patient with acute myeloid leukemia (AML) who developed CMV-ME after cord blood stem cell transplantation (CBT), and could, for the first time, confirm the presence of donor-derived CMV-specific CD8 T cells in the cerebrospinal fluid (CSF), using the dextramer staining procedure.

Case report

A 59-year-old Japanese man with AML (M6) evolving from myelodysplastic syndrome received a chemotherapy consisting of aclarubicin and cytarabine, and achieved complete remission morphologically, which was, however, considered as returning to refractory anemia because of the existence of deletion chromosome 20 in 17 of 20 cells in the karyotype analysis of bone marrow (BM) samples. The patient therefore was referred to our hospital for allogeneic SCT. Since there was no suitable donor in related and unrelated donor pools, we decided to perform CBT using reduced-intensity conditioning regimen, which consisted of fludarabine (FLU) 30 mg/m^2/day (day −6 to −2, total 150 mg), cyclophosphamide (CY) 50 mg/kg/day on day −6, and total body irradiation (TBI) 3 Gy on day −7 [2]. Graft-versus-host disease (GVHD) prophylaxis consisted of continuous infusion of cyclosporine (CsA) (target blood

concentration was 300–400 ng/ml), and mycophenolate mofetil (MMF) 30 mg/kg, both of which started from day −3. Cord blood (CB) graft was female and contained 6.66×10^7 nucleated cells/kg and 4.77×10^5 $CD34^+$ cells/kg. The HLA profiles of the patient and CB unit were as follows: patient HLA A*24:02 *02:01, B*35:01 *56:01, DRB1*04:10 *09:01, and CB HLA A*24:02 *02:01, B*35:01 *40:01, DRB1*04:03 *09:01, which means mutual HLA 1 antigen mismatch in B locus. The clinical course of the patient is shown in Fig. 1. Hematopoietic engraftment was rapidly achieved, with absolute neutrophil count $>0.5 \times 10^9$/l on day 15 and platelet count $>50 \times 10^9$/l on day 29. On day 17, complete donor chimerism was confirmed in both $CD3^+$ and myeloid fractions of peripheral blood (PB) using informative short tandem repeat-PCR technique. The patient started having spiking fever >39 °C on day 7, which was considered as pre-engraftment immune reaction (PIR) [3]. Prednisolone was started at a dose of 5 mg/day on day 9 and increased to 10 mg/day on day 12. On day 12, the patient developed acute cutaneous GVHD (stage 1), which subsided a few days later without the need for any additional treatment. Foscarnet (FCN) 80 mg/kg/day was also started on day 8 as the prophylaxis of CMV or human herpes virus 6 (HHV6) infection. The fever subsided by day 20, but reappeared on day 32. CMV pp65 antigenemia, which was monitored weekly, continued to be negative from day −4 to day 87. Reactivation status for CMV, HHV6, adenovirus (ADV), and BK virus (BKV) was also monitored weekly using real-time PCR analysis of plasma samples. No viral reactivation was observed, except for a positive result for CMV (7.7×10^7 copy/μg DNA) on day 42.

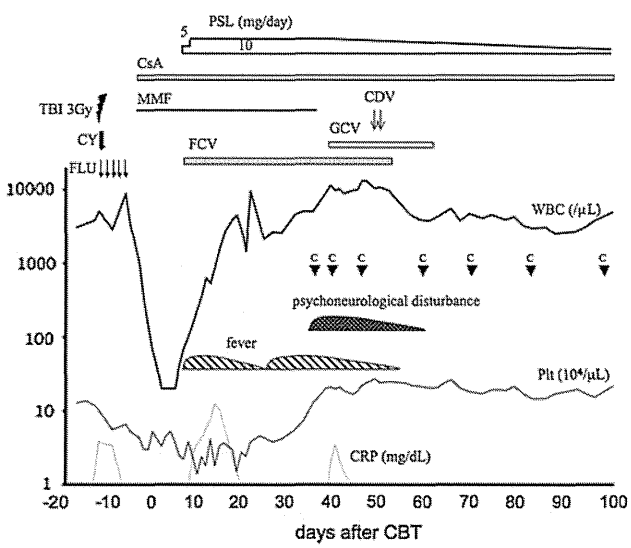

Fig. 1 Clinical course. *Bold*, *thin*, and *dotted lines* denote white blood cells (WBC) (/μL), platelets (10^4/μL), and CRP (mg/dL), respectively. *Flu* fludarabine, *CsA* cyclosporine, *MMF* mycophenolate mofetil, *PSL* prednisolone, *CY* cyclophosphamide, *TBI* total body irradiation, *FCN* foscarnet, *GCV* ganciclovir, *CDV* cidofovir, *C* CSF sample analysis

On the night of day 37, the patient's behavior suddenly became abnormal, such as cutting the infusion line with a pair of scissors or urinating on the bed board. Next morning, the patient was barely able to make even a simple conversation and his consciousness level was decreased, with the occurrence of general convulsion for a few minutes. An analysis of CSF sample on day 38 revealed that the cell number was 3464/3 μl (normal range 0–15/3 μl) consisting of polymorphonuclear leukocytes (PMN) 83 % and mononuclear cells (NMC) 17 %. The biochemical data of the CSF sample were: protein 261 mg/dl (normal range 40–75 mg/dl), Cl 116 mmol/l (normal range 120–130 mmol/l), and LDH 95 U/l (normal range 8–50 U/l). These results suggested that the patient had bacterial meningitis, but no bacterium was cultured in the CSF sample. MRI of the brain on day 39 showed no abnormal findings. Meropenem 3 g/day and ganciclovir (GCV) 3 mg/kg/day were started, with the administration of an increased dose of immunoglobulin. Real-time PCR data revealed that the CSF sample on day 38 was positive for CMV and negative for HHV6, while the PB sample was negative for the 2 viruses. There was no sign of CMV disease in other organs [4]. Follow-up data of the CSF are shown in Table 1. The CSF cell components turned to an MNC-dominant status after day 42. Although the reason why PMN was dominant in the CSF sample on day 38 is unknown, we speculate that PMN might have reflected a hyperacute inflammatory response in the central nervous system (CNS). Cidofovir 1 mg/kg/day was administered on days 50 and 52, and discontinued due to the elevation of serum creatinine level. His psychological and neurological symptoms gradually improved and completely disappeared on day 60. Follow-up brain MRI showed also normal results. He was discharged without any sequelae on day 104.

