

ORIGINAL ARTICLE

Clinical characteristics and outcome of human herpesvirus-6 encephalitis after allogeneic hematopoietic stem cell transplantation

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In this retrospective analysis using the Transplant Registry Unified Management Program, we identified 145 patients with human herpesvirus (HHV)-6 encephalitis among 6593 recipients. The cumulative incidences of HHV-6 encephalitis at 100 days after transplantation in all patients, recipients of bone marrow or PBSCs and recipients of cord blood were 2.3%, 1.6% and 5.0%, respectively. Risk factors identified in multivariate analysis were male sex, type of transplanted cells (relative risk in cord blood transplantation, 11.09, $P < 0.001$; relative risk in transplantation from HLA-mismatched unrelated donor, 9.48, $P < 0.001$; vs transplantation from HLA-matched related donor) and GvHD prophylaxis by calcineurin inhibitor alone. At 100 days after transplantation, the overall survival rate was 58.3% and 80.5% among patients with and without HHV-6 encephalitis, respectively ($P < 0.001$). Neuropsychological sequelae remained in 57% of 121 evaluated patients. With both foscarnet and ganciclovir, full-dose therapy (foscarnet ≥ 180 mg/kg, ganciclovir ≥ 10 mg/kg) was associated with better response rate (foscarnet, 93% vs 74%, $P = 0.044$; ganciclovir, 84% vs 58%, $P = 0.047$). HHV-6 encephalitis is not rare not only in cord blood transplant recipients but also in recipients of HLA-mismatched unrelated donors. In this study, development of HHV-6 encephalitis was associated with a poor survival rate, and neurological sequelae remained in many patients.

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INTRODUCTION

Reactivation of human herpesvirus (HHV)-6 is observed in 30–70% of allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients.^{1–3} Accumulating evidence supports a causal association between HHV-6 reactivation and encephalitis.^{4–6}

Previous analyses have suggested that the mortality rates attributable to HHV-6 encephalitis are high,^{7–12} and many survivors display cognitive sequelae.¹³ HHV-6 encephalitis is therefore increasingly recognized as an important complication of allo-HSCT; however, many questions remain unanswered. Reported incidence of HHV-6 encephalitis among allo-HSCT recipients ranges from 0.96 to 11.6%,⁵ mainly owing to small cohorts of cases. Although umbilical cord blood transplantation (CBT) represents a risk factor for HHV-6 encephalitis,^{5,12,14} other risk factors remain unconfirmed. Treatment recommendations have been made based on *in vitro* data,^{15–17} but few data are available on the clinical effectiveness. Our study aimed to define the epidemiology, clinical characteristics and outcome of HHV-6 encephalitis, and the effect of antiviral treatment on the outcome.

PATIENTS AND METHODS

Data source and patient selection

For this retrospective observational study, clinical data of patients ≥ 16 years old who received allo-HSCT between 1 January 2007 and 31 December 2011 were obtained from the Japan Society of Hematopoietic Cell Transplantation (JSHCT) and the Japanese Data Center for Hematopoietic Cell Transplantation (JDCHCT), using the Transplant Registry Unified Management Program (TRUMP).^{18,19} Patient data in TRUMP including survival and long-term complications are followed longitudinally using an annual follow-up form. Additional data, including detailed conditions, treatments and outcomes, were collected for this study. The study was approved by the data management committee of the JSHCT and by the ethics committee of Oita University Faculty of Medicine.

Definitions

HHV-6 encephalitis was diagnosed if the patient satisfied all of the following three criteria: (1) presence of central nervous system (CNS) dysfunction; (2) a positive PCR result for HHV-6 DNA in cerebrospinal fluid (CSF); and (3) absence of other identified causes of CNS dysfunction, including other infectious agents. CNS dysfunction was defined as the presence of disorientation as to time and place, loss of consciousness,

