Risk Factors for HHV-6 Encephalitis

UCBT and HCT from human leukocyte antigen (HLA)-mismatched donors were significantly associated with development of HHV-6 encephalitis by univariate analysis. Figure 2*C* shows the cumulative incidence curve of HHV-6 encephalitis in recipients of UCBT and non-UCBT. Multivariate analysis for the risk factors of HHV-6 encephalitis was not performed, because the number of patients who developed HHV-6 encephalitis was small and HLA mismatch is associated with UCBT. HLA allelic mismatch was not significantly associated with the incidence of HHV-6 encephalitis among recipients of UCBT ($P = .50$) and recipients of non-UCBT ($P = .12$).

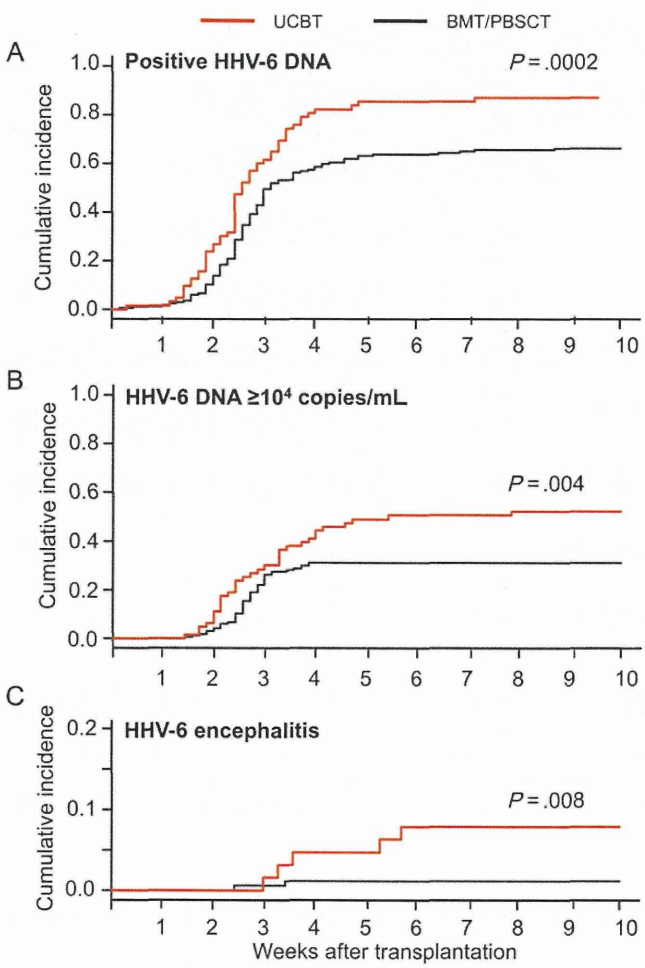

Figure 2. Cumulative incidence curves for patients who received umbilical cord blood transplantation (red line) versus bone marrow transplantation or peripheral blood stem cell transplantation (black line). *A*, First detection of positive human herpesvirus 6 (HHV-6) DNA. *B*, First detection of plasma HHV-6 DNA $\geq 10^4$ copies/mL. *C*, HHV-6 encephalitis. Abbreviations: BMT/PBSCT, bone marrow transplantation or peripheral blood stem cell transplantation; HHV-6, human herpesvirus 6; UCBT, umbilical cord blood transplantation.

Kinetics of Plasma HHV-6 DNA in Patients Who Developed HHV-6 Encephalitis

Figure 3 shows the kinetics of HHV-6 DNA in plasma and CSF in patients who developed HHV-6 encephalitis. CNS dysfunction developed concomitant to peak HHV-6 DNA. Median maximum plasma HHV-6 load among patients who developed HHV-6 encephalitis was 121 436 copies/mL (range, 21 656–433 639 copies/mL). Median duration between first detection of positive plasma HHV-6 DNA and development of CNS dysfunction was 4 days (range, 1–14 days).