We performed the immunological characterization of CSF cells. The CSF cells on day 61 were of 100 % donor (CB) origin on chimerism analysis using STR-PCR. MNCs in the CSF mainly consisted of T cells: $CD3^+$ $CD4^+$ T cells 48.2 % and $CD3^+$ $CD8^+$ T cells 23.7 %, NK cells ($NKp46^+$ cells) 21.4 %, and B cells 0.8 %. The patient and CB shared HLA A*24:02 and A*02:01. We tested the presence of CMV-specific CD8 T cells in the PB and CSF samples on day 70 using CMV-specific HLA A*24:02-restricted and HLA A*02:01-restricted dextramers (Immudex, Copenhagen, Denmark). Dextramer staining was performed according to the manufacturer's protocol. Cells were stained with phycoerythrin-Cy7-conjugated anti-CD8, phycoerythrin-Cy5-conjugated anti-CD3 (Beckman Coulter Inc., Fullerton, CA, USA), and phycoerythrin- conjugated dextrameric-HLA A*02:01-restricted NLVPMVATV peptide complex or fluorescein isothiocyanate-conjugated

Table 1 Laboratory and viral PCR data of the CSF

Post CBT day	38	42	49	61	70	84	98
Cell number (/3 μl)	3464	1136	1336	861	376	277	217
PMN/MNC (%)	83/17	2/98	4/96	0/100	1/99	0/100	1/99
Protein (mg/dl)	261	268	289	118	107	100	96
CMV in CP CSF (copy/μg DNA)	2.8×10^6	7.5×10^5	2.0×10^4	1.6×10^2	NT	–	–
CMV in whole CSF (copy/ml)	2.8×10^6	4.9×10^7	9.1×10^5	6.7×10^2	NT	–	–
HHV6 in CP CSF (copying DNA)	–	–	–	–	NT	–	–
HHV6 in whole CSF (copy/ml)	–	–	–	–	NT	–	–
ADV in whole CSF (copy/ml)	–	–	–	–	NT	–	–
BKV in whole CSF (copy/ml)	–	–	–	–	NT	–	–

The amount of DNA of viruses, including CMV, HHV6, ADV, and BKV, in the whole or centrifuged pellet (CP) samples of the CSF was measured using real-time PCR. There was no PCR data on day 70 because most of the CSF sample was used for the flow cytometry and dextramer assay

PMN polymorphonuclear cells, *MNC* mononuclear cells, *CMV* cytomegalovirus, *HHV6* human herpes virus 6, *ADV* adenovirus, *BKV* BK virus, *NT* not tested

dextrameric-HLA A*24:02-restricted QYDPVAALF peptide complex (Immudex, Copenhagen, Denmark). After lysing red blood cells and washing twice with bovine serum albumin containing phosphate-buffered saline, cells were examined on a flow cytometer (Cytomics FC 500, Beckman Coulter, Inc., USA). More than 100,000 cells were acquired in the lymphocyte gate and analyzed using CXP software. The percentage of CMV-specific dextramer-positive cells in the $CD3^+$ $CD8^+$ fraction is shown in Table 2. The dextramer-negative control value in the CSF was a little high; however, these data suggest that the percentage of CMV-specific T cells is higher in the CSF than in the PB at least for A*02:01 dextramer. CMV-specific CD8 T cells seemed to be dominantly HLA A*02:01-restricted, but direct comparison was limited due to the difference in the efficacy of the two dextramers. Of note, CSF cell numbers were maintained still at high levels even after CMV DNA became undetectable (Table 1).

Table 2 CMV-specific T cells (%) in the $CD3^+$ $CD8^+$ fraction

	A*02:01 dextramer	A*24:02 dextramer	Dextramer (−)
PB	0.01	0.26	0.01
CSF	1.19	0.47	0.19

Discussion

Cytomegalovirus disease of the CNS is a rare complication after allogeneic SCT in patients. Reddy et al. [4] recently summarized 11 cases of CMV disease of the CNS after SCT. According to their report, all cases developed CMV CNS disease at late onset (occurring 166 or more days after transplantation), were ganciclovir resistant, and ten of them expired despite antiviral combination therapy. Drug resistance was pointed out to be a key factor in the occurrence of CMV CNS disease [5]. In our case, the CMV disease was also suggested to be relatively FCN resistant, since CMV-ME developed during prophylactic FCN administration. In accordance with the previous report [4], there was no evidence of CMV disease in organs other than CNS. The patient did not even show CMV antigenemia or positive PCR test for CMV DNA using the plasma samples except for one (PCR data on day 42). The occurrence of CMV CNS lesion in an isolated form may reflect a relatively low penetration of FCN, as described by Reddy et al. CMV disease of the CNS is reported to develop at late onset because drug resistant virus appears after a relatively long period of drug therapy. On the other hand, CMV-ME in our case that developed in a form as related to PIR in the engraftment period is similar to post-transplant HHV-6 encephalitis, which was reported to develop in association with the production of inflammatory cytokines such as interleukin-6 [6]. Furthermore, in our case, the absence of abnormal findings of MRI of the brain may have resulted in complete recovery of this serious complication.

