

ARTICLE



Risk factors and long-term outcomes for human herpesvirus 6 encephalitis in the early period after allogeneic stem cell transplantation

Yi Yu^{1,2,3,4}, Weihao Chen^{1,2,3,4}, Huarui Fu^{1,2,3}, Jimin Shi^{1,2,3}, Yi Luo^{1,2,3}, Jian Yu^{1,2,3}, Xiaoyu Lai^{1,2,3}, Lizhen Liu^{1,2,3}, Yishan Ye^{1,2,3}, Congxiao Zhang^{1,2,3}, He Huang^{1,2,3,4}✉ and Yanmin Zhao^{1,2,3,4}✉

© The Author(s), under exclusive licence to Springer Nature Limited 2024

Human herpesvirus 6 (HHV6) encephalitis is a rare but life-threatening complication for patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, reports on susceptibility factors and clinical outcomes are limited. We enrolled HHV6 encephalitis patients following allo-HSCT between 2018 and 2022, then conducted a 1:4 nested case-control cohort study to evaluate risk factors and long-term outcomes. Among 1350 patients, 20 (1.48%) developed HHV6 encephalitis, with a median onset time of 25.5 days after HSCT. Patient age <30 (odds ratio [OR], 3.24, $P = 0.016$) and NK cell count <115/ul at 21 days (OR, 6.07, $P = 0.018$) were identified as independent risk factors in multivariate analysis. Moreover, the HHV6 encephalitis group was significantly associated with higher incidence of grade II-IV graft-versus-host disease (aGVHD) (hazard ratio [HR], 5.52, $P < 0.001$) and transplant-associated microangiopathy (HR, 9.86, $P < 0.001$), and demonstrated a significantly higher non-relapse mortality (NRM) (HR, 5.28, $P = 0.004$) and a lower overall survival (HR, 4.34, $P = 0.001$) or progression-free survival (HR, 3.94, $P = 0.001$) compared to control group. In conclusion, patients <30 years old or with delayed NK cell recovery are more susceptible to HHV6 encephalitis after allo-HSCT, and patients with HHV6 encephalitis after transplantation have poorer clinical outcomes.

Bone Marrow Transplantation; <https://doi.org/10.1038/s41409-024-02332-0>

INTRODUCTION

Human herpesvirus 6 (HHV6) was first identified in 1986 [1], encoding several conserved homologous genes with cytomegaloviruses identified in herpesvirus families [2]. The virus is generally ubiquitous, mostly acquired in the earliest stages of life or transmitted through saliva, and exhibits a preference for infecting activated CD4⁺ T cells. It can be divided into two non-overlapping subgroups: HHV6A and HHV6B [3, 4]. Unlike other herpesviruses, this virus has a 1% chance of integrating into the telomeres of host cell chromosomes, passing it on to the next generation (known as ciHHV-6, chromosomally integrated HHV-6), which has been confirmed to be completely inactivated [5]. Besides, three main routes are involved in causing an active HHV6 infection: primary infection, reactivation, or exogenous infection [4]. However, the enduring latency characteristic of the virus and the observation that most infected individuals remain asymptomatic frame the issue in a relatively less alarming light [5]. But it differs in allogeneic hematopoietic stem cell transplantation (HSCT) patients, who exhibit a human herpesvirus 6 (HHV6) reactivation rate of up to 50% under circumstances where testing is not performed routinely. Upon reactivation, variable clinical manifestations could be observed [1], while HHV6 encephalitis is rare. HHV6 encephalitis was observed more frequently among

patients receiving umbilical cord blood transplantation (4.9–21.4%), and less frequently in bone marrow/peripheral blood stem cell transplantation (0–11.6%) [2]. Other factors for developing HHV6 encephalitis include T-cell-depleted allografts, mismatched or unrelated donors, aGVHD, and treatment with glucocorticoids [6]. To date, the prevalence of HHV6 encephalitis following T-cell replete allogeneic peripheral blood stem cell transplantation (PB-HSCT), as well as its relationship with transplantation outcomes, remains unclear. Aiming to resolve this issue, we initiated a retrospective nested-control study through the transplant cohort of our center to identify potential risk factors, onset characteristics, complications, and the long-term prognosis of HHV6 encephalitis.

MATERIALS AND METHODS

Patients

We reviewed all consecutive patients who underwent PB-HSCT for hematological malignancies and were ≥ 12 years old between January 2018 and June 2022 at our center. Written informed consent was obtained from each patient or a relative before transplantation. The exclusion criteria were: (1) secondary or more than secondary transplantation; (2) death during the conditioning period. A total of 1350 patients were enrolled. Twenty of these patients met the criteria for the diagnosis of

¹Bone Marrow Transplantation Center of The First Affiliated Hospital & Liangzhu Laboratory, Zhejiang University School of Medicine, Hangzhou, Zhejiang, PR China. ²Institute of Hematology, Zhejiang University, Hangzhou, Zhejiang, PR China. ³Zhejiang Province Engineering Research Center for Stem Cell and Immunity Therapy Zhejiang Province Engineering Laboratory for Stem Cell and Immunity Therapy, Hangzhou, Zhejiang, PR China. ⁴These authors contributed equally: Yi Yu, Weihao Chen, He Huang, Yanmin Zhao. ✉email: huanghe@zju.edu.cn; yanminzhao@zju.edu.cn

Received: 15 January 2024 Revised: 11 June 2024 Accepted: 13 June 2024

Published online: 27 June 2024

HHV6 encephalitis. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of the First Affiliated Hospital of Zhejiang University. The authors had full access to the data and assumed responsibility for its authenticity. The end of the follow-up was August 30, 2023.

