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Lymphocyte Area Under the Curve as a Predictive Factor for Viral Infection after Allogenic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Viral infection is a serious complication that can greatly affect patient mortality and morbidity after allogenic hematopoietic stem cell transplantation (allo-HSCT). For the early identification of patients at high risk for viral infection, we evaluated the impact of lymphocyte area under the curve (AUC) value as a new predictive factor for early immune reconstitution after allo-HSCT against viral infection. This study included 286 patients who underwent their first allo-HSCT at Kyoto University Hospital between 2005 and 2017. Lymphocyte AUC from day 0 to day +15 was calculated in the analysis of human herpesvirus 6 (HHV-6), and lymphocyte AUC from day 0 to day +30 was calculated in the analysis of other viruses (cytomegalovirus [CMV], adenovirus, BK virus, JC virus, and varicella zoster virus). The risk factors for each viral reactivation/infection were assessed by multivariate analysis. The median age at transplantation was 51 years (range, 17 to 68 years). The median lymphocyte AUC was 63/μL (range, 0 to 5620/μL) at day +15 and 3880 (range, 0 to 118,260/μL) at day +30. An increase in lymphocyte AUC was significantly associated with a high frequency of HHV-6 reactivation ($P = .033$) and a low frequency of CMV antigenemia ($P = .014$). No apparent association was found between lymphocyte AUC and reactivation/infection of other viruses. Aplastic anemia as a primary disease (hazard ratio [HR], 5.34; $P < .001$) and cord blood as a donor source (HR, 3.05; $P = .006$) were other risk factors for HHV-6 reactivation. Other risk factors for CMV antigenemia included the occurrence of acute graft-versus-host disease (HR 2.21; $P < .001$) and recipient age (HR 1.55; $P = .017$). Higher lymphocyte AUC at day +30 was significantly associated with low treatment-related mortality (HR, .47; $P = .045$). Lymphocyte AUC may be a good predictive factor for immune reconstitution against CMV reactivation. It also provides valuable information for predicting HHV-6 reactivation and treatment-related mortality.

INTRODUCTION

Viral infections continue to be serious complications that negatively impact patient survival after allogenic hematopoietic stem cell transplantation (allo-HSCT). After allo-HSCT, patients often develop reactivation of and infection by various latent viruses, including cytomegalovirus (CMV), varicella zoster virus (VZV), human herpesvirus 6 (HHV-6), adenovirus (ADV), BK virus (BKV), and JC virus (JCV), owing to their prolonged and strongly immunosuppressed background [1].

Given the increasing number of transplantations from various stem cell sources, such as cord blood units, and the number of transplantations performed for high-risk patients, the management of viral infection is becoming increasingly important to improve the clinical outcomes of HSCT. However, preventive measures and effective treatments against these viruses remain limited and are largely dependent on immune reconstitution in the recipients themselves. As seen with the prophylactic administration of acyclovir/valacyclovir against VZV [1,2] and preemptive therapies against CMV infections diagnosed via serum antigen or real-time polymerase chain reaction (PCR) [3,4], early intervention leads to favorable outcomes. It is important to identify high-risk patients for viral infection in the early stage after HSCT. Thus, in the present study, we assessed a new biomarker, lymphocyte area under the curve (AUC), as a new predictive factor for immune reconstitution after allo-HSCT by evaluating its impact on viral reactivation/infection.

METHODS

Data Collection

A total of 286 patients who underwent their first allogeneic HSCT for hematologic disease at a single center of Kyoto University Hospital between 2005 and 2017 were reviewed. Lymphocyte AUC is defined as the sum of serial absolute lymphocyte counts under the lymphocyte count-time curve [5]. In the analysis of HHV-6 reactivation, lymphocyte AUC values from day 0 to day +15 post-HSCT were calculated in patients who survived for >15 days, because most cases of HHV-6 virus reactivation occurred between day +15

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and day +30. For the analysis of other viruses (CMV, ADV, BKV, JCV, and VZV), lymphocyte AUC values from day 0 to day +30 were calculated in patients who survived for >30 days after transplantation, because infection by these viruses mostly occurred after 30 days post-HSCT.

This study was approved by the Institutional Review Board of Kyoto University Hospital, and written informed consent was obtained from each participating patient.

Viral Detection and Treatment

CMV Antigenemia and CMV Virus Infection

CMVpp65 antigen was examined once weekly in each patient after an increase in the neutrophil count was ascertained and was also examined in patients with suspicious signs and symptoms of CMV diseases. Most of the patients were examined via the C10/C11 method, whereas some patients were assessed via the C7-HRP method. The results of these 2 methods are known to be highly correlated [6]. Both methods were performed as described previously [7-10]. In patients in whom >2 positive cells within 2 slides (within 50,000 WBC in C10/C11) were detected, preemptive therapy was provided, followed by close monitoring of CMV antigen [6,8].

