Human herpesvirus-6 in hematopoietic cell transplant recipients

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Human herpesvirus (HHV)-6 reactivation is common (30–50%) after allogeneic hematopoietic cell transplantation (allo-HCT), and is linked to various clinical manifestations. HHV-6 has been recognized as an important pathogen that can cause encephalitis after allo-HCT. HHV-6 encephalitis typically develops around 2–6 weeks after allo-HCT, and is characterized by short-term memory loss, loss of consciousness, and seizures. Magnetic resonance imaging typically shows bilateral signal abnormalities in the limbic system. Umbilical cord blood transplantation is associated with increased risk of HHV-6 encephalitis. While antiviral therapy using ganciclovir or foscarnet is recommended as a first-line therapy for HHV-6 encephalitis, mortality rates attributable to this pathology remain high. Even among survivors, many patients display cognitive sequelae. Establishment of optimal strategies is urgently needed to prevent HHV-6 encephalitis. Besides encephalitis, associations between HHV-6 and various important post-transplant complications have been reported, including pneumonitis, gastroenterocolitis, hepatitis, bone marrow suppression/graft failure, graft-versus-host disease, and cytomegalovirus infection. Further investigations are needed to determine the roles of HHV-6 in these manifestations.

Introduction

Human herpesvirus (HHV)-6 is a member of the Roseolovirus genus in the Betaherpesvirinae subfamily of human herpesviruses. Two variants of HHV-6 (A and B) have been identified. The clinical significance of HHV-6A remains unclear. HHV-6B is the causative agent of exanthema subitum. HHV-6 is transmitted through saliva and infects virtually all children within the first few years of life. Like the other herpesviruses, HHV-6 is capable of persisting in the host after primary infection, and can reactivate from latency under conditions of immunosuppression. Most HHV-6 infections in immunosuppressed patients are due to reactivation of HHV-6B.

The accumulated data indicate that HHV-6 represents an emerging and potentially life-threatening pathogen for recipients of allogeneic hematopoietic cell transplantation (allo-HCT). Although the full spectrum of the clinical syndrome for HHV-6-associated complications remains controversial, many recent studies have indicated causal associations between HHV-6 and central nervous system (CNS) disease after allo-HCT. This article reviews findings regarding the clinical syndrome accompanying HHV-6 reactivation, with a particular focus on HHV-6 encephalitis, after allo-HCT.

Diagnosis of HHV-6 reactivation

Serological determination of either immunoglobulin (Ig) G or IgM has no significance for the diagnosis of active HHV-6 infection or disease in HCT recipients. Several methods have been used to diagnosis HHV-6 reactivation, including: virus isolation; PCR assay; and antigenemia assay. Currently, direct detection of HHV-6 DNA using PCR
is the most commonly used method for detecting HHV-6 reactivation. Viral DNA loads provide important information about the significance of the reactivation, and thus should be quantified. The European Conference on Infections in Leukemia (ECIL) recommends quantitative PCR for HHV-6A and -6B DNA in peripheral blood (whole blood or plasma/serum) for the diagnosis of HHV-6 infection. The kinetics of HHV-6 DNA differ substantially between whole blood and plasma, and positive results for HHV-6 DNA in whole blood can persist for a relatively long time (sometimes, more than 60 days) after the disappearance of plasma HHV-6 DNA. The persistence of positive result in whole blood may reflect latent infection in leukocytes. Although approaches using whole blood may still offer some advantages, detection of HHV-6 DNA in plasma or serum correlates well with active replication and may be more directly interpretable.

Careful interpretation is needed for results of HHV-6 DNA in peripheral blood. First, the unique phenomenon of HHV-6 chromosomal integration may account for abnormally high levels of HHV-6 DNA. This condition has been termed "chromosomally integrated HHV-6" (ciHHV-6) or "inherited HHV-6". The complete HHV-6 genome becomes integrated into the host germ in an individual with ciHHV-6 and is vertically transmitted in a Mendelian manner. The condition is found in less than 1% of controls in the United States and United Kingdom. Although there are suggestions that ciHHV-6 can be induced to a state of lytic viral replication in vitro, little is known about the pathogenic role of ciHHV-6 in allo-HCT recipients. The possibility of ciHHV-6 should be considered when high viral DNA loads in peripheral blood persist despite an absence of clinical symptoms of HHV-6-associated disease. In situations where a donor has ciHHV-6, HHV-6 DNA load in the recipient will increase with engraftment. The most practical method to confirm ciHHV-6 status is quantitative PCR using whole blood. If a donor has ciHHV-6, the recipient will show one or more copies per white blood cell after engraftment, corresponding to $5^{> \cdot 5 \log_{10}}$ copies/ml of whole blood. Second, as shown later, HHV-6 reactivation is common after allo-HCT, and most reactivations are asymptomatic. It is important to consider multiple potential causes, because the presence of HHV-6 DNA in peripheral blood does not necessarily prove causality. Third, the duration of positive HHV-6 DNA in plasma is short after allo-HCT. If sampling time is delayed for several days after the onset of HHV-6-associated complications, the HHV-6 DNA load may already be in decline. Fourth, methods of real-time PCR to quantify HHV-6 DNA loads have not been standardized. Results for HHV-6 viral loads in the same specimen thus now differ considerably among several laboratories.

