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Guidelines from the 2017 European Conference on Infections in Leukaemia for management of HHV-6 infection in patients with hematological malignancies and after hematopoietic stem cell transplantation

by Katherine N. Ward, Joshua A. Hill, Petr Hubacek, Rafael de la Camara, Roberto Crocchiolo, Hermann Einsele, David Navarro, Christine Robin, Catherine Cordonnier, and Per Ljungman

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Guidelines from the 2017 European Conference on Infections in Leukaemia for management of HHV-6 infection in patients with hematological malignancies and after hematopoietic stem cell transplantation.

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Runing heads: Guidelines for HHV-6 infections

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HHV-6B encephalitis is an important cause of morbidity and mortality after allogeneic hematopoietic stem cell transplant. Guidelines for the management of HHV-6 infections in patients with hematological malignancies or post-transplant were prepared a decade ago but there have been no other guidelines since then despite significant advances in the understanding of HHV-6 encephalitis, its therapy, and other aspects of HHV-6 disease in this patient population. Revised guidelines prepared at the 2017 European Conference on Infections in Leukaemia covering diagnosis, preventative strategies and management of HHV-6 disease are now presented.

INTRODUCTION

Over the past 10 years it has been recognized that HHV-6A and HHV-6B are distinct species[1], HHV-6B not HHV-6A is the most frequent cause of encephalitis post-hematopoietic stem cell transplant (HSCT) and that chromosomally integrated HHV-6 (CIHHV-6) is clinically significant. Revised European Conference on Infections in Leukemia (ECIL) HHV-6 guidelines were prepared after a literature review by a group of experts, and discussed at a plenary session on September 22, 2017 until consensus. Those guidelines specifically applying to treatment were graded according to pre-ordained criteria (Table 1) for level of evidence and strength of recommendation; participants were hematologists, microbiologists and infectious disease specialists with expertise on infectious complications in hematology. A final slide set was posted on the ECIL website (www.ecil-leukaemia.com) on October 2, 2017 and made available for open consultation.

BACKGROUND

HHV-6A & HHV-6B: The two species of HHV-6, HHV-6A and HHV-6B infect and establish latency in different cell types including CD4 positive T lymphocytes, monocytes, and other epithelial, fibroblastic and neuronal cells [2]. No disease has been causally linked to HHV-6A, and its natural history is unknown. In contrast, HHV-6B primary infection is ubiquitous in the first two years of life sometimes causing *exanthema subitum;* subsequent viral latency gives the potential for reactivation and disease.

CIHHV-6: As well as the almost universal postnatal acquisition of HHV-6B, in about 1% of humans the complete genome of HHV-6A or HHV-6B is integrated into a chromosomal telomere in every nucleated cell in the body and is transmitted through Mendelian inheritance [3;4]. Although HHV-6A is rare in the general population, HHV-6A and HHV-6B are encountered in approximately one-third and two-thirds of individuals with CIHHV-6, respectively [5]. Telomeric integration sites have been identified on different chromosomes using fluorescent in situ hybridization (FISH) [6]. Integration is normally restricted to a particular chromosome per individual but very rarely two sites, if inherited from both parents [3].

HHV-6 DNA detected in blood usually indicates virus replication. However, in individuals with CIHHV-6, viral DNA in latent form originating from human chromosomal DNA is persistently detected at high levels in whole blood as well as in “cell free” samples such as serum and cerebrospinal fluid (CSF), since the latter contain cellular DNA released from damaged cells during sample preparation [7;8]. Although HHV-6B encephalitis is an accepted albeit rare complication of primary HHV-6B infection in young children, HHV-6 DNA in the CSF of older immunocompetent children and adults is most likely due to latent virus originating from CIHHV-6 rather than central nervous system (CNS) infection [8;9].

CIHHV-6 & potential for disease post-HSCT: There is limited evidence of symptomatic reactivation of CIHHV-6. One report demonstrated CIHHV-6A reactivation in a child with severe combined immunodeficiency and hemophagocytic syndrome pre-HSCT and thrombotic microangiopathy post-HSCT [10]. Two other reports from settings other than HSCT give evidence for symptomatic reactivation in a patient treated with a histone deacetylase inhibitor [11] and a patient who received a liver transplant from a donor with CIHHV-6A [12].