HHV-6 Preventive Measures, Reactivation, and Infection

After transplantation, the HHV-6 viral load was determined quantitatively by multiplex PCR designed for multiple viral detection [11] whenever a patient developed symptoms suspicious of HHV-6 reactivation. In patients who underwent cord blood transplantation (CBT) within the previous 7 years, PCR was performed consistently (every 1 to 2 weeks up to 2 months post-transplantation).

For patients who had undergone CBT within the previous 3 years, foscarnet infusion was started at a maintenance dose (90 mg/kg/day, adjusted based on kidney function) to prevent severe HHV-6 reactivation when patients were administered systemic steroids for an immune reaction, such as engraftment syndrome or acute graft-versus-host disease (GVHD). Foscarnet at a curative dose (180 mg/kg/day, adjusted based on kidney function) was injected when HHV-6 infection, including HHV-6 encephalitis, was diagnosed [12]. For patients with only HHV-6 reactivation who were diagnosed as serum HHV-6 positive without any symptoms, treatment was initiated at the physician's discretion, considering the detected viral dose (approximately 10^3 copies/mL) and the patient's background.

ACV, BKV, and JCV Infections

When symptoms indicative of urinary tract infection, such as hematuria, emerged, serum and urinary levels of ADV, BKV, and JCV were examined by multiplex PCR [11]. For ADV, patients were also subjected to additional examinations when they developed hepatitis, fever, or other symptoms of infection of undetectable origin. For patients in whom ADV and BKV were detected in serum, systemic cidofovir injection was initiated at 1 mg/kg, 3 three times a weekly. Meanwhile, for those in whom BKV and ADV were detected only in the urine, bladder instillation of cidofovir was preferred at 5 mg/kg for 2 consecutive days was preferred [13-15].

Endpoints

The primary study endpoint was the occurrence of reactivation and infection with various viruses (CMV, VZV, HHV-6, ADV, BKV, and JCV) diagnosed within 180 days after HSCT.

Statistical Analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Viral reactivation/infection, treatment-related mortality, and disease relapse occurring by day +180 were calculated based on cumulative incidence curves [16,17]. Overall survival was evaluated by the Kaplan-Meier method. The competing event was death without a diagnosis of viral reactivation/infection. Lymphocyte AUC was estimated by collecting the AUC of lymphocyte counts in each patient from day 1 until either day +15 for HHV-6 or day +30 for the other viruses. These landmark days (days +15 and +30) were determined based on a preceding analysis in which >75% of new-onset cases were detected between day +15 and day +30 in HHV-6 reactivation and after day +30 in CMV antigenemia. The Fine and Gray proportional hazards model [18] was used to evaluate the impact of lymphocyte AUC on viral reactivation/infection in each patient. The following possible covariates were considered: recipient sex, age at transplantation (<50 years or ≥50 years), disease diagnosis (myeloid malignancies, lymphoid malignancies, and others), disease status (complete remission or non-complete remission), donor type (bone marrow transplantation from unrelated donor, peripheral blood stem cell transplantation from related donor, or CBT), conditioning regimen (reduced intensity or myeloablative), GVHD prophylaxis (tacrolimus or cyclosporine in addition to mycophenolate mofetil or methotrexate), and the occurrence of acute GVHD by day +30 (only for CMV antigenemia). All covariate factors with a variable retention criterion of $P < .05$ in the univariate analysis were selected and analyzed together with lymphocyte AUC in the multivariate analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) [19].

RESULTS

Patient Characteristics

A total of 286 patients were reviewed in the analysis of HHV-6 reactivation, and 283 patients were examined for other

Table 1
Patient Characteristics at Day +15

Characteristic	Total (N = 286)	Low/Middle AUC (N = 189)		High AUC (N = 97)		*P* Value
Age, yr, median (range)*	51 (17-68)	52 (18-68)		50 (17-68)		.581
Sex, n (%)						
Male	168	108	(57.1)	60	(61.9)	.526
Female	118	81	(42.9)	37	(38.1)	
Donor source, n (%)						
Sibling	78	51	(27.0)	27	(27.8)	<.05
Unrelated BM	129	99	(52.4)	30	(30.9)	
Unrelated CB	79	39	(20.6)	40	(41.2)	
Disease, n (%)						
AML/MDS	172	115	(60.8)	57	(58.8)	.838
ALL/other leukemias	61	41	(21.7)	20	(20.6)	
Malignant lymphoma	45	25	(13.2)	20	(20.6)	
Aplastic anemia	8	8	(4.2)	0	(0)	
Disease status, n (%)						
CR	130	79	(41.8)	51	(52.6)	.068
Non-CR	156	110	(58.2)	46	(47.4)	
Conditioning intensity, n (%)						
Myeloablative	149	101	(53.4)	48	(49.5)	.535
Reduced intensity	137	88	(46.6)	49	(50.5)	
Acute GVHD prophylaxis, n (%)						
CI	23	7	(3.7)	16	(16.5)	<.05
CI + MMF	56	28	(14.8)	28	(28.9)	
CI + MTX	161	119	(63.0)	42	(43.3)	
CI + MMF + MTX	44	34	(18.0)	10	(10.3)	
ATG-containing regimens	2	1	(.5)	1	(1.0)	