**HHV-6 reactivation after allo-HCT**

Overall, HHV-6 has been shown to reactivate in 30–50% of patients undergoing allo-HCT. HHV-6 appears most frequently around 2–4 weeks after allo-HCT, and 0–9 days after neutrophil engraftment. HHV-6 can reactivate to high levels within a week, but the duration of HHV-6 reactivation is usually short. Most HHV-6 infections are due to reactivation of HHV-6B, with type A isolated in 2–3% by HHV-6 subtyping. Figure 1 shows the incidence of positive plasma HHV-6 DNA and the associated kinetics in 178 patients who underwent allo-HCT in the Oita Group (Oita University Hospital and Oita Prefectural Hospital). As previously reported, HHV-6 reactivation was typically detected 2–4 weeks after transplantation.

Several risk factors are associated with developing HHV-6 reactivation, including: younger age; underlying diseases; sex mismatch; HLA mismatch; steroid treatment; unrelated transplants; umbilical cord blood transplantation (UCBT); and low anti-HHV-6 IgG titer before transplantation. Among these, steroid administration and UCBT are also associated with higher-level HHV-6 reactivation.

**Pathogenicity of HHV-6**

The host tissue range of HHV-6 in vivo includes peripheral blood mononuclear cells, salivary glands, brain tissue, liver cells, lymph nodes, and endothelial cells. Candidate sites for latency are salivary glands, brain tissue, monocytes, and early bone marrow progenitor cells. These broad tissue ranges suggest potential pathogenicity for various posttransplant complications.

HHV-6 is recognized as an important virus that modulates immune responses. HHV-6 shows a predominant tropism for CD4-positive T cells, exhibiting cytopathic effects on these cells. HHV-6 inhibits the lymphoproliferative responses of monocytes. HHV-6 infection is associated with selective suppression of interleukin 12 production, an important cytokine for antiviral immune response. As a result, this suppression drives the immune balance toward a
Th2 response. HHV-6 impairs intracellular signaling through Toll-like receptor. Disruption of antiviral immune response may represent a strategy by which viruses can evade the immune surveillance system, and this mechanism may facilitate immunosuppression in allo-HCT recipients. Unsurprisingly, then, HHV-6 reactivation is also associated with subsequent cytomegalovirus (CMV) reactivation.

In contrast to the in vitro evidence of HHV-6-induced immunosuppressive effects, HHV-6 reactivation in allo-HCT may induce pro-inflammatory response. Recent large-scale studies have demonstrated associations between HHV-6 reactivation and subsequent graft-versus-host disease (GVHD). In those studies, the authors speculated that immune-mediated responses such as activation of alloreactive T cell or the pro-inflammatory or type I immune responses induced by HHV-6 reactivation play a role in the development of GVHD. Another report showed an association between HHV-6 reactivation and elevated serum cytokine levels. Immune-mediated responses to HHV-6 may promote the development of subsequent immunological complications.

HHV-6 is recognized as an important pathogen affecting the nervous system. The possible pathogenicity of HHV-6 in the development of encephalitis following allo-HCT is discussed in the “HHV-6 encephalitis” section.

**HHV-6-associated complications**

Many clinical observations have shown associations between incidence of various complications and HHV-6 reactivation in a subset of allo-HCT recipients. HHV-6 is now recognized as an important pathogen that causes encephalitis after allo-HCT. Whether many potential complications other than encephalitis represent causal relationships remains...
undermined and careful interpretation of disease associations is still required.

1. HHV-6 encephalitis

Currently, both terms “HHV-6 encephalitis” and “HHV-6 encephalopathy” are widely used. This may be because the pathogenicity of HHV-6 for CNS disease has not been elucidated in sufficient detail; some investigators may feel that using “encephalitis” requires evidence of tissue inflammation by HHV-6. In this article, “HHV-6 encephalitis” is used, because this term seems to be more commonly used. Characteristics of HHV-6 encephalitis following allo-HCT are summarized in Table 1.

**Clinical presentation:** Zerr performed a review of 48 allo-HCT recipients with HHV-6-associated encephalitis previously described in the literature. Onset of encephalitis began on a median of day 24. Symptoms were characterized by short-term memory loss, depressed consciousness, confusion, disorientation and clinical seizure. Magnetic resonance imaging (MRI) studies showed abnormal findings in 70% of patients, most commonly observed within the medial temporal lobes. Muta et al. reported a retrospective analysis of 23 patients with HHV-6 encephalitis in Japan. Encephalitis developed at a median of day 22 after allo-HCT. Symptoms included disturbance of consciousness (91%), loss of short-term memory (74%), and convulsions (70%). MRI showed abnormal findings within the hippocampus, or temporal lobes in all 18 patients who underwent examination. Results shown in both analyses were similar and therefore indicate characteristics of HHV-6 encephalitis. In summary, HHV-6 encephalitis typically develops around 3 weeks (2–6 weeks) after allo-HCT, the typical initial symptom is short-term memory loss with subsequent progression to loss of consciousness, confusion, and seizure. MRI typically showed bilateral signal abnormalities in the limbic system. Figure 2 shows MRI findings of the brain in a patient who developed HHV-6 encephalitis in our institute. Focal abnormalities in limbic system have been identified.