Control selection

Once the HHV6 encephalitis patient was identified, patients for the control group were chosen at a ratio of 1:4. The selection of the control group is mainly based on two points: (1) a male-to-female ratio of 1:1; (2) the transplantation time is within a week before or after the transplantation time of the case, but if in certain extreme circumstances where sex matching cannot be accomplished as described, priority will be afforded to those with matching transplantation times. MRI and psychiatric consultation are performed before HSCT to exclude preexisting central nervous system (CNS) diseases, and all the controls enrolled are free of any abnormal mental symptoms during the time.

Definitions

Patients were classified as standard and high-risk according to the criteria described previously [7, 8], on the basis of cytogenetic abnormalities, white blood cell (WBC) count at diagnosis, response to induction chemotherapy, and relapse after first complete remission (CR1). HHV-6 encephalitis was diagnosed if the patient satisfied all the following criteria [9]: (1) presence of neurological symptoms, such as disorientation as to time and place, loss of consciousness, abnormal behaviors, memory loss, convulsions, or dysesthesia not attributable to peripheral neuropathy (2) HHV-6 DNA detection in CSF, and (3) absence of other identified causes of CNS dysfunction. Neutrophil engraftment was defined as the achievement of an absolute neutrophil count $\geq 500/\mu\text{l}$ for 3 consecutive days. Platelet engraftment was defined as a platelet count $\geq 20,000/\mu\text{l}$ for three consecutive days and no transfusion dependence [9]. Acute GVHD was defined and graded based on the Mount Sinai Acute GVHD International Consortium consensus [10]. Hematopoietic stem cell transplant-associated microangiopathy (TMA) was diagnosed according to the International Working Group (IWG) consensus [11]. Overall survival (OS) was defined as the duration from transplant until death from any cause. Progression-free survival (PFS) was defined as the time from transplant until death or relapse. Relapse was defined as the reappearance of leukemia in patients who had previously achieved complete remission. Complete remission was

defined as $<5\%$ leukemic cells in the bone marrow and the absence of leukemia cells in peripheral blood or extramedullary sites. Non-relapse mortality (NRM) was defined as death resulting from causes unrelated to underlying malignancy relapse.

Transplantation protocols

Human leukocyte antigen (HLA) allele typing was performed at HLA-A, HLA-B, HLA-C, HLA-DQ, and HLA-DRB1. The conditioning regimens utilized at our institution have been described in detail previously [12]. All patients received peripheral blood stem cell transplantation mobilization by recombinant human granulocyte-colony stimulating factor (G-CSF) (donors used G-CSF 5–10 $\mu\text{g}/(\text{kg} \times \text{d})$ continuous subcutaneous injection for 4–5 days and then collected peripheral blood stem cells. The GVHD prophylaxis regimen consisted of cyclosporine, short-course methotrexate, and mycophenolate mofetil. Patients developing aGVHD were treated with methylprednisolone as the first-line therapy. Second-line therapies included ruxolitinib, TNF-alpha receptor inhibitors, and mesenchymal stem cells. Supportive care included G-CSF administration until neutrophil engraftment and prophylaxis with levofloxacin, fluconazole or posaconazole, and acyclovir for infections.

Treatment of HHV6 encephalitis and response

Immediately after diagnosis, antiviral drugs like ganciclovir, or sodium foscarnet, or a combination of both will be applied. A complete response was defined by the resolution of all clinical signs and symptoms and radiologic findings, partial response was defined by CTCAE grade ≥ 1 and no worsening radiologic and clinical findings and failure of therapy was defined by worsening disease.

Statistical analysis

Continuous variables were compared using the Mann–Whitney U test or t-test, while the comparisons of categorical variables were performed by the chi-square or Fisher's exact test. The cutoff values for continuous variables were determined by receiver operating characteristic (ROC) curve analysis and Youden's index. Cumulative incidences (CI) were estimated for aGVHD, TMA, NRM, and relapse to accommodate competing risks. Relapse was a competing risk for NRM. Relapse and death from any cause were competing risks for HHV-6 encephalitis, aGVHD, and TMA. The Gray method was applied to compare the cumulative incidence of competing-risk endpoints. OS and PFS (progression-free survival) curves were plotted

