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High human herpesvirus 6 viral load in pediatric allogeneic hematopoietic stem cell transplant patients is associated with detection in end organs and high mortality

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Abstract

Human Herpes Virus 6 (HHV-6) reactivation occurs in approximately half of patients following allogeneic hematopoietic stem cell transplant (HSCT). While encephalitis and delayed engraftment are well-documented complications of HHV-6 following HSCT, the extent to which HHV-6 viremia causes disease in children is controversial. We performed a retrospective review of HHV-6 reactivation and possible manifestations in pediatric allogeneic HSCT patients at a single institution. Of 89 children and young adults who underwent allogeneic HSCT over a three-and-a-half-year period, 34 patients reactivated HHV-6 early post-transplant. Unrelated donor stem cell source and lack of antiviral prophylaxis were risk factors for the development of HHV-6 viremia. Viremia correlated with the presence of acute graft-versus-host disease, but not chronic graft-versus-host disease. We identified two subgroups within the viremic patients—a high-risk viremic

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

LEW: Developed the concept and design of the study, performed data analysis, and wrote the manuscript; RP and JST: Performed statistical analyses of the data; JB: Collected and tabulated research data; BAP: Reviewed the PCR data and helped with interpretation as well as critical review of the manuscript; JJW: Wrote the PCR methodology and critically reviewed the manuscript; and SK: Designed the study, performed data analysis and interpretation, and revised the manuscript.

and tissue-positive group that reactivated HHV-6 and had suspected end-organ disease and a low-risk viremic but asymptomatic group that reactivated HHV-6 but did not exhibit symptoms or signs of end-organ disease. Peak viral load was found to be strongly associated with mortality. Prospective studies in larger numbers of patients are needed to further investigate the role of HHV-6 in causing symptomatic end-organ disease as well as the association of viral load with mortality.

Keywords

allogeneic hematopoietic stem cell transplantation; end-organ disease; human herpesvirus-6; pediatric

1 INTRODUCTION

HHV-6 is a member of the herpes family of viruses, which are known to persist in the host and remain latent in leukocytes after primary infection.¹ Herpesvirus reactivation is associated with severe complications among patients on immunosuppressive therapy after HSCT. HHV-6 reactivation occurs in approximately half to two-thirds of allogeneic HSCT recipients, mainly during the first month post-transplantation.^{2, 3} Often herpesviruses, especially HHV-6 and CMV, coexist.⁴

Patients who receive myeloablative preparative regimens, cord blood transplants, second transplants, or corticosteroids are at higher risk of HHV-6 reactivation.^{5–7} HHV-6 reactivation is associated with acute limbic encephalitis,^{8–10} interstitial pneumonia,^{11–13} and delayed engraftment.⁵ Some studies suggest a relationship between HHV-6 reactivation and acute GVHD^{3, 7, 14–18}; however, the relationship with GVHD as well as the effect of HHV-6 on outcome remains controversial.

Antiviral agents have demonstrated activity against HHV-6 in vitro. In addition, antiviral prophylaxis has been shown to decrease the frequency of HHV-6 reactivation¹⁹; however, the decision about when antiviral administration is warranted must be weighed against the side effects of these medications, which include myelosuppression and nephrotoxicity. Routine surveillance for HHV-6 suggests that a subset of patients is asymptomatic and viremia resolves spontaneously without any treatment.²⁰ On the other hand, manifestations of HHV-6, such as graft failure and encephalitis, can be life-threatening.⁶ It is not clear whether antiviral prophylaxis may be beneficial in a subset of high-risk patients; the optimal prophylaxis and treatment as well as duration of therapy remain uncertain.

We report the results of a single-institution retrospective cohort study that investigated the incidence, risk factors, and impact of HHV-6 reactivation in children, adolescents, and young adults undergoing allogeneic HSCT over a three-and-a-half-year period.

2 PATIENTS AND METHODS

Eighty-nine patients aged 0–26 years who received allogeneic HSCT at a single center between January 1, 2010, and July 1, 2013, had serial DNA PCR testing for HHV-6. Patients received conditioning for transplant and prophylaxis of GVHD per institutional protocols.

We collected the following data: age, donor, stem cell source, HLA match, underlying diagnosis/transplant indication, conditioning regimen, antiviral prophylaxis and treatment, details of herpesvirus reactivation, development of GVHD, and mortality. The collection of these data was retrospective and was approved by the Stanford Institutional Review Board.