Calcineurin inhibitors include tacrolimus and cyclosporin. Low/middle AUC, lymphocyte AUC <230/μL; high AUC, lymphocyte AUC ≥230/μL.
AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marrow; CB, cord blood; CI, calcineurin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin.
* Age indicates patient age at transplantation.

viral reactivation/infection (3 patients died between day +15 and day +30). Transplantation was performed with grafts from a related donor in 78 patients, with unrelated bone marrow grafts in 129 patients, and with unrelated cord blood units in 79 patients. Their median age at transplantation was 51 years (range, 17 to 68 years). The median lymphocyte AUC was 63 (range, 0 to 5620/μL) at day +15 and 3880/μL (range, 0 to 118,260/μL) at day +30. No apparent difference in lymphocyte AUC was seen across the different donor sources.

We categorized the patients into 3 groups according to their lymphocyte AUC count at day +15 and day +30. However, in the analysis of HHV-6 reactivation, the first tertile was 0/μL, given that 129 patients showed no lymphocyte recovery by day +15. Thus, we used the second tertile of 230/μL as a threshold to categorize patients into 2 groups in the analysis of lymphocyte AUC by day +15: lymphocyte AUC $\leq$230/μL (n = 189) and lymphocyte AUC $>$230/μL (n = 97) (Table 1). In the analysis of CMV antigenemia and infection, patients were categorized into 3 groups according to the first (2710/μL) and second (5250/μL) tertiles: low lymphocyte AUC (n = 93), middle lymphocyte AUC (n = 93), and high lymphocyte AUC (n = 97) (Table 2).

HHV-6 Reactivation/Infection

HHV-6 reactivation was detected in 48 of the 286 patients (cumulative incidence, 17.5% on day +180), of whom 8 patients developed virologically diagnosed HHV-6 encephalitis with typical neurologic symptoms and viral detection in spinal fluid with or without positive findings in magnetic resonance imaging. Nine patients received foscarnet injection as prophylaxis from week 1 to week 4 after CBT, of whom 5 were diagnosed with HHV-6 viremia after cessation of foscarnet.

Multivariate analysis showed that high lymphocyte AUC was significantly associated with HHV-6 reactivation (high AUC group versus low/middle AUC group: HR, 1.83; $P = .048$) (Figure 1). Other risk factors detected were aplastic anemia as a primary disease (HR, 5.34; $P < .001$) and cord blood as a donor source (HR, 3.05; $P = .006$) (Table 3). The subanalysis of patients with a history of HHV-6 viremia revealed no significant difference in lymphocyte AUC between the HHV-6 encephalitis group and no-encephalitis group (median lymphocyte AUC value: encephalitis group, 530/μL; no-encephalitis group, 249/μL; $P = .248$). Foscarnet treatment had no prophylactic effect on HHV-6 viremia (incidence in patients with foscarnet prophylaxis versus those without, 55.6% versus 37.1%).

Because HHV-6 reactivation has been suggested to be epidemiologically associated with immune reactions before engraftment, including preengraftment immune reaction in CBT [20], we performed an additional analysis to examine the association between lymphocyte AUC and the occurrence of immune-related reactions by day +15. High lymphocyte AUC was associated with the occurrence of immune-related reactions (odds ratio, 2.02; $P = .015$). However, in a stratification analysis, high-lymphocyte AUC was significantly associated with HHV-6 reactivation in patients both with and without an immune reaction by day +15 (high AUC group versus low/middle AUC group, patients with immune reaction: HR, 2.41; $P = .047$; patients without immune reaction: HR, 2.51; $P = .018$). Meanwhile, in another stratification analysis, immune-related reactions showed no apparent association with HHV-6 reactivation in patients with a high lymphocyte AUC and those with a low/middle lymphocyte AUC (patients with an immune reaction versus patients without an immune reaction, high AUC group: HR, 1.73; $P = .160$; low/middle AUC group: HR, 1.83; $P = .169$).