Despite the above case of reactivation with accompanying morbidity post-HSCT [10], this has not been reported in the few other cases where CIHHV-6 was identified in the donor or recipient [13-16] and the frequency and type of diseases caused by CIHHV-6 in HSCT recipients remain unknown. A recent study of 87 patients with CIHHV-6 in HSCT donors and/or recipients demonstrated an association with acute graft-versus-host disease (GVHD) and CMV reactivation, but there was no effect on overall or non-relapse mortality [17]. Neither has an increased frequency of CIHHV-6 been identified in a range of hematological malignancies [17-21]. None of these studies were designed to address the likelihood that integration into different chromosomal sites might have different pathological consequences and vary according to HHV-6 species.

HHV-6 & disease in patients with hematological malignancies or post-HSCT: In patients with hematological malignancies without HSCT, there is little evidence that HHV-6 causes disease. Post-HSCT the high frequency of HHV-6B reactivation plus the difficulty of identifying CIHHV-6 causes substantial challenges in determining the pathogenic role of HHV-6 in disease. For autologous transplants there are insufficient data for a causal association with end-organ disease. However, after allogeneic HSCT, HHV-6B is associated with several syndromes and is a well recognised cause of encephalitis with high morbidity and mortality.

DEFINITIONS

Primary HHV-6 infection: This is defined as the first detection of HHV-6 replication in an individual with no evidence of previous infection. Normally this would be accompanied by HHV-6 seroconversion, but severely immunocompromised HSCT recipients may not develop antibodies. Donor-derived CIHHV-6 must be excluded.

HHV-6 reactivation: Given the difficulty distinguishing between reactivation of latent virus (endogenous) and reinfection (exogenous), the term HHV-6 reactivation is applied to both scenarios in clinical practice and is defined as new detection of HHV-6 in individuals with evidence of previous infection; this latter can be assumed in individuals older than 2 years. The diagnosis usually relies on the presence of HHV-6 DNA in peripheral blood but other methods and samples are sometimes used. Reactivation is not proven if newly detected HHV-6 DNA is due to donor- or recipient-derived CIHHV-6 since latently-integrated viral DNA cannot be distinguished from replicating virus DNA. See below for tests for CIHHV-6 and its reactivation.

HHV-6 DIAGNOSTIC TESTING

Antibody tests cannot distinguish between HHV-6A and HHV-6B and are not indicated in HSCT patients. Table 2 gives an overview of possible diagnostic tests.

DNA tests: PCR is the mainstay of HHV-6 diagnosis and a variety of real-time PCR assays for HHV-6 DNA load are available [22;23]. Not all differentiate between HHV-6A and HHV-6B, and agreement between laboratories for HHV-6 DNA levels is poor [22;24]. However, a WHO standard for HHV-6B DNA is now available (http://www.nibsc.org/documents/ifu/15-266.pdf).

- **Quantitative PCR that distinguishes between HHV-6A and HHV-6B DNA is recommended for diagnosis of infection.**

- **For a given patient, repeat HHV-6 DNA testing should be performed using the same DNA extraction method, quantitative PCR and type of specimen.**

Interpretation of DNA testing post-HSCT in the presence of CIHHV-6 **(Table 3):** If a HSCT donor has CIHHV-6, HHV-6 DNA load in blood will increase post-HSCT in parallel with leukocyte engraftment [13;16;25] , and antivirals will have no effect on the quantity of the latently integrated viral DNA [26]. Alternatively, if the recipient has CIHHV-6, high levels of HHV-6 DNA will be detected pre-HSCT in blood and will decrease alongside recipient leukocytes post-transplant [14;27]. Importantly in this latter situation HHV-6 DNA will continue to be detected at high levels in non-hematopoietic tissue throughout the body [28].

Tests for CIHHV-6: Currently there is no indication routinely to test HSCT donors or recipients for CIHHV-6. However, in clinically ambiguous cases such testing can be important to avoid unnecessary, potentially toxic, antiviral therapy.

CIHHV-6 should be suspected in the donor and/or recipient if HHV-6 DNA detection follows one of the patterns described in Table 3 or if HHV-6A is detected. Where necessary, CIHHV-6 can easily be excluded by a negative HHV-6 DNA test on a blood/serum sample taken pre-transplant from the recipient or at any time from the donor. Individuals with CIHHV-6 have characteristic persistently high levels of HHV-6 DNA in whole blood (>5.5 $\log_{10}$ copies/ml) and in serum (100-fold lower than that in whole blood for a given patient) [5;7]. The level of DNA detected in plasma varies depending on the timing of separation from whole blood [29].