There have been no reports showing the presence of CMV-specific CTLs in the CFS of patients with CMV-ME. Regarding the detection of virus-specific CTLs in the CSF, JC virus-specific CTLs in patients with progressive multifocal leukoencephalopathy [7], and HIV-specific $CD8^+$ T cells in antiretroviral therapy-naïve HIV-positive subjects [8], have been reported. These studies suggest that the presence of virus-specific CTLs in the CSF has a beneficial effect in controlling these viral CNS diseases. Likewise, the presence of CMV-specific CTLs in the CSF in our case may have exerted some beneficial effects, although ganciclovir and/or cidofovir are considered to have contributed to controlling CMV-ME. In the present report, we first showed the existence of CMV-specific T cells in CSF samples of the patient with CMV-ME. In addition, we

underlined that CMV-specific T cells were of donor origin (CB derived), and that the frequency of CMV-specific T cells was higher in CSF than in PB. In macaques, activated T cells were reported to preferentially enter the intrathecal compartment and increase in frequency early after acute simian immunodeficiency virus infection [9]. Furthermore, rodent data suggest that the expression of viral antigens in the brain may upregulate endothelial cell major histocompatibility complex class I expression, contributing to $CD8^+$ T cell migration into the brain [10]. Taken together with these findings, we consider in our case that CB-derived CMV-specific T cells may develop early in transplantation and enter the intrathecal compartment.

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Conflict of interest The authors declare no competing financial interests.

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Short communication

Nephrotoxicity of concomitant use of tacrolimus and teicoplanin in allogeneic hematopoietic stem cell transplant recipients

T. Mori, T. Shimizu, J. Kato, T. Kikuchi, S. Kohashi, Y. Koda, T. Toyama, M. Saburi, O. Iketani, S. Okamoto. Nephrotoxicity of concomitant use of tacrolimus and teicoplanin in allogeneic hematopoietic stem cell transplant recipients.
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Abstract: Both tacrolimus and glycopeptide antibiotics are known to be nephrotoxic, and are often concomitantly given after hematopoietic stem cell transplantation (HSCT) or solid organ transplantation. The aim of this study is to evaluate the nephrotoxicity of concomitant use of tacrolimus and glycopeptide antibiotics in HSCT recipients. We retrospectively evaluated 67 patients who received intravenous tacrolimus and teicoplanin concomitantly for >4 days after allogeneic HSCT for hematologic diseases. Therapeutic drug monitoring (TDM) was performed in all patients for both tacrolimus and teicoplanin. The median age of the patients was 48 years (range: 16–62), and the median duration of the co-administration of tacrolimus and teicoplanin was 11 days (range: 4–40). The mean serum creatinine (sCr) level tended to be elevated after the co-administration (from 0.69 ± 0.26 to 0.75 ± 0.30 mg/dL; $P = 0.08$); however, a 2-fold or greater increase in sCr was observed only in 2 (3.0%) patients. Increased sCr was reversible, and no patient required hemodialysis. These results suggest that the incidence of clinically significant nephrotoxicity can be minimized if the TDM of each drug is properly applied.

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Key words: tacrolimus; teicoplanin; nephrotoxicity; serum creatinine; therapeutic drug monitoring

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Tacrolimus, a calcineurin inhibitor, has been widely used for the prophylaxis and treatment of graft-versus-host disease in allogeneic hematopoietic stem cell transplantation (HSCT) and graft rejection in solid organ transplantation (1, 2). Among the various types of tacrolimus toxicity, nephrotoxicity is the most common and clinically problematic. Therefore, it is recommended that the concomitant use of other nephrotoxic agents be avoided, in order to prevent the development of serious nephrotoxicity (3).

However, transplant recipients remain highly susceptible to a variety of infectious complications and often require nephrotoxic antimicrobial agents, such as glycopeptide antibiotics. Glycopeptide antibiotics, such as vancomycin and teicoplanin, are active against beta-lactam-resistant gram-positive organisms, and have been increasingly used early after HSCT for the treatment of opportunistic infections caused by these organisms (4). Although both drugs are nephrotoxic, teicoplanin is reported to be less toxic than vancomycin (4–6). However, the nephrotoxicity of the concomitant use of tacrolimus and teicoplanin in this setting has yet to be evaluated. Therefore, we retrospectively evaluated the incidence and severity of nephrotoxicity in HSCT recipients who concomitantly received intravenous tacrolimus and teicoplanin.

Patients and methods

Patients

Among the patients who underwent allogeneic HSCT for hematologic diseases at Keio University Hospital

(Tokyo, Japan) between December 2004 and April 2012, the patients who fulfilled the following criteria were included in this study: (i) intravenous teicoplanin was given during the continuous infusion of tacrolimus within 30 days after allogeneic HSCT; (ii) teicoplanin was given concomitantly with tacrolimus for >4 days; (iii) therapeutic drug monitoring (TDM) of both tacrolimus and teicoplanin was performed; and (iv) no amphotericin B, liposomal amphotericin B, or foscarnet were administered during the evaluated co-administration period. Data were collected from the institutional database and the medical records of Keio University Hospital.

Drug administration and measurement of blood concentrations

For prophylaxis against graft-versus-host disease, all patients received tacrolimus in combination with short-term methotrexate, as we have previously described (2). Tacrolimus was continuously infused with an initial dose of 0.03 mg/kg starting 1 day before transplantation, and the dose was adjusted to maintain a whole-blood concentration between 10 and 20 ng/mL (2). Teicoplanin was administered intravenously with a loading dose of 800 mg in 2 divided doses on the first day, which was followed by a daily dose of 400 mg/day in 1 dose. The dose of teicoplanin was adjusted to maintain its trough level between 10 and 20 mg/mL. The whole-blood concentration of tacrolimus was measured either by a standard microparticle enzyme immunoassay or chemiluminescent immunoassay. The serum concentration of teicoplanin was measured by fluorescence polarization immunoassay, basically according to the previous methods (7). Concentrations were measured at least 3 times and twice a week for tacrolimus and teicoplanin, respectively. The frequency of TDM was increased at the discretion of the physician or pharmacist.