2.1 Antiviral prophylaxis

Patients were given acyclovir for CMV, HSV, and VZV prophylaxis in allogeneic HSCT patients through June 2012 as per institutional guidelines. However, in the following period, CMV prophylaxis was modified in high-risk patients, defined as those patients who were CMV-seropositive and/or had donors who were CMV-seropositive and with one of the following characteristics: umbilical cord blood, mismatched unrelated donor, patients receiving serotherapy including antithymocyte globulin and alemtuzumab, patients on high-dose steroids, and patients with acute or chronic graft-versus-host disease. These high-risk patients received ganciclovir during conditioning (through day-2) followed by high-dose acyclovir (500 mg/m² 3 times daily) until engraftment, and finally valganciclovir prophylaxis following engraftment.²¹ No antiviral prophylaxis was used if patients and donors were CMV-seronegative or patients were seronegative for HSV and VZV.

2.2 Specimen collection

Routine viral surveillance in blood was performed with weekly PCR monitoring for viral reactivation following HSCT. If a patient was found to have reactivated a virus, monitoring twice per week was instituted. Tissues obtained either via biopsy from sinuses, liver, gastrointestinal tract, and gallbladder following removal were tested for the presence of opportunistic pathogens. Additionally, body fluids including cerebrospinal fluid, pericardial fluid, and bone marrow were also tested for opportunistic pathogens (including CMV, EBV, and HHV-6) as clinically indicated. Infectious workup included routine bacterial, fungal, and viral culture in addition to PCR for various viruses, including HHV-6. Tissues were also sent to pathology to evaluate for histologic evidence of GVHD.

2.3 Detection of HHV-6 DNA by PCR

Nucleic acid extraction was performed on plasma using the Qiagen EZ1 Virus Mini Kit v2.0 on the Qiagen EZ1 instrument. HHV-6 DNA was detected using a lab-developed, real-time PCR assay targeting the HHV-6 U66 gene. The HHV-6 assay was performed using the QuantiFast Pathogen +IC kit on the Rotor-Gene Q instrument. Primers (HHV6Q_FWD: GAACACGTGGGTCAGATAGTTGAT; HHV6Q_REV: CATCGCCGTCACCAAACCTT) and hydrolysis probe (HHV6Q_Probe: FAM-CACGATTGGCTAAAGC-MGB) were added. Samples in which HHV-6 and exogenous internal control DNA did not amplify were considered to have failed extraction or to contain inhibitors. One thousand copies/mL is the lower limit of quantitation for the assay in the plasma. Detection of HHV-6 in tissues and

fluids was performed as a qualitative PCR test with the disclaimer that the sensitivity and specificity have only been validated for CSF. Immunohistochemistry for HHV-6 and evaluation for HHV-6 viral integration were not routinely performed at our institution.

2.4 HHV-6 treatment

Patients with persistent HHV-6 viremia defined as >1000 copies/mL (>3 logs) for 2 consecutive weeks who also received an unrelated donor transplant were treated preemptively with antiviral therapy until viremia resolved and they were asymptomatic. Antiviral therapy was chosen based on engraftment status with foscarnet used prior to engraftment due to myelosuppressive effects associated with ganciclovir and ganciclovir used post-engraftment. In asymptomatic patients, antiviral therapy was discontinued after 2 consecutive weeks of undetectable viral load.

2.5 Study group definitions

We divided patients into three groups: (i) no reactivation, (ii) asymptomatic viremic patients, and (iii) viremic, tissue-positive patients. The no reactivation group consisted of those who did not have any evidence of HHV-6 reactivation on routine monitoring. The asymptomatic viremic group consisted of those who reactivated HHV-6 (with a minimum of one positive serum PCR) but did not exhibit symptoms suggesting end-organ disease attributable to HHV-6. Finally, those with HHV-6 reactivation who subsequently developed symptoms that suggested end-organ disease underwent fluid sampling such as BAL, CSF, or bone marrow aspirate or tissue sampling from the GI tract (gastric/intestinal biopsy, liver biopsy, or gallbladder removal) and sinuses. These signs and symptoms included nausea or vomiting associated with weight loss, severe abdominal pain, profuse diarrhea, persistent unexplained cough, respiratory distress, intractable headache, neurologic symptoms, or other signs or symptoms concerning for graft-versus-host disease. If the end organ demonstrated the qualitative presence of HHV-6, these patients were classified into the viremic, tissue-positive group.

2.6 Statistical methods

Wilcoxon Mann-Whitney test was performed to assess whether the viral load differed between the asymptomatic and symptomatic patients. Fisher's exact test for association was used to determine whether patients with asymptomatic reactivation systematically differed from those with tissue positivity for HHV-6. Based on the previous literature, we analyzed seven risk factors for developing viremia: gender, diagnosis (malignant, non-malignant), stem cell source (bone marrow, cord blood, peripheral blood), stem cell donor (related, unrelated), human leukocyte antigen matching (matched, mismatched), conditioning (myeloablative, other), and antiviral prophylaxis (yes, no).^{6, 10, 14, 15, 22} We used stepwise logistic regression with a *P*-value of .05 for a risk factor to either enter or leave the model. We used hierarchical log-linear models to perform an association analysis between groups (control, viremia), acute graft-versus-host disease (present, absent), and chronic graft-versus-host disease (present, absent). A ROC curve was generated to evaluate the association of peak viral load with mortality. We used the Kaplan-Meier method to compute the survival curves for the asymptomatic and tissue-positive viremic groups and the log-rank test of homogeneity to compare across strata.