Association Between Plasma HHV-6 DNA Load and HHV-6 Encephalitis

Associations between maximum plasma HHV-6 DNA load in each of the 230 patients and development of HHV-6 encephalitis were evaluated. Incidences of HHV-6 encephalitis were 0% and 8.1% among patients with peak plasma HHV-6 DNA $<10^4$ copies/mL (n = 144) and $\geq 10^4$ copies/mL (n = 86), respectively ($P = .0009$). Among patients displaying $\geq 10^5$ HHV-6 DNA copies/mL plasma (n = 25), HHV-6 encephalitis occurred in 4 patients, and the incidence was 16%. For identifying HHV-6 encephalitis, plasma HHV-6 DNA $\geq 10^4$ copies/mL offered 100% sensitivity and 64.6% specificity, and plasma HHV-6 DNA $\geq 10^5$ copies/mL offered 57.1% sensitivity and 90.6% specificity.

DISCUSSION

This large-scale, prospective, multicenter study evaluated the epidemiology and morbidity of HHV-6 reactivation and HHV-6 encephalitis and analyzed these associations. Although HHV-6 reactivation was common after allogeneic HCT, HHV-6 encephalitis sporadically developed at the time of HHV-6

Table 3. Multivariate Analysis of Factors Affecting Human Herpesvirus 6 Reactivation

Variable	Unfavorable Factors	HR (95% CI)	*P*
Positive plasma HHV-6 DNA			
Conditioning regimen	MAC	1.5 (1.1–2.0)	.01
Type of transplanted cells	CB	1.8 (1.3–2.5)	.0003
HHV-6 DNA ≥10 000 copies/mL			
Conditioning regimen	MAC	1.9 (1.2–2.9)	.004
Sex	Male	1.6 (1.0–2.5)	.04
Type of transplanted cells	CB	2.0 (1.3–3.0)	.003

Abbreviations: CB, cord blood; CI, confidence interval; HHV-6, human herpesvirus 6; HR, hazard ratio; MAC, myeloablative conditioning.

Table 4. Clinical Features of Patients Who Developed Central Nervous System Dysfunction

Patient	Age (Years)/Sex	Stem Cell Source	Days to CNS Dysfunction[a]	Plasma HHV-6 DNA (copies/mL)[b]	Initial Symptoms	Etiology	Possible Cause[c]	CSF Findings
1	22/F	U	28	692 885	Visual disturbance	PRES		ND
2	63/M	R	15	435 927	Convulsion	Undetermined	Liver disease	ND
3	26/M	R	17	433 639	Memory loss	HHV-6 encephalitis		HHV-6 DNA 48 906 copies/mL
4	59/M	U	24	415 308	Consciousness loss	HHV-6 encephalitis		HHV-6 DNA 20 000 copies/mL
5	34/F	C	21	129 130	Consciousness loss	HHV-6 encephalitis		HHV-6 DNA 28 700 copies/mL
6	54/F	C	26	124 310	Consciousness loss	Undetermined		ND
7	66/F	U	26	123 007	Consciousness loss	Undetermined		ND
8	52/F	C	23	121 436	Memory loss	HHV-6 encephalitis		HHV-6 DNA 52 754 copies/mL
9	29/F	C	25	39 601	Consciousness loss	HHV-6 encephalitis		HHV-6 DNA 16 128 copies/mL
10	60/F	U	22	33 050	Memory loss	Undetermined	Opioid use	ND
11	56/M	C	40	28 386	Dysesthesia	HHV-6 encephalitis		HHV-6 DNA 11 647 copies/mL
12	64/M	C	37	21 656	Memory loss	HHV-6 encephalitis		HHV-6 DNA 117 530 copies/mL
13	51/F	R	20	11 387	Consciousness loss	Undetermined		ND
14	28/M	U	45	1042	Convulsion	Undetermined	TMA	ND
15	34/M	U	20	663	Delirium	Drug (due to opioid use)		ND
16	56/M	R	41	531	Memory loss	Undetermined		Negative HHV-6 DNA
17	35/F	R	8	176	Headache	PRES		ND
18	34/M	R	19	123	Tremor	Drug (drug-induced parkinsonism)		ND
19	23/F	U	53	<50	NI	Cerebral bleeding		ND
20	56/M	R	52	<50	NI	Cerebral bleeding		NI
21	48/M	U	0	<50	Headache,	Cerebral bleeding		ND
22	25/M	R	41	<50	Convulsion	PRES		ND
23	69/M	R	10	<50	NI	Uremic encephalopathy		ND
24	62/F	U	20	<50	Weakness	CNS relapse		Leukemia cells
25	63/M	U	9	<50	Consciousness loss	Drug (due to opioid use)		ND
26	65/F	U	68	<50	Consciousness loss	Undetermined		Negative HHV-6 DNA
27	60/M	U	17	<50	Consciousness loss	Undetermined		Negative HHV-6 DNA
28	55/F	U	27	<50	Sensory disturbance, weakness	Undetermined		Negative HHV-6 DNA
29	56/F	U	15	<50	Disorientation	Undetermined		Negative HHV-6 DNA
30	61/F	U	15	<50	Confusion	Undetermined		Negative HHV-6 DNA
31	39/M	U	11	<50	Delirium	Undetermined	Opioid use	ND
32	64/F	U	18	<50	Consciousness loss	Undetermined		Negative HHV-6 DNA
33	17/M	R	10	<50	Delirium	Undetermined		ND