Table 2
Patient Characteristics at Day +30

Characteristic	Total (N = 283)	Low AUC (N = 93)		Middle AUC (N = 93)		High AUC (N = 97)		*P* Value
Age, yr, median (range)*	51 (17-68)	52 (20-68)		51 (18-68)		49 (17-68)		.581
Sex, n (%)								
Male	117	29	(31.2)	44	(47.3)	44	(45.4)	.050
Female	166	64	(68.8)	49	(52.7)	53	(54.6)	
Donor source, n (%)								
Sibling	77	19	(20.4)	22	(23.7)	36	(37.1)	<.05
Unrelated BM	128	30	(32.3)	44	(47.3)	54	(55.7)	
Unrelated CB	78	44	(47.3)	27	(29.0)	7	(7.2)	
Disease, n (%)								
AML/MDS	169	59	(63.4)	56	(60.2)	54	(55.7)	.208
ALL/other leukemias	61	15	(16.1)	22	(23.7)	24	(24.7)	
Malignant lymphoma	45	13	(14.0)	14	(15.1)	18	(18.6)	
Aplastic anemia	8	6	(6.5)	1	(1.1)	1	(1.0)	
Disease status, n (%)								
CR	130	33	(35.5)	52	(55.9)	45	(46.4)	<.05
Non-CR	153	60	(64.5)	41	(44.1)	52	(53.6)	
Conditioning intensity, n (%)								
Myeloablative	146	47	(50.5)	48	(51.6)	51	(52.6)	.908
Reduced intensity	137	46	(49.5)	45	(48.4)	46	(47.4)	
GVHD prophylaxis, n (%)								
CI	22	10	(10.8)	10	(10.8)	2	(2.1)	<.05
CI + MMF	55	27	(29.0)	20	(21.5)	8	(8.2)	
CI + MTX	161	45	(48.4)	44	(47.3)	72	(74.2)	
CI + MMF + MTX	43	11	(11.8)	19	(20.4)	13	(13.4)	
ATG-containing regimens	2	0	(0)	0	(0)	2	(2.1)	
GVHD by day +30, grade at onset, n (%)								
I	18	4	(4.3)	6	(6.5)	8	(8.2)	.577
II	51	13	(14.0)	22	(23.7)	16	(16.5)	
III	10	3	(3.2)	2	(2.2)	5	(5.2)	
IV	2	0	(0)	2	(2.2)	0	(0)	

Calcineurin inhibitors include tacrolimus and cyclosporin. Low AUC, lymphocyte AUC <2710/μL; middle AUC, lymphocyte AUC of $\geq$2710/μL and <5250/μL; high AUC, lymphocyte AUC $\geq$5250/μL.

* Age indicates patient age at transplantation.

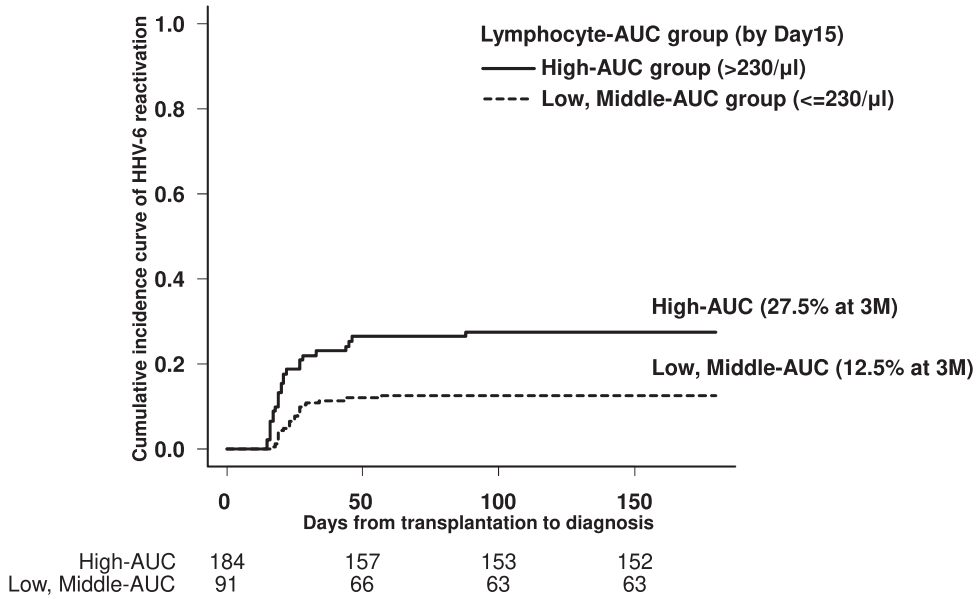

Figure 1. Cumulative incidence of HHV-6 reactivation.

CMV Antigenemia

CMV antigenemia was detected in 146 of the 284 patients (cumulative incidence, 54.7% by day +180). Nine cases of CMV end-organ infection occurred, 6 of which were diagnosed as CMV-related colitis/gastritis and 1 each were diagnosed as retinitis, hepatitis, and pneumonia. In 9 patients, foscarnet was administered as HHV-6 prophylaxis and was discontinued after day +30. No other agents were used for HHV-6 or CMV prophylaxis in the remaining 277 patients.