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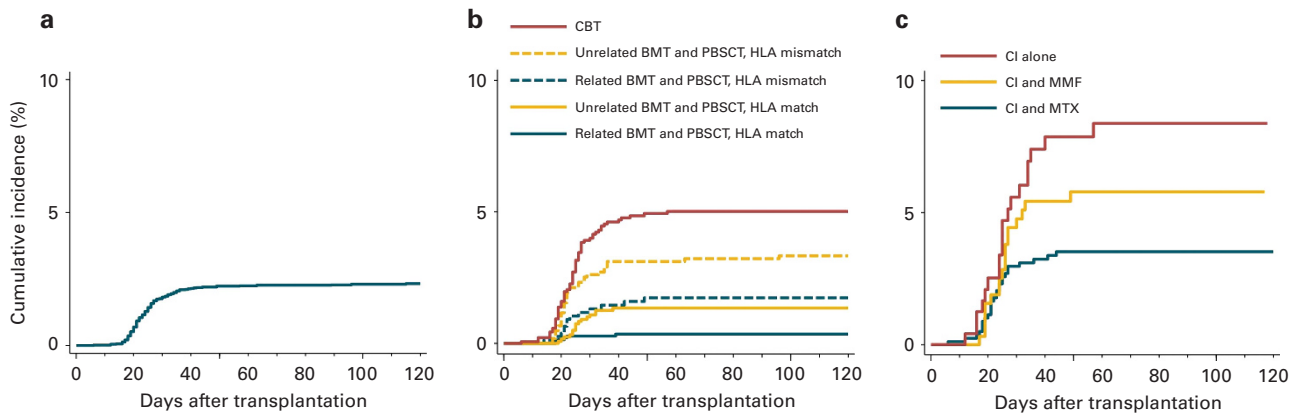


Figure 1. Cumulative probabilities of HHV-6 encephalitis. **(a)** All cases. Cumulative incidence of HHV-6 encephalitis at 100 days after HSCT was 2.3%. **(b)** Stratified by donor source: related or unrelated bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT), HLA match or mismatch. Cumulative incidence of HHV-6 encephalitis at 100 days after HSCT was 0.36%, 1.7%, 1.3%, 3.3% and 5.0% in patients receiving related BMT or PBSCT (HLA match), related BMT or PBSCT (HLA mismatch), unrelated BMT or PBSCT (HLA match), unrelated BMT or PBSCT (HLA mismatch) and CBT, respectively. **(c)** Stratified by method of GvHD prophylaxis in CBT recipients: CI alone, CI with mycophenolate mofetil (MMF) or CI with methotrexate (MTX). Cumulative incidence of HHV-6 encephalitis at 100 days after CBT was 3.5%, 5.8% and 8.4% in patients who received GvHD prophylaxis using CI with MTX, CI with MMF and CI alone, respectively.

Table 1. Univariate analysis of risk factors for HHV-6 encephalitis

Risk factor	N	No. of patients who developed HHV-6 encephalitis ^a	Cumulative incidence at day 100 (%)	p ^b
<i>Patient age</i>				
< 50 Years	3544	75	2.2	0.60
≥ 50 Years	3049	69	2.4	
<i>Patient sex</i>				
Female	2778	40	1.5	< 0.001
Male	3815	104	2.9	
<i>Performance status</i>				
0–1	5641	126	2.3	0.80
2–4	902	18	2.5	
<i>Disease phase</i>				
Standard	2446	50	2.1	0.065
Advanced	2177	60	3.0	
<i>Lineage of neoplasm</i>				
Myeloid	3692	78	2.2	0.84
Lymphoid	2631	61	2.4	
<i>Type of transplanted cells</i>				
Related BM or PB, HLA match	1439	5	0.36	< 0.001
Related BM or PB, HLA mismatch	833	13	1.7	
Unrelated BM or PB, HLA match	1230	16	1.3	
Unrelated BM or PB, HLA mismatch	1066	34	3.3	
CB	1464	67	5.0	
<i>Conditioning regimen</i>				
MAC	3416	74	2.3	0.76
RIC	3149	69	2.3	
<i>GvHD prophylaxis: type of CI</i>				
CyA	2652	41	1.6	0.002
Tac	3794	103	2.9	
<i>GvHD prophylaxis: combination regimen</i>				
CI+MTX	5021	96	2.0	< 0.001
CI+MMF	578	23	4.3	
CI alone	481	19	4.4	

Abbreviations: BM = bone marrow; CB = cord blood; CI = calcineurin inhibitor; CyA = cyclosporine; HHV-6 = human herpesvirus 6; MAC = myeloablative conditioning; MMF = mycophenolate mofetil; MTX = methotrexate; PB = peripheral blood stem cell; RIC = reduced-intensity conditioning; Tac = tacrolimus.
^aData for date of onset of HHV-6 encephalitis missing for one patient. ^bLog-rank test.

