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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.

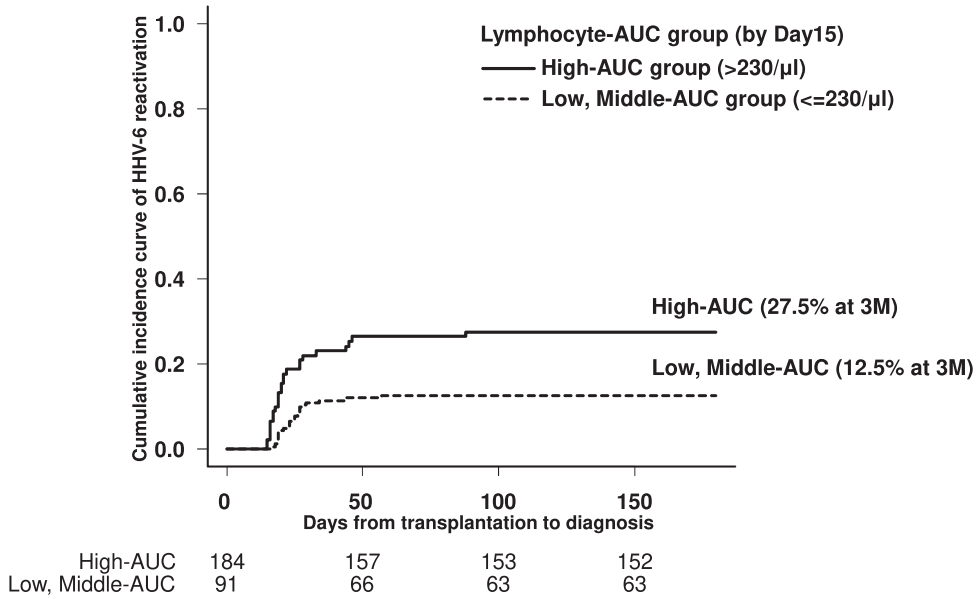

Figure 1. Cumulative incidence of HHV-6 reactivation.

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

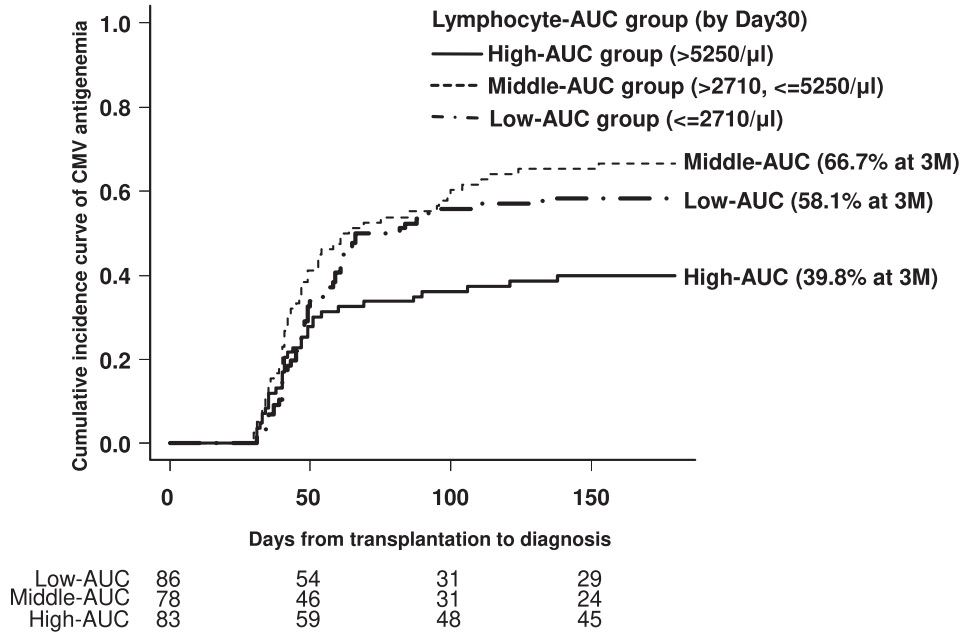

Figure 2. Cumulative incidence of CMV antigenemia.