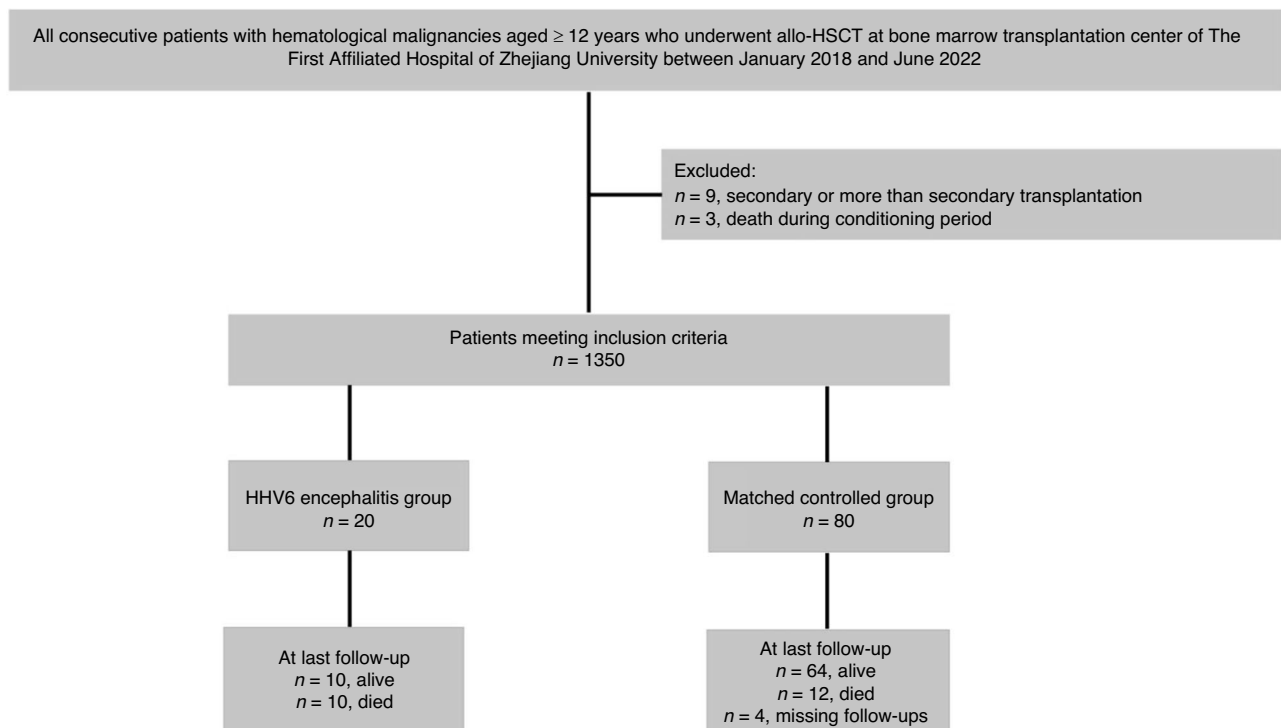


Fig. 1 Consort diagram. Enrolled procedures of the patients.

Table 1. Comparison of clinical characteristics between HHV6 encephalitis patients and control patients.

	Total (n = 100)	HHV-6 encephalitis group (n = 20)	Control group (n = 80)
Median age (years, range)	36 (12–67)	28 (18–58)	40 (12–67)
Sex, n (%)			
Female	53 (53)	13 (65)	40 (50)
Male	47 (47)	7 (35)	40 (50)
Primary disease, n (%)			
AML/MDS	65 (65)	10 (50)	55 (68.8)
ALL/Lymphoma	32 (32)	9 (45)	23 (28.7)
CML/MPN	3 (3)	1 (5)	2 (2.5)
Risk classification, n (%)			
Standard	65 (65)	12 (60)	53 (66.3)
High risk	35 (35)	8 (40)	27 (33.7)
HCT-CI			
0–2	87 (87)	17 (85)	70 (87.5)
>2	13 (13)	3 (15)	10 (12.5)
HLA match, n (%)			
HLA-identical sibling transplant	10 (10)	0 (0)	10 (12.5)
10/10 Unrelated transplant	10 (10)	3 (15)	7 (8.8)
Haploidentical transplant	80 (80)	17 (85)	63 (78.7)
Median age of donors (years, range)	33 (12–56)	36 (14–53)	33 (12–56)
Donor–patient sex matching, n (%)			
Female–male	14 (14)	5 (25)	9 (11.3)
Others	86 (86)	15 (75)	71 (88.7)
GVHD prophylaxis, n (%)			
ATG+	91 (91)	20 (100)	71 (88.8)
MMF+	87 (87)	18 (90)	69 (86.3)
Conditioning regimen, n (%)			
MAC	87 (87)	19 (95)	68 (85)
RIC	13 (13)	1 (5)	12 (15)
Remission status at time of HCT, n (%)			
CR1	76 (76)	15 (75)	61 (76.3)
≥CR2	18 (18)	5 (25)	13 (16.2)
No CR	6 (6)	0 (0)	6 (7.5)
MNCs in transplant ($\times 10^8$ /kg, range)	12.1 (3.7–38.0)	10.7 (3.7–19.9)	12.4 (5.3–38.0)
CD34 ⁺ cells in transplant ($\times 10^6$ /kg, range)	5.2 (1.7–17.3)	4.2 (1.7–9.3)	5.3 (1.9–17.3)
CD3 ⁺ cells at 21 days after HSCT (μ l, range)	158 (0–3075)	123 (1–496)	164 (0–3075)
CD19 ⁺ cells at 21 days after HSCT (μ l, range)	8 (0–44)	12 (0–42)	8 (0–44)

Table 1. continued

	Total (n = 100)	HHV-6 encephalitis group (n = 20)	Control group (n = 80)
NK cells at 21 days after HSCT (μ l, range)	104 (0–728)	34 (0–152)	121 (0–728)
Median time of platelet engraftment (days, range)	13 (7–54)	13 (10–54)	13 (7–45)
Median time of neutrophil engraftment (days, range)	13 (9–26)	12 (10–26)	12 (9–20)

HHV6 human herpesvirus type 6, AML acute myeloid leukemia, MDS myelodysplastic syndrome, ALL acute lymphoblastic leukemia, CML chronic myelogenous leukemia, MPN myeloproliferative neoplasms, HCT-CI hematopoietic cell transplantation-specific comorbidity index, HLA human leukocyte antigen, GVHD graft versus host disease, ATG anti-thymocyte globulin, MMF mycophenolate mofetil, MAC myeloablative conditioning, RIC reduced intensity conditioning, HCT/HSCT hematopoietic stem cell transplantation, CR complete remission, CR1 first complete remission, CR2 second complete remission, MNCs mononuclear cells, CD cluster of differentiation, NK cells natural killer cells.

using the Kaplan–Meier method and the log-rank test. All variables with a $P < 0.1$ in the univariate analysis were included in the multivariate analysis. In multivariate analysis, a Cox proportional hazard regression model was adopted for OS and PFS, while the Fine-Gray proportional hazard regression model was constructed for competing-risk endpoints. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 26 (Chicago, IL, USA) and R software (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Incidence of HHV-6 encephalitis and patient characteristics

The patient selection process is illustrated in Fig. 1. A total of 1350 eligible patients between January 2018 and January 2023 were included, 20 (1.48%) were finally diagnosed with HHV-6 encephalitis. The patients' characteristics are listed in Table 1. The HHV6 encephalitis group encompassed 13 males and 7 females, with a median age of 28 years (range, 18–58), including 6 with acute myeloid leukemia (AML), 4 with myelodysplastic syndromes (MDS), 6 with acute lymphoblastic leukemia (ALL), 3 with lymphoma, and 1 with chronic myelogenous leukemia (CML). According to the disease risk stratification before HSCT, twelve patients (60%) were classified as standard risk and eight (40%) as high risk. Of the 20 patients with HHV6 encephalitis, the majority (17, 85%) underwent haploidentical transplantation, and 3 (15%) underwent unrelated donor HSCT, while none received HLA-identical sibling transplantation. The usage ratio of ATG and MMF for GVHD prophylaxis was 20 (100%) and 18 (90%), respectively. The median onset of HHV-6 encephalitis was 25.5 (range, 21–56) days after HSCT.