### Table 1. Summary of HHV-6 encephalitis following allo-HCT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Findings and comments</th>
<th>Supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Onset</td>
<td>Around 3 weeks (2-6 weeks) after transplantation</td>
<td>Generally within several days after engraftment. In UCBT recipients, HHV-6 encephalitis may tend to develop before engraftment</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Short-term memory loss, confusion, convulsions, and loss of consciousness</td>
<td>Memory loss is a typical initial symptom</td>
</tr>
<tr>
<td>Brain MRI</td>
<td>Abnormal findings in limbic system</td>
<td>Frequently bilateral, T2-weighted FLAIR and DWI are useful for early detection</td>
</tr>
<tr>
<td>CSF</td>
<td>Positive HHV-6 DNA</td>
<td>Minority of patients had obvious pleocytosis</td>
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<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. UCBT</td>
<td></td>
<td>UCBT is strongly associated with increased risk of HHV-6 encephalitis</td>
</tr>
<tr>
<td>2. HCT from unrelated donor or HLA-mismatched donor</td>
<td></td>
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<tr>
<td>3. Immune reaction such as PIR, engraftment syndrome, and GVHD</td>
<td></td>
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<td>4. Steroid treatment</td>
<td></td>
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<tr>
<td>5. Two or more allo-HCT</td>
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<td>6. Use of alemtuzumab</td>
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<td><strong>Secondary complications</strong></td>
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<tr>
<td>Syndrome of inappropriate secretion of ADH</td>
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<td></td>
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<tr>
<td><strong>Diagnosis</strong></td>
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<tr>
<td>Presence of neurological symptoms, positive HHV-6 DNA in the CSF, and the absence of other identified etiologies of CNS dysfunction</td>
<td>Differential diagnosis includes encephalitis due to other pathogens, PRES, TMA, and adverse drug effects</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>First-line therapy: PFA or GCV</td>
<td>Early initiation of antiviral will improve outcomes</td>
</tr>
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</table>

UCBT, umbilical cord blood transplantation; MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery; DWI, diffusion-weighted imaging; CSF, cerebrospinal fluid; PIR, pre-engraftment immune reaction; CNS, central nervous system; PRES, posterior reversible encephalopathy syndrome; TMA, thrombotic microangiopathy; PFA, foscarnet; GCV, ganciclovir; CDV, cidofovir
HHV-6 encephalitis typically develops several days after bone marrow engraftment. In UCBT recipients, however, HHV-6 encephalitis may tend to develop before engraftment. A single center study analyzing 1344 allo-HCT recipients (19 of whom developed HHV-6 encephalitis) showed onset of HHV-6 encephalitis symptoms before engraftment in 7 of 10 patients who underwent UCBT, compared to only 1 of 9 adult donor recipients.

**Incidence and risk factors:** Several studies that performed monitoring of HHV-6 viral load have demonstrated associations between systemic HHV-6 reactivation and CNS dysfunction. Incidence ranged from 3.6% to 8%. Retrospective observations conducted by several Japanese allo-HCT units have shown a high incidence of HHV-6-associated CNS dysfunction in each institute (4–11.4% of allo-HCT recipients).

The incidence of HHV-6 encephalitis is strongly dependent on the selection of stem cell source. A review of allo-HCT recipients with HHV-6 encephalitis showed that approximately 85% of these cases developed in recipients of unrelated or HLA mismatched-related allo-HCT. In particular, UCBT has been recognized as a strong risk factor for HHV-6 encephalitis. A single center retrospective study in Japan analyzing 228 allo-HCT recipients showed a significant correlation between HHV-6 encephalitis/myelitis and UCBT (15.7% in UCBT; 2.8% in bone marrow or peripheral blood stem cells, P = 0.005). In a single-center study analyzing 1344 patients undergoing allo-HCT, the incidence of HHV-6 encephalitis was 9.9% after UCBT and 0.7% after adult-donor HCT (adjusted hazard ratio [aHR], 20.0; P < 0.001).

Post-transplant immune reactions, such as pre-engraftment immune reaction (PIR), engraftment syndrome, and GVHD,
may be associated with the development of HHV-6 encephalitis.\textsuperscript{52,53,55} Mori et al. reported that all of 13 patients with HHV-6 encephalitis/myelitis presented with PIR prior to the onset of encephalitis.\textsuperscript{55} Another study reported among patients with high-level HHV-6 reactivation, GVHD or engraftment syndrome was a significant risk factor for subsequent development of HHV-6 encephalitis.\textsuperscript{52} That study showed elevated serum interleukin-6 concentration preceding the development of HHV-6 encephalitis.\textsuperscript{53} A single-center study analyzing 1344 patients\textsuperscript{52} demonstrated time-dependent acute GVHD grade II to IV as a significant risk factor (aHR, 7.5; \( P < 0.001 \)) for HHV-6 encephalitis. These findings suggest that post-transplant immune reactions in the early phase may play a role in the development of HHV-6 encephalitis.

Several other factors may be associated with development of HHV-6 encephalitis, including steroid treatment,\textsuperscript{52} 2 or more allo-HCTs using cord blood,\textsuperscript{55} and use of alemtuzumab.\textsuperscript{59} The roles of these factors remain undetermined.