3 RESULTS

3.1 Patient characteristics

Of the 89 consecutive patients included in this study, two-thirds were transplanted for malignant disease, and the majority received myeloablative conditioning (as shown in Table 1). The median age was 10 years (range: 0–26 years). Fifty-six received acyclovir prophylaxis and nine received ganciclovir prophylaxis, while the remaining quarter received no viral prophylaxis. Thirty-four of the 89 patients in this cohort developed HHV-6 viremia following allogeneic HSCT, yielding an incidence of 38%. The asymptomatic viremic and tissue-positive viremic groups had significantly different median viral loads ($P = .029$). The median viral load in the asymptomatic group was found to be 5140 copies/mL (3.71 log copies/mL) while the median was 136 788 copies/mL (5.14 log copies/mL) in the tissue-positive group.

3.2 Risk factors for viremia

Logistic regression revealed that the only statistically significant risk factors for viremia were stem cell donor type ($P = .0034$) and antiviral prophylaxis ($P = .036$). Patients who received unrelated donor transplants were 4.9 times more likely (95% CI 1.8–15.3) than those that received related donor transplants to develop HHV-6 viremia. Likewise, a patient who did not receive antiviral prophylaxis was 3.4 times more likely (95% CI 1.1–12.0) than a patient who did receive antiviral prophylaxis to develop viremia. Patients who developed acute GVHD were 3.1 times more likely (95% CI 1.1–8.3) than patients without acute GVHD to also have viremia.

3.3 Viremia with tissue positivity

Overall, 14 patients in this cohort exhibited severe symptoms and signs that led to further investigation to elucidate etiology, including tissue biopsy and/or fluid sampling. A summary of patients who had PCR positivity in tissues/fluids is shown in Table 2. Figure 1 illustrates various sites of HHV-6 detection. Four patients were found to have coexisting CMV in tissues as well. These four patients had statistically significant lower peak HHV-6 viral loads (<2000 copies/mL or 3.3 logs). In two of these four patients, CMV was detected in the same tissues as HHV-6.

Four of the 14 patients had encephalitis evidenced by detection of HHV-6 in the CSF. Three of the four patients with encephalitis died; however, their deaths were not directly attributable to encephalitis. One died due to relapse, one due to ARDS, and one due to multi-organ system failure thought to be secondary to complications of GVHD.

When compared to patients who were viremic and asymptomatic, patients who were viremic and tissue-positive did not exhibit any statistically significant differences with respect to gender, transplant indication, HLA matching, stem cell donor, stem cell source, intensity of conditioning regimen, and use of antiviral prophylaxis by logistic regression. However, there was a trend toward increased use of HLA-mismatched donor and cord blood as stem cell source in this group that could have contributed to increased non-relapse mortality and decreased survival (Table 1). The 95% confidence intervals were very wide, most likely

reflecting the small sample size. While peak viral load was significantly associated with tissue positivity ($P = .04$), following incorporation of these confounding variables into the multivariable logistic regression model, the effect was no longer statistically significant ($P = .08$).

3.4 Survival

Peak viral load was strongly associated with mortality ($P = .008$). However, following multivariable logistic regression factoring in diagnosis, stem cell source, donor, and conditioning regimen, the association was attenuated ($P = .058$). An ROC curve was generated to further evaluate the association of peak viral load with mortality. The AUC was 0.842 ($P < .0001$).

However, the significance of HHV-6 viremia and positivity in tissues is not well understood. Figure 2 shows survival curves of the asymptomatic viremic group and tissue-positive viremic group. The asymptomatic viremic group demonstrated 79% probability of surviving at least 12 months compared to 63% probability of surviving at least 12 months among the tissue-positive group. While the survival curves qualitatively appear quite divergent, the survival difference between the asymptomatic and tissue-positive viremic groups was not statistically significant by log-rank comparison ($P = .079$) (Figure 3).

4 DISCUSSION

This single-center retrospective cohort study of pediatric allogeneic HSCT patients demonstrates that the incidence of HHV-6 reactivation in this population was 38%. Unrelated donor stem cell source and lack of antiviral prophylaxis were found to be risk factors for developing viremia. While the presence of viremia was found to be significantly associated with acute GVHD, no such association was found with chronic GVHD. Within the viremic group, tissue positivity and presumed end-organ disease developed in 14 of 34 (41%) patients and this unique subgroup had higher NRM at 40% when compared to NRM in the control and asymptomatic groups, which was 11%; however, this difference did not reach statistical significance in our small dataset.