In a multivariate analysis, the high lymphocyte AUC group (AUC $\geq$5250/μL) had a lower risk for CMV antigenemia than the low lymphocyte AUC group (HR, .61; P = .052). Meanwhile, the risk for CMV antigenemia was not significantly different between the middle lymphocyte AUC group ($<$5250/μL) and

Table 3
Univariate and Multivariate Analyses of HHV-6 Reactivation

Variable		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age*							
	<50 yr	1.00		Reference			
	≥50 yr	.63	.32-1.28	.201			
Sex							
	Male	1.00		Reference			
	Female	1.29	.95-1.75	.102			
Donor source							
	Sibling	1.00		Reference	1.00		Reference
	Unrelated BM	.54	.21-1.40	.204			
	Unrelated CB	4.53	2.17-9.45	<.001	3.05	1.38-6.72	.006
Disease							
	AML/MDS	1.00		Reference	1.00		Reference
	ALL/other leukemias	.78	.36-1.70	.527			
	Malignant lymphoma	1.12	.52-2.42	.779			
	Aplastic anemia	3.24	1.29-8.16	.012	5.34	2.38-12.00	<.001
Disease status							
	CR	1.00		Reference			
	Non-CR	.65	.39-1.08	.096			
Conditioning regimen							
	Myeloablative	1.00		Reference			
	Reduced intensity	.91	.52-1.60	.749			
GVHD prophylaxis							
	CI	1.00		Reference	1.00		Reference
	CI + MMF	2.37	.91-6.16	.077			
	CI + MTX	.26	.09-.75	.013	.35	.15-.84	.019
	CI + MMF + MTX	.67	.21-2.12	.493			
	ATG-containing regimens	2.07	.35-12.33	.421			
Lymphocyte AUC group							
	Low/middle AUC	1.00		Reference	1.00		Reference
	High AUC	2.44	1.40-4.23	.002	1.83	1.01-3.34	.048

Calcineurin inhibitors include tacrolimus and cyclosporin. Low/middle AUC, lymphocyte AUC <230/μL; high AUC, lymphocyte AUC ≥230/μL.
* Age indicates patients' age at transplantation.

the low lymphocyte AUC group (HR, 1.13; $P = .560$) (Figure 2). Other risk factors detected in the multivariate analysis were age ≥50 years (versus <50 years; HR, 1.55; $P = .017$) and the occurrence of acute GVHD by day +30 (versus no occurrence of acute GVHD: HR, 2.21; $P < .001$) (Table 4).

There was no association between preceding HHV-6 reactivation and the occurrence of CMV antigenemia (cumulative incidence of CMV reactivation after day +30: patients with history of HHV-6 reactivation by day +30 versus those without, HR 1.07; $P = .746$).

Reactivation of Other Viruses

A total of 27 cases in 20 patients were diagnosed as various viral reactivations, including ADV viremia (n = 7), BKV viremia (n = 13), JCV viremia (n = 5), VZV viremia (n = 1), and EBV viremia (n = 1). Nine cases represented multiple viral coinfections (ADV/BKV, n = 4; BKV/JCV, n = 4; and ADV/BKV/JCV, n = 1). No apparent association was noted between these viral infections and lymphocyte AUC.

Regarding the frequencies of sequential infections of these viruses, 6 of 45 patients with a history of HHV-6 viremia by day +30 experienced a subsequent infection with ADV, BKV, or JCV, compared with 3 of 238 patients without a history of HHV-6 viremia. The cumulative incidence of ADV, BKV, or JCV reactivation after day +30 was significantly higher in patients with a history of HHV-6 reactivation by day +30 compared with patients without this history (HR, 11.1; $P = .001$).

Overall Survival, Relapse, and Treatment-Related Mortality

No apparent associations between lymphocyte AUC at day +15 and overall survival (high AUC group versus low/middle AUC group: HR, .81; $P = .386$), relapse (high AUC group versus low/middle AUC group:, HR, 1.01; $P = .974$) or treatment-related mortality (high AUC group versus low/middle AUC group: HR, .77; $P = .477$) were found.

Also, neither overall survival (high AUC group versus low AUC group: HR, .66; $P = .110$; middle AUC group versus low AUC group: HR, .63; $P = .095$) nor relapse (high AUC group versus low AUC group: HR, .821; $P = .581$; middle AUC group versus low AUC group: HR, 1.25; $P = .512$) was significantly associated with lymphocyte AUC at day +30. However, treatment-related mortality was associated with lymphocyte AUC at day +30 (high AUC group versus low AUC group: HR, .47; $P = .045$; middle-AUC group versus low-AUC group: HR, .33; $P = .013$).