Statistical analysis

In each patient, serum creatinine (sCr) at the end of the co-administration of tacrolimus and teicoplanin was compared with that before initiating the co-administration. The Wilcoxon signed-rank test was used to compare the difference in sCr before and after the co-administration. *P*-values <0.05 were accepted as statistically significant.

Results

Patients

In total, 67 patients (median age: 48 years) fulfilled the criteria and were enrolled in the analysis. Patient characteristics are shown in Table 1. All but 5 patients with aplastic anemia received HSCT for hematologic malignancies. Most of the patients received bone marrow from unrelated donors, and about two-thirds of the patients received total body irradiation (12 Gy)-based myeloablative conditioning.

Co-administration of tacrolimus and teicoplanin and its effects on sCr levels

The median sCr level before the co-administration of tacrolimus and teicoplanin was 0.7 mg/dL (range: 0.3–2.1), and sCr was >2.0 mg/dL in 1 patient (Fig. 1). The median duration of the co-administration was 11 days (range: 4–40). Fifty-seven (85%) of the patients underwent the co-administration for 7 days or longer. The mean whole-blood concentration of tacrolimus during the co-administration was 16.3 ± 1.7 ng/mL. The mean sCr level tended to be elevated after the

Patient characteristics (*n* = 67)

Median age (range)	48 (16–62)
Gender	
Male/Female	43/24
Disease	
Acute myeloid leukemia	18
Acute lymphoblastic leukemia	12
Myelodysplastic syndrome	10
Non-Hodgkin's lymphoma	8
Multiple myeloma	8
Aplastic anemia	5
Chronic myelogenous leukemia	3
Myeloproliferative neoplasms	2
Chronic lymphocytic leukemia	1
Type of donor and stem cell sources	
Unrelated, bone marrow	63
Related, bone marrow	2
Unrelated, cord blood	2
Conditioning	
Myeloablative	39
Reduced-intensity	28

Table 1

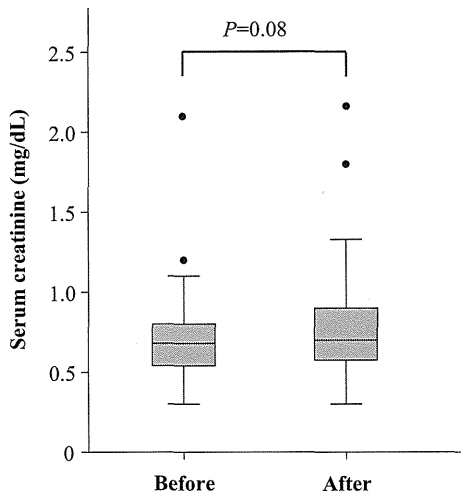

Fig. 1. Effect of co-administration of tacrolimus and teicoplanin on the serum creatinine level. The serum creatinine level was not statistically elevated after the co-administration ($P = 0.08$).

co-administration, as compared with that before (0.69 ± 0.26 vs. 0.75 ± 0.30 mg/dL; $P = 0.08$; Fig. 1). On an individual basis, a 50% or greater increase in sCr, compared with that before initiating teicoplanin, was observed only in 5 (7.5%) of 67 patients, including 2 (3.0%) with a 2-fold or greater increase. Increased sCr levels were reversible in all patients by appropriate hydration and adjusting the blood concentration of tacrolimus to between 10 and 15 ng/mL, and no patient required hemodialysis. The administration of teicoplanin was not discontinued, and its dose was not modified solely because of the increased sCr levels in any of the patients.

Discussion

In this study, we have shown a low incidence of nephrotoxicity associated with the co-administration of tacrolimus and teicoplanin, even early after allogeneic HSCT for hematologic diseases. These results strongly suggest that teicoplanin could be safely administered concomitantly with tacrolimus. However, it should be emphasized that TDM was universally performed in all the patients in this study. TDM is essential for both tacrolimus and teicoplanin to maximize their efficacy while minimizing their several types of toxicity, especially nephrotoxicity. In contrast to tacrolimus, however, TDM for teicoplanin has not been widely practiced, as teicoplanin has a favorable toxicity profile and a relationship between its blood concentration and toxicity has not been established (8, 9). TDM has been routinely performed for teicoplanin in our institution because of the institutional policy and the increasing evidence for the use of TDM in increasing the efficacy of teicoplanin (10, 11). It should be emphasized that all patients in this study were placed on the target blood concentration of teicoplanin under a strict TDM. Therefore, the results of this study should be interpreted while taking the efficacy of TDM into consideration.

In general, concomitant use of nephrotoxic agents should be avoided when possible; in some cases, however, and especially in seriously ill patients, such treatment cannot be avoided. HSCT and solid organ transplant recipients are highly susceptible to infectious complications, and routinely receive calcineurin inhibitors such as tacrolimus and cyclosporin A (CsA). Therefore, calcineurin inhibitors are often co-administered with nephrotoxic antimicrobial agents in this patient population. Glycopeptide antibiotics are among the most widely recognized nephrotoxic agents. Although teicoplanin has been demonstrated to be less nephrotoxic than vancomycin, it should be used cautiously to avoid causing renal insufficiency being caused (4). Only 1 report has been published of nephrotoxicity associated with the concomitant use of calcineurin inhibitors and glycopeptides; in that case (12), CsA with vancomycin was found to be more nephrotoxic than CsA with teicoplanin, resulting in higher mortality. Tacrolimus, especially its intravenous formulation, is known to be highly nephrotoxic, and thus the safety of concomitant use of tacrolimus and glycopeptides should be fully examined. However, to the best of our knowledge, this kind of evaluation has not been reported in the literature. At our institution, teicoplanin is recommended as the first-line antimicrobial agent for infection caused by beta-lactam-resistant gram-positive organisms after allogeneic HSCT, and thus teicoplanin was selected for the cases included in this study. In our study, by retrospectively analyzing the accumulated data of multiple cases, we were able to evaluate the safety of this co-administration. In a future study, co-administration of tacrolimus with more widely used vancomycin should also be evaluated.