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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ORIGINAL ARTICLE

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Impact of cumulative steroid dose on infectious diseases after allogenic hematopoietic stem cell transplantation

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Abstract

Background: Systemic steroid is used to treat various transplant-related complications after allogenic hematopoietic stem cell transplantation (allo-HSCT). However, measures to evaluate its impact on infections are still limited. Hence, we examined the cumulative steroid dose used within 30 days after transplant as a predictor of future risk of infections.

Methods: This study included 226 patients who underwent their first allo-HSCT at Kyoto University Hospital between 2005 and 2015.

Results: Sixty-one patients received transplantation from related donors, 106 received unrelated BMT and 59 received unrelated single-unit CBT. Patients were categorized into three groups according to the cumulative steroid dose in terms of prednisolone: no-steroid group (n = 174), low-dose group (≤7 mg/kg) (n = 22) and high-dose group (>7 mg/kg) (n = 30). In a multivariate analysis, high-dose steroid administration was associated with cytomegalovirus (CMV) antigenemia (HR 1.91, $P = 0.037$) and bacteremia (HR 2.59, $P = 0.053$). No impact was found on the occurrence of invasive fungal infection.

Conclusion: High-dose cumulative steroid could predict high risks of bacteremia and CMV antigenemia. Additional anti-bacterial agents for fever and regular measurement of CMV antigen are recommended for whom with systemic steroid administration even after neutrophil engraftment.

1 | INTRODUCTION

Systemic steroid is frequently used as a primary treatment for transplant-related complications such as graft-vs-host disease (GVHD) and non-infectious pulmonary complications after allogeneic hematopoietic stem cell transplantation (HSCT).[1-3] The use of systemic steroid along with the occurrence of GVHD has been suggested to be a risk factor for various infectious diseases,[4-8] which are main causes of transplant-related mortality. Since the number of HSCTs with a higher risk of complications, such as cord blood transplantations (CBT) and HLA mismatch transplantations in older patients, has been increasing,[9-12] it is important to evaluate the effect of steroid use on clinical outcomes.

The associations between the cumulative dose of steroid and the occurrence of side effects have been discussed in patients with non-hematologic diseases who receive systemic steroid for a prolonged period. The impact of the cumulative dose of steroid on infectious complications has been controversial, although a positive association was noted in patients taking immunosuppressive agents after solid organ transplantations.[13-16] Similarly, in recipients of HSCT, steroid administration could increase the risk of infectious complications because of the concomitant use of calcineurin inhibitors and

the delay of immune reconstitution after HSCT. However, there is little information available regarding the steroid dose. Hence, in the present study, we examined the impact of the cumulative steroid dose on the risk of infectious diseases after HSCT in a single transplant center.

2 | METHODS

2.1 | Data collection

A total of 238 patients who underwent their first allogeneic HSCT for hematologic diseases at Kyoto University Hospital from 2005 to 2015 and survived at least 30 days after transplantation were included. Patients who had already started to receive steroid before transplantation were excluded. Patients who had active bacterial, fungal, or viral infection at transplantation or who had had history of invasive fungal infection before transplantation were also excluded. The Institutional Review Board of Kyoto University Hospital, where this study was organized, approved this study.