DISCUSSION

In this study, we evaluated lymphocyte AUC at days +15 and +30 post-HSCT as a predictive factor for reactivation of and infection by several viruses. HHV-6 and CMV are the 2 major viruses that cause various complications during the management of HSCT, negatively affecting patient mortality and morbidity. We found that lymphocyte AUC can be used to identify patients at high risk for reactivation of these viruses.

In the analysis of HHV-6 reactivation, high lymphocyte AUC was strongly associated with viral reactivation. Because early intervention with antiviral agents is necessary to reduce HHV-6 reactivation and subsequent virus-related complications [21-24], regular examination of the plasma level of HHV-6 viral load is strongly recommended for all patients, especially in those who show rapid growth of lymphocytes by day +15. In previous studies, HHV-6 reactivation was associated with a myeloablative conditioning regimen, cord blood transplantation, and immune reactions [21,25]. Contrary to our expectations, an early immune reaction before engraftment had less of an impact on HHV-6 reactivation than lymphocyte AUC despite the temporary administration of systemic steroids to treat it. This finding that HHV-6 reactivation occurred with the rapid growth of lymphocytes regardless of an immune reaction and the preceding use of systemic steroids by day +15 might provide insights into the mechanism of HHV-6 growth after transplantation. Although it is not known whether the preceding HHV-6 growth increased the lymphocyte counts or the rapid growth of lymphocytes stimulated HHV-6 growth, HHV-6 expansion was accompanied by lymphocyte growth. This is consistent with previous reports suggesting that an inflammatory background caused by various sources of pathogenesis and the up-regulation of several chemokines were associated

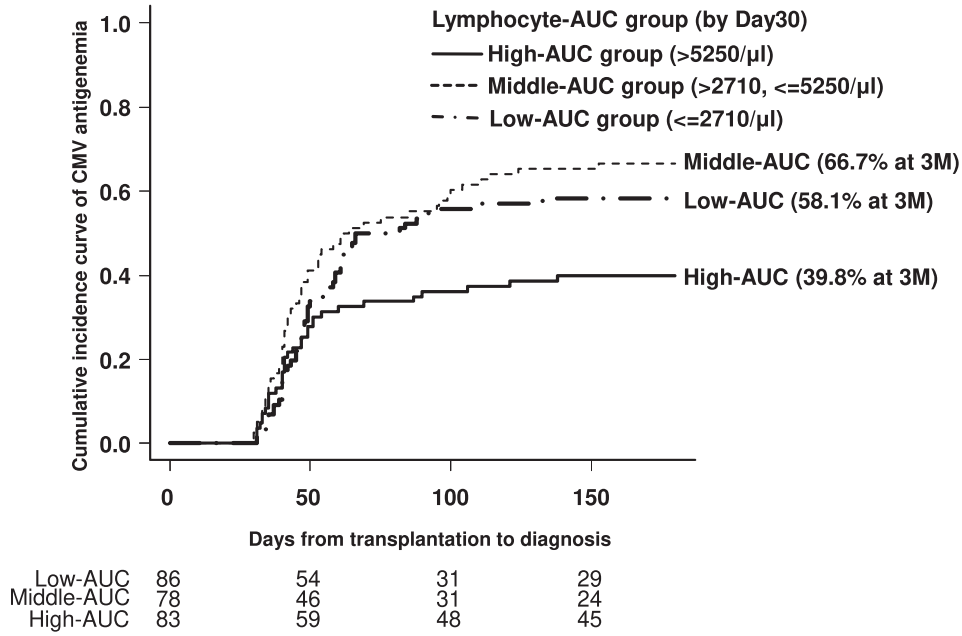

Figure 2. Cumulative incidence of CMV antigenemia.

Table 4
Univariate and Multivariate Analysis of CMV Antigenemia

Variable		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age*[1]							
	<50 yr	1.00		Reference	1.00		Reference
	≥50 yr	1.46	1.01-2.09	.042	1.55	1.08-2.21	.017
Sex							
	Male	1.00		Reference			
	Female	.90	.77-1.08	.273			
Donor source							
	Sibling	1.00		Reference			
	Unrelated BM	1.08	.71-1.63	.731			
	Unrelated CB	1.47	.22-1.78	.075			
Disease							
	AML/MDS	1.00		Reference			
	ALL/other leukemias	1.30	.84-2.00	.237			
	Malignant lymphoma	.93	.54-1.60	.800			
	Aplastic anemia	1.13	.41-3.07	.817			
Disease status							
	CR	1.00		Reference			
	Non-CR	1.01	.75-1.35	.960			
Conditioning regimen							
	Myeloablative	1.00		Reference			
	Reduced intensity	.98	.70-1.36	.882			
GVHD prophylaxis							
	CI	1.00		Reference			
	CI + MMF	.83	.44-1.54	.549			
	CI + MTX	.66	.37-1.17	.154			
	CI + MMF + MTX	1.06	.56-2.00	.847			
	ATG-containing regimens	1.14	.67-1.93	.618			
aGVHD by day +30							
	No	1.00		Reference	1.00		Reference
	Occurrence	1.94	1.37-2.75	<.001	2.21	1.49-3.29	<.001
Lymphocyte AUC group							
	Low AUC	1.00		Reference	1.00		Reference
	Middle AUC	1.27	.87-1.84	.212	1.13	.74-1.73	.560
	High AUC	.63	.40-.98	.041	.61	.37-1.01	.052

Calcineurin inhibitors include tacrolimus and cyclosporin. Low AUC, lymphocyte AUC <2710/μL; middle AUC, lymphocyte AUC of ≥2710/μL and <5250/μL; high AUC, lymphocyte AUC ≥5250/μL.