The reported reactivation rate of HHV-6 in children varies between 48% and 67% with rates as high as 80% reported within the first 30 days following HSCT.^{2, 23} Our use of antiviral prophylaxis for other herpesviruses, including CMV, likely decreased the rate of reactivation of HHV-6 when compared with these previous reports. Umbilical cord blood as stem cell source, HLA-mismatched transplants, younger patient age, and use of glucocorticoids have been reported as risk factors for HHV-6 reactivation.^{7, 10} Likewise, the use of myeloablative regimens was associated with increased risk of HHV-6 reactivation in a cohort of 108 adults who underwent HSCT.¹⁴ In addition, HHV-6 reactivation in HSCT patients has been found to be associated with an increase in non-relapse mortality as well as grade II to IV acute GVHD and chronic GVHD.^{2, 3} In our study, unrelated donor stem cell source and lack of antiviral prophylaxis were risk factors for development of viremia. We also found an association of HHV-6 reactivation with acute GVHD but not chronic GVHD.

Encephalitis is the foremost cause of serious morbidity and mortality secondary to HHV-6 in HSCT patients.^{24–28} Other clinical complications well known to occur with HHV-6 reactivation are fever, rash, delayed engraftment following HSCT, and pneumonia.^{7, 23, 29–32} A recent report found that HHV-6 was detected in a third of cases previously classified as idiopathic pneumonia syndrome, suggesting that HHV-6 may be an unidentified cause of some previously described clinical syndromes.¹³ Diarrhea, hepatitis, and hemorrhagic cystitis secondary to HHV-6 reactivation have been described in case reports.^{23, 29, 33, 34} Moreover, delayed complications with HHV-6 have also been described including pure red cell aplasia and secondary graft failure.³⁵

In our examination of the tissue-positive viremic group, there was a wide spectrum of end organs involved. In addition to the central nervous system, lungs, and bone marrow, HHV-6 was found in pericardial fluid, gallbladder, sinuses, and the gastrointestinal tract. Six of fourteen patients had more than one site that tested positive for HHV-6, while the remainder had single organ involvement. Interestingly, at the time of tissue detection of HHV-6, with the exception of one patient, the virus was only detectable at very low copy numbers or not at all in blood, as has also been described by Greco et al.³⁶ We conclude that while preemptive antiviral treatment may enable clearance of the virus from blood, HHV-6 seems to persist in tissues.

We investigated the coexistence of CMV and HHV-6 in this cohort as these two herpesviruses have been reported to be present together,^{4, 37} and found that only four of 14 symptomatic patients had coexistent CMV and HHV-6 positivity. Notably, the four patients with coexistent CMV had lower peak HHV-6 viral loads when compared to those without simultaneous CMV viremia. In two of these four patients, CMV was detected in the same tissues as HHV-6. The relationship between the two viruses is not well understood and remains controversial.^{15, 38–40}

Previously published studies have reported that high viral loads are associated with delayed platelet engraftment^{29, 41} and encephalitis.⁷ Additionally, use of high-dose intravenous gamma globulin (IVIG) was found to be associated with lower viral load in one study.²⁹

We found that patients with high peak viral loads, a previously described predictor of mortality,⁴² were at higher risk of mortality compared to those with lower peak viral loads in our cohort as well. Peak viral loads were also higher in patients who had tissue-positive viremia. The strong relationship between viral load and mortality demonstrated in the ROC curve in figure 2 suggests that those with a high viral load represent a particularly high-risk population, warranting special attention.

Little is known about prophylaxis of HHV-6 reactivation. However, some studies have reported that ganciclovir and foscarnet are safe and possibly effective in preventing HHV-6 reactivation.^{19, 43} In our cohort, antiviral prophylaxis was not implemented for HHV-6 but for other herpesviruses, and we found that use of prophylaxis was associated with a lower rate of HHV-6 reactivation than previously reported. This finding is particularly surprising given that the majority of patients received acyclovir, which is not active against HHV-6.

However, due to small numbers of patients who received ganciclovir as part of prophylaxis, the efficacy of specific prophylactic agents could not be compared.

The indication and appropriate regimen for treatment of HHV-6 reactivation are not well defined. Ganciclovir, foscarnet, and cidofovir have been proposed as agents for treatment of HHV-6 infection.^{19, 44–46} In our cohort, patients with HHV-6 reactivation were preemptively treated. The duration of treatment varied based on response to treatment as defined by persistence of viremia and symptomatology. In our experience, there was increased non-relapse mortality in the tissue-positive viremic group, even with the use of antiviral treatment.