2.2 | Treatment policy and definition

2.2.1 | Definitions

Neutrophil engraftment was diagnosed when an absolute neutrophil count over 500/μL was observed for 3 days in a row. Acute GVHD was diagnosed and classified by each physician according to traditional criteria.[17]

2.2.2 | Invasive fungal infections

βD-glucan was examined once a week, and imaging inspection and blood culture were examined for fever or other suspicious conditions. Diagnoses of invasive fungal infections were categorized into three types; possible, probable, and proven, based on the practice guidelines from the Infectious Diseases Society of America (IDSA) and Japanese guidelines.[18-20]

In our hospital, antifungal prophylaxis was administered in all patients who underwent allo-HSCT. The antifungal agents that were generally used as prophylaxis were oral fluconazole, voriconazole, micafungin and liposomal amphotericin B injection, according to each patient's history of fungal infection.

All patients were hospitalized in a cleanroom of ISO Class 5 (ISO 14644-1)[21] before and in the early period after day 0 and moved to a cleanroom of ISO Class 6 (ISO 14644-1)[21] after they achieved neutrophil engraftment.

2.2.3 | CMV antigenemia and CMV disease

CMVpp65 antigen examinations were performed using C10/11[22] method or C7-HRP[23] method once a week for every patient after transplantation and examined additionally for suspicious symptoms of cytomegalovirus (CMV) diseases.

In cases with more than three positive cells in two slides (C10/C11 method) or more than two positive cells out of 50 000 WBC (C7-HRP method), pre-emptive therapy was given followed by close CMV-antigen monitoring.[22,23] Diagnosis of CMV end-organ diseases were diagnosed according to published definitions.[24]

2.2.4 | Other viremias

Patients were examined by viral PCR detection at the timing of fever of unknown origin or any other symptoms of infection based on the judgment of each physician in charge. Viruses examined in PCR included adenovirus, BK virus, JC virus, varicella zoster virus, human herpes simplex, EB virus and other viruses according to each patient's symptoms.

2.2.5 | Bacteremia

Two sets of blood culture were examined for each patient with fever or any other symptoms suggesting infectious diseases. As our policy, antibacterial prophylaxis was not applied in every patient, except for those who were at high risk of bacterial infection, such as those with a history of repeated severe bacterial infection or a long history of chemotherapeutic treatment.

2.3 | Endpoints

The endpoint of this study was the incidence of various infectious diseases including invasive fungal infection, CMV antigenemia, and bacteremia diagnosed from 30 days to 6 months after HSCT. The cumulative steroid dose was calculated as the total amount administered per patient within 30 days after transplantation, since the first steroid administration mainly began within this period as a treatment for pre-engraftment or engraftment syndrome and for acute GVHD.

2.4 | Statistical analysis

Descriptive statistics were used to summarize variables related to patient characteristics. We calculated the cumulative steroid dose within 30 days after HSCT. The landmark day was set at 30 days after transplantation. Prednisolone-equivalent conversion was performed in accordance to the general formula.[25] Episodes of infectious diseases (invasive fungal infection, CMV antigenemia or disease, and bacteremia) were calculated based on cumulative incidence curves. A competing event was death without infectious disease. Cumulative incidences in the groups were compared using the Gray test. Fine and Gray's proportional hazards model was used to evaluate the effect of cumulative steroid dose on the occurrence of infectious diseases.[26] The following covariates were considered; recipient's sex, age (<50 or ≥50 years old), disease diagnosis (myeloid malignancies, lymphoid malignancies, or others), year of transplantation (2005-2009 or 2010-2016), disease status