Abbreviations: C, cord blood; CNS, central nervous system; CSF, cerebrospinal fluid; HHV-6, human herpesvirus 6; ND, not done; NI, not informative; PRES, posterior reversible encephalopathy syndrome; R, bone marrow of peripheral blood stem cell from related donor; TMA, thrombotic microangiopathy; U, bone marrow from unrelated donor.

[a] No. of days between transplantation and onset of CNS dysfunction.

[b] Peak HHV-6 DNA load in plasma between 7 days before and 7 days after onset of CNS dysfunction.

[c] Interpretation of physician.

Table 5. Demographics and Clinical Presentation of Patients Who Developed Human Herpesvirus 6 Encephalitis

			Brain MRI		CSF				
Patient[a]	Antivirals Used to Prevent HHV-6 Encephalitis	Main Neurological Symptoms	Latent Period (days)[b]	Findings	Latent Period (days)[c]	Protein/ glucose (mg/dL)	Cell Count (/μL)	Virus Study Other Than HHV-6	Culture
3	No	Memory loss	0	Normal	2	30/61	0	ND	Negative
		Disorientation							
4	No	Consciousness loss	0	Normal	1	70/80	4	ND	Negative
		Disorientation							
5	No	Consciousness loss	0	Normal	1	29/54	2	ND	Negative
		Involuntary labial movement							
8	No	Memory loss	1	T2 hyperintensity in medial temporal lobes	1	57/86	4	ND	Negative
		Disorientation							
9	Foscarnet[d]	Consciousness loss	1	Normal	2	128/155	2	Negative PCR for HSV, VZV, and CMV	Negative
		Abducens nerve palsy							
		Seizure							
11	Ganciclovir	Dysesthesia		ND	0	45/78	0	Negative PCR for HSV, VZV, and CMV	Negative
		Hyperalgesia							
12	Foscarnet[e]	Memory loss	3	T2 hyperintensity in bilateral hippocampal area	2	79/105	2	Negative PCR for HSV, VZV, and CMV	Negative
		Seizure							

Patient	Treatment	Survival Post-encephalitis, Days (for Alive Patients, Days From Onset of Encephalitis to Date Last Seen Alive)	Cause of Death	Sequelae of HHV-6 Encephalitis
3	Foscarnet	Alive (567)		None
4	Ganciclovir	Alive (724)		Memory disturbance
5	Foscarnet	282	Leukemia	None
8	Foscarnet and ganciclovir	Alive (375)		None
9	Foscarnet and ganciclovir	34	Lymphoma	None
11	Ganciclovir	506	Leukemia	None
12	Foscarnet and ganciclovir	Alive (617)		Memory disturbance

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; HHV-6, human herpesvirus 6; HSV, herpes simplex virus; MRI, magnetic resonance imaging; ND, not done. PCR, polymerase chain reaction; VZV, varicella zoster virus.