Risk factors of HHV-6 encephalitis

We performed a nested case-control study to identify the risk factors. The control cohort of 80 recipients was successfully matched as previously described. The baseline clinical characteristics of patients with HHV6 encephalitis and their controls are listed in Table 1. There was no significant difference in diagnosis of the primary disease, disease risk classification, hematopoietic cell transplantation-specific comorbidity index (HCT-CI), HLA match, donor-patient sex matching, acute GVHD prophylaxis regimens,

Table 2. Univariate analysis and multivariate analysis of different factors between patients of both groups.

	Univariate	Multivariate	
	P-value	Odds ratio (95% CI)	P-value
Age < 30	0.012	3.24 (1.25–8.42)	0.016
Sex	0.201		
Female			
Male			
Primary disease	0.380		
AML/MDS			
ALL/Lymphoma			
CML/MPN			
Risk classification, n (%)	0.637		
Standard			
High risk			
HCT-CI	0.754		
0–2			
>2			
HLA match	0.836		
HLA-identical sibling transplant			
10/10 Unrelated transplant			
Haploidentical transplant			
Median age of donors (years, range)	0.451		
Donor–patient sex matching	0.083		
Female–male		1.00 [Reference]	
Others		0.94 (0.34–2.60)	0.900
GVHD prophylaxis			
ATG+	0.696		
MMF+	0.712		
Conditioning regimen	0.250		
MAC			
RIC			
Remission status at time of HCT	0.642		
CR1			
≥CR2			
No CR			
MNCs in transplant	0.044	0.91 (0.80–1.03)	0.140
CD34 ⁺ cells in transplant	0.026	0.85 (0.65–1.10)	0.220
CD3 ⁺ cells at 21 days after HSCT	0.340		
CD19 ⁺ cells at 21 days after HSCT	0.233		
NK cells < 115 at 21 days after HSCT	0.006	6.07 (1.37–26.99)	0.018
Median time of platelet engraftment	0.545		
Median time of neutrophil engraftment	0.592		

Bold values highlight the factors that have statistical significance.

AML acute myeloid leukemia, MDS myelodysplastic syndrome, ALL acute lymphoblastic leukemia, CML chronic myelogenous leukemia, MPN myeloproliferative neoplasms, HCT-CI hematopoietic cell transplantation-specific comorbidity index, HLA human leukocyte antigen, GVHD graft versus host disease, ATG anti-thymocyte globulin, MMF mycophenolate mofetil, MAC myeloablative conditioning, RIC reduced intensity conditioning, HCT/HSCT hematopoietic stem cell transplantation, CR complete remission, CR1 first complete remission, CR2 second complete remission, MNCs mononuclear cells, CD cluster of differentiation, NK cells natural killer cells.

conditioning regimens, or remission status at the time of HSCT, while patients with HHV-6 encephalitis tended to be younger than the controls (median age, 28 versus 40 years, $P = 0.012$). The donor's age is almost the same (median age, 36 versus 33, $P = 0.451$).

Compared with the control group, the HHV6 encephalitis group shows a slightly lower median amount of MNCs ($\times 10^8/\text{kg}$, 10.7 versus 12.4, $P = 0.044$) in grafts, as the CD34⁺ cells ($\times 10^6/\text{kg}$, 4.2 versus 5.3, $P = 0.026$) do. We assessed the immune recovery by measuring cell counts 21 days post-HSCT. The counts of CD3⁺ cells (per μl , 123 versus 164, $P = 0.340$) and CD19⁺ cells (per μl , 12 versus 8, $P = 0.233$) in recipients were comparable between HHV6 encephalitis and the control group, while NK cells displayed a completely different situation ($P = 0.006$), with 34/ μl (range, 0–152) for the HHV6 group but 121/ μl (range, 0–728) for the control, which raised our strong interest. HHV6 encephalitis didn't affect the median time for both platelet and neutrophil engraftment (13 days versus 13 days, $P = 0.545$; 12 days versus 12 days, $P = 0.592$, respectively).

In the univariate analysis shown in Table 2, age under 30 years, low MNCs and CD34⁺ cell counts in grafts, and NK cell counts <115 per μl of recipient blood at 21 days after HSCT were associated with HHV6 encephalitis incidence after allo-HSCT. Factors with a p-value < 0.1 and factors that were strongly related to HHV6 encephalitis according to previous studies were subsequently included in the multivariate analysis, by which two independent risk factors were identified: age under 30 years ($P = 0.016$; odds ratio [OR], 3.24; 95% confidence interval (CI), 1.25 to 8.42), and counts of NK cells <115 per μl at 21 days after HSCT ($P = 0.018$; OR, 6.07; 95% CI, 1.37–26.99).

Clinical manifestation and clinical outcomes of HHV-6 encephalitis

Figure 2 illustrates the key clinical manifestations observed in our case cohort. Among the 20 patients with HHV6 encephalitis, fever is the most prevalent symptom, occurring in 70.0% of cases, followed by confusion, accounting for 55% of cases. Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH), sensory disorders, and short-term memory loss all present with a similar prevalence, each affecting approximately 50.0%, 45.0%, and 45.0% of the population, respectively. Seizures constitute the least commonly observed symptom, presenting in only 20% of cases, but they are the most serious. These findings underscore the need for a heightened level of vigilance in HHV6 infections, given the broader spectrum of clinical symptoms.