TABLE 1 Patient characteristics

Group by cumulative steroid dose within 30 d		No administration (n = 174)		Low (≤7 mg/kg PSL) (n = 22)		High (>7 mg/kg PSL) (n = 30)		
	Total	Value		Value		Value		Variance
	n[a]	n	%[b]	n	%	n	%	*P*-value
Age[c] median(range)		51 (17-66)		47 (21-66)		48 (20-66)		0.651
Gender								
Male	126	103	59.2	9	40.9	14	46.7	0.144
Female	100	71	40.8	13	59.1	16	53.3	
Donor source								
Sibling	61	47	27.0	7	31.8	7	23.3	0.930
Unrelated BM	106	81	46.6	9	40.9	16	53.3	
Unrelated CB	59	46	26.4	6	27.3	7	23.3	
Disease								
AML/MDS	134	113	64.9	11	50.0	10	33.3	0.015
ALL/other leukemias	50	30	17.2	8	36.4	12	40.0	
Malignant lymphoma	35	25	14.4	3	13.6	7	23.3	
Aplastic anemia	7	6	3.4	0	0.0	1	3.3	
Disease status								
CR	94	72	41.4	11	50.0	11	36.7	0.652
Non CR	132	102	58.6	11	50.0	19	63.3	
Conditioning intensity								
Myeloablative	112	86	49.4	11	50.0	15	50.0	1.000
Reduced intensity	114	88	50.6	11	50.0	15	50.0	
Neutrophil engraftment at day 30								
No	20	18	10.5	1	4.5	1	3.4	0.477
Yes	203	154	89.5	21	95.5	28	96.6	
Levofloxacin prophylaxis								
No	181	142	84.0	14	63.6	25	89.3	0.079
Yes	38	27	16.0	8	36.4	3	10.7	
GVHD prophylaxis								
CI	19	12	6.9	2	9.1	5	16.7	0.755
CI + MMF	35	27	15.5	4	18.2	4	13.3	
CI + MTX	137	107	61.5	13	59.1	17	56.7	
CI + MMF + MTX	35	28	16.1	3	13.6	4	13.3	
GVHD grade at onset								
I	38	30	34.5	6	30.0	2	7.4	0.092
II	76	47	54.0	11	55.0	18	66.7	
III	14	6	6.9	2	10.0	6	22.2	
IV	6	4	4.6	1	5.0	1	3.7	
Cytomegalovirus resopositivity								
Donor+/Recipient+	86	63	41.2	9	45.0	14	53.8	0.527
Donor+/Recipient−	10	9	5.9	0	0.0	1	3.8	
Donor−/Recipient+	85	64	41.8	10	50.0	11	42.3	
Donor−/Recipient−	18	17	11.1	1	5.0	0	0.0	

(Continues)

TABLE 1 (Continued)

Group by cumulative steroid dose within 30 d		No administration (n = 174)		Low (≤7 mg/kg PSL) (n = 22)		High (>7 mg/kg PSL) (n = 30)		
	Total	Value		Value		Value		Variance
	n[a]	n	%[b]	n	%	n	%	*P*-value
Reason for steroid								
Acute GVHD	32			13	59.1	19	63.3	
Engraftment syndrome	9			3	13.6	6	20.0	
Others	11			6	27.3	5	16.7	

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CB, cord blood; CI, Calcinerin inhibitor; CR, complete remission; GVHD, graft-vs-host disease; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; PSL, prednisolone.
Calcinerin inhibitors include Tacrolimus and Cyclosporin.
[a]n indicates the number of patients with each characteristics.
[b]% indicates the percentage of patients in each steroid group.
[c]Age indicates patients' age at transplantation.