* Age indicates patient age at transplantation.

with HHV-6 reactivation [26-28]. The viral latency of HHV-6 and its interaction with lymphocytes and chemokines in growth mechanisms remain to be disclosed. Our limited data (n = 49) on lymphocyte subsets examined from day +15 to day +21 after transplantation failed to clarify which constituent of lymphocytes contributed to the growth of HHV-6 (data not shown); however, our data suggest that rapid and early growth of lymphocytes is a predictor of HHV-6 reactivation after HSCT.

Regarding CMV antigenemia, only the high lymphocyte AUC group (≥5250/μL) showed a low predicted risk of virus reactivation, indicating that sufficient recovery of lymphocytes is required for immunity against CMV reactivation. CMV antigen must be screened regularly if the lymphocyte AUC remains low, regardless of whether a single-point blood count at day +30 shows apparent immune recovery. Our findings also showed that the occurrence of acute GVHD was associated with CMV reactivation, which is consistent with previous reports [29,30].

In the analysis of viral infections other than HHV-6 and CMV, HHV-6 reactivation influenced the subsequent occurrence of ADV, BKV, and/or JCV, which is compatible with the findings in a previous study [31]. This suggests that HHV-6 infection may directly influence subsequent ADV/BKV/JCV infection or may simply reflect the severity of the immunocompromised status. Further prospective analysis is needed to tackle this clinically important topic of coinfection and sequential viral infection in patients after HSCT.

As for overall survival and treatment-related mortality, only a low lymphocyte AUC <2710/μL was suggested to be associated with an elevated risk for treatment-related mortality. The 2 major causes of treatment-related mortality after HSCT are the occurrence of GVHD and complications caused by various pathogens, including bacteria, viruses, and fungi. Considering that lymphocyte AUC at day +30 was not associated with the occurrence of acute GVHD or chronic GVHD (data not shown), the high risk of treatment-related mortality for low lymphocyte AUC seems to reflect the immature immune reconstitution. Our study suggests that lymphocyte AUC at day +30 may be a good predictor of general immune reconstitution, including antiviral immunity against CMV antigenemia.

This study has several limitations, however. First, data on lymphocyte subsets were limited. Because various lineages of lymphocyte reconstitution have been suggested to be associated with HHV-6 reactivation [32,33], they should be evaluated more precisely to further clarify the interaction between HHV-6 and lymphocytes. Second, because the number of cases with HHV-6 infection such as encephalitis in our hospital was limited, the impact of lymphocyte AUC on HHV-6 infection was not examined. Studies with a larger cohort are needed to examine the impact of lymphocyte AUC on symptomatic HHV-6 reactivation.

In conclusion, increases in lymphocyte AUC at days +15 and +30 may help identify patients who are at high risk for HHV-6 reactivation and low risk for CMV reactivation and treatment-

related mortality. A prospective clinical study of preemptive therapy with antiviral agents against HHV-6 for patients with high lymphocyte AUC at day +15 is expected in the future.