**Pathogenesis:** How HHV-6 contributes to CNS disease pathology is not well known, and the processes may include direct or immune-mediated destruction of the CNS. Several autopsy studies of patients who died with HHV-6 encephalitis have demonstrated the involvement of HHV-6 in the brain.\textsuperscript{6,60-63} Regions of disease were the frontal lobe,\textsuperscript{60} parietal lobe,\textsuperscript{62} and hippocampus.\textsuperscript{60-63} On microscopic examination, diffuse edema, accumulation of inflammatory cells in the tissue,\textsuperscript{6} necrosis, loss of myelin,\textsuperscript{60} loss of neurons,\textsuperscript{60,61,63} reactive astrocytosis,\textsuperscript{61,63} and clusters of reactive microglia\textsuperscript{61} have been observed. Presence of HHV-6 in the brain was demonstrated by PCR\textsuperscript{6,60,62} or immunohistochemical examination.\textsuperscript{60,61} The hippocampus demonstrated strong reactivity against HHV-6,\textsuperscript{60-62} and HHV-6 displayed tropism for astrocytes in hippocampus.\textsuperscript{61,62} These pathological findings are consistent with the clinical presentations of HHV-6 encephalitis, in which the typical initial symptom is short-term memory loss. Localization of HHV-6 to a pathologically damaged region suggests that HHV-6 play a central role in the development of HHV-6 encephalitis. An in vitro study has shown HHV-6-induced dysregulation of glutamate uptake and expression of glutamate transporters in HHV-6-infected astrocytes,\textsuperscript{64} and this mechanism may be associated with seizure development.

Nevertheless, several opinions have been put forward regarding pathogenic mechanisms other than direct destruction by HHV-6 for the development of HHV-6 encephalitis. MRI of patients with HHV-6 encephalitis commonly shows bilaterally symmetrical findings in the region of the limbic system (Fig. 2). Such findings suggest the existence of systemic processes rather than local infection.\textsuperscript{51} HHV-6 limbic encephalitis bears a clinical resemblance to paraneoplastic limbic encephalitis,\textsuperscript{63} a sub acute illness associated with paraneoplastic autoantibodies such as anti-Hu.\textsuperscript{65} Not only high-level HHV-6 reactivation, but also a hypercytokinemic state preceding HHV-6 reactivation is associated with progression to HHV-6 encephalitis.\textsuperscript{53} Furthermore, HHV-6-negative post-transplant acute limbic encephalitis has been reported.\textsuperscript{63} Such findings suggest the existence of multiple pathogenic mechanisms, including immune attack, underlying the CNS manifestations of HHV-6 encephalitis.\textsuperscript{51} Further exploration of the pathogenesis of HHV-6 encephalitis is needed.

**Diagnosis:** In general, HHV-6 encephalitis is defined as: the presence of neurological symptoms; positive PCR results for HHV-6 in the cerebrospinal fluid (CSF) (cHHV-6 should be excluded); and the absence of other identified etiologies of CNS dysfunction.\textsuperscript{66} Wang et al. detected HHV-6 DNA in CSF samples from 5 of 22 cases (23%) with CNS symptoms and in CSF specimens from 1 of 107 controls (0.9%).\textsuperscript{6} These data, the results of retrospective analysis,\textsuperscript{49} and many case reports\textsuperscript{25} are strongly suggestive of the clinical importance of demonstrating HHV-6 DNA in CSF for the diagnosis of HHV-6 encephalitis. CSF examination for HHV-6, as well as other pathogens, must be performed for patients displaying CNS symptoms, particularly featuring short-term memory loss, amnesia, and seizures. Many differential diagnoses must be considered for CNS dysfunction, including encephalitis due to other pathogens (other herpesviruses, JC virus, toxoplasma, bacteria or fungi), vascular diseases such as thrombotic microangiopathy, posterior reversible encephalopathy syndrome, adverse drug effects, and many others.\textsuperscript{67-70}

In patients showing distinctive CNS symptoms, high levels of plasma HHV-6 DNA are suggestive of HHV-6 encephalitis, and may allow earlier initiation of treatment.\textsuperscript{17,18,50,53} A study of weekly monitoring for plasma HHV-6 DNA in 111 HSCT recipients\textsuperscript{53} showed that incidences of HHV-6 encephalitis were 0%, 33.3%, and 66.7% in patients with peak HHV-6 DNA levels of \(< 10^4 \) copies/ml, \(10^4-10^5 \) copies/ml, and \(\geq 10^5 \) copies/ml, respectively. In a recent study including 185 patients tested for HHV-6 DNA, a plasma HHV-6 viral load \(\geq\)
10^5 copies/ml offered 71% sensitivity and 94% specificity for a diagnosis of HHV-6 encephalitis. These findings indicate the potential diagnostic performance of plasma HHV-6 DNA in identifying cases of HHV-6 encephalitis. However, careful interpretation is needed. The duration of positive HHV-6 DNA in plasma is short. Without routine monitoring, interpretation is needed. The duration of positive HHV-6 DNA in identifying cases of HHV-6 encephalitis. However, careful interpretation is needed. The duration of positive HHV-6 DNA in identifying cases of HHV-6 encephalitis. However, careful interpretation is needed.

HHV-6 encephalitis is typically accompanied by an MRI signature of hyperintense lesions on T2-weighted, fluid attenuation inversion recovery, and diffusion-weighted imaging of bilateral medial temporal lobes, primarily affecting the hippocampus and amygdala (Fig. 2). Such findings confirmed in HSCT recipients suggest the diagnosis of HHV-6 encephalitis.