[a] These numbers correspond to patient number listed in Table 4.

[b] Days from onset of neurological symptoms to scan.

[c] Days from onset of neurological symptoms to puncture.

[d] Intermitted 3 days before development of HHV-6 encephalitis due to renal toxicity.

[e] Intermitted 15 days before development of HHV-6 encephalitis due to renal toxicity.

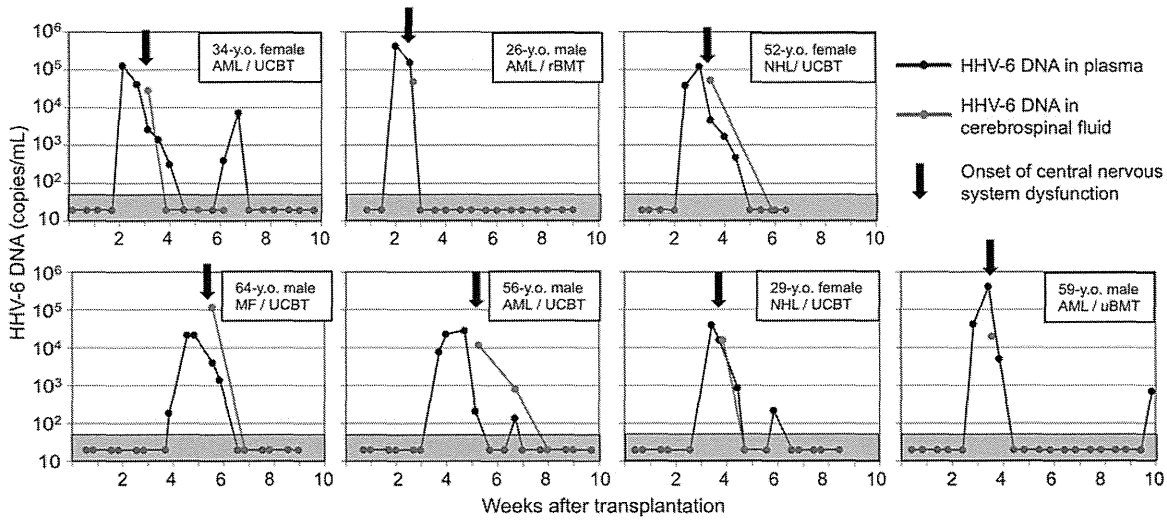

Figure 3. Kinetics of human herpesvirus 6 (HHV-6) DNA in 7 patients who developed HHV-6 encephalitis. Abbreviations: AML, acute myeloid leukemia; HHV-6, human herpesvirus 6; MF, myelofibrosis; NHL, non-Hodgkin lymphoma; rBMT, related bone marrow transplantation; uBMT, unrelated bone marrow transplantation; UCBT, umbilical cord blood transplantation.

reactivation if the reactivation level was high. Our data provide evidence of the effect of HHV-6 reactivation on the development of HHV-6 encephalitis after allogeneic HCT.

The significance of HHV-6 reactivation detected by blood HHV-6 DNA for the development of HHV-6 encephalitis has not been sufficiently determined. Some epidemiological studies have not found a significant relationship between HHV-6 reactivation and CNS diseases [23, 24]. A recent, well-designed, large prospective study [19] identified HHV-6 reactivation as a predictor of CNS dysfunction. However, the etiology of CNS dysfunction was not sufficiently determined because most patients did not undergo CSF examination. The present study required the detection of HHV-6 DNA in CSF for diagnosis of HHV-6 encephalitis and showed that higher levels of plasma HHV-6 DNA were associated with increased risk of developing HHV-6 encephalitis.