There are several limitations of our study. Firstly, it is a retrospective single-center cohort study with limited numbers, which has limited power to detect relationships to the risk factors evaluated. Moreover, although we did not find any statistically significant differences between those with asymptomatic viremia and those with tissue positivity, the large point estimates, particularly for stem cell source, suggest that there may be an association. This association may suggest confounding and could decrease the strength of the relationships presented. Secondly, while we presume that a detectable viral load is due to HHV-6 reactivation, it is possible that a small subset of our patients actually had primary infection with HHV-6. We did not test for HHV-6 prior to transplant. Chromosomal integration of HHV-6, defined as HHV-6 DNA copy levels $>1 \times 10^6$ genomes/mL of whole blood, has been described in less than 1% of patients undergoing solid organ transplant and HSCT.^{47, 48} Only one of our patients exceeded the threshold of 1 million copies/mL; given that he was both symptomatic and demonstrated a prompt decrease in plasma viral load following initiation of antiviral agents, the clinical situation is not consistent with chromosomal integration of HHV-6. There is also the possibility that HHV-6 was detected in tissues due to blood contamination in the six patients who had a detectable viral load in the plasma at the time of end-organ sampling.

Finally, the significance of detection of HHV-6 in tissues still remains a matter of debate,⁴² and it is not completely clear that the virus had a causal role in producing the symptoms that initiated tissue sampling. We did not consistently obtain tissue samples from those patients who were asymptomatic to verify that the finding of tissue positivity was only present in those patients who were symptomatic. However, detection of HHV-6 and severe symptoms with associated high non-relapse mortality is certainly concerning and suggests that further investigation is warranted. While deposition of HHV-6 in the tissues could have occurred at any point, the temporal relationship, in which viremia preceded the development of symptoms, and the improvement that followed the initiation of antiviral treatment reinforces the role HHV-6 may play in these tissues. In two patients, CMV coexisted in the tissues with HHV-6 and could have caused or contributed to symptoms.

In conclusion, this is the first study to report the presence of HHV-6 by PCR in end organs in a high-risk group of pediatric HSCT patients with a high NRM at 1 year. Due to limited numbers of patients, our study did not show a significant difference in survival in this subgroup compared to patients with viremia who did not demonstrate tissue positivity. However, these findings are compelling enough to warrant prospective studies to better

understand the clinical implications of HHV-6 reactivation, and particularly to identify a potentially high-risk group of immunocompromised patients who might need more aggressive monitoring and treatment. Additionally, we show that peak viral load is an important factor linked to overall survival. Prophylactic strategies should be investigated in these high-risk patients and high viral loads warrant prompt attention. Better diagnostic modalities, prophylaxis, and treatment guidelines may need to be established based on the findings of larger trials in this area.

Abbreviations

ARDS	acute respiratory distress syndrome
AUC	area under the curve
CI	confidence interval
CMV	cytomegalovirus
CSF	cerebrospinal fluid
GI	gastrointestinal
GVHD	graft-versus-host disease
HHV-6	human herpesvirus 6
HSCT	hematopoietic stem cell transplant
HSV	herpes simplex virus
NRM	non-relapse mortality
ROC	receiver operating characteristic
VZV	varicella zoster virus

References

1. Gompels UA, Macaulay HA. Characterization of human telomeric repeat sequences from human herpesvirus 6 and relationship to replication. *J Gen Virol*. 1995; 76:451–458. [PubMed: 7844567]
2. de Pagter PJ, Schuurman R, Visscher H, et al. Human herpes virus 6 plasma DNA positivity after hematopoietic stem cell transplantation in children: an important risk factor for clinical outcome. *Biol Blood Marrow Transplant*. 2008; 14:831–839. [PubMed: 18541204]
3. Verhoeven DH, Claas EC, Jol-van der Zijde CM, et al. Reactivation of human herpes virus-6 after pediatric stem cell transplantation: risk factors, onset, clinical symptoms and association with severity of acute graft-versus-host disease. *Pediatr Infect Dis J*. 2015; 34:1118–1127. [PubMed: 26132825]
4. Humar A, Asberg A, Kumar D, et al. An assessment of herpesvirus co-infections in patients with CMV disease: correlation with clinical and virologic outcomes. *Am J Transplant*. 2009; 9:374–381. [PubMed: 19120074]
5. Chevallier P, Hebia-Fellah I, Planche L, et al. Human herpes virus 6 infection is a hallmark of cord blood transplant in adults and may participate to delayed engraftment: a comparison with matched unrelated donors as stem cell source. *Bone Marrow Transplant*. 2010; 45:1204–1211. [PubMed: 19935727]