**Treatment:** Antiviral drugs for HHV-6 are the same as those used against CMV. Ganciclovir (GCV), foscarnet (PFA), and cidofovir (CDV) have been shown to exert in vitro effects against HHV-6.

Currently, no antiviral agents have been approved by either the Ministry of Health, Labour and Welfare in Japan, or the Food and Drug Administration in the United States for the treatment of HHV-6 infection. However, many case reports and observations have strongly suggested that such drugs are active against HHV-6 encephalitis. The International Herpes Management Forum, ECIL, the Infectious Disease Society of America, and the Japan Society for Hematopoietic Cell Transplantation all recommend PFA or GCV as first-line therapies for HHV-6 encephalitis. CDV is not recommended as a first-line agent for use in the treatment of HHV-6 encephalitis because of drug-related renal toxicity and because the ability to penetrate the CNS has been poorly studied. The main adverse effects are bone marrow suppression for GCV and dose-dependent nephrotoxicity for PFA.

Clinical superiority of either GCV or PFA has not been established. However, a study of the in vitro efficacy of these antiviral drugs identified PFA as showing the highest selectivity as an anti-HHV-6 compound. Patients who developed HHV-6 encephalitis during GCV treatment (10 mg/kg/day) as therapy for CMV infection have been reported. HHV-6 encephalitis typically developed around the time of engraftment, and GCV therapy around this period may be problematic due to toxicity against the hematopoietic function. PFA may therefore be more suitable for the treatment of HHV-6 encephalitis.

Although the optimal schedule for PFA treatment against HHV-6 encephalitis has not been established, available data suggest that several approaches are important to rescue patients with this serious complication: starting PFA as soon as possible; not reducing the PFA dose except in response to renal dysfunction (if creatinine clearance is >0.4 mL/min/kg, 180 mg/kg/day of PFA should be administered); and not shortening the therapeutic course. Neurological symptoms usually progress rapidly in patients who develop HHV-6 encephalitis. A review of reported cases of patients with HHV-6 encephalitis showed that 25% of patients died within 1–4 weeks of diagnosis. Delayed initiation of antiviral therapy will give HHV-6 time to replicate in the brain. A clinical study of prophylactic low-dose PFA therapy against HHV-6 reactivation showed that low-dose PFA (50 mg/kg/day) appears insufficient, but PFA at 180 mg/kg/day is sufficient to suppress HHV-6 replication. Figure 3 shows the clinical course of patients who developed HHV-6 encephalitis. Plasma HHV-6 DNA levels continued increasing despite prophylactic administration of 50 mg/kg PFA in Cases 1 and 3. After treatment with 180 mg/kg of PFA, HHV-6 DNA in both plasma and CSF decreased rapidly. A detailed autopsy study of patients who died with HHV-6 encephalitis showed that viral DNA, RNA and antigen were still detectable in the brain despite HHV-6 DNA in CSF and serum having decreased to low levels before or at autopsy. Such findings suggest that the therapeutic course should not be shortened early just because HHV-6 DNA results in CSF become negative.

Supportive therapy represents an important basis of management. Patients with HHV-6 encephalitis should have access to a care unit equipped with mechanical ventilators. Seizures should be controlled with anticonvulsants such as phenytoin, phenobarbital, diazepam, or midazolam. Careful monitoring must be performed to maintain respiration, viral, and fluid balances. Aspiration pneumonia and inappropriate antidiuretic hormone secretion are sometimes observed as secondary complications in the course of HHV-6 encephalitis.

In general, steroid pulse therapy has been observed to be beneficial in a small number of immunocompetent patients with acute viral encephalitis and progressive disturbances of consciousness. Little is known, however, about the role of steroid therapy in post-transplant HHV-6 encephalitis.
doses of corticosteroids as an adjunct treatment for HHV-6 encephalitis may be associated with increased risk of subsequent infectious diseases.

**Prognosis:** Some of the available data regarding the outcomes of HHV-6 encephalitis are summarized in Table 2. Most patients have received GCV or PFA therapy for the treatment of HHV-6 encephalitis. Despite the appropriate treatment, success rates were not high. Not only encephalitis, but also various identified causes including GVHD, infectious diseases, or graft failure were associated with patient death.
HHV-6 may directly or indirectly affect the courses of these subsequent complications through immunomodulatory effects (see “pathogenicity of HHV-6” section).

Even among the survivors, many patients are left with neurological compromise. Sakai et al. reported that 4 of 5 surviving patients were unable to return to society because of neuropsychological disorders at the end of follow-up, including anterograde amnesia and seizures. In that report, prominent hippocampal atrophy in the late phase was demonstrated on MRI.

Prevention: ECIL does not recommend antiviral prophylaxis against HHV-6, due to the low risk of HHV-6 disease and the toxicity associated with available antiviral drugs. Actually, no available data have demonstrated any significant efficacy of prophylactic antiviral therapy to prevent HHV-6 encephalitis in allo-HCT recipients. However, HHV-6 encephalitis is not rare, particularly among UCBT recipients. In that report, prominent hippocampal atrophy in the late phase was demonstrated on MRI.

Preemptive approaches against asymptomatic CMV reactivation can successfully prevent the development of CMV pneumonia. Two small trials have provided data on the efficacy of plasma HHV-6 DNA-guided preemptive therapy to prevent HHV-6 encephalitis. However, these preemptive approaches cannot prevent the development of HHV-6 encephalitis due to the dynamic kinetics of plasma HHV-6 DNA.