Table 2. Multivariate analysis of risk factors for HHV-6 encephalitis

Risk factor	Total		BMT or PBSCT		CBT	
	Relative risk (95% confidence interval)	P-value	Relative risk (95% confidence interval)	P-value	Relative risk (95% confidence interval)	P-value
<i>Patient sex</i>						
Female	Reference		Reference			
Male	1.94 (1.31–2.87)	0.001	2.65 (1.87–3.75)	< 0.001		
<i>Type of transplanted cells</i>						
Related BM or PB, HLA match	Reference		Reference			
Related BM or PB, HLA mismatch	5.35 (1.81–15.78)	0.002	2.95 (1.47–5.94)	0.002		
Unrelated BM or PB, HLA match	3.91 (1.42–10.74)	0.008	2.62 (1.33–5.19)	0.006		
Unrelated BM or PB, HLA mismatch	9.48 (3.67–24.48)	< 0.001	5.32 (2.71–10.47)	< 0.001		
CB	11.09 (4.48–27.50)	< 0.001				
<i>GvHD prophylaxis: type of CI</i>						
CyA			Reference			
Tac			1.62 (1.03–2.54)	0.037		
<i>GvHD prophylaxis: combination regimen</i>						
CI+MTX	Reference				Reference	
CI+MMF	1.41 (0.87–2.27)	0.16			1.71 (0.94–3.10)	0.078
CI alone	1.69 (1.02–2.81)	0.042			2.33 (1.30–4.18)	0.005

Abbreviations: BM = bone marrow; BMT = bone marrow transplantation; CB = cord blood; CBT = cord blood transplantation; CI = calcineurin inhibitor; CyA = cyclosporine; HHV-6 = human herpesvirus 6; MMF = mycophenolate mofetil; MTX = methotrexate; PB = peripheral blood stem cell; PBSCT = peripheral blood stem cell transplantation; RIC = reduced-intensity conditioning; Tac = tacrolimus.

personality change, convulsions, memory loss or dysesthesia not attributable to peripheral neuropathy.^{11,20} Diagnosis and grading of acute GvHD were based on clinical criteria described elsewhere.²¹ Engraftment syndrome was defined according to the proposal for a uniform definition by Spitzer.²² Pre-engraftment immune reaction was defined as CBT recipients who satisfied criteria described elsewhere.^{23,24} Steroid treatment was defined as treatment with ≥ 0.5 mg/kg of prednisolone or ≥ 0.4 mg/kg of methylprednisolone. Disease phase was evaluated against patients with hematological neoplasms. Early stage was defined as acute leukemia in a first to second remission, CML in a first chronic phase or myelodysplastic syndrome classified as refractory anemia without excess blasts; all others were considered non-early stage. We defined the following dosages as reduced-intensity conditioning regimens: TBI ≤ 5 Gy in a single fraction or 5–8 Gy in multiple fractions; busulfan < 9 mg/kg (IV busulfan < 7.2 mg/kg); or melphalan ≤ 140 mg/m². Donor and recipient pairs were considered HLA matched when the HLA-A, HLA-B and HLA-DRB1 loci were matched in HSCT from a related donor or CBT. For unrelated HSCT other than CBT, matching at the HLA-A, HLA-B, HLA-Cw and HLA-DRB-1 loci was considered indicative of matching. HLA mismatches were defined by the presence of ≥ 1 disparity among HLA loci. Regarding the effects of antiviral therapy, response was defined as disappearance or obvious improvement of CNS symptoms after starting treatment. With respect to the outcomes of HHV-6 encephalitis, ‘return to society’ meant that the patient was able to return to work, school or household activities.

Statistical considerations

The cumulative incidence of HHV-6 encephalitis was estimated by considering competing risks regression models according to the method of Fine and Gray.²⁵ Death without HHV-6 encephalitis was treated as competing risk. Factors associated with at least borderline significance ($P < 0.1$) in the univariate analyses were subjected to multivariate analysis. The Fine and Gray²⁶ proportional hazards model was used for the multivariate analysis. The cumulative incidence of acute GvHD was estimated by considering competing risks regression models, and treating death without GvHD as a competing risk. The probability of overall survival was estimated using the Kaplan–Meier method. To evaluate the influence of development of HHV-6 encephalitis on survival, proportional hazard modeling was used, treating the development of HHV-6 encephalitis as a time-dependent covariate. Survival estimates were compared using the log-rank test. Factors associated with outcome of HHV-6 encephalitis were

analyzed using Fisher’s exact test and Cox modeling. Proportional hazard modeling was used to evaluate the effect of antivirals on survival, treating the start of antiviral therapy as a time-dependent covariate. All statistical tests were two sided and performed using a 5% level of significance and were carried out using Stata 13 software (StataCorp LP, College Station, TX, USA).