6. Mori Y, Miyamoto T, Nagafuji K, et al. High incidence of human herpes virus 6-associated encephalitis/myelitis following a second unrelated cord blood transplantation. *Biol Blood Marrow Transplant.* 2010; 16:1596–1602. [PubMed: 20685258]
7. Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clin Infect Dis.* 2005; 40:932–940. [PubMed: 15824982]
8. Singh N, Paterson DL. Encephalitis caused by human herpesvirus-6 in transplant recipients: relevance of a novel neurotropic virus. *Transplantation.* 2000; 69:2474–2479. [PubMed: 10910264]
9. Miyashita N, Endo T, Onozawa M, et al. Risk factors of human herpesvirus 6 encephalitis/myelitis after allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis.* 2017; 19:e12682.
10. Yamane A, Mori T, Suzuki S, et al. Risk factors for developing human herpesvirus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its association with central nervous system disorders. *Biol Blood Marrow Transplant.* 2007; 13:100–106. [PubMed: 17222758]
11. Buchbinder S, Elmaagacli AH, Schaefer UW, Roggendorf M. Human herpesvirus 6 is an important pathogen in infectious lung disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 2000; 26:639–644. [PubMed: 11035370]
12. Cone RW, Hackman RC, Huang ML, et al. Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. *N Engl J Med.* 1993; 329:156–161. [PubMed: 8390614]
13. Seo S, Renaud C, Kuypers JM, et al. Idiopathic pneumonia syndrome after hematopoietic cell transplantation: evidence of occult infectious etiologies. *Blood.* 2015; 125:3789–3797. [PubMed: 25918347]
14. de Pagter PJ, Schuurman R, Keukens L, et al. Human herpes virus 6 reactivation: important predictor for poor outcome after myeloablative, but not non-myeloablative allo-SCT. *Bone Marrow Transplant.* 2013; 48:1460–1464. [PubMed: 23771003]
15. Jeulin H, Agrinier N, Guery M, et al. Human herpesvirus 6 infection after allogeneic stem cell transplantation: incidence, outcome, and factors associated with HHV-6 reactivation. *Transplantation.* 2013; 95:1292–1298. [PubMed: 23514960]
16. Pichereau C, Desseaux K, Janin A, et al. The complex relationship between human herpesvirus 6 and acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2012; 18:141–144. [PubMed: 21801705]
17. Hentrich M, Oruzio D, Jager G, et al. Impact of human herpesvirus-6 after haematopoietic stem cell transplantation. *Br J Haematol.* 2005; 128:66–72. [PubMed: 15606551]
18. Takemoto Y, Takatsuka H, Wada H, et al. Evaluation of CMV/human herpes virus-6 positivity in bronchoalveolar lavage fluids as early detection of acute GVHD following BMT: evidence of a significant relationship. *Bone Marrow Transplant.* 2000; 26:77–81. [PubMed: 10918408]
19. Tokimasa S, Hara J, Osugi Y, et al. Ganciclovir is effective for prophylaxis and treatment of human herpesvirus-6 in allogeneic stem cell transplantation. *Bone Marrow Transplant.* 2002; 29:595–598. [PubMed: 11979309]
20. Cone RW, Huang ML, Corey L, Zeh J, Ashley R, Bowden R. Human herpesvirus 6 infections after bone marrow transplantation: clinical and virologic manifestations. *J Infect Dis.* 1999; 179:311–318. [PubMed: 9878013]
21. Milano F, Pergam SA, Xie H, et al. Intensive strategy to prevent CMV disease in seropositive umbilical cord blood transplant recipients. *Blood.* 2011; 118:5689–5696. [PubMed: 21937692]
22. Ogata M, Satou T, Kadota J, et al. Human herpesvirus 6 (HHV-6) reactivation and HHV-6 encephalitis after allogeneic hematopoietic cell transplantation: a multicenter, prospective study. *Clin Infect Dis.* 2013; 57:671–681. [PubMed: 23723198]
23. Dulery R, Salleron J, Dewilde A, et al. Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study. *Biol Blood Marrow Transplant.* 2012; 18:1080–1089. [PubMed: 22212513]
24. Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. *N Engl J Med.* 1994; 330:1356–1360. [PubMed: 8152447]