In the present study, the nephrotoxicity associated with the co-administration of tacrolimus and teicoplanin was evaluated when tacrolimus was infused intravenously. As mentioned above, intravenously administered tacrolimus has been shown to be more nephrotoxic than orally administered tacrolimus, although the blood concentration of the drug also plays an important role (1). In the present work, the blood concentration was maintained at a high level (a median of >16 ng/mL) to maximize its efficacy. Thus, this

condition is suitable for the evaluation of the true toxicity of tacrolimus and its co-administration with other nephrotoxic agents. The infrequent relevant nephrotoxicity observed under these conditions strongly suggested that tacrolimus and teicoplanin could be safely co-administered. However, as compared to our study, higher doses and blood concentration of teicoplanin (>20 mg/mL) have recently been recommended for severe infections such as bloodstream infection and musculoskeletal infection (13–16). Therefore, future studies should focus on the safety of the co-administration of tacrolimus and teicoplanin when the higher blood concentration of teicoplanin is applied. In addition, as mentioned above, TDM has not been globally practiced (8, 9). In this situation, therefore, the results of a future study comparing the nephrotoxicity between the groups with and without TDM for teicoplanin would be of great clinical benefit.

When a 50% or greater increase in sCr level compared to the baseline was observed during the co-administration of tacrolimus and teicoplanin, patients were initially managed with appropriate hydration in this study. If sCr continued to increase, the dose of tacrolimus was then adjusted to decrease the blood concentration of tacrolimus, without adjusting the dose of teicoplanin. Such management could have avoided the progression of renal insufficiency and the requirements of hemodialysis. Although the results of our study suggest the safety of the co-administration of tacrolimus and teicoplanin, a careful monitoring of sCr levels along with appropriate management is still required.

In conclusion, these results suggest that the incidence of clinically significant nephrotoxicity caused by the co-administration of tacrolimus and teicoplanin can be minimized if TDM of each drug is properly applied. Future studies should focus on the nephrotoxicity associated with the co-administration of tacrolimus and nephrotoxic agents other than teicoplanin.

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MAJOR ARTICLE

Human Herpesvirus 6 (HHV-6) Reactivation and HHV-6 Encephalitis After Allogeneic Hematopoietic Cell Transplantation: A Multicenter, Prospective Study

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Background. The epidemiology of human herpesvirus 6 (HHV-6) encephalitis after allogeneic hematopoietic cell transplantation (HCT) and its relationship with HHV-6 reactivation have not been sufficiently characterized.

Methods. This prospective, multicenter study of 230 allogeneic HCT recipients investigated the epidemiology of HHV-6 reactivation and HHV-6 encephalitis. Plasma HHV-6 DNA load was prospectively evaluated twice weekly until 70 days after HCT.

Results. Cumulative incidence of positive HHV-6 DNA and high-level HHV-6 reactivation (plasma HHV-6 DNA $\geq 10^4$ copies/mL) at day 70 after HCT was 72.2% and 37.0%, respectively. Multivariate analysis identified myeloablative conditioning (hazard ratio [HR], 1.9; $P = .004$), umbilical cord blood transplantation (UCBT) (HR, 2.0; $P = .003$), and male sex (HR, 1.6; $P = .04$) as risk factors for displaying high-level HHV-6 reactivation. HHV-6 encephalitis occurred in 7 patients, and cumulative incidence at day 70 was 3.0%. None of the144 patients without high-level HHV-6 reactivation and 7 of 86 patients (8.1%) with high-level HHV-6 reactivation developed HHV-6 encephalitis ($P = .0009$). Prevalence of HHV-6 encephalitis was significantly higher among patients receiving UCBT than in patients with other sources (cumulative incidence at day 70, 7.9% vs 1.2%, $P = .008$). In each of 7 patients with HHV-6 encephalitis, central nervous system (CNS) symptoms developed concomitant with peak plasma HHV-6 DNA (range, 21 656–433 639 copies/mL).

Conclusions. High levels of plasma HHV-6 DNA are associated with higher risk of HHV-6 encephalitis. UCBT is a significant risk factor for HHV-6 encephalitis. HHV-6 encephalitis should be considered if CNS dysfunction develops concomitant to high-level plasma HHV-6 DNA after allogeneic HCT.

Keywords. human herpesvirus 6; allogeneic hematopoietic cell transplantation; HHV-6 encephalitis; HHV-6 reactivation; viral load.

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Human herpesvirus 6 (HHV-6) belongs to the *Betaherpesvirinae* subfamily and persists latently in the majority of the general population. HHV-6 comprises 2 forms, A and B. In 2012, HHV-6A and HHV-6B were officially considered as distinct species [1]. The clinical significance of HHV-6A remains unclear, whereas HHV-6B is the causative agent for exanthema subitum. In

recipients of allogeneic hematopoietic cell transplantation (HCT), reactivations of HHV-6 are common [2, 3] and related to various post-transplant complications [4–6]. Most HHV-6 reactivation in the setting of HCT is due to HHV-6B [2, 6]. HHV-6 encephalitis is a serious post-transplant complication that is sporadically caused by HHV-6 reactivation [3, 5, 7, 8]. Mortality rates attributable to this pathology remain high [7, 9–14], and even among survivors, many patients display cognitive sequelae [15].

Despite the poor outcome, the effects of systemic HHV-6 reactivation on the development of HHV-6 encephalitis and the morbidity of HHV-6 encephalitis have not been sufficiently characterized. This makes preventative options difficult to establish. The European Conference on Infections in Leukemia does not recommend antiviral prophylaxis against HHV-6 reactivation because of the low risk of HHV-6 disease and the toxicity of available antiviral drugs [16]. Recently, high incidences of HHV-6 encephalitis (5.7%–8.0%) have consistently been reported from HCT units in Japan [3, 8, 12, 14, 15, 17]. However, these studies have been retrospective, using less-stringent definitions of HHV-6 encephalitis and small populations. Some studies have suggested associations between high-level HHV-6 reactivation and development of central nervous system (CNS) dysfunction, but these associations were evaluated retrospectively [3, 8, 18]. A recent prospective study demonstrated an independent association between HHV-6 reactivation and subsequent delirium [19]. However, determining the proportion of cases with HHV-6 encephalitis is difficult, because cerebrospinal fluid (CSF) was obtained in only a small subset (4 of 19) of patients, and 2 of those 4 patients did not show detectable levels of HHV-6 DNA in CSF [19].