RESULTS

Data collection

Using the TRUMP database, we identified a total of 574 recipients, aged ≥ 16 years, who received allo-HSCT between January 2007 and December 2011 and whose records showed they had viral infections owing to HHV-6. Most patients were not monitored for HHV-6 reactivation. A questionnaire was mailed to each associated transplant institution ($n = 128$) to collect additional patient data. Questionnaires were returned from 372 recipients with HHV-6 infection (data collection rate, 65%) from 82 transplant institutions (institution participation rate, 63%). Patients comprised 239 with HHV-6-associated diseases and 133 with asymptomatic reactivation of HHV-6. Among the 239 patients with HHV-6-associated disease, 213 (89%) had been diagnosed with HHV-6 encephalitis by an attending physician (Supplementary Table S1). Among these, 145 fulfilled the criteria for HHV-6 encephalitis.

Incidence and risk factors of HHV-6 encephalitis

Cumulative incidence of HHV-6 encephalitis in the overall study population (patients at institutes from whom questionnaires were returned who were ≥ 16 years old and had received allo-HSCT between January 2007 and December 2011; $n = 6$ 593) at 100 days after HSCT was 2.3% (Figure 1a). Cumulative incidence of HHV-6 encephalitis at day 100 was significantly higher in patients receiving CBT than in those receiving another stem cell source (5.0% vs 1.6%, $P < 0.001$). By univariate analysis, patient sex, type of transplanted cells (Figure 1b) and measures of GvHD prophylaxis were associated with incidence of HHV-6 encephalitis (Table 1). GvHD prophylaxis by calcineurin inhibitor (CI) alone was

Table 3. Characteristics of HHV-6 encephalitis (n = 145)

Variable	Value
<i>Onset (data available, N = 144)</i>	
Days after HSCT, median (range)	23 (6–115)
<i>Conditions 0–8 days before onset of HHV-6 encephalitis</i>	
PIR or ES or GvHD (one or more, either)	82 (57%)
Steroid treatment	77 (53%)
<i>Symptoms at diagnosis^a</i>	
Loss of consciousness	90 (62%)
Disorientation	76 (52%)
Memory disturbance	69 (48%)
Seizure	46 (32%)
Dysesthesia	32 (22%)
Autonomic symptoms	9 (6.2%)
Other ^b	18 (15%)
<i>HHV-6 DNA in CSF</i>	
Demonstrated by quantitative PCR	125
HHV-6 DNA in CSF (copies/ml), median (range)	17 000 (100–7 400 000)
Demonstrated by qualitative PCR	17
Positive, but details not available	3
<i>HHV-6 DNA in peripheral blood at time of developing encephalitis (data available, N = 84)</i>	
Positive	84 (100%)
Negative	0
<i>Species of HHV-6 in CSF (data available, N = 136)</i>	
HHV-6A	0
HHV-6B	48
Undifferentiated	88
<i>Brain MRI (performed, N = 131)</i>	
Normal findings	37 (28%)
Abnormal findings in limbic system	72 (55%)
Abnormal findings in limbic system and other area	6 (4.6%)
Abnormal findings in other than limbic system ^c	16 (12%)
Intubation after onset of HHV-6 encephalitis	40 (28%)

Abbreviations: CSF = cerebrospinal fluid; ES = engraftment syndrome; HHV-6 = human herpesvirus 6; HSCT = hematopoietic stem cell transplantation; MRI = magnetic resonance imaging; PIR = pre-engraftment immune reaction. Data are given as n (%), unless otherwise indicated. ^aSome overlap of symptoms is present. ^bThese included involuntary movement (n = 7), changes in personality and behavior (n = 3), restlessness (n = 3), paralysis (n = 2), bladder and rectal disturbance (n = 1), visual disturbance (n = 1) and central respiratory dysfunction (n = 1). ^cThese included basal ganglia (n = 5), occipital lobe (n = 3), temporal lobe (n = 3), parietal lobe (n = 2), meninges (n = 2), spinal cord (n = 3), hypothalamus (n = 2), frontal lobe (n = 1) and periventricular area (n = 1).

associated with increased incidence of HHV-6 encephalitis among CBT recipients (Supplementary Table S2, Figure 1c) but was not a significant risk factor in non-CBT (Supplementary Table S3). Multivariate analysis (Table 2) revealed that male sex, HSCT from other than an HLA-matched related donor and GvHD prophylaxis by CI alone were significant risk factors for HHV-6 encephalitis. Among CBT recipients, GvHD prophylaxis by CI alone was the only significant factor for HHV-6 encephalitis. Regarding the associations between GvHD prophylactic methods and incidence of GvHD in CBT recipients, cumulative incidences of grade 2–4 acute GvHD at 100 days were 37.1%, 43.0% and 54.4% in patients receiving CI with methotrexate, CI with mycophenolate mofetil and CI alone, respectively ($P < 0.001$).