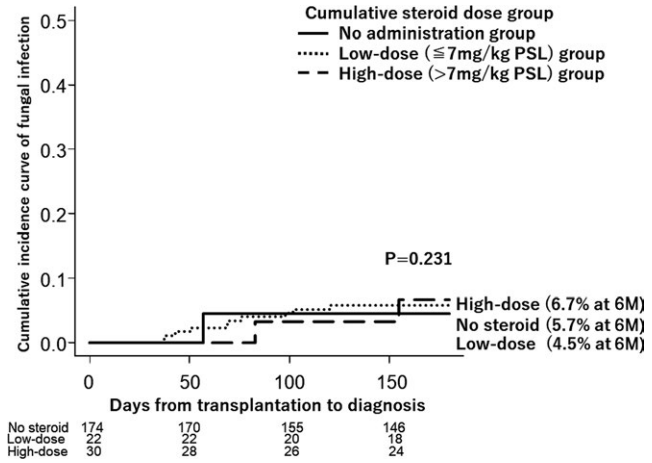

FIGURE 1 Cumulative incidence of invasive fungal infection

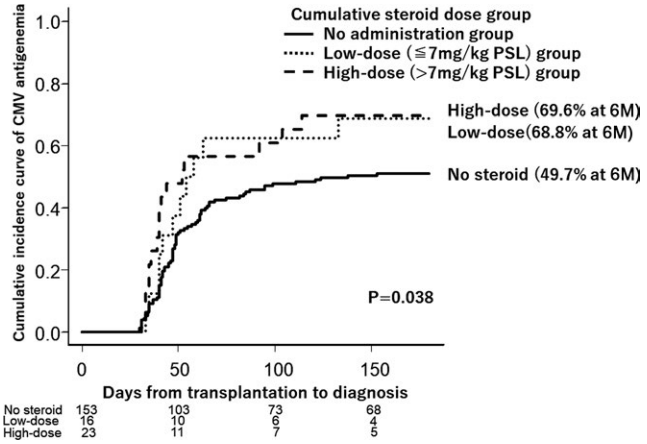

FIGURE 2 Cumulative incidence of cytomegalovirus antigenemia

(complete remission [CR] or non-CR), 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), presence or absence of neutrophil engraftment at day 30, and prophylactic administration of levofloxacin. All factors, in addition to the main effect, were selected with a variable retention criterion of $P < 0.05$ in the univariate analysis and analyzed in the multivariate analysis.

Although acute GVHD has been suggested to be a risk factor for infectious diseases after HSCT, we did not include acute GVHD because there was a correlation between acute GVHD and steroid administration (data not shown), and it would be inappropriate to include both in the same model.

All statistical analyses were performed with Stata version 14 (Stata Corp, College Station, TX) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.1.1, Vienna, Austria).

3 | RESULTS

3.1 | Patient characteristics

Sixty-one patients received transplantation from a related donor, 106 received unrelated bone marrow grafts, and 59 received unrelated cord blood units. Their median age was 51 years (range, 17-66). Neutrophil engraftment was achieved in 203 patients (90%) by day 30 and mean neutrophil engraftment day from transplantation in each graft were 21 in bone marrow transplantation, 17 in peripheral blood stem cell transplantation and 25 in cord blood transplantation.

Patients were categorized into three groups according to the cumulative steroid dose within 30 days: no steroid group (n = 174), low-dose cumulative steroid group (7 mg/kg or less of prednisolone-equivalent dose, n = 22), and high-dose cumulative steroid group (over 7 mg/kg of prednisolone-equivalent dose, n = 30). The cutoff value of 7 mg/kg of prednisolone-equivalent dose approximately stands for initial steroid treatment against acute GVHD in Japan (1 mg/kg during 7 days at

TABLE 2 Univariate and multivariate analysis of cytomegalovirus antigenemia

	Univariate analysis			Multivariate analysis		
Variables	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Age[a]						
<50	1.00		Reference	1.00		Reference
≧50	1.46	1.01-2.09	0.042	1.62	1.14-2.30	0.007
Gender						
Male	1.00		Reference			
Female	0.88	0.60-1.28	0.499			
Year of transplant						
2005-2009	1.00		Reference			
2010-2015	1.19	0.81-1.74	0.373			
Donor source						
Sibling	1.00		Reference	1.00		Reference
Unrelated BM	1.10	0.68-1.78	0.687			
Unrelated CB	1.64	1.00-2.68	0.047	1.62	1.09-2.40	0.018
Disease						
AML/MDS	1.00		Reference			
ALL/other leukemias	1.43	0.88-2.31	0.140			
Malignant lymphoma	0.93	0.50-1.75	0.824			
Aplastic anemia	1.46	0.54-3.93	0.453			
Disease status						
CR	1.00		Reference			
Non CR	1.18	0.80-1.75	0.394			
Conditioning regimen						
Myeloablative	1.00		Reference			
Reduced intensity	1.19	0.81-1.74	0.369			
GVHD prophylaxis						
CI	1.00		Reference			
CI + MMF	1.00	0.50-1.99	0.994			
CI + MTX	0.65	0.35-1.21	0.178			
CI + MMF + MTX	1.10	0.54-2.22	0.792			
Neutrophil engraftment at day 30						
No	1.00					
Yes	1.46	0.86-2.50	0.164			
Steroid group						
No administration	1.00		Reference	1.00		Reference
Low-dose[b]	1.58	0.87-2.87	0.140	1.64	0.91-2.96	0.100
High-dose[c]	1.78	1.02-3.12	0.044	1.91	1.04-3.50	0.037

