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Impact of Human Herpesvirus-6 Reactivation on Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation

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Human herpesvirus-6 (HHV-6) is known to reactivate after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and may be associated with development of acute graft-versus-host disease (GVHD) and non-relapse mortality (NRM). However, the clinical significance of HHV-6 reactivation after allo-HSCT remains unclear. Therefore, we conducted a retrospective analysis to elucidate the impact of HHV-6 reactivation on transplantation outcomes. Of 236 patients who underwent allo-HSCT, 138 (58.5%) developed HHV-6 reactivation and 98 (41.5%) did not. Univariate analysis indicated that at 3 years, patients with HHV-6 reactivation had significantly higher NRM (27.7% versus 13.7%, $P = .003$) and worse overall survival (42.1% versus 59.0%, $P = .008$) than those without reactivation. In multivariate analysis, HHV-6 reactivation was associated with higher incidence of acute GVHD (hazard ratio [HR], 1.87; $P = .01$), cytomegalovirus reactivation (HR, 2.24; $P < .001$), and NRM (HR, 2.73; $P = .007$). Subgroup analysis stratified according to conditioning intensity indicated that a significant impact of HHV-6 reactivation on acute GVHD was observed only in patients who received myeloablative conditioning (MAC). These results indicate that HHV-6 reactivation was associated with development of acute GVHD, cytomegalovirus reactivation, and NRM. Furthermore, adverse impact of HHV-6 reactivation on transplantation outcomes was prominent in the setting of MAC.

INTRODUCTION

Human herpesvirus-6 (HHV-6) ubiquitously infects healthy individuals during early childhood [1]. Primary HHV-6 infection causes exanthema subitum and acute febrile illness [2]. Like other herpes viruses, HHV-6 establishes a latent infection after the primary infection [3]. The latent infection can reactivate during severe immunosuppression, such as that induced under allogeneic hematopoietic stem cell transplantation (allo-HSCT). Previous studies revealed that HHV-6 reactivates in approximately one half of patients after allo-HSCT and reactivation causes skin rash [4-7], fever, and encephalitis [8-14]. HHV-6 reactivation also has been associated with poor outcomes, including acute graft-versus-host disease (GVHD) [15-20], cytomegalovirus (CMV) reactivation [6,7,15,16], delayed platelet recovery [9,15,19], and increased mortality. However, the actual clinical significance of HHV-6 reactivation remains unclear.

Therefore, we conducted a single-center study of 236 patients who underwent allo-HSCT in our hospital. The purpose of the current study was to determine the clinical impact of HHV-6 reactivation on early complications and outcomes after allo-HSCT.

MATERIALS AND METHODS

Study Population

Clinical data were collected from the clinical records of Kanagawa Cancer Center. Patients aged 16 years or older who underwent a first allo-HSCT between April 2004 and December 2013 were evaluated for analysis. Of 242 patients, HHV-6 monitoring was performed for 236 patients. Plasma samples were collected weekly from 1 or 2 weeks until 4 weeks after transplantation. HHV-6 DNA copy numbers were measured using real-time polymerase chain reaction, as described previously [12]. Since 2008, foscarnet sodium 3 g/body was started when HHV-6 DNA exceeded 125 copies/mL. We retrospectively analyzed the clinical impact of HHV-6 reactivation on outcomes of allo-HSCT. This study was approved by the institutional review board of the Kanagawa Cancer Center.

Statistical Analysis

HHV-6 reactivation was defined as a detection of HHV-6 DNA at any level. *CMV reactivation* was defined as more than 1 cell per 3.0×10^4 peripheral blood mononuclear cells positive for CMV pp65 antigen.

A myeloablative conditioning (MAC) regimen was defined as regimen having the following dosage level: total body irradiation > 8 Gy, oral

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busulfan ≥ 9 mg/kg (or equivalent intravenous doses), or melphalan > 140 mg/m^2. Other regimens were classified as reduced-intensity conditioning (RIC) [21].

Low-risk disease status included the following: first and second complete remission in acute leukemia and lymphoma, refractory anemia and refractory anemia with ringed sideroblasts (myelodysplastic syndromes), and first and second chronic phase of chronic myeloid leukemia and nonmalignant disorders. Other diseases were classified as high-risk disease status.

Overall survival (OS) was defined as the number of days from allo-HSCT until death from any cause. The *incidence of relapse* was defined as the number of days from allo-HSCT to relapse of the underlying disease. *Non-relapse mortality* (NRM) was defined as the number of days from allo-HSCT to death without relapse. Any patient who was alive at the last follow-up date was censored.

Neutrophil engraftment was defined as the continuous achievement of neutrophil counts of 500 × 10^6/L or higher. *Platelet recovery* was defined as continuous achievement of platelet counts of 20 × 10^9/L or higher without transfusion.

In univariate analysis, the effect of HHV-6 reactivation on outcomes was analyzed using landmark methods. OS and NRM were analyzed among patients who survived more than 30 days after transplantation and relapse was analyzed among those who survived without relapse more than 30 days after transplantation. In multivariate analysis, the impact of HHV-6 reactivation on other outcomes was studied as a time-dependent variable. OS, NRM, acute GVHD, and hematopoietic recovery were analyzed in all patients; chronic GVHD was analyzed in patients who survived more than 100 days after transplantation.