A ratio of one copy of HHV-6 DNA/cellular genome confirms the diagnosis of CIHHV-6. Droplet digital PCR [29] is the most accurate method as it gives an absolute number. Comparison of two quantitative real-time PCR results (one for HHV-6 and one for a human gene present in all nucleated cells) is also acceptable albeit with a significant margin of error due to inherent assay imprecision [7]. HHV-6 DNA is present in hair follicles and nails exclusively in persons with CIHHV-6 [4;19].

- **If CIHHV-6 is suspected, whole blood or serum or cellular samples or leftover DNA taken from donor and/or recipient pre-HSCT should be tested by quantitative PCR that distinguishes between HHV-6A and HHV-6B DNA. Testing plasma is not recommended.**

- **CIHHV-6 can be confirmed by evidence of one copy of viral DNA/cellular genome, or viral DNA in hair follicles/nails, or by FISH demonstrating HHV-6 integrated into a human chromosome.**

Tests for CIHHV-6 reactivation: This must be confirmed by virus culture plus viral genome sequencing to confirm identity of the viral isolate with the integrated virus.

HHV-6B END-ORGAN DISEASE & OTHER OUTCOMES POST-HSCT

HHV-6B primary infection versus reactivation: Only two cases of primary HHV-6B infection after allogeneic HSCT have been reported, which were in very young children accompanied by fever and rash [30;31]. In contrast, various end-organ diseases and other complications post-HSCT have been associated with HHV-6B reactivation, but apart from encephalitis and fever with rash, the evidence for causation is moderate or weak (Table 4).

HHV-6B encephalitis & its definition: The first described encephalitis case [32] was followed by many confirmatory reports [33]. Zerr and Ogata analyzed the accumulated published data and provided evidence for a causal association between HHV-6 and encephalitis using the Bradford Hill criteria [34].

The most frequent cause of encephalitis after allogeneic transplant is HHV-6. When the species is identified, it is almost invariably HHV-6B. Of the only three reported patients with HHV-6A encephalitis, one had an atypical presentation and the other two had unrecognized CIHHV-6 [9]. In one of these two, testing of archived samples confirmed CIHHV-6A pre-HSCT [35] but the question remained as to whether the virus reactivated causing encephalitis or an alternative unidentified cause was responsible. Whether CIHHV-6B can reactivate causing encephalitis is theoretically possible but requires viral culture and sequencing to distinguish childhood-acquired HHV-6B from integrated virus.

HHV-6B encephalitis typically presents early as post-transplant acute limbic encephalitis (PALE). CSF protein and cell counts are often unremarkable – see Table 5 for further clinical features. Although magnetic resonance imaging may be negative at the start of the disease, changes in the temporal lobe are demonstrated in approximately 60% of cases [36]. However, similar observations occur in limbic encephalitis caused by other infectious agents [37]. Extrahippocampal abnormalities may occur in areas such as the entorhinal cortex or amygdala [38]. Temporal lobe seizures are relatively frequent but focal neurological deficits rare. Computed tomography of the brain is often normal. Electroencephalograms are usually diffusely abnormal sometimes involving the temporal region. Autopsy reveals hippocampal disease with HHV-6 protein in astrocytes and neurons suggesting local virus reactivation [32] rather than an indirect effect of virally-induced neuroinflammation. Notably, a retrospective study [39] showed that only one-third of HHV-6 encephalitis patients had the typical features of PALE.

Different studies have used different definitions of HHV-6 encephalitis [40]. Ideally the definition would require proof of HHV-6 infection in tissue samples from the affected part of the brain. However, given the impracticality of such an approach and the epidemiological evidence, the definition below can replace the need for brain biopsy.