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marow; CB, cord blood; CI, Calcinerin inhibitor; CR, complete remission; GVHD, graft-vs-host disease; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; PSL, prednisolone.

Calcinerin inhibitors include Tacrolimus and Cyclosporin.

[a]Age indicates patients' age at transplantation.

[b]Low-dose indicates group who undertook low cumulative dose of steroid (≤7 mg/kg of prednisolone).

[c]High-dose indicates group who undertook high cumulative dose of steroid (>7 mg/kg of prednisolone).

maximum). The reason for steroid administration was treatment for GVHD in 33 patients, engraftment syndrome in 9, and other reasons including lung complications in 10. Grade II-IV acute GVHD was diagnosed in 96 patients in total. There was no obvious difference in background among the different donor sources. (Table 1).

3.2 | Invasive fungal infection

We observed 13 cases of invasive fungal infection, including one proven case with candida bloodstream infection and two probable and 10 possible cases of pneumonia. The cumulative incidence of invasive fungal infection at 6 months was 5.7%, 4.5%, and 6.7% in the no-administration, low-dose, and high-dose groups, respectively ($P = 0.231$, Gray test) (Figure 1). Multivariate analysis showed no association between steroid administration and the occurrence of invasive fungal infection. We found no other significant risk factor.

3.3 | CMV antigenemia and diseases

Eighty-six HSCT were performed from CMV-antibody (Ab) positive donors to CMV-Ab positive recipients, 10 were from CMV-Ab positive donors to CMV negative recipients and the other 103 were from CMV-Ab negative donors (Table 1).

A total of 105 (46%) patients were diagnosed as CMV antigenemia and 81 (78%) received Ganciclovir as a pre-emptive antiviral therapy. Seven patients were pathologically diagnosed as CMV disease including colitis and hepatitis, all of whom were positive for CMV antigenemia. There were four cases of CMV antigenemia with clinically suspected CMV diseases, although they were not definitely diagnosed due to a lack of pathological evidence. No patient died of CMV-related complications. The cumulative incidences of CMV antigenemia at 6 months in the no-administration, low-dose, and high-dose groups were 49.7%, 68.8%, and 69.6%, respectively ($P = 0.038$) (Figure 2). Reason for steroid initiation had little impact on the occurrence of CMV antigenemia (GVHD vs other reasons: HR 2.119, $P = 0.089$). Multivariate analysis showed that both a low-dose and high-dose of cumulative steroid administration were associated with CMV reactivation, although the association in the low-dose group was not statistically significant (low-dose vs no-administration group: HR 1.64, $P = 0.100$, high-dose vs no-administration group: HR 1.91 $P = 0.037$). Other risk factors detected were cord blood unit as a donor source (cord blood unit vs sibling donor: HR 1.62, $P = 0.018$) and recipient age over 50 years at transplantation (age ≥ 50 vs <50: HR 1.62, $P = 0.007$) (Table 2).

3.4 | Viral infections other than CMV

A total of 15 cases were diagnosed as viremia including Adenovirus in one patient, BK virus in 2, Epstein Barr virus in 1, Varicella Zoster virus in 3, and human herpes virus 6 in 7. Ten patients were in the no-administration group and there was no association between viremia and the cumulative steroid dose.