The present study investigated the epidemiology and morbidity of HHV-6 encephalitis in allogeneic HCT recipients, focusing on the relationship between plasma HHV-6 load and development of HHV-6 encephalitis using data on prospectively monitored HHV-6 load.

PATIENTS AND METHODS

Study Design and Patients

This prospective, multicenter trial was approved by the ethics committees of each contributing center. All patients who underwent allogeneic HCT between July 2010 and December 2011 were registered to this study. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The primary endpoint of the study was HHV-6 encephalitis episodes. The observation period for assessing HHV-6 reactivation and CNS dysfunction was from 1 to 70 days after transplantation.

Patient Care

Transplantation regimens, including selection of stem cell source, conditioning regimens, antiviral prophylaxis, and prophylaxis of acute graft-versus-host disease (GVHD) were determined according to the protocol of each institute and participation in this study had no effect on these clinical decisions. All patients were administered oral acyclovir at 600–1000 mg/day from day 7 to day 35 to prevent infection with herpes simplex virus. Cytomegalovirus prevention consisted of weekly antigenemia surveillance and preemptive therapy with ganciclovir. The decision to administer antivirals prophylactically to prevent HHV-6 encephalitis was made at the discretion of the attending physician. If high-level HHV-6 DNA ($\geq 10^4$ copies/mL plasma) was confirmed by HHV-6 monitoring in this study, physicians were able to start administering antivirals against HHV-6 reactivation.

Specimen Collection

After transplantation, EDTA-treated peripheral blood was collected prospectively and basically twice a week until 70 days after transplantation or discharge. Within 3 hours after sampling, separation of plasma from whole blood was performed by centrifugation ($1500 \times g$ for 10 minutes) at each institute. Plasma samples were immediately frozen at −30°C, and sent to Oita University Hospital on the day of collection in a frozen state for HHV-6 DNA testing. Shipping time was 2–3 days in most cases.

HHV-6 DNA Quantification

HHV-6 DNA copy numbers in plasma samples were measured using real-time polymerase chain reaction (PCR) methods, as described elsewhere [3]. This PCR system detects both HHV-6A and HHV-6B. Real-time PCR was performed basically twice a week (Monday and Thursday) at Oita University Hospital and HHV-6 DNA loads were reported to each institute on the day of quantification.

Definitions

Disease phase was evaluated against patients with hematological neoplasms. Early stage was defined as: acute leukemia in first to second remission; chronic myeloid leukemia in first chronic phase; or myelodysplastic syndrome classified as refractory anemia without excess of blasts. All others were considered non-early stage. Myeloablative conditioning (MAC) regimens were defined as: total body irradiation (TBI) with 1 fraction of >5 Gy or fractionated doses of >8 Gy, busulfan doses >9 mg/kg, or melphalan doses >140 mg/m^2. Reduced-intensity conditioning (RIC) regimens were defined as follows: TBI doses ≤5 Gy in a single fraction; busulfan doses ≤9 mg/kg; melphalan doses ≤140 mg/m^2; and fludarabine-based regimens without myeloablative doses of TBI, busulfan, or melphalan [20]. Diagnosis and grading of acute GVHD were based on the clinical criteria described elsewhere [21]. Steroid treatment was defined as treatment with ≥0.5 mg/kg of prednisolone or methylprednisolone for >1 week. HHV-6 reactivation was defined as detection

of HHV-6 DNA in plasma at any level. High-level HHV-6 reactivation was defined as a plasma HHV-6 DNA $\geq 10^4$ copies/mL. This was set based on our findings of retrospective studies that the threshold level for the development of HHV-6 encephalitis was 10^4 copies/mL plasma under our real-time PCR assay [3, 8]. In addition, this level was close to the median maximum plasma HHV-6 load among patients with positive results in the present study (11 387 copies/mL). CNS dysfunction was defined as the presence of lethargy or apathy, disorientation regarding time of place, personality change, systemic convulsions, loss of consciousness, or memory loss that persisted for >24 hours. HHV-6 encephalitis was defined as the presence of CNS dysfunction, a positive PCR result for HHV-6 DNA in CSF, and the absence of other identified causes of CNS dysfunction.

Statistical Analyses

All censored criteria were calculated from the time of transplantation. Probabilities of the first incidence of HHV-6 reactivation, high-level HHV-6 reactivation, CNS dysfunction, and HHV-6 encephalitis were calculated based on cumulative incidence curves. Competing events were discontinuance of HHV-6 DNA monitoring before the 10th week after transplantation for HHV-6 reactivation and high-level HHV-6 reactivation, death without experiencing the events for CNS dysfunction for CNS dysfunction, and death without HHV-6 encephalitis for HHV-6 encephalitis. Groups were compared using Grey test. The Fine-Grey proportional hazards model was used to evaluate the effects of confounding variables on HHV-6 reactivation. Comparisons of proportion for HHV-6 encephalitis between groups were made using Fisher exact test. All statistical tests were 2-sided and performed using a 5% level of significance. Statistical analyses were carried out using EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (R Foundation for Statistical Computing, ver. 2.13.0) [22].