- **Diagnosis of HHV-6B encephalitis should be based on HHV-6 DNA in CSF coinciding with acute-onset altered mental status (encephalopathy), or short term memory loss, or seizures.**

- **Other likely infectious or non-infectious causes must be excluded.**

- **CIHHV-6 in donor & recipient should be excluded.**

- **If CIHHV-6 is detected, evidence for CIHHV-6 reactivation in the CSF or brain tissue is necessary to implicate CIHHV-6.**

Other CNS dysfunction: Apart from encephalitis post-HSCT, HHV-6 has been associated with CNS disease ranging from headache to delirium and neurocognitive decline [41-43]; patients whose donors or recipients had CIHHV-6 were excluded in two of these studies [42;43]. HHV-6 has also been associated with myelitis, pruritis and dysesthesia in Japanese patients [44]. Notably, HHV-6 DNA can be found in CSF in patients without CNS symptoms [42].

Risk factors for HHV-6B encephalitis: HHV-6B reactivation in blood (i.e. viremia) is a major risk factor and occurs in approximately half of allogeneic transplant recipients in the first few weeks post-HSCT [45;46]. The highest rates are seen after umbilical cord blood transplantation (CBT); in a prospective cohort of 125 cord blood recipients, HHV-6B reactivation was documented in 94% [47]. In a multicenter prospective study, Ogata [48] showed that reactivation precedes or coincides with HHV-6 encephalitis and that ≥10,000 copies/ml in plasma correlated with onset of disease with 100% sensitivity and 64.6% specificity. Similar values of 100% and 81% respectively were obtained in a much larger retrospective study [49].

However, not all patients develop encephalitis when the plasma HHV-6 DNA level is high and other factors are involved usually related to poor T-cell function, such as T-cell depleted allografts, CBT, a mismatched or unrelated donor, acute GVHD and treatment with glucocorticoids [50]. A retrospective cohort study of 1344 patients showed CBT is a major risk factor (adjusted hazard ratio (aHR) 20.0; p<.001), as well as acute GVHD grades II-IV (aHR

7.5; p<.001) and use of mismatched unrelated donors (aHR 4.3; p <.04) [49]. A subsequent systematic review and meta-analysis of all relevant HSCT studies also demonstrated the incidence of HHV-6 encephalitis was significantly higher post-CBT than other stem cell sources (8.3% versus 0.5%; p<.001) [40]. Ogata [36] used the Japanese Adult Transplant Registry and identified 145 patients with HHV-6 encephalitis; the relative risk for CBT was 11.09, p<0.001, and 9.48, p<0.001 for HLA-mismatched unrelated donors. Haploidentical transplant recipients may also be at high risk of HHV-6B encephalitis based on a combined report of two small studies [51] where, in an attempt to improve engraftment and reduce GVHD, donor cells were depleted of naive T cells and NK cells but memory T cells remained. Finally, pre-engraftment syndrome might be a risk factor for HHV-6 encephalitis [50].

Prognosis of HHV-6B encephalitis: Zerr [33] reviewed the outcome in the many prior detailed descriptions of individual patients; 11/44 (25%) died within 1-4 weeks of diagnosis, 6 (14%) showed improvement but died with various unrelated medical problems, 8 (18%) improved but with lingering neurological compromise and 19 (43%) appeared to make a full recovery. In a single retrospective study, Hill [49] reported 19 patients with PALE; attributable mortality was higher after CBT (5/10) than in recipients of adult donor stem cells (0/9). In a much larger number of allogeneic HSCT recipients [36] neuropsychological sequelae were reported in 57% of encephalitic patients with an overall survival rate of 58.3% in those with encephalitis as opposed to 80.5% in those without.

Other retrospective surveys of small numbers of patients have reported variable outcomes in terms of mortality and neurological sequelae including temporal lobe epilepsy (TLE) [50]. Long-term consequences of HHV-6 encephalitis post-HSCT in children may include a new syndrome, involving generalized epilepsy (as opposed to TLE in adults) together with cognitive regression and delayed intellectual development [52;53].

HHV-6B myelosuppression & allograft failure: Evidence for a causal association is moderate (Table 4). HHV-6B infects hematological progenitor cells *in vitro* thereby reducing colony formation [54]. Virus reactivation post-HSCT has been frequently associated with myelosuppression and delayed engraftment, particularly involving platelets [46;55;56] and also allograft failure [57;58].

- **If there is failed engraftment, blood or bone marrow should be tested for HHV-6B DNA.**
- **Other likely infectious or non-infectious causes must be excluded.**

- **CIHHV-6 in donor & recipient should be excluded.**

Other end-organ diseases: Evidence for a causal association of HHV-6 with other disease post-HSCT is weak (Table 4). Viral DNA in tissue is not diagnostic as it may reflect HHV-6 DNAemia or inflammation with consequent infiltrating HHV-6 infected lymphocytes.