A study of allo-HCT recipients with unrelated bone marrow or UCBT comparing consecutive cohorts of patients not receiving prophylaxis (cohort 1, n = 51) with those receiving PFA prophylaxis (cohort 2, n = 67) has recently been reported. The prophylactic regimen was 50 mg/kg/day of PFA for 10 days after engraftment. No significant reduction in high-level reactivation (HHV-6 DNA ≥ 10^4 copies/ml) by day 70 was seen in Cohort 2 (19.4%) compared with Cohort 1 (33.8%, P = 0.095). Breakthrough HHV-6 encephalitis occurred following PFA prophylaxis in 3 patients (Figure 3), and the incidence of HHV-6 encephalitis did not differ between Cohort 1 (9.9%) and Cohort 2 (4.5%, P = 0.24). The results of that study showed that 50 mg/kg/day of PFA for 10 days after engraftment did not effectively suppress HHV-6 reactivation and did not prevent all cases of HHV-6 encephalitis.

Clinical trials using an increased dose of prophylactic PFA, or using other compounds such as lipid esters of CDV, for a longer period in high-risk patients may be warranted. Alternative approaches based on the pathogenesis

### Table 2. Summary of reported outcomes for HHV-6 encephalitis

<table>
<thead>
<tr>
<th>References</th>
<th>Study type</th>
<th>N</th>
<th>Use of GCV/VGCV or PFA for HHV-6 encephalitis</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujimaki et al.</td>
<td>Multicenter, retrospective</td>
<td>11</td>
<td>11 (100%)</td>
<td>Full / good recovery: 0, Alive with cognitive sequelae: 0, Died due to problems other than encephalitis: 7 (64%), Died of encephalitis: 2 (18%), Details unknown: 2 (18%)</td>
</tr>
<tr>
<td>Zerr et al.</td>
<td>Review of case reports and case series</td>
<td>44</td>
<td>38 of 48 (79%)</td>
<td>Full / good recovery: 19 (43%), Alive with cognitive sequelae: 8 (18%), Died due to problems other than encephalitis: 6 (14%), Died of encephalitis: 11 (25%), Details unknown: 0</td>
</tr>
<tr>
<td>Seeley et al.</td>
<td>Single center, retrospective</td>
<td>9</td>
<td>9 (100%)</td>
<td>Full / good recovery: 1 (11%), Alive with cognitive sequelae: 3 (33%), Died due to problems other than encephalitis: 5 (56%), Died of encephalitis: 0, Details unknown: 0</td>
</tr>
<tr>
<td>Muta et al.</td>
<td>Nationwide surveillance</td>
<td>23</td>
<td>23 (100%)</td>
<td>Full / good recovery: 11 (48%), Alive with cognitive sequelae: 10 (43%), Died due to problems other than encephalitis: 0, Died of encephalitis: 2 (9%), Details unknown: 0</td>
</tr>
<tr>
<td>Mori et al.</td>
<td>Single center, retrospective</td>
<td>13</td>
<td>12 (92%)</td>
<td>Full / good recovery: 0, Alive with cognitive sequelae: 3 (23%), Died due to problems other than encephalitis: 9 (69%), Died of encephalitis: 1 (8%), Details unknown: 0</td>
</tr>
<tr>
<td>Sakai et al.</td>
<td>Multicenter, retrospective</td>
<td>8</td>
<td>8 (100%)</td>
<td>Full / good recovery: 1 (12%), Alive with cognitive sequelae: 4 (50%), Died due to problems other than encephalitis: 3 (38%), Died of encephalitis: 0, Details unknown: 0</td>
</tr>
<tr>
<td>Hill et al.</td>
<td>Single center, retrospective</td>
<td>19</td>
<td>18 (95%)</td>
<td>Full / good recovery: 4 (22%), Alive with cognitive sequelae: 9 (50%), Died due to problems other than encephalitis: 0, Died of encephalitis: 5 (28%), Details unknown: 0</td>
</tr>
</tbody>
</table>

GCV, ganciclovir; VGCV, valaganciclovir; PFA, foscarnet.

Seeley et al. Information on prognosis was obtained for 44 of the reported 48 patients.

Sakai et al. Data represent outcomes of encephalitis in the early period. Seven patients died due to various reasons after onset of HHV-6 encephalitis.
of HHV-6 encephalitis will be required in the next step.

2. CNS dysfunction other than encephalitis due to HHV-6 infection

A well-designed, prospective study of 315 allo-HCT recipients demonstrated an independent association between HHV-6 reactivation and subsequent delirium, as indicated by neuropsychiatric screening. HHV-6 reactivation was also independently associated with subsequent neurocognitive decline at approximately 3 months after allo-HCT, as measured by baseline and follow-up neurocognitive testing. In that study, no typical imaging abnormalities associated with HHV-6 encephalitis were confirmed in any of the 9 patients who underwent brain MRI. Those findings suggest that HHV-6 may lead to CNS dysfunction in the absence of encephalitis.