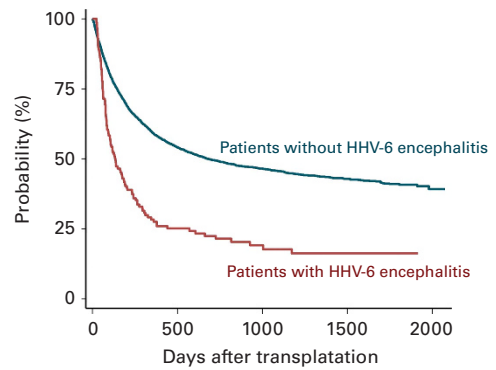


Figure 2. Effect of HHV-6 encephalitis on survival.

Characteristics of HHV-6 encephalitis

Characteristics of HHV-6 encephalitis are shown in Table 3. A total 125 (84.4%) of the 145 included patients developed HHV-6 encephalitis during weeks 3–5 (15–35 days) after HSCT. Among CBT recipients, criteria-defined pre-engraftment immune reaction was observed 0–8 days before development of HHV-6 encephalitis in 35 (52%) of 67 patients. Differentiation of HHV-6 was performed for 48 patients, all of whom had HHV-6B. Limbic encephalitis was demonstrated in a total of 78 (60%) of 131 patients who underwent brain magnetic resonance imaging examination.

Outcome

The probabilities of overall survival on day 100 and day 365 after HSCT were 58.3% (95% confidence interval (CI): 49.6–66.1%) and 27.5% (95% CI: 20.3–35.2%) in patients who developed HHV-6 encephalitis and 80.5% (95% CI: 79.5–81.5%) and 58.7% (95% CI: 57.5–59.9%) in those who did not develop HHV-6 encephalitis, respectively ($P < 0.001$; Figure 2). Cumulative incidence of the mortality attributable to HHV-6 encephalitis on day 30 and day 100 after development of HHV-6 encephalitis was 2.1% and 12.9%, respectively.

Supplementary Table S4 shows proportional hazards modeling of the mortality risk after development of HHV-6 encephalitis. In the final multivariable Cox model, a positive but statistically nonsignificant relationship was confirmed between male sex and death after the development of HHV-6 encephalitis.

Figure 3 shows outcomes for those patients who developed HHV-6 encephalitis. Among patients who were evaluated for CNS dysfunction after completing antiviral therapy (n = 121), a total of 69 patients (57%) were considered to have retained various neuropsychological dysfunctions. The most frequent sequela was memory disturbance.

Effect of antiviral treatment

Among 145 patients with HHV-6 encephalitis, information about response to antiviral therapy was available for 133 patients. The reasons for missing data are as follows: response was unable to be evaluated (n = 9), no therapy (n = 1) and treatment data not available (n = 2). A total of 123 patients received foscarnet or ganciclovir monotherapy, and the other 10 patients were treated with combination foscarnet and ganciclovir therapy.

Response rates of neurological symptoms after starting antiviral therapy were 83.8% and 71.4% with foscarnet monotherapy and ganciclovir monotherapy, respectively. This difference between groups was not significant ($P = 0.10$, Fisher's exact test). With both foscarnet and ganciclovir, the response rate of full-dose monotherapy (foscarnet ≥ 180 mg/kg, ganciclovir ≥ 10 mg/kg) was significantly higher than that of non-full-dose monotherapy (foscarnet, 93% vs 74%, $P = 0.044$; ganciclovir, 84% vs 58%, $P = 0.047$, Fisher's exact test; Figure 4). The response rate of