RESULTS

Patient Characteristics

Between July 2010 and December 2011, a total of 260 patients were preliminarily enrolled in this study. Among these, 30 patients were excluded from analysis, because of insufficient sampling (<3 samples during the first 21 days after transplantation, n = 8), registration after receiving transplantation (n = 3), results consistent with chromosomally integrated HHV-6 (ciHHV-6; persistent levels ≥100 copies/mL in ≥80% of subsequent plasma samples after day 14 after transplantation [19], ciHHV-6 in recipient, n = 2; ciHHV-6 in donor, n = 1; origin undetermined, n = 1), or receiving second or more allogeneic HCT (n = 15). Kinetics of plasma HHV-6 DNA in patients with presumed ciHHV-6 is shown in the Supplementary Figure. None of the 4 patients with presumed ciHHV-6 developed encephalopathy. Characteristics of the 230 patients who were included in the analyses are summarized in Table 1. Eighty-three patients (36.1%) received antiviral agents active against HHV-6 (ganciclovir, valganciclovir, or foscarnet) within 30 days after transplantation.

Table 1. Patient Characteristics

Characteristics	No.	%
Age, years		
Median	49	
Range	15–71	
Male sex	135	58.7
Underlying disease		
Acute myeloid leukemia	111	48.3
Acute lymphoblastic leukemia/ lymphoblastic lymphoma	35	15.2
Acute leukemias of ambiguous lineage	4	1.7
Chronic myeloid leukemia	3	1.3
Myelodysplastic syndrome	14	6.1
Myelodysplastic/ myeloproliferative neoplasms	5	2.2
Malignant lymphoma	26	11.3
Adult T-cell leukemia	14	6.1
Multiple myeloma/plasma cell leukemia	7	3.0
Chronic lymphocytic leukemia/ prolymphocytic leukemia	2	0.9
Aplastic anemia	8	3.5
Langerhans cell histiocytosis	1	0.4
Disease phase at transplantation[a]		
Early	89	40.1
Non-early	133	59.9
Preconditioning regimen		
MAC	94	40.9
RIC	136	59.1
Transplant type		
Related BM/PBSC	66	28.7
Unrelated BM	101	43.9
CB	63	27.4
HLA match		
Allele match/allele mismatch	96/134	41.7/58.3
Antigen match/antigen/ mismatch	131/99	57.0/43.0
Antivirals used within 30 days after transplantation		
Acyclovir	230	100
Ganciclovir, valganciclovir, or foscarnet	83	36.1

Abbreviations: BM, bone marrow; CB, cord blood; HLA, human leukocyte antigen; MAC, myeloablative conditioning; PBSC, peripheral blood stem cells; RIC, reduced-intensity conditioning.

[a] Eight patients with aplastic anemia were not included.

Among these, 27 patients (umbilical cord blood transplantation [UCBT] recipients, n = 23; non-UCBT recipients, n = 4) received these antivirals to prevent development of HHV-6 encephalitis, and 18 patients received them in response to confirmation of high-level HHV-6 reactivation. The remaining patients received these antivirals against cytomegalovirus reactivation.

Patient Samples

In total, 3537 samples were examined using real-time PCR. Blood-sample collection was started at a median of 3 days after transplantation (range, 1–15 days) and lasted at a median of 59.5 days after transplantation (range, 12–70 days). Collection intervals between samples were as follows: 1–2 days, n = 176; 3–4 days, n = 2843; 5–6 days, n = 207; 7–8 days, n = 45; 9–10 days, n = 17; 11–12 days, n = 11; and 13–15 days, n = 8. The median number of blood samples per patient was 16 (range, 3–21).

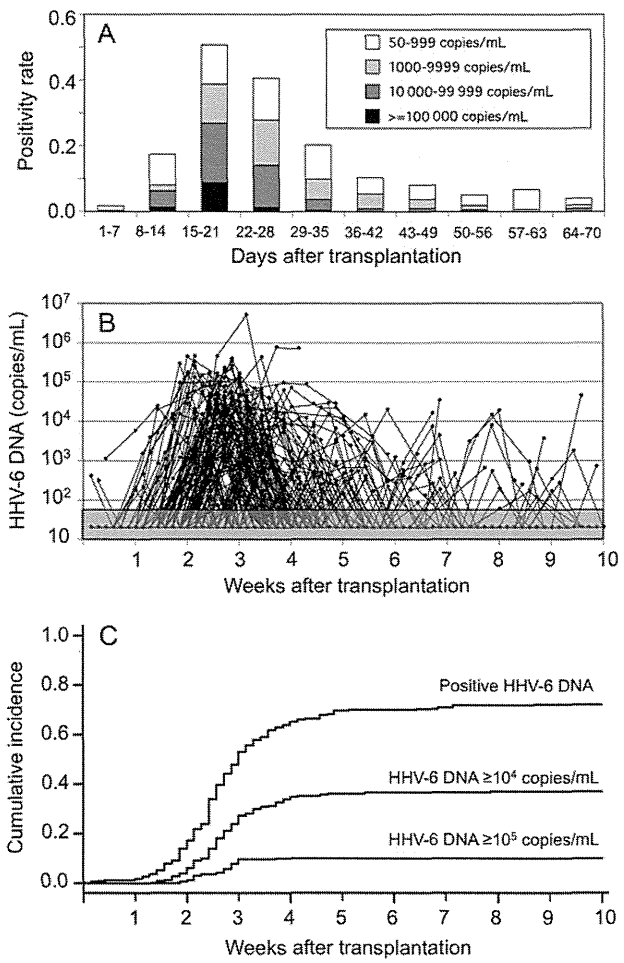

Figure 1. *A*, Positive rate for human herpesvirus 6 (HHV-6) polymerase chain reaction (PCR) results in each post-transplantation period. *B*, Kinetics of HHV-6 DNA copy numbers in plasma among patients who displayed positive HHV-6 results on PCR. Shaded area, values below the threshold for detection (ie, <50 copies/mL plasma). *C*, Cumulative incidence of first detection of plasma HHV-6 DNA, by number of copies per milliliter of plasma. Abbreviation: HHV-6, human herpesvirus 6.