The Fisher's exact test and the Mann-Whitney test were used for comparison of categorical and continuous variables, respectively. OS was estimated by the Kaplan-Meier method and was compared using a log-rank test. Relapse and NRM were considered competing risk events for each other and were compared using Gray's test. The cumulative neutrophil and platelet recoveries and incidences of grades 2 to 4 acute GVHD and chronic GVHD were also estimated and compared by Gray's test considering death without these events as a competing risk. In a multivariate analysis, the Cox proportional hazard model was used for all events and the clinical impact of HHV-6 reactivation on other outcomes was always studied as a time-dependent variable. The clinical impact of HHV-6 was adjusted for variables with a P value of less than .20 in a univariate analysis. The following variables were compared by univariate analysis: age at allo-HSCT, sex, primary disease, disease risk, conditioning regimen, and donor source. P values were 2-sided and differences were considered to be statistically significant when $P < .05$. All statistical analyses were performed using EZR (R version 2.13.0) [22].

RESULTS

Patient Characteristics

Table 1 shows patients' characteristics. Of 236 patients, 138 (58.5%) developed HHV-6 reactivation (reactivation group) and 98 (41.5%) did not (control group). The median maximum DNA level was 326.5 copies/mL (range, <125 to 290,000) in the reactivation group. Of the 3 patients who received conditioning containing antithymocyte globulin, 1 (33.3%) developed HHV-6 reactivation. Age at allo-HSCT, sex, background disease, and conditioning regimen were comparable in both groups. The proportion of patients with high-risk disease tended to be higher in the reactivation group ($P = .08$). A higher number in the reactivation group received HLA-mismatched and cord blood transplantations compared with the control group ($P < .001$).

Table 1
Patient Characteristics

Characteristic	HHV-6 Reactivation		*P* Value
	No (n = 98)	Yes (n = 138)	
Age, median (range), yr	46 (17-65)	48 (18-67)	.10
Sex			
Male	61 (62.2%)	78 (56.5%)	
Female	37 (37.8%)	60 (43.5%)	.42
Disease			
AML	59 (60.2%)	83 (60.1%)	
ALL	57 (21.4%)	34 (24.6%)	
Others	51 (18.4%)	21 (15.2%)	.76
Disease risk			
High	31 (31.6%)	60 (43.5%)	
Low	67 (68.4%)	78 (56.5%)	.08
Conditioning			
MAC	61 (62.2%)	78 (56.5%)	
RIC	37 (37.8%)	60 (43.5%)	.42
Donor source			
M-RD	50 (51.0%)	17 (12.3%)	
MM-RD	3 (3.1%)	10 (7.2%)	
WPM-URD	26 (26.5%)	35 (25.4%)	
MM-URD	7 (7.1%)	24 (17.4%)	
UR-CB	12 (12.2%)	52 (37.7%)	<.001

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; M-RD, matched related donor; MM-RD, mismatched related donor; WPM-URD, well or partially matched unrelated donor; MM-URD, mismatched unrelated donor; UR-CB, unrelated cord blood.

OS

The probability of OS of the reactivation group was significantly lower than that of the control group (45.7% versus 59.7%, respectively, at 3 years; $P = .003$) (Figure 1A). In subgroup analysis stratified according to conditioning intensity, the negative impact of HHV-6 reactivation was seen in the MAC group (42.1% for reactivation group versus 59.0% for control group at 3 years, $P = .008$) (Figure 2A). In contrast with univariate analysis, multivariate analysis of all patients indicated HHV-6 reactivation had no significant impact on OS after adjusted covariates (Table 2). On the other hand, multivariate analysis of subgroups stratified by conditioning intensity revealed that HHV-6 reactivation was a risk factor for worse OS with borderline significance in the MAC group (hazard ratio [HR], 1.62; 95% confidence interval [CI], .97 to 2.73; $P = .07$) (Table 2).

Relapse

The cumulative incidences of relapse were similar between both groups (29.0% for reactivation group versus 30.7% for control group at 3 years, $P = .97$) (Figure 1B). Similarly, HHV-6 reactivation did not affect relapse in subgroup analysis stratified according to conditioning intensity (Figure 2C,D). In multivariate analysis, HHV-6 reactivation had no significant effect on the relapse rate after adjusting for covariates (Table 2).

NRM

The cumulative incidence of NRM in the reactivation group was significantly higher compared with the control group (27.7% for reactivation group versus 13.7% for control group at 3 years, $P = .003$) (Figure 1C). In subgroup analysis stratified by conditioning intensity, the cumulative incidence of NRM in the reactivation group was significantly higher in the MAC group (29.8% for reactivation group versus 13.3% for control group at 3 years, $P = .01$) and tended to be higher in the RIC group (22.4% for reactivation group versus 12.6% for control group at 3 years, $P = .08$) (Figure 2E,F). Multivariate analysis of all patients showed that risk of NRM was higher in the reactivation group compared with in the control group (HR, 2.73; 95% CI, 1.31 to 5.68; $P = .007$) (Table 2). In multivariate analysis of subgroups stratified by conditioning intensity, HHV-6 reactivation had an adverse impact on the NRM in the MAC group (HR, 3.04; 95% CI, 1.31 to 7.04; $P = .009$). HHV-6 reactivation affected NRM with borderline significance in the RIC group (HR, 2.88; 95% CI, .94 to 8.77; $P = .06$) (Table 2).

Hematopoietic Recovery and Incidence of Acute GVHD

Multivariate analysis of all patients indicated that HHV-6 reactivation was associated with a higher incidence of grades