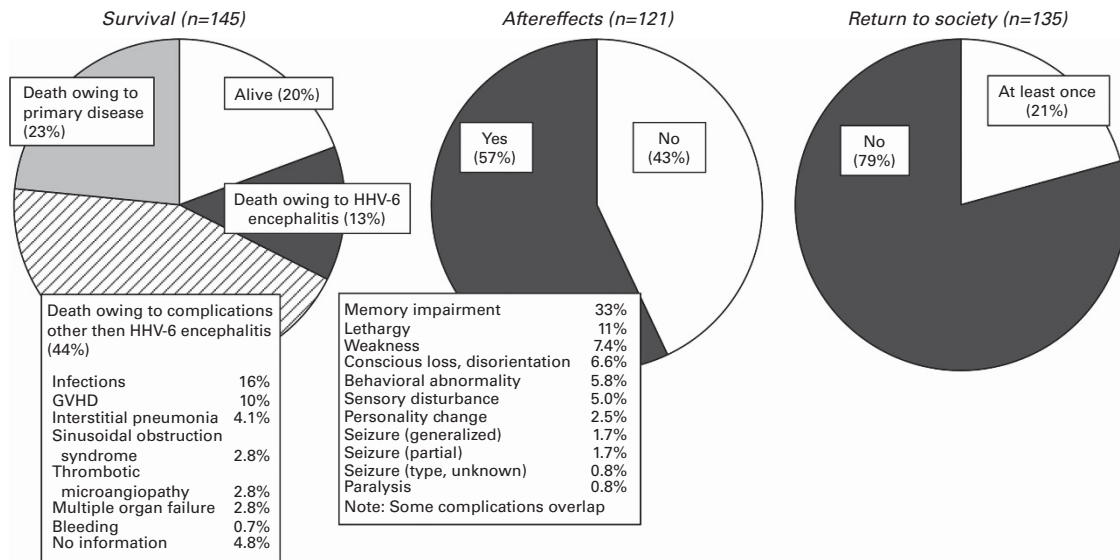


Figure 3. Outcome for patients who developed HHV-6 encephalitis. For patients with HHV-6 encephalitis, median follow-up after development of HHV-6 encephalitis was 1651 days for surviving patients (range, 649–2680 days). Sequelae of HHV-6 encephalitis could not be evaluated in 24 patients owing to early death ($n = 20$) or intubation ($n = 4$). Information on the return to society was not obtained for 3 surviving patients and 7 patients who died.

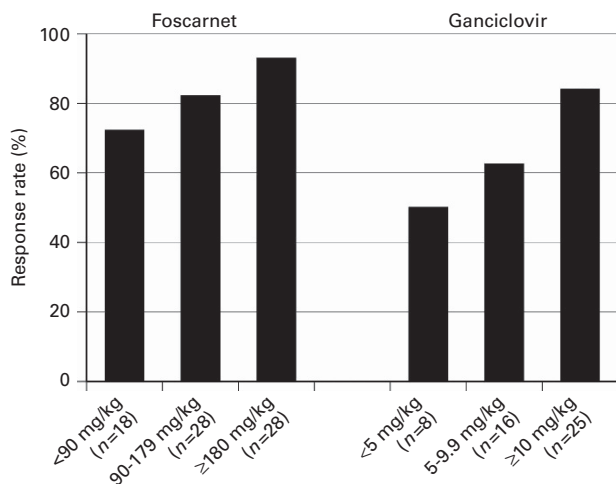


Figure 4. Response rate of patients receiving monotherapy with foscarnet or ganciclovir stratified by antiviral dose per body weight.

patients receiving combination therapy using various doses of foscarnet and ganciclovir was 100%.

The rate of sequelae or death owing to HHV-6 encephalitis was significantly lower in patients who received full-dose therapy than in those who did not receive full-dose therapy (ganciclovir < 10 mg/kg and foscarnet < 180 mg/kg; Table 4). The rate of death from any cause within 30 days after the development of HHV-6 encephalitis was significantly lower in patients who received foscarnet therapy and significantly higher in patients who received ganciclovir therapy (Table 4). Using Kaplan–Meier analysis, the probabilities of overall survival on day 30 and day 100 after development of HHV-6 encephalitis were 88.3% (95% CI: 79.4–93.6%) and 60.8% (95% CI: 49.7–70.2%) in patients who were treated with foscarnet as first-line therapy, and 67.0% (95% CI: 52.3–78.0%) and 49.3% (95% CI: 35.0–62.0%) in those who were not treated with foscarnet as first-line therapy, respectively ($P=0.14$); these probabilities were 83.3% (95% CI: 71.2–90.7%) and 62.1% (95% CI: 48.7–72.9%) in patients who were treated with

full-dose therapy, and 78.2% (95% CI: 67.4–85.9%) and 52.1% (95% CI: 40.5–62.6%) in those who were not treated with full-dose therapy, respectively ($P=0.15$).