3.5 | Bacteremia

The cumulative incidences of bacteremia at 6 months in the no-administration, low-dose, and high-dose groups were 9.3%, 15.8%, and 21.7%, respectively ($P = 0.224$) (Figure 3). Detected microbes at the first onset of bacteremia were gram-negative rods in 10 cases, gram-positive cocci in 12 cases, and gram-positive rods in one case. Reason for steroid initiation had little impact on the occurrence of bacteremia (GVHD vs other reasons: HR 4.89, $P = 0.14$). Administration of levofloxacin showed no apparent prophylactic effect on bacteremia (HR 0.73, $P = 0.574$).

Multivariate analysis showed that the high-dose group was marginally associated with an increased risk of bacteremia (low-dose vs no-administration group: HR 2.13, $P = 0.240$, high-dose vs no-administration group: HR 2.59, $P = 0.053$). Regarding the microbes detected, there was no significant difference among the three groups. The other major risk factor for bacteremia was a recipient age over 50 years at transplantation, which had a HR of 2.69 (age ≥ 50 vs <50: $P = 0.021$) (Table 3).

3.6 | Other bacterial infections

The other infectious events proven as bacterial complications were four cases *Clostridium difficile* colitis, two cases of pneumonia (one of *Pseudomonas aeruginosa*, one of *Stenotrophomonas maltophilia*), one cellulitis of *Coagulase-negative staphylococcus*, and one endophthalmitis of *Coagulase-negative staphylococcus*.

4 | DISCUSSION

In the present study, we examined the impact of the cumulative dose of steroid on infectious complications after HSCT and found associations between steroid dose and both CMV and bacterial infections following HSCT.

Although acute GVHD and systemic steroid have been reported to be risk factors for invasive fungal infection after HSCT,[27-30] the cumulative steroid dose was not associated with fungal infection in our study. All patients in our hospital continued prophylactic treatment with antifungal drugs according to the risk of fungal infection, following Japanese and European guidelines.[31] Only 13 of 226 patients had invasive fungal infection over 10 years, although our cohort included a relatively large number of cord blood transplantations. This