A high incidence of HHV-6 encephalitis has continuously been reported from several Japanese groups [3, 8, 12, 14, 15, 17]. However, epidemiological evidence of incidence and risk factors of HHV-6 encephalitis is limited, due to a less-stringent definition of HHV-6 encephalitis, retrospective design, and small study population. In the present prospective study, 7 of 230 patients developed HHV-6 encephalitis. Selection of cord blood as a stem cell source represents a significant risk factor for the development of HHV-6 encephalitis. The high incidence of HHV-6 encephalitis in patients undergoing UCBT is likely associated with the higher frequency and degree of HHV-6 reactivation.

Two recent studies have also shown higher incidences of HHV-6 encephalitis after UCBT. In a single-center retrospective study, the incidence of HHV-6 encephalitis was 9.9% after UCBT and 0.7% after adult-donor HCT [13]. In a literature review, incidence of HHV-6 encephalitis was 8.3% after UCBT and 0.5% after adult-donor HCT [25]. Prospective evaluation in this study also showed a similar incidence. The high incidence of HHV-6 encephalitis in UCBT recipients may be associated with the amount and function of T cells, as cord blood transplants contain only a small number of T cells that are immunologically immature in the absence of HHV-6-specific T cells.

In patients who developed HHV-6 encephalitis, median duration between first detection of plasma HHV-6 DNA and development of CNS dysfunction was only 4 days. This indicates that monitoring plasma HHV-6 DNA is not useful for predicting the development of HHV-6 encephalitis. In patients showing distinctive CNS symptoms, however, high levels of plasma HHV-6 DNA are suggestive of HHV-6 encephalitis and may allow for earlier initiation of treatment. In fact, all patients who developed HHV-6 encephalitis in this study were able to receive antivirals against HHV-6 early based on the plasma HHV-6 DNA results. No patients with HHV-6 encephalitis died as a direct result of the encephalitis, and 5 of the 7 patients did not retain neuropsychological disorders.

Among patients who developed CNS dysfunction concomitant with high-plasma HHV-6 DNA levels, CSF was obtained from 7 of 13 patients. HHV-6 DNA was demonstrated in all 7 patients. In another 6 patients for whom CSF was not obtained, etiologies of 5 patients were not determined. These proportions suggest that CNS dysfunction in some of these patients may also be caused by HHV-6. Actually, lumbar puncture is sometimes difficult to perform for patients who developed CNS dysfunction in the early phase of transplantation due to severe

thrombocytopenia or experienced rapid exacerbation of general condition. One practical management for such patients may be that HHV-6 encephalitis should be considered as a cause of CNS dysfunction if blood HHV-6 DNA is high at the time of developing CNS dysfunction.

Several limitations must be considered in the interpretation of the present results. One is the diagnosis of CNS dysfunction. This study recommended the demonstration of HHV-6 DNA as a criterion for diagnosing HHV-6 encephalitis. CSF was not obtained from 8 of the 15 patients with unknown etiology of CNS dysfunction. This raises the possibility of underestimating the frequency of HHV-6 encephalitis. Another limitation is that our PCR system cannot distinguish between HHV-6A and HHV-6B. Currently, the methods for real-time PCR to evaluate HHV-6 DNA loads have not been standardized [26]. We used one of the recommended PCR assays that provided results closest to the expected value [26], although the assay detects both HHV-6A and HHV-6B. We have previously performed DNA sequencing of amplified viral DNA in samples containing high-level HHV-6 DNA to distinguish between HHV-6A and HHV-6B. As a result, more than 99% of samples contained HHV-6B only. In this study, DNA sequencing to differentiate HHV-6A from HHV-6B was not performed to allow processing of a large quantity of specimens.

In conclusion, the present results demonstrate an association between high-level HHV-6 reactivation and development of HHV-6 encephalitis. UCBT is a significant risk factor for both HHV-6 reactivation and HHV-6 encephalitis. Monitoring of plasma HHV-6 DNA is useful to identify HHV-6 encephalitis in patients with CNS dysfunction. Based on the results, we propose a clinical trial of prophylactic antiviral intervention in UCBT recipients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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