DISCUSSION

In this study, we determined the epidemiology, characteristics and outcomes of HHV-6 encephalitis by accessing a nationwide database. We found that the prevalence of HHV-6 encephalitis was unexpectedly high in patients receiving HSCT from an HLA-mismatched unrelated donor. The finding is consistent with a prior study.¹² We also showed risk factors that were previously unconfirmed, including GvHD prophylaxis by CI alone in CBT. The prognosis of patients who developed HHV-6 encephalitis was poor and many surviving patients retained psychophysiological dysfunctions.

Although CBT is an established risk factor for HHV-6 encephalitis, considerable differences in incidence can be found among centers.^{11,27} Methods of GvHD prophylaxis were shown to be associated with the development of HHV-6 encephalitis in CBT; previous reports have supported the clinical plausibility of such a relationship. An inflammatory milieu early after allo-HSCT, such as pre-engraftment immune reaction,¹¹ engraftment syndrome²⁸ and GvHD,^{12,28} represents risk factors for subsequent development of HHV-6 encephalitis. In fact, more than half (52%) of CBT recipients in this study displayed pre-engraftment immune reaction before developing HHV-6 encephalitis. GvHD prophylaxis without methotrexate is known to be associated with a high incidence of post-CBT immune reaction.^{24,29} In this cohort, prophylaxis using CI alone was a significant risk factor for acute GvHD. We suggest that better prevention of GvHD and related immune reactions will reduce HHV-6 encephalitis incidence.

This study clearly showed a poor prognosis for patients who developed HHV-6 encephalitis. Not only encephalitis but also various other conditions were associated with patient death, including GvHD. Reactivated HHV-6 may affect such subsequent complications.³⁰ Even among survivors, more than half of patients retained some degree of neurological compromise; the proportion of patients who could return to society at least once was only 21%.

Table 4. Factors associated with HHV-6 encephalitis-related events

Variables	No. of data available	Death owing to HHV-6 encephalitis		Sequelae or death owing to HHV-6 encephalitis		Death from any cause within 30 days of HHV-6 encephalitis development		
		N (%)	P-value	N (%)	P-value	No. of data available ^a	N (%)	P-value
Age								
< 50 Years	75	7 (9.3)	0.32	51 (68)	0.73	75	14 (19)	0.84
≥ 50 Years	70	11 (16)		45 (64)		69	14 (20)	
Sex								
Male	105	13 (12)	1.00	73 (70)	0.18	104	22 (21)	0.49
Female	40	5 (13)		23 (58)		40	6 (15)	
Transplanted cells								
CBT	67	8 (12)	1.00	47 (70)	0.38	67	13 (19)	1.00
BMT/PBSCT	78	10 (13)		49 (63)		77	15 (19)	
HHV-6 DNA in CSF								
< 17 000 copies/ml	63	8 (13)	1.00	42 (67)	1.00	63	13 (20)	0.65
≥ 17 000 copies/ml	62	7 (11)		42 (68)		61	10 (16)	
Onset, days after HSCT								
< 23 Days	66	9 (14)	0.80	47 (71)	0.29	66	11 (17)	0.53
≥ 23 Days	78	9 (12)		48 (62)		78	17 (22)	
Conditioning regimen								
MAC	75	7 (9.3)	0.44	47 (63)	0.48	74	15 (20)	0.68
RIC	69	10 (14)		48 (70)		69	12 (17)	
Antiviral prophylaxis using active agents against HHV-6								
Yes	47	6 (13)	1.00	33 (70)	0.58	46	11 (24)	0.37
No	98	12 (12)		63 (64)		98	17 (17)	
Seizure at onset of encephalitis								
Yes	46	8 (17)	0.28	33 (72)	0.35	45	9 (20)	1.00
No	99	10 (10)		63 (64)		99	19 (19)	
Presence of GvHD, ES or PIR 0–8 days before onset of HHV-6 encephalitis								
Yes	82	12 (15)	0.45	55 (67)	0.86	81	15 (19)	0.83
No	63	6 (9.5)		41 (65)		63	13 (21)	
Use of foscarnet								
Yes	91	10 (11)	0.60	58 (64)	0.59	91	11 (12)	0.008
No	53	8 (15)		37 (70)		52	16 (31)	
Use of ganciclovir								
Yes	65	9 (14)	0.80	44 (68)	0.73	64	18 (28)	0.017
No	78	9 (12)		50 (64)		78	9 (12)	
Antiviral treatment using full dose of foscarnet (≥180 mg/kg/day) or ganciclovir (≥10 mg/kg/day)								
Yes	66	5 (7.6)	0.13	37 (56)	0.022	66	11 (17)	0.67
No	77	13 (17)		59 (75)		77	16 (21)	