Myelitis may occur due to HHV-6 reactivation. Mori et al. reported two patients who developed HHV-6-associated myelitis after UCBT. A single-center study analyzing 228 allo-HCT recipients identified a total of 11 patients with HHV-6 myelitis; 4 patients presented only with dysesthesia and pruritus, and 7 patients showed both symptoms of encephalitis and myelitis. All 4 patients presenting only with myelitis symptoms were UCBT recipients. HHV-6 myelitis may be associated with UCBT.

3. Bone marrow suppression/graft failure

Many studies have shown that HHV-6 reactivation is associated with bone marrow suppression or delayed platelet engraftment. Ljungman et al. demonstrated that HHV-6 viral load is significantly correlated with time to platelet engraftment and number of platelet transfusions. Direct infection of bone marrow progenitors, suppressive effects of HHV-6 on granulocyte-macrophage progenitors, erythroid progenitors, and megakaryocyte progenitors may involve myelosuppressive activity. Vascular endothelial injury, or thrombotic microangiopathy due to HHV-6 reactivation may also be associated with delayed platelet recovery.

Suppressive effects of HHV-6 on bone marrow may lead to secondary graft failure. A few cases of patients who developed HHV-6-related secondary graft failure have been reported. A single-center study showed that among 5 patients who underwent UCBT and died of HHV-6...
encephalitis, four never achieved engraftment. HHV-6 may be considered as a differential diagnosis for secondary graft failure after allo-HCT.

4. Lung disease

HHV-6 may be a potential cause of unexplained interstitial pneumonia after allo-HCT. In patients who developed interstitial pneumonia after allo-HCT, HHV-6 infection of the lung was demonstrated by immunohistochemical staining on lung tissue. PCR study of lung biopsy specimens or PCR study of bronchoalveolar lavage samples reported the computed tomography (CT) findings of HHV-6 pneumonitis: beginning as perihilar and bilateral septal thickening, reticulation, and ground-glass opacity and extending from both central areas, turning into a crazy-paving pattern of infiltration followed by lung parenchymal consolidation. Figure 4 shows lung CT from a patient who developed HHV-6 pneumonitis in our institute. HHV-6 DNA load in bronchoalveolar lavage fluid was 6.8 \times 10^6 copies/ml, and no other pathogens were identified. Differentiation between HHV-6 pneumonitis and idiopathic pneumonia syndrome may be important, because specific antiviral therapies may influence the course of HHV-6 pneumonitis. To date, only a small number of definitive HHV-6-associated cases have been reported. Further investigation is needed to clarify the incidence and significance of HHV-6 pneumonitis after allo-HCT.

5. Gastrointestinal disease

Hentrich et al. have reported that HHV-6 was detected in gastric (n = 14) and duodenal (n = 11) biopsies in 23 patients complaining of gastrointestinal symptoms. Amo et al. reported that HHV-6B DNA was demonstrated in the nuclei of goblet cells by in situ hybridization in patients with severe diarrhea. A recent retrospective single-center analysis including 50 patients with severe vomiting and diarrhea after allo-HCT showed that HHV-6 DNA was detected in 51% of initial biopsy specimens of gastrointestinal tract. That study showed detection of HHV-6 DNA in biopsy specimens was not associated with overall survival and antiviral therapy against HHV-6 provided no beneficial effects. PCR for HHV-6 DNA in biopsy specimens may be too sensitive to diagnose clinically relevant HHV-6 infection. Further studies investigating HHV-6 antigen expression on pathological mucosa are needed to elucidate whether HHV-6 causes gastrointestinal disease in HSCT recipients, as seen in CMV infection.

6. Liver disease

A few allo-HCT recipients with HHV-6-associated liver disease have been reported. In one patient, HHV-6 was detected in a liver biopsy along with histopathological changes suggestive of viral hepatitis, high levels of HHV-6 and no other explanations for the hepatitis. However, demonstrating HHV-6 as a causative pathogen for liver dysfunction in allo-HCT recipients is difficult, given the many potential causes of liver dysfunction, including GVHD, drug, and infectious agents. Carefully designed studies are needed to determine the roles of HHV-6 in liver disease after allo-HCT.

7. GVHD

Many previous studies have reached conflicting conclusions regarding the role of HHV-6 in acute GVHD. Recent epidemiological studies analyzing 235 consecutive patients revealed a significant association between acute GVHD and HHV-6 reactivation. This association was stronger for high-grade acute GVHD, and remained significant in patients who experienced HHV-6 reactivation before initiation of salvage immunosuppression for acute GVHD. In that study, the authors suggested inflammatory responses to HHV-6 reactivation, effects of HHV-6 against the host immune system through various mechanisms, and activation of alloreactive T cells may have triggered acute GVHD. More recently, Zerr et al. performed a large prospective study to define HHV-6-associated sequelae after allo-HCT. That study revealed high-level HHV-6 was associated with subsequent grade II-IV GVHD (HR, 2.7; P = 0.02). The authors speculated that HHV-6 reactivation causes a pro-inflammatory or type I immune response, which would play a role in the development of acute GVHD.