Pneumonitis remains a leading cause of morbidity and mortality post-HSCT, and HHV-6 has been implicated as a potential cause [59]. Studies using heterogeneous populations and methods, including patients with hematological malignancies with and without HSCT, have produced variable results [60-62]. A recent study applied molecular testing for 28 pathogens in bronchoalveolar lavage samples from HSCT recipients previously diagnosed with idiopathic pneumonia syndrome. HHV-6 was the most common pathogen (29% of cases) identified, and it was the only pathogen in about half of these [63]. However, the clinical significance of this finding remains to be determined.

Although there are many reports of HHV-6B-associated hepatitis after liver transplantation, this has only been well documented in 2 cases post-HSCT [64;65], both of which describe acute hepatitis successfully treated with ganciclovir. HHV-6B DNA was demonstrated in hepatic tissue by immunohistochemistry.

- **In suspected end-organ disease other than failed engraftment or encephalitis, tissue from the affected organ should be tested for HHV-6 infection by culture, immunochemistry, in situ hybridization or reverse transcription PCR for mRNA.**

- **PCR for HHV-6 DNA in tissue is not recommended for documentation of HHV-6 disease.**

- **Other likely infectious or non-infectious causes must be excluded.**

- **CIHHV-6 in donor & recipient should be excluded.**

HHV-6B & CMV reactivation: HHV-6B reactivation has been associated with an increased risk of subsequent CMV reactivation and disease post-HSCT [45;66], although this was not replicated in another study [67]. One study suggests that HHV-6 reactivation may indicate cellular immunosuppression which also predisposes to CMV reactivation [68] . *In vitro* studies of HHV-6 reactivation demonstrate that HHV-6B infection might contribute to CMV reactivation through inhibition of IL-12 production [69;70].

HHV-6B - acute GVHD & increased all-cause mortality: A well-designed study established an association between HHV-6B reactivation and subsequent acute GVHD [71]. A meta-analysis of 11 such studies demonstrated a statistically significant association between HHV-6B and subsequent grade II to IV acute GVHD (hazard ratio, 2.65; 95% confidence interval, 1.89 to 3.72; $P < .001$) [72].

HHV-6B reactivation has also been associated with increased all-cause mortality post-HSCT [45;46;73;74]. However, whether HHV-6B directly or indirectly impacts on mortality in the absence of clinically apparent end-organ disease remains unclear.

TREATMENT STRATEGIES

Antiviral drugs & immunotherapy: Ganciclovir, foscarnet, and cidofovir inhibit HHV-6 replication *in vitro* [75]. Whilst *in vitro* studies support the potential for HHV-6 to develop resistance to the above antiviral agents, very few case reports have described the emergence of drug-resistant isolates, specifically to ganciclovir and after lengthy exposure in the clinical setting [76-79]. Additionally, the use of valganciclovir or ganciclovir treatment for CMV disease did not result in the emergence of drug-resistant HHV-6 mutants in a large prospective trial of solid organ transplant patients [80].

New treatment modalities for HHV-6 are needed due to the nephrotoxic and myelosuppressive properties of the available agents. Brincidofovir (or CMX-001) has high *in vitro* activity against HHV-6 species[81] but has significant gastrointestinal toxicity [82]; an intravenous formulation in development may be better tolerated [83]. However, this drug is not currently available for clinical use. Adoptive immunotherapy with virus-specific T-cells is an exciting new therapeutic approach for HHV-6 [84;85]. This approach appears to be safe and potentially effective in small, uncontrolled studies.

Prevention of HHV-6B encephalitis: HHV-6 DNA screening during the high-risk period post-HSCT is impractical as HHV-6 reactivation often coincides with the onset of disease [48]. Effective pre-emptive or prophylactic strategies have not been identified. Three prospective, non-randomized studies of *prophylactic* foscarnet (pre- or post-engraftment) did not significantly lower the incidence of encephalitis[86-88]. Similarly, two prospective, non-randomized studies of *pre-emptive* ganciclovir or foscarnet did not reduce the incidence of HHV-6B encephalitis [89;90]. Failure of these approaches may be a result of inadequate dosing due to concerns about toxicity and resultant insufficient drug penetration into the CSF. Thus, routine HHV-6 DNA screening is not recommended for pre-emptive or prophylactic therapy, in any context.