25. Hill JA, Koo S, Guzman Suarez BB, et al. Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6-associated acute limbic encephalitis: a cohort analysis. *Biol Blood Marrow Transplant*. 2012; 18:1638–1648. [PubMed: 22564265]
26. Zerr DM, Gooley TA, Yeung L, et al. Human herpesvirus 6 reactivation and encephalitis in allogeneic bone marrow transplant recipients. *Clin Infect Dis*. 2001; 33:763–771. [PubMed: 11512080]
27. Vu T, Carrum G, Hutton G, Heslop HE, Brenner MK, Kamble R. Human herpesvirus-6 encephalitis following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2007; 39:705–709. [PubMed: 17401392]
28. Tanaka M, Taguchi J, Hyo R, et al. Human herpesvirus-6 encephalitis after unrelated cord blood transplantation. *Leuk Lymphoma*. 2005; 46:561–566. [PubMed: 16019484]
29. Ljungman P, Wang FZ, Clark DA, et al. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J Haematol*. 2000; 111:774–781. [PubMed: 11122137]
30. Cone RW, Huang ML, Hackman RC. Human herpesvirus 6 and pneumonia. *Leuk Lymphoma*. 1994; 15:235–241. [PubMed: 7866272]
31. Cone RW. Human herpesvirus 6 as a possible cause of pneumonia. *Semin Respir Infect*. 1995; 10:254–258. [PubMed: 8668853]
32. Yata K, Nakajima M, Takemoto Y, et al. [Pneumonitis with a bronchiolitis obliterans organizing pneumonia-like shadow in a patient with human herpes virus-6 viremia after allogeneic bone marrow transplantation]. *Kansenshogaku Zasshi*. 2002; 76:385–390. [PubMed: 12073575]
33. Kim YJ, Kim DW, Lee DG, et al. Human herpesvirus-6 as a possible cause of encephalitis and hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. *Leukemia*. 2002; 16:958–959. [PubMed: 11986964]
34. Kuribayashi K, Matsunaga T, Iyama S, et al. Human herpesvirus-6 hepatitis associated with cyclosporine-A encephalitis after bone marrow transplantation for chronic myeloid leukemia. *Intern Med*. 2006; 45:475–478. [PubMed: 16679705]
35. Lagadinou ED, Marangos M, Liga M, et al. Human herpesvirus 6-related pure red cell aplasia, secondary graft failure, and clinical severe immune suppression after allogeneic hematopoietic cell transplantation successfully treated with foscarnet. *Transpl Infect Dis*. 2010; 12:437–440. [PubMed: 20561301]
36. Greco R, Crucitti L, Noviello M, et al. Human herpesvirus 6 infection following haploidentical transplantation: immune recovery and outcome. *Biol Blood Marrow Transplant*. 2016; 22:2250–2255. [PubMed: 27697585]
37. Razonable RR, Rivero A, Brown RA, et al. Detection of simultaneous beta-herpesvirus infections in clinical syndromes due to defined cytomegalovirus infection. *Clin Transplant*. 2003; 17:114–120. [PubMed: 12709076]
38. Zerr DM, Boeckh M, Delaney C, et al. HHV-6 reactivation and associated sequelae after hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2012; 18:1700–1708. [PubMed: 22641196]
39. Tormo N, Solano C, de la Camara R, et al. An assessment of the effect of human herpesvirus-6 replication on active cytomegalovirus infection after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2010; 16:653–661. [PubMed: 20005968]
40. Hill JA, Mayer BT, Xie H, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. *Blood*. 2017; 129:2316–2325. [PubMed: 28209721]
41. Violago L, Jin Z, Bhatia M, et al. Human herpesvirus-6 viremia is not associated with poor clinical outcomes in children following allogeneic hematopoietic cell transplantation. *Pediatr Transplant*. 2015; 19:737–744. [PubMed: 26329541]
42. Hill JA, Boeckh MJ, Sedlak RH, Jerome KR, Zerr DM. Human herpesvirus 6 can be detected in cerebrospinal fluid without associated symptoms after allogeneic hematopoietic cell transplantation. *J Clin Virol*. 2014; 61:289–292. [PubMed: 25066883]

43. Ishiyama K, Katagiri T, Ohata K, et al. Safety of pre-engraftment prophylactic foscarnet administration after allogeneic stem cell transplantation. *Transpl Infect Dis.* 2012; 14:33–39. [PubMed: 21794043]
44. Ishiyama K, Katagiri T, Hoshino T, Yoshida T, Yamaguchi M, Nakao S. Preemptive therapy of human herpesvirus-6 encephalitis with foscarnet sodium for high-risk patients after hematopoietic SCT. *Bone Marrow Transplant.* 2011; 46:863–869. [PubMed: 20838386]
45. Ogata M, Satou T, Inoue Y, et al. Foscarnet against human herpesvirus (HHV)-6 reactivation after allo-SCT: breakthrough HHV-6 encephalitis following antiviral prophylaxis. *Bone Marrow Transplant.* 2013; 48:257–264. [PubMed: 22750998]
46. Pohlmann C, Schetelig J, Reuner U, et al. Cidofovir and foscarnet for treatment of human herpesvirus 6 encephalitis in a neutropenic stem cell transplant recipient. *Clin Infect Dis.* 2007; 44:e118–e120. [PubMed: 17516391]
47. Lee SO, Brown RA, Razonable RR. Clinical significance of pretransplant chromosomally integrated human herpesvirus-6 in liver transplant recipients. *Transplantation.* 2011; 92:224–229. [PubMed: 21629177]
48. Lee SO, Brown RA, Razonable RR. Chromosomally integrated human herpesvirus-6 in transplant recipients. *Transpl Infect Dis.* 2012; 14:346–354. [PubMed: 22321264]

FIGURE 1.