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REFERENCES

1. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant*. 2009;15:1143–1238.
2. Styczynski J, Reusser P, Einsele H, et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. *Bone Marrow Transplant*. 2009;43:757–770.
3. Ljungman P. CMV infections after hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2008;42(Suppl 1):S70–S72.
4. El Chaer F, Shah DP, Chemaly RF. How I treat resistant cytomegalovirus infection in hematopoietic cell transplantation recipients. *Blood*. 2016;128:2624–2636.
5. Kimura SI, Wada H, Sakamoto K, et al. L-index as a novel index to evaluate both the intensity and duration of lymphopenia after allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis*. 2012;14:364–373.
6. The Japan Society for Hematopoietic Cell transplantation, Japanese Guideline for CMV infection. https://www.jshct.com/guideline/pdf/guideline_CMV_2.pdf. 2011; Accessed April 9, 2018.
7. Takenaka K, Gondo H, Tanimoto K, et al. Increased incidence of cytomegalovirus (CMV) infection and CMV-associated disease after allogeneic bone marrow transplantation from unrelated donors. Fukuoka Bone Marrow Transplantation Group. *Bone Marrow Transplant*. 1997;19:241-248.
8. Kanda Y, Mineishi S, Saito T, et al. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. *Bone Marrow Transplant*. 2001;27:437–444.
9. Boeckh M, Bowden RA, Goodrich JM, Pettinger M, Meyerst JD. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. *Blood*. 1992;80:1358-1364.
10. Mori T, Okamoto S, Matsuoka S, et al. Risk-adapted pre-emptive therapy for cytomegalovirus disease in patients undergoing allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2000;25:765-769.
11. Inazawa N, Hori T, Hatakeyama N, et al. Large-scale multiplex polymerase chain reaction assay for diagnosis of viral reactivations after allogeneic hematopoietic stem cell transplantation. *J Med Virol*. 2015;87:1427–1435.
12. Ljungman P, de la Camara R, Cordonnier C, et al. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplant*. 2008;42:227–240.
13. Cesaro S, Hirsch HH, Faraci M, et al. Cidofovir for BK virus-associated hemorrhagic cystitis: a retrospective study. *Clin Infect Dis*. 2009;49:233–240.
14. Sakurada M, Kondo T, Umeda M, Kawabata H, Yamashita K, Takaori-Kondo A. Successful treatment with intravesical cidofovir for virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation: a case report and a review of the literature. *J Infect Chemother*. 2016;22:495–500.
15. Nagafuji K, Aoki K, Henzan H, et al. Cidofovir for treating adenoviral hemorrhagic cystitis in hematopoietic stem cell transplant recipients. *Bone Marrow Transplant*. 2004;34:909–914.
16. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695–706.
17. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. 1988;16:1141-1154.
18. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. 1999;94:496-509.
19. Kanda Y. Investigation of the freely available easy-to-use software EZR for medical statistics. *Bone Marrow Transplant*. 2013;48:452–458.
20. Miyashita N, Endo T, Onozawa M, et al. Risk factors of human herpesvirus 6 encephalitis/myelitis after allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis*. 2017;19:1–10.
21. Ogata M, Satou T, Kadota J, et al. Human herpesvirus 6 (HHV-6) reactivation and HHV-6 encephalitis after allogeneic hematopoietic cell transplantation: a multicenter, prospective study. *Clin Infect Dis*. 2013;57:671–681.
22. Dulery R, Salleron J, Dewilde A, et al. Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study. *Biol Blood Marrow Transplant*. 2012;18:1080–1089.
23. Aoki J, Numata A, Yamamoto E, Fujii E, Tanaka M, Kanamori H. Impact of human herpesvirus-6 reactivation on outcomes of allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2015;21:2017–2022.
24. Pichereau C, Desseaux K, Janin A, et al. The complex relationship between human herpesvirus 6 and acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2012;18:141–144.
25. Jeulin H, Agrinier N, Guery M, et al. Human herpesvirus 6 infection after allogeneic stem cell transplantation: incidence, outcome, and factors associated with HHV-6 reactivation. *Transplantation*. 2013;95:1292–1298.
26. Sashihara J, Tanaka-Taya K, Tanaka S, et al. High incidence of human herpesvirus 6 infection with a high viral load in cord blood stem cell transplant recipients. *Blood*. 2002;100:2005–2011.
27. Shimazu Y, Kondo T, Ishikawa T, Yamashita K, Takaori-Kondo A. Human herpesvirus-6 encephalitis during hematopoietic stem cell transplantation leads to poor prognosis. *Transpl Infect Dis*. 2013;15:195–201.
28. Razonable RR. Infections due to human herpesvirus 6 in solid organ transplant recipients. *Curr Opin Organ Transplant*. 2010;15:671–675.
29. Osarogiagbon RU, Defor TE, Weisdorf MA, Erice A, Weisdorf DJ. CMV antigenemia following bone marrow transplantation: risk factors and outcomes. *Biol Blood Marrow Transplant*. 2000;6:280–288.
30. George B, Kerridge IH, Gilroy N, et al. A risk score for early cytomegalovirus reactivation after allogeneic stem cell transplantation identifies low-, intermediate-, and high-risk groups: reactivation risk is increased by graft-versus-host disease only in the intermediate-risk group. *Transpl Infect Dis*. 2012;14:141–148.
31. Quintela A, Escuret V, Roux S, et al. HHV-6 infection after allogeneic hematopoietic stem cell transplantation: from chromosomal integration to viral co-infections and T-cell reconstitution patterns. *J Infect*. 2016;72:214–222.
32. de Koning C, Admiraal R, Nierkens S, Boelens JJ. Human herpesvirus 6 viremia affects T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. *Blood Adv*. 2018;2:428–432.
33. Eliassen E, Di Luca D, Rizzo R, Barao I. The interplay between natural killer cells and human herpesvirus-6. *Viruses*. 2017;9:14–16.