- **Routine screening of HHV-6 DNA in blood post-HSCT is not recommended (DIIu)**

- **Anti-HHV-6 prophylactic or pre-emptive therapy is not recommended for the prevention of HHV-6B reactivation or encephalitis post-HSCT (DIIu)**

Treatment of HHV-6B encephalitis: Zerr [91] demonstrated a response of HHV-6 to ganciclovir or foscarnet as measured by DNA in the CSF or serum of allogeneic HSCT patients. Ljungman [92] reported reductions in the HHV-6 load in saliva in patients receiving ganciclovir for pre-emptive therapy of CMV. Vu et al [93] described positive responses in four of five patients treated with foscarnet.

On the basis of the above results, foscarnet or ganciclovir were recommended for treatment of HHV-6 encephalitis post-HSCT [94]. Since then a substantial amount of additional evidence supports the use of ganciclovir and foscarnet. Hill et al [49] treated 18 patients with HHV-6 PALE with foscarnet 180mg/kg/day and symptoms improved in most. Schmidt-Hieber [95] reported a response rate of 63% with either foscarnet or ganciclovir therapy for HHV-6 encephalitis. Most recently data comparing the use of ganciclovir with foscarnet in Japanese patients [36] showed response rates of neurological symptoms were 83.8% and 71.4% with foscarnet monotherapy and ganciclovir monotherapy, respectively (p=.10, Fisher's exact test). Full-dose therapy with foscarnet (≥180 mg/kg) or ganciclovir (≥10 mg/kg) was associated with a better response rate than treatment with lower doses (foscarnet, 93% vs 74%, p=.044; ganciclovir, 84% vs 58%, p=.047). The response rate of 10 patients receiving combination therapy with various doses of foscarnet and ganciclovir was 100%. However, the small sample size limits conclusions regarding whether combination therapy is superior to monotherapy, and drug toxicity is an important consideration. Death from any cause within 30 days after development of HHV-6 encephalitis was significantly lower in patients who received foscarnet and significantly higher in patients who received ganciclovir but this was in unadjusted descriptive analyses.

Information on the clinical use of cidofovir for the treatment of HHV-6 encephalitis is limited to two case reports [96;97]; in one cidofovir was interrupted due to drug toxicity and in the other the drug was combined with foscarnet.

- **Intravenous foscarnet or ganciclovir are recommended for treatment of HHV-6B encephalitis. Drug selection should be dictated by the drug's side effects and the patient's comorbidities (AIIu).**

- **The recommended doses are 90mg/kg b.d. for foscarnet and 5mg/kg b.d. for ganciclovir (AIIu).**

- **Antiviral therapy should be for at least 3 weeks and until testing demonstrates clearance of HHV-6 DNA from blood and, if possible, CSF (CIII).**

- **Combined ganciclovir & foscarnet therapy can be considered (CIII).**

- **Immunosuppressive medications should be reduced if possible (BIII).**

- **There are insufficient data on the use of cidofovir to make a recommendation.**

Treatment of HHV-6B associated end-organ diseases other than encephalitis: Since the strength of associations with other end-organ diseases is moderate or weak, there are insufficient data to guide a recommendation for antiviral treatment.

- **No recommendation can be made.**

CONCLUSIONS

HHV-6B is the primary cause of infectious encephalitis after allogeneic HSCT. Studies of prevention and treatment strategies for this disease are urgently required to improve outcomes using novel therapeutic approaches, such as new antiviral drugs and immunotherapy.

As regards other possible HHV-6B end-organ diseases post-HSCT, improved RNA diagnostic tests are necessary to demonstrate active viral replication (in situ hybridization and/or reverse transcription PCR).

Understanding the pathogenic potential of HHV-6 and CIHHV-6 necessitates that all prospective studies on HSCT patients and health outcomes use tests on both donor and recipient that distinguish HHV-6A from HHV-6B.