With the intervention of antiviral drugs, 65.0% of the HHV6 encephalitis patients (13/20) achieved the standard of complete remission (CR), as shown by controlled fever, dissipated itching, improved mental symptoms, or others. Four (20.0%, 4/20) patients reached PR, and the remaining three patients (15.0%, 3/20) resulted in NR.

Unfortunately, with a median follow-up time of 516 (35–1936) days, only half of the 20 HHV6 encephalitis patients stay alive. Among the mortalities, four died from disease relapse, three from TMA, one from primary graft failure, one from severe infection, and the last one succumbed to a combined complication of both TMA and GVHD.

The impact of HHV6 encephalitis on transplant outcomes

As previously highlighted, HHV6 encephalitis is closely associated with acute GVHD. Our study further supports this assertion, showing a significantly increased incidence of grade II-IV aGVHD - at a striking 70% compared to a mere 13.8% within the control group ($P < 0.001$). The incidence of TMA was also higher in the HHV6 encephalitis group than controls (35% versus 2.5%, $P < 0.001$), but there was no statistically significant difference in relapse, with rates of 25.6% versus 16.1% ($P = 0.182$). Patients with HHV6 encephalitis experienced significantly more non-relapsing

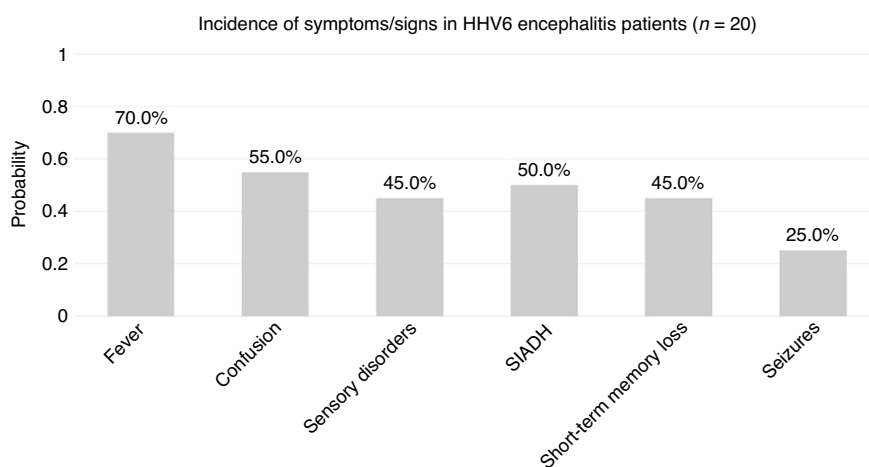


Fig. 2 Key clinical manifestations. The key clinical manifestations and incidences observed in the case after HHV6 encephalitis. (n = 20).

Table 3. The incidences of different clinical outcomes for HHV6 encephalitis group and control group.

	HHV-6 encephalitis group	Control group	P-value
aGVHD II-IV (CI)	70.0%	13.8%	<0.001
TMA (CI)	35.0%	2.5%	<0.001
NRM (CI)	30.0%	7.3%	0.002
Relapse (CI)	25.6%	16.1%	0.182
OS	40.0%	81.8%	<0.001
PFS	44.4%	78.9%	<0.001

Bold values highlight the factors that have statistical significance.

HHV6 human herpesvirus type 6, aGVHD acute graft versus host disease, TMA hematopoietic stem cell transplant-associated microangiopathy, NRM non-relapse mortality, OS overall survival, PFS progression-free survival.

mortality (NRM) than the control group (30.0% versus 7.3%, $P = 0.002$), resulting in a lower OS (40% versus 81.8%, $P < 0.001$) and PFS (44.4% versus 78.9%, $P < 0.001$). All data are shown in Table 3 and Fig. 3.

We further conducted a Cox regression model to assess the impact of HHV6 encephalitis on transplant outcomes. As illustrated in Fig. 4, it is not surprising to observe that HHV6 encephalitis emerges as an independent risk factor associated with the occurrence of II-IV aGVHD (hazard ratio [HR], 5.52; 95% CI, 2.19–13.91, $P < 0.001$) and TMA (HR, 9.86; 95% CI, 1.34–72.47, $P = 0.024$). Moreover, HHV6 encephalitis has been identified as one of the independent prognostic factors for NRM (HR, 5.28; 95% CI, 1.70–16.50, $P = 0.004$), OS (HR, 4.34; 95% CI, 1.84–10.25, $P = 0.001$), and PFS (HR, 3.94; 95% CI, 1.77–8.76, $P = 0.001$), providing additional evidence that HHV6 encephalitis significantly impacts HSCT outcomes.

DISCUSSION

Given the rare prevalence of HHV-6 encephalitis after HSCT, previous studies have not been powered to evaluate the risk factors and prognostic significance. Our findings have clearly revealed that factors like age and NK cell population would influence the occurrence of HHV6 encephalitis. Primary infection directly correlates with young age; however, few studies have worked on HHV6 encephalitis and pediatric HSCT. Two pediatric studies reported infection rates of 3.4% and 7.0% [13, 14], respectively, while they would reach 18% when employing a T-

cell-depleted transplant approach [15]. Compared with our lower infection rate of 1.48%, it suggests a higher prevalence among pediatric allo-HSCT patients. Therefore, it is reasonable to conclude that age is a significant risk factor. In our cohort, none of the cases of HHV6 encephalitis were identified in the context of HLA-matched allo-HSCT without the administration of ATG. This finding aligns with previous demonstrations that T-cell-depleted allo-HSCT increases the odds of HHV6 encephalitis [16], emphasizing the role of CD3⁺ cells in preventing HHV6 infection after allo-HSCT. In addition, we have learned that early T-cell reconstitution plays a crucial role in improving HSCT outcomes [17–19]. However, our center didn't screen T-cell subset counts as frequently as the referenced article, so the data may not reflect a more comprehensive T-cell reconstruction. With a longer follow-up period, we would be able to draw more accurate conclusions.