HHV-6 Reactivation

HHV-6 DNA was most frequently apparent in plasma at 15–21 days (3rd week) after HCT (Figure 1*A* and 1*B*). Cumulative incidences for the first detection of positive HHV-6 DNA, HHV-6 DNA $\geq 10^4$ copies/mL plasma, and HHV-6 DNA $\geq 10^5$ copies/mL plasma at 70 days after HCT were 72.2%, 37.0%, and 10.0%, respectively (Figure 1*C*). Median maximum plasma HHV-6 load among patients with positive results was 11 387 copies/mL (range, 70.5–4 991 563.5 copies/mL). Although plasma HHV-6 DNA level exceeded 1 000 000 copies/mL in one patient, that patient was not considered as having ciHHV-6 because the HHV-6 DNA load eventually decreased to an undetectable level.

Risk Factors for HHV-6 Reactivation

By univariate analysis, UCBT, MAC, conditioning without antithymocyte globulin (ATG), GVHD ≥grade 2, and steroid treatment after HCT were associated with increased risk of HHV-6 reactivation, while male sex, UCBT, MAC, conditioning without ATG, and GVHD ≥ grade 2 were associated with high-level HHV-6 reactivation (Table 2). Figure 2*A* and 2*B* show the cumulative incidence curves for first HHV-6 reactivation and high-level HHV-6 reactivation in recipients of UCBT and non-UCBT. Multivariate analysis for the pretransplant parameter revealed MAC and UCBT as risk factors for HHV-6 reactivation and MAC, male sex, and UCBT as risk factors for high-level HHV-6 reactivation (Table 3).

CNS Dysfunction

During the observation period 70 days after HCT, 33 of 230 patients (14.3%) developed CNS dysfunction. Etiologies of CNS dysfunctions were HHV-6 encephalitis (n = 7), cerebral bleeding (n = 3), posterior reversible encephalopathy syndrome (n = 3), drug-induced encephalopathy (n = 3, due to opioid or major tranquilizer), uremic encephalopathy (n = 1), CNS relapse (n = 1), and unknown (n = 15). Cumulative incidences of CNS dysfunction and HHV-6 encephalitis at 70 days after HCT were 14.4% and 3.0%, respectively. Characteristics of patients who developed CNS dysfunctions are summarized in Table 4. We grouped these patients with CNS dysfunction into 2 groups: Cases 1–13 as the high-plasma HHV-6 DNA group (HHV-6 DNA $\geq 10^4$ copies/mL plasma); and cases 14–33 as the low- or negative plasma HHV-6 DNA group (HHV-6 DNA $< 10^4$ copies/mL plasma). This was set based on the findings from the present study that median maximum plasma HHV-6 load among patients with positive results was 11 387 copies/mL. Among patients in whom HHV-6 DNA in CSF was evaluated

Table 2. Risk Factors for Human Herpesvirus 6 (HHV-6) Reactivation and HHV-6 Encephalitis

Variables	No.	Positive Plasma HHV-6 DNA		HHV-6 DNA ≥10 000 copies/mL		HHV-6 Encephalitis	
		CI at Day 70 (%)	P^a	CI at Day 70 (%)	P^a	CI at Day 70 (%)	P^a
Age, years							
<50 years	119	73.9	.22	39.5	.33	2.5	.64
≥50 years	111	70.3		34.2		3.6	
Sex							
Male	135	70.4	.73	42.2	.04	3.0	.93
Female	95	74.7		29.5		3.2	
Disease phase at transplantation[b]							
Early	89	78.7	.20	38.2	.98	2.2	.54
Non-early	133	68.4		38.3		3.8	
Lineage of tumor cells[c]							
Myeloid	133	71.4	.85	38.3	.55	3.8	.58
Lymphoid	84	72.6		35.7		2.4	
Type of transplanted cells							
BM/PBSC	167	66.5	.0002	31.1	.004	1.2	.008
CB	63	87.3		52.4		7.9	
Matching of HLA							
Allele match	96	69.8	.18	29.2	.06	0.0	.02
Allele mismatch	134	73.9		42.5		5.2	
Antigen-match	131	68.7	.12	31.3	.07	0.8	.02
Antigen-mismatch	99	76.8		44.4		6.1	
Conditioning regimen							
MAC	94	78.7	.02	46.8	.005	3.2	.90
RIC	136	67.6		30.1		2.9	
ATG in conditioning							
Yes	39	56.4	.04	20.5	.03	0.0	.23
No	191	75.4		40.3		3.7	
GVHD ≥grade 2							
Yes	96	85.4	.0003	46.9	.02	5.2	.11
No	134	62.7		29.9		1.5	
Steroid administration after HCT							
Yes	109	78.0	.04	41.3	.22	4.6	.20
No	121	66.9		33.1		1.7	

Abbreviations: ATG, antithymocyte globulin; BM, bone marrow; CB, cord blood; CI, cumulative incidence; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; MAC, myeloablative conditioning; PBSC, peripheral blood stem cells; RIC, reduced-intensity conditioning.

[a] Grey test.

[b] Eight patients with aplastic anemia were not included.

[c] Thirteen patients were not included (8 patients with aplastic anemia, 4 patients with acute leukemias of ambiguous lineage, and 1 patient with Langerhans cell histiocytosis).

around the time of developing CNS dysfunction, HHV-6 DNA was demonstrated in all of the 7 patients with high-plasma HHV-6 DNA and none of the 7 patients with low- or negative plasma HHV-6 DNA. Diagnosis of HHV-6 encephalitis was therefore made based on positive HHV-6 DNA in CSF in 7 of 13 patients (53.8%) in the high-plasma HHV-6 DNA group, and none of the 20 patients (0%) in the low- or negative plasma HHV-6 DNA group ($P = .0004$). Detailed findings for the 7 patients diagnosed with HHV-6 encephalitis are shown in Table 5. In 5 of 6 patients in the high-plasma HHV-6 DNA group who did not receive CSF examination, the etiology of CNS dysfunction has not been determined.