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Table 1. ESCMID (European Society of Clinical Microbiology and Infectious Diseases) grading system

Strength of a recommendation	
Grade A	ESCMID strongly supports a recommendation for use
Grade B	ESCMID moderately supports a recommendation for use
Grade C	ESCMID marginally supports a recommendation for use
Grade D	ESCMID supports a recommendation against use
Quality of evidence	
Level I	Evidence from at least one properly designed randomised, controlled trial
Level II *	Evidence from at least one well-designed clinical trial, without randomisation; from cohort or case-controlled analytical studies (preferably from more than one centre); from multiple time series; or from dramatic results of uncontrolled experiments
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

***Added index for level II quality of evidence**

• r: meta-analysis or systematic review of randomised controlled trials
• t: transferred evidence, that is, results from different patient cohorts, or similar Immune-status situation
• h: comparator group is a historical control
• u: uncontrolled trial
• a: published abstract (presented at an international symposium or meeting)

Table 2. HHV-6 diagnostic tests

Method	Use & limitations
Virus culture*	Diagnosis of infection - gold standard, specialised, labour-intensive
Viral antigen test (immunohistochemical staining)*	Diagnosis of infection - limited sensitivity, slow turn-around time
Detection of viral mRNA by reverse transcription PCR*	Late gene transcripts to confirm virus replication No international standardization or specific thresholds for virus replication, especially for CIHHV-6
Quantitative viral DNA PCR	Longitudinal studies, comparison of HHV-6 DNA levels in blood v. organs Can discriminate HHV-6A and HHV-6B*
Droplet digital PCR*	Precise method for DNA levels, identification of CIHHV-6
Fluorescent in situ hybridization*	Confirmation of CIHHV-6

*Not available to most diagnostic laboratories

CIHHV-6, chromosomally integrated HHV-6

Table 3. HHV-6 test results after allogeneic hematopoietic stem cell transplantation that indicate naturally acquired HHV-6 infection versus chromosomally integrated HHV-6 (CIHHV-6).

Laboratory observations	HHV-6 status			
	Prior childhood infection*	Donor CIHHV-6 positive	Recipient CIHHV-6 positive	Donor & recipient CIHHV-6 positive
One HHV-6 copy/leucocyte	No	Yes**	No	Yes **
One HHV-6 copy/non-hematopoietic cell	No	No	Yes §	Yes §
HHV-6 species	B	A or B	A or B	A or B
Persistent HHV-6 DNA in blood	No	Yes	+/-***	Yes
Response of HHV-6 DNA level to antiviral drugs	Yes	No	No	No

* HHV-6B primary infection usually occurs in childhood.

** HHV-6 found persistently in hematopoetic tissue e.g. blood, bone marrow, spleen.

§ HHV-6 found persistently at extremely high levels in all nucleated non-hematopoietic cells.

*** A low level in peripheral blood if organ damage and cell death or hematological malignancy relapse.

Table 4. HHV-6B reactivation after allogeneic hematopoietic stem cell transplantation HSCT: disease associations.

	Epidemiological associations	**Level of *in vitro* or *in vivo* support for causation**
HHV-6B end-organ disease	Encephalitis (predominantly limbic)	Strong
	Non-encephalitic central nervous system dysfunction e.g. delirium, myelitis	Moderate
	Myelosuppression, allograft failure	Moderate
	Pneumonitis	Weak
	Hepatitis	Weak
Other	Fever & rash	Strong
	Acute graft-versus-host disease	Moderate
	CMV reactivation	Moderate
	Increased all-cause mortality	Weak

Adapted from Table 29.2 [98]

Table 5. Clinical features of HHV-6B encephalitis

Disease onset	Usually 2-6 weeks post-HSCT but can be later
Symptoms/signs	Confusion, encephalopathy, short term memory loss, SIADH, seizures, insomnia
Brain MRI[a]	Often normal. Typically but not exclusively, circumscribed, non-enhancing, hyperintense lesions in the medial temporal lobes (especially hippocampus & amygdala)
Cerebrospinal fluid	HHV-6B DNA, +/- mild protein elevation, +/- mild lymphocytic pleocytosis
Prognosis	Memory defects & neuropsychological sequelae in 20 - 60% Death due to progressive encephalitis in up to 25% of all HSCT recipients & up to 50% of cord blood recipients

HSCT, hematopoietic stem cell transplantation; SIADH, syndrome of inappropriate antidiuretic hormone secretion; MRI, magnetic resonance imaging

[a] Features of T2, fluid attenuation, inversion recovery (FLAIR) & diffusion weighted-imaging (DWI) sequences

Modified from Hill & Zerr [99]