As the key participant in the innate immune system, NK cells are the first donor-derived lymphocyte population to recover following allo-HSCT. They play a critical role in antiviral defense against viruses such as the herpesvirus, poxvirus, papillomavirus, etc. An in vitro assay illuminated the elimination ability of NK cells when co-cultured with HHV-6B CD4⁺ T cells at a ratio of 2:1 [15]. A subsequent clinical study conducted in Spain, involving the infusion of NK cells on day 7 after CD45RA⁺ T-cell-depleted allo-HSCT in children, demonstrated that despite some patients experiencing virus reactivation, early adoptive NK cell infusion was still effective in preventing HHV-6B encephalitis [20]. The newly reconstituted NK cells post-allo-HSCT are reported to consist of more CD56^{bright} cells (50%) than in health donors (10%). During maturation, these cells express higher levels of NKG2A, NKG2C, KIR, and NCRs, which contributes to the immune system's ability to recognize and eliminate the HHV6-infected cells. This explains why NK cells are highlighted in our data. However, further research is needed to understand the underlying mechanisms for efficiently reconstituting NK cells [21, 22].

Regarding the prognostic significance of HHV6 encephalitis on HSCT outcomes, our study observed that patients tend to develop aGVHD and TMA. The latter was usually related to severe illness and non-relapse mortality. The etiology may be mainly due to the virus causing tissue damage or endothelial injury, promoting pro-inflammatory or type I immune responses, and delaying the immune reconstruction with CD4⁺ T cells, especially Tregs [23–27].

Nevertheless, our study has limitations. Firstly, our data is center-specific, offering a partial perspective without incorporating data from other transplantation centers. To address this, a multi-center study could validate our conclusions and explore additional risk possibilities. Secondly, due to the impracticality of

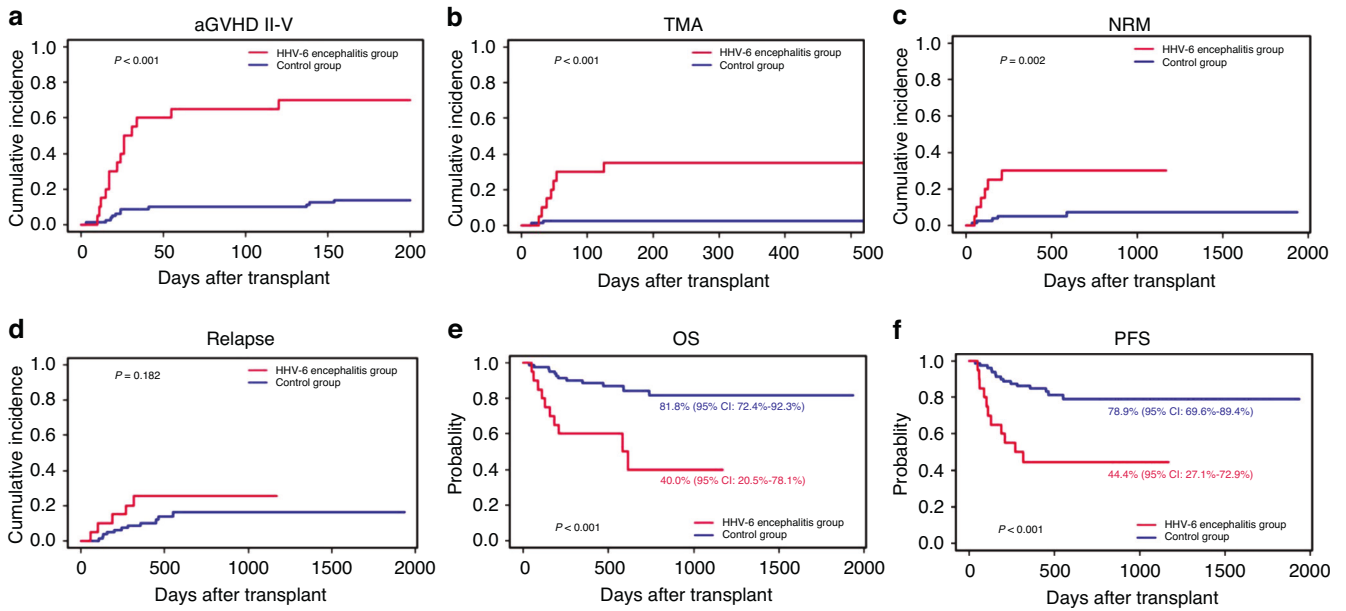


Fig. 3 Key clinical outcomes. a–d aGVHD, TMA, NRM, relapse for patients. e, f OS and PFS by Kaplan-Meier method for HHV6 encephalitis patients (n = 20) and control (n = 80) patients.