Figure 1 shows organs and tissues that were positive for HHV-6 by PCR. CSF, cerebrospinal fluid; GI, gastrointestinal

FIGURE 2.

Receiver operating characteristic (ROC) curve demonstrating a strong association between peak viral load and mortality with an area under the curve (AUC) of 0.84 ($P < .0001$)

FIGURE 3. Survival of asymptomatic viremic (red) and symptomatic tissue-positive viremic (blue) groups ($P = .072$)

TABLE 1

Patient characteristics

	Total N	No reactivation n (%)	Asymptomatic viremia n (%)	Viremia associated with tissue positivity n (%)	Odds ratio ^a	95% CI	P-value ^a
Gender							
Male	49	26 (47)	12 (60)	11 (79)	0.42	0.06–2.35	.3
Female	40	29 (53)	8 (40)	3 (21)			
Indication							
Malignant	60	36(65)	14 (70)	10 (71)	0.94	0.15–5.25	1.0
Non-malignant	29	19(35)	6 (30)	4 (29)			
HLA							
Matched	50	35 (64)	10 (50)	5 (36)	1.77	0.36–9.37	.50
Mismatched	39	20 (36)	10 (50)	9 (64)			
Donor							
Related	33	28 (51)	3 (15)	2 (14)	1.05	0.10–14.48	1.0
Unrelated	56	27 (49)	17 (85)	12 (86)			
Source							
Bone marrow	59	41 (75)	13 (65)	5 (36)	2.83	0.59–15.19	.18
Cord blood	28	12 (22)	7 (35)	9 (64)			
Other	3	2 (3)	1 (5)	0 (0)			
Conditioning				13 (93)			
Myeloablative	76	47 (85)	16 (80)		0.32	0.01–3.75	.40
Non-myeloablative	13	8 (15)	4 (20)	1 (7)			
Antiviral prophylaxis							
Acyclovir ^b	56	33 (60)	14 (70)	9 (64)	0.95	0.07–9.65	1.0
Ganciclovir	9	3 (5)	3 (15)	3 (21)			
None	24	19 (35)	3 (15)	2 (14)			
Total		55	20	14			

^aPatients with viremia and tissue positivity were compared to asymptomatic viremic patients to evaluate whether there was any association with any of these risk factors.

9.
Of note, acyclovir is not thought to be active against HHV-6.
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TABLE 2

Summary of 14 patients with viremia and tissue positivity

Subject	Age (y)/Gender	Source	HLA	Peak viral load in copies/mL (log scale)	Day of peak	Site of end-organ disease (day)	Viral load at sampling	Outcome
1	12/M	Cord	Mismatched	1136 471(6.06)	13	Encephalitis (22)	344 488 (5.54)	Relapse
2	11/M	Cord	Mismatched	28 485 (4.45)	181	Encephalitis (230)	<500 ^a (<2.70) to 1155 (3.06)	Multi-organ system failure
3	18/M	Cord	Mismatched	225 899 (5.35)	15	Encephalitis (50)	<500 ^a (<2.70)	Respiratory failure
4	5/F	Marrow	Matched	<1000(<3) ^a	41	Pericarditis (90)	0	Recovered
5	4/M	Marrow	Mismatched	183 371 (5.26)	17	Encephalitis (22)	0	Recovered
6	3/M	Marrow	Mismatched	<1000(<3) ^a	14	Cholecystitis (248)		Recovered
7	10/M	Cord	Mismatched	114 575 (5.06)	15	Pneumonitis (286, 342)	0 to 11 100 (4.05)	Pulmonary hemorrhage and bacteremia
						Pericardial fluid (286)		
						Marrow (427)		
8	2/M	Cord	Matched	159 000 (5.20)	13	Red cell aplasia (265)	0	Recovered
9	7/F	Cord	Matched	1810 (3.26)	33	Pneumonitis (97)	0	Recovered
						Gastritis (97)		
10	6/M	Cord	Mismatched	381 000 (5.58)	15	Pneumonitis (42, 68)	0 to <1000(<3) ^a	Respiratory failure
						Gastritis (42)		
						Bone marrow (30, 60)		
11	60/F	Cord	Matched	566 000 (5.75)	11	Delayed engraftment (30)	0	Recovered
12	3/M	Marrow	Mismatched	6 600 000 (6.82)	22	Delayed engraftment (51)	0 to <1000(<3) ^a	Recovered
						Pericardial effusion (48)		
						Gastritis (51)		
13	16/M	Marrow	Matched	1060 (3.03)	11	Cholecystitis (71)	0	Recovered
14	18/M	Cord	Mismatched	30 600 (4.49)	15	Colitis (22)	0	Recovered

^aDenotes a sample in which HHV-6 was detected, but at a level that could not be quantified.