8. Rash

Early studies have shown associations between HHV-6 reactivation and rash. Immunohistological examinations against of skin specimens from patients displaying rash concomitant with positive HHV-6 DNA in plasma or skin did not demonstrate expression of HHV-6 antigen. Distinguishing HHV-6-associated skin rash from rash due to acute GVHD may be difficult.
9. CMV infection

Several reports have shown associations between HHV-6 reactivation and CMV reactivation.45, 110, 111 A recent prospective study45 analyzing 315 allo-HCT recipients demonstrated that HHV-6 reactivation was independently associated with subsequent CMV reactivation (aHR, 1.9; P = 0.002). The same study also demonstrated that high-level HHV-6 reactivation was strongly associated with increased risk of subsequent high-level CMV reactivation (aHR, 3.11; P = 0.002). Whether HHV-6 induces CMV reactivation though its immunomodulatory effects, or whether more severe immunosuppression after allo-HCT accounts for the reactivation of both HHV-6 and CMV remains uncertain.

Future need

Over the past decade, HHV-6 encephalitis has become well known as a complication accompanying allo-HCT. Currently, HHV-6 encephalitis may be recognized as the most feared CNS complication in UCBT recipients. Despite appropriate diagnosis and early initiation of treatment, the attributable mortality rate continues to be high (Table 2). Even in survivors, many patients are left with neurological compromise, making it difficult to return to society.57 We should recognize that currently available treatments are inadequate for patients who have developed HHV-6 encephalitis. New treatment options for HHV-6 encephalitis are urgently needed. One strategy may be the use of novel compounds against HHV-6 encephalitis. However, whether use of more selective compounds against HHV-6 improves prognosis in patients who have developed HHV-6 encephalitis is uncertain. As several investigators have asserted,50, 55, 57, 81, 82 the establishment of preventative methods against HHV-6 encephalitis represents an important challenge for allo-HCT physician.

To date, no effective methods have been shown to prevent HHV-6 encephalitis. Preemptive treatment failed to prevent HHV-6 encephalitis50, 85 because the success of preemptive therapy depends on the presence of HHV-6 DNA in the blood before the onset of encephalitis. Prophylactic administration of low-dose PFA (50 mg/kg/day) cannot prevent HHV-6 encephalitis,51 probably because this dose is insufficient to suppress HHV-6 replication. An increased dose of prophylactic PFA for a longer period, or prophylaxis using novel compounds such as lipid esters of CDV82 warrant evaluation in patients at higher risk of HHV-6 encephalitis. Furthermore, alternative approaches based on the pathogenesis of HHV-6 encephalitis will be required. If immune reactions in the early phase of transplantation play a role in subsequent HHV-6 encephalitis51-53, 55 control of the inflammatory state (such as intensive prophylaxis against GVHD) may reduce the incidence of HHV-6 encephalitis.

HHV-6 may be associated with various important complications in allo-HCT recipients. Associations between HHV-6 reactivation and GVHD or CMV infection were recently demonstrated in a large-scale prospective study.45 However, whether those associations are causal remains undetermined. Determination of the spectrum of HHV-6-associated diseases in allo-HCT recipients will be important, because antiviral agents can manage HHV-6 reactivation. If HHV-6 reactivation plays a causative role in the development of acute GVHD or CMV infection, reducing HHV-6 reactivation using antiviral agents for HHV-6 might lower the incidence of these complications.

Executive summary

Methods for diagnosing HHV-6 reactivation
1. HHV-6 DNA loads in plasma or serum correlate well with indicators of active HHV-6 replication.
2. ciHHV-6 should be excluded.

HHV-6 reactivation after allo-HCT
1. HHV-6 reactivates in 30–50% of patients undergoing allo-HCT.
2. HHV-6 is most frequently apparent at about 2–6 weeks after transplantation, around the time of engraftment.
3. Receiving UCBT, unrelated transplants, treatment with steroids, and HLA mismatches are all associated with increased risk of HHV-6 reactivation.

HHV-6-associated complications
1. HHV-6 causes encephalitis in a subset of allo-HCT recipients.
2. Various post-transplant complications, including delirium, myelitis, bone marrow suppression/graft failure, GVHD, rash, CMV infection, and complications affecting the lungs, gastrointestinal tract, and liver have also been associated with HHV-6 reactivation.
3. Whether these associations other than encephalitis represent causal relationships remains undermined.

HHV-6 encephalitis, general
1. A significant, life-threatening complication accompanied by HHV-6 reactivation in allo-HCT.
2. Typically develops around 3 weeks (2–6 weeks) after allo-HCT.
3. Short-term memory loss and clinical seizures are common.
4. MRI studies typically show bilateral signal abnormalities in
the limbic system.  
5. Receiving UCBT is a significant risk factor for HHV-6 encephalitis.  
6. Unrelated or HLA mismatched-related allo-HCT, 2 or more allo-HCTs, steroid treatment, and use of alemtuzumab may also be risk factors for HHV-6 encephalitis.  
7. Post-transplant immune reaction, such as PIR, engraftment syndrome, and GVHD may be associated with the development of HHV-6 encephalitis.  
8. Demonstration of HHV-6 DNA in CSF is essential for the diagnosis of HHV-6 encephalitis. 

**HHV-6 encephalitis, treatment and prognosis**

1. PFA or GCV is recommended as first-line therapy for HHV-6 encephalitis.  
2. Not only encephalitis, but also various causes including GVHD, infectious diseases, and graft failure are associated with patient death.  
3. Even among survivors, many patients are left with neurological compromise.  
4. Antiviral therapy should be started early to prevent brain damage.  
5. Appropriate preemptive or prophylactic therapies have not yet been established.

### Conflict of interest

The author declares no conflict of interest.

### References

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