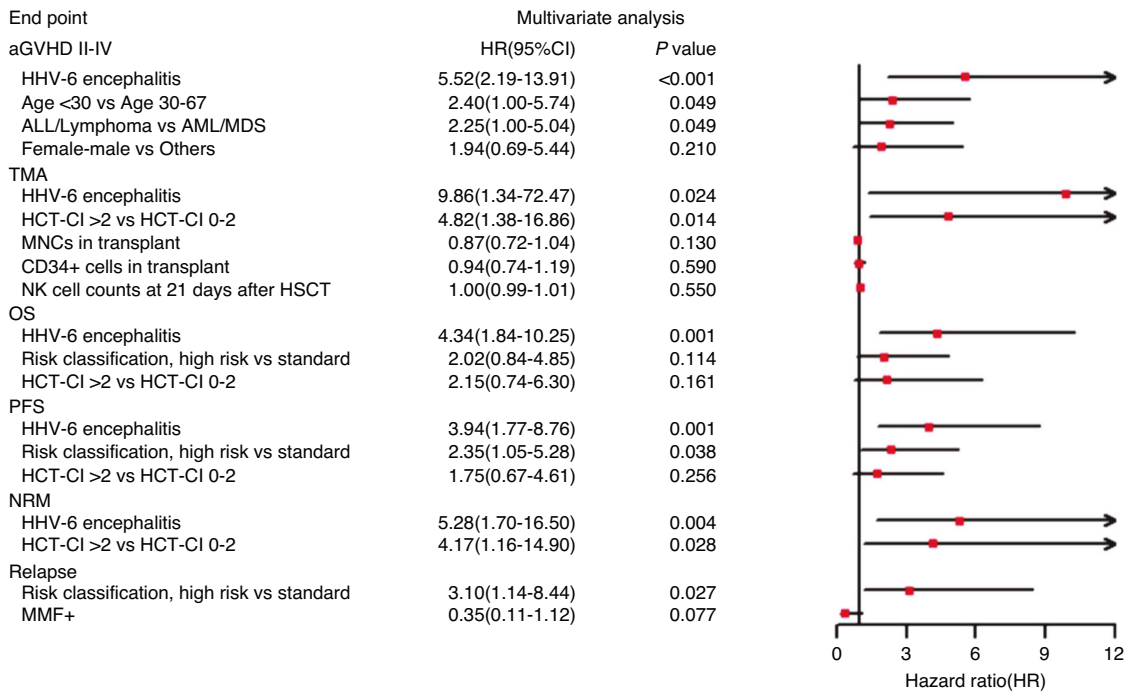


Fig. 4 Forest plot showing multivariate analysis for clinical outcomes of HHV6 encephalitis patients. The multivariate analysis show HHV6 encephalitis is an independent risk factor for patients developing II-IV aGVHD and TMA, affecting the patients’ OS, PFS, and NRM. HHV6 human herpesvirus type 6; GVHD graft versus host disease; ALL acute lymphoblastic leukemia; TMA hematopoietic stem cell transplant-associated microangiopathy; HCT-CI hematopoietic cell transplantation-specific comorbidity index; MNCs mononuclear cells; CD cluster of differentiation; NK cells natural killer cells; OS overall survival; MMF mycophenolate mofetil; PFS progression-free survival; NRM non-relapse mortality.

brain biopsy, we rely on lumbar puncture upon symptom development, with HHV6 detection through next-generation sequencing (NGS). While not a guideline gold standard, NGS’s effectiveness is widely acknowledged. However, in atypical cases lacking typical symptoms or with low CSF viral loads, potential misdiagnosis exists. Dynamic monitoring of the virus in serum/plasma, CSF/blood replication ratio, or qualitative/quantitative CSF

assays, as recommended in other studies, could complement ciHHV6 diagnosis after excluding alternative causes [28, 29]. The delayed recovery of NK cells post-HSCT is closely linked to HHV6 encephalitis. Early recognition based on risk factors and typical symptoms is crucial due to its variable symptoms and poor prognosis. Establishing consensus on accurate diagnosis, suitable treatments, and optimal intervention timing is imperative.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

- Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science*. 1986;234:596–601. <https://doi.org/10.1126/science.2876520>.
- Caserta MT, Hall CB. Human herpesvirus-6. *Annu Rev Med*. 1993;44:377–83. <https://doi.org/10.1146/annurev.me.44.020193.002113>.
- Takahashi K, Sonoda S, Higashi K, Kondo T, Takahashi H, Takahashi M, et al. Predominant CD4 T-lymphocyte tropism of human herpesvirus 6-related virus. *J Virol*. 1989;63:3161–3. <https://doi.org/10.1128/JVI.63.7.3161-3163.1989>.
- Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin Microbiol Rev*. 2015;28:313–35. <https://doi.org/10.1128/CMR.00122-14>.
- Pantry SN, Medveczky PG. Latency, integration, and reactivation of human herpesvirus-6. *Viruses*. 2017;9. <https://doi.org/10.3390/v9070194>.
- Ogata M, Fukuda T, Teshima T. Human herpesvirus-6 encephalitis after allogeneic hematopoietic cell transplantation: what we do and do not know. *Bone Marrow Transplant*. 2015;50:1030–6. <https://doi.org/10.1038/bmt.2015.76>.
- Luo Y, Xiao H, Lai X, Shi J, Tan Y, He J, et al. T-cell-replete haploidentical HSCT with low-dose anti-T-lymphocyte globulin compared with matched sibling HSCT and unrelated HSCT. *Blood*. 2014;124:2735–43. <https://doi.org/10.1182/blood-2014-04-571570>.
- Zhao Y, Gao F, Shi J, Luo Y, Tan Y, Lai X, et al. Incidence, risk factors, and outcomes of primary poor graft function after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2019;25:1898–907. <https://doi.org/10.1016/j.bbmt.2019.05.036>.
- Ogata M, Oshima K, Ikebe T, Takano K, Kanamori H, Kondo T, et al. Clinical characteristics and outcome of human herpesvirus-6 encephalitis after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2017;52:1563–70. <https://doi.org/10.1038/bmt.2017.175>.
- Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22:4–10. <https://doi.org/10.1016/j.bbmt.2015.09.001>.
- Ruutu T, Barosi G, Benjamin RJ, Clark RE, George JN, Gratwohl A, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica*. 2007;92:95–100. <https://doi.org/10.3324/haematol.10699>.
- Gao Y, Wu H, Shi Z, Gao F, Shi J, Luo Y, et al. Prognostic factors and clinical outcomes in patients with relapsed acute leukemia after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2023;58:863–73. <https://doi.org/10.1038/s41409-023-01989-3>.
- Howell KB, Tiedemann K, Haeusler G, Mackay MT, Kornberg AJ, Freeman JL, et al. Symptomatic generalized epilepsy after HHV6 posttransplant acute limbic encephalitis in children. *Epilepsia*. 2012;53:e122–6. <https://doi.org/10.1111/j.1528-1167.2012.03494.x>.
- Cheng FW, Lee V, Leung WK, Chan PK, Leung TF, Shing MK, et al. HHV-6 encephalitis in pediatric unrelated umbilical cord transplantation: a role for ganciclovir prophylaxis? *Pediatr Transplant*. 2010;14:483–7. <https://doi.org/10.1111/j.1399-3046.2009.01253.x>.
- Perruccio K, Sisinni L, Perez-Martinez A, Valentin J, Capolsini I, Massei MS, et al. High incidence of early human herpesvirus-6 infection in children undergoing haploidentical manipulated stem cell transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2018;24:2549–57. <https://doi.org/10.1016/j.bbmt.2018.07.033>.
- Schmidt-Hieber M, Schwender J, Heinz WJ, Zabelina T, Kuhl JS, Mousset S, et al. Viral encephalitis after allogeneic stem cell transplantation: a rare complication with distinct characteristics of different causative agents. *Haematologica*. 2011;96:142–9. <https://doi.org/10.3324/haematol.2010.029876>.
- Admiraal R, de Koning CCH, Lindemans CA, Bierings MB, Wensing AMJ, Versluis AB, et al. Viral reactivations and associated outcomes in the context of immune reconstitution after pediatric hematopoietic cell transplantation. *J Allergy Clin Immunol*. 2017;140:1643–50.e1649. <https://doi.org/10.1016/j.jaci.2016.12.992>.
- Greco R, Crucitti L, Novello M, Racca S, Mannina D, Forcina A, et al. Human herpesvirus 6 infection following haploidentical transplantation: immune recovery and outcome. *Biol Blood Marrow Transplant*. 2016;22:2250–5. <https://doi.org/10.1016/j.bbmt.2016.09.018>.
- Noviello M, Lorentino F, Xue E, Racca S, Furnari G, Valtolina V, et al. Human herpesvirus 6-specific T-cell immunity in allogeneic hematopoietic stem cell transplant recipients. *Blood Adv*. 2023;7:5446–57. <https://doi.org/10.1182/bloodadvances.2022009274>.
- Gasior M, Ferreras C, de Paz R, Bueno D, Mozo Y, Sisinni L, et al. The role of early natural killer cell adoptive infusion before engraftment in protecting against human herpesvirus-6B encephalitis after naive T-cell-depleted allogeneic stem cell transplantation. *Transfusion*. 2021;61:1505–17. <https://doi.org/10.1111/trf.16354>.
- Ullah MA, Hill GR, Tey SK. Functional reconstitution of natural killer cells in allogeneic hematopoietic stem cell transplantation. *Front Immunol*. 2016;7:144. <https://doi.org/10.3389/fimmu.2016.00144>.
- Rizzo R, Di Luca D. Human herpesvirus 6A and 6B and NK cells. *Acta Microbiol Immunol Hung*. 2018;65:119–25. <https://doi.org/10.1556/030.65.2018.010>.
- Aoki J, Numata A, Yamamoto E, Fujii E, Tanaka M, Kanamori H. Impact of human herpesvirus-6 reactivation on outcomes of allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2015;21:2017–22. <https://doi.org/10.1016/j.bbmt.2015.07.022>.
- Zerr DM, Boeckh M, Delaney C, Martin PJ, Xie H, Adler AL, et al. HHV-6 reactivation and associated sequelae after hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2012;18:1700–8. <https://doi.org/10.1016/j.bbmt.2012.05.012>.
- Phan TL, Pritchett JC, Leifer C, Zerr DM, Koelle DM, Di Luca D, et al. HHV-6B infection, T-cell reconstitution, and graft-vs-host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2018;53:1508–17. <https://doi.org/10.1038/s41409-018-0225-2>.
- Belford A, Myles O, Magill A, Wang J, Myhand RC, Waselanko JK. Thrombotic microangiopathy (TMA) and stroke due to human herpesvirus-6 (HHV-6) reactivation in an adult receiving high-dose melphalan with autologous peripheral stem cell transplantation. *Am J Hematol*. 2004;76:156–62. <https://doi.org/10.1002/ajh.20068>.
- Vasu S, Bostic M, Zhao Q, Sharma N, Puto M, Knight S, et al. Acute GVHD, BK virus hemorrhagic cystitis and age are risk factors for transplant-associated thrombotic microangiopathy in adults. *Blood Adv*. 2022;6:1342–9. <https://doi.org/10.1182/bloodadvances.2021004933>.
- Zhu H, Ali A, Woan KV, Tam E, Yaghmour G, Flores A, et al. Unique challenges to diagnosing human herpesvirus-6 (HHV-6) encephalitis following post-hematopoietic stem cell transplant: a case and brief review. *Cell Transplant*. 2022;31:9636897221119734. <https://doi.org/10.1177/09636897221119734>.
- Berzeto G, Campanini G, Vegezzi E, Paoletti M, Pichiecchio A, Simoncelli AM, et al. Human herpesvirus 6 encephalitis in immunocompetent and immunocompromised hosts. *Neurol Neuroimmunol Neuroinflamm*. 2021;8. <https://doi.org/10.1212/NXI.0000000000000942>.

AUTHOR CONTRIBUTIONS

H Huang and YM Zhao had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: H Huang, YM Zhao. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Yi Yu. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis and graphing: WH Chen. Obtained funding: YM Zhao, PX Qian. Supervision: H Huang, YM Zhao.

FUNDING

This work was supported by grants from the National Key R&D Program of China, Stem Cell and Translation Research (grant nos. 2022YFA1103500), and the National Natural Science Foundation of China (grant nos. 82170210).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to He Huang or Yanmin Zhao.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.