Abbreviations: BMT = bone marrow transplantation; CBT = cord blood transplantation; CSF = cerebrospinal fluid; ES = engraftment syndrome; HHV-6 = human herpesvirus 6; HSCT = hematopoietic stem cell transplantation; MAC = myeloablative conditioning; PBSCT = peripheral blood stem cell transplantation; PIR = pre-engraftment immune reaction; RIC = reduced-intensity conditioning. ^aData on exact date of onset not available for 1 patient, resulting in data for a total of 144 patients.

We showed that full-dose antiviral therapy was associated with lower incidences of sequelae and death owing to HHV-6 encephalitis, and the use of foscarnet, but not ganciclovir, was associated with a low incidence of early death. Some of these findings are biologically plausible. Previous *in vitro* studies have shown that foscarnet offers better *in vitro* activity against HHV-6 than ganciclovir.^{31,32} Antiviral CSF concentrations reach the therapeutic range in patients receiving full-dose foscarnet;³³ a case report suggested that this may not be the case in patients receiving full-dose ganciclovir.³⁴ Although the reasons why ganciclovir is associated with an increased risk of early death are

unknown, myelosuppression owing to ganciclovir might increase the risk of infectious diseases. Combination therapy with foscarnet and ganciclovir seemed to be most effective in our study, but its evaluation is difficult because only 10 patients received the combination therapy and the doses of foscarnet and ganciclovir varied.

Several limitations must be considered in the interpretation of our results. First, optimal evaluation of CNS dysfunction and monitoring of HHV-6 reactivation after transplantation could not be performed in this retrospective study. Although we used strict criteria for the presence of HHV-6 DNA to define HHV-6

encephalitis, many recipients who actually develop HHV-6 encephalitis may not receive CSF examination in clinical practice. A lack of opportunities for optimal evaluation of CNS dysfunction and stringent diagnostic criteria will cause underestimation of the incidence of HHV-6 encephalitis. Second, we could not rule out the possibility that positive HHV-6 DNA in the CSF was due to chromosomally integrated HHV-6 (ciHHV-6).³⁵ Population-based studies have estimated the prevalence of ciHHV-6 to be ~1% in the general population.^{36,37} In our study population ($n=6593$), a total of 412 patients showed CNS dysfunction, based on the TRUMP database. Assuming that all patients with CNS dysfunction were tested for the presence of HHV-6 in the CSF and that incidence of ciHHV-6 in both donors and recipients was 1%, the positive results for HHV-6 DNA in CSF from about 8 patients with CNS dysfunction were thought to be owing to ciHHV-6. Therefore, we consider that there is some bias owing to the participation of patients with ciHHV-6, leading to misdiagnosis. Third, results of the analysis of antiviral effects require careful interpretation as data on the presence of organ dysfunction at the time of developing HHV-6 encephalitis were not collected in this study. There may be some patients who did not receive full-dose antiviral therapy owing to renal dysfunction or poor general status. Prospective studies of antiviral therapy with pre-specific dose and schedules are warranted to demonstrate the effect of antivirals for the treatment of HHV-6 encephalitis.

In summary, we found risk factors for HHV-6 encephalitis, including male sex, type of transplanted cells and GvHD prophylaxis by CI alone for CBT recipients. The incidence of HHV-6 encephalitis was unexpectedly high in patients receiving HSCT from an HLA-mismatched unrelated donor. The prognosis of HHV-6 encephalitis is poor, but we showed an association between appropriate antiviral treatment and better outcomes. The results of this large-scale study serve to improve understanding regarding the clinical impact of HHV-6 encephalitis in allo-HSCT and serve as a benchmark for future trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MO designed the study, analyzed the data and wrote the manuscript. KO, TI and KT designed the study, analyzed the data and revised the manuscript. HK, TK, YU, TM, HH, HO, TE, TU and TM collected data and revised the manuscript. TI revised the manuscript and was responsible for data management for the JDCHCT. YA managed the registry database and revised the manuscript. TF designed the study, revised the manuscript and was responsible for the Complication Working Group.

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