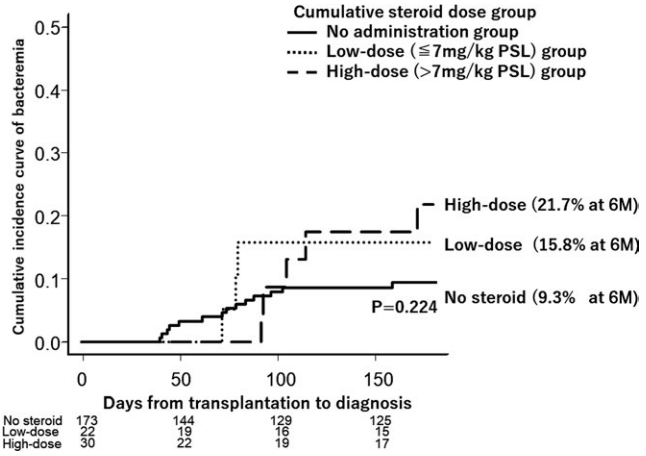

FIGURE 3 Cumulative incidence of bacteremia

TABLE 3 Univariate and multivariate analysis of bacteremia

	Univariate analysis			Multivariate analysis		
Variables	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Age[a]						
<50	1.00		Reference	1.00		Reference
≧50	2.40	1.07-5.38	0.034	2.69	1.16-6.22	0.021
Gender						
Male	1.00		Reference			
Female	1.20	0.52-2.78	0.671			
Year of transplant						
2005-2009	1.00		Reference			
2010-2015	1.11	0.48-2.53	0.813			
Donor source						
Sibling	1.00		Reference			
Unrelated BM	1.28	0.46-3.55	0.633			
Unrelated CB	1.18	0.37-3.72	0.778			
Disease						
AML/MDS	1.00		Reference			
ALL/other leukemias	1.06	0.36-2.77	0.917			
Malignant lymphoma	1.52	0.52-3.96	0.419			
Aplastic anemia						
Disease status						
CR	1.00		Reference			
Non CR	1.63	0.66-3.98	0.287			
Conditioning regimen						
Myeloablative	1.00		Reference			
Reduced intensity	1.70	0.72-4.03	0.226			
GVHD prophylaxis						
CI	1.00		Reference			
CI + MMF	1.61	0.31-9.13	0.568			
CI + MTX	0.72	0.15-3.37	0.674			
CI + MMF + MTX	1.16	0.24-6.58	0.864			
Neutrophil engraftment at day 30						
No	1.00					
Yes	3.86	0.23-64.05	0.346			
Levofloxacin prophylaxis						
No	1.00					
Yes	0.73	0.250-2.159	0.574			
Steroid group						
No administration	1.00		Reference	1.00		Reference
Low-dose[b]	1.74	0.50-6.07	0.390	2.13	0.60-7.51	0.240
High-dose[c]	2.27	0.87-5.93	0.097	2.59	0.99-6.78	0.053

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marow; CB, cord blood; CI, Calcinerin inhibitor; CR, complete remission; GVHD, graft-vs-host disease; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; PSL, prednisolone.

Calcinerin inhibitors include Tacrolimus and Cyclosporin.

[a]Age indicates patients' age at transplantation.

[b]Low-dose indicates group who undertook low cumulative dose of steroid (≤7 mg/kg of prednisolone).

[c]High-dose indicates group who undertook high cumulative dose of steroid (>7 mg/kg of prednisolone).

suggests that fungal infection could be avoided regardless of the occurrence of acute GVHD, steroid use, and donor source by appropriate clinical practice.

With regard to CMV-related complications, steroid use was strongly associated with CMV antigenemia regardless of the cumulative dose, which is similar to previous reports.[4,32,33] Almost all the patients in our cohort were seropositive before transplant and thus CMV antigen levels must be measured regularly after HSCT. Another risk factor for CMV antigenemia was cord blood unit as a donor source, although the HR was lower than previously reported and there were no CMV-related deaths. Older patients also had a higher risk of CMV antigenemia. Contrary to a previous report,[4] myeloablative conditioning was not found to be a risk factor for CMV antigenemia, which is probably due to the difference in the conditioning regimen or the medication used for GVHD prophylaxis.

High-dose, but not low-dose, cumulative steroid administration was a risk factor for bacterial infection. Anti-bacterial prophylaxis and preemptive therapies for fever of undetected origin might be better considered for patients after HSCT receiving a high cumulative dose of steroid, regardless of their neutrophil count. An advanced age at transplant was another risk factor for bacterial infection after HSCT, which was consistent with previous reports.[34]

The present study has several limitations. First, this is a retrospective study of small population with heterogeneous background in a single transplant center. Second, the loads of viruses other than CMV were not regularly measured and the timing of the examination was determined by each physician in charge. Finally, information on blood sugar levels was not collected, although blood sugar levels were checked regularly and treated by continuous intravenous insulin infusion, which minimized the effect of hyperglycemia on bacterial infections.

In conclusion, our study confirmed that the cumulative steroid dose could be a good prognostic marker for CMV antigenemia and bacterial infection after HSCT. These post-transplant complications must be detected and managed in the early period, particularly in elderly patients who are receiving a high cumulative dose of steroid.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHORS CONTRIBUTION

MW and JK designed the research; JK organized the project; MW and JK performed the statistical analysis; MH, TK, KY, and AT-K interpreted the data; MW wrote the first draft and all other authors critically reviewed the draft and approved the final version for publication.

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