Human Herpes Virus-6 Following Pediatric Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT: **Background:** Human herpes virus-6 (HHV-6) reactivation after hematopoietic stem cell transplantation (HSCT) is well known and has been linked with several clinical manifestations. The significance of HHV-6 viremia and related complications in this setting is still unclear.

Objective: To estimate the incidence of HHV-6 reactivation and associated morbidity in children undergoing allogeneic HSCT.

Methods: Blood samples obtained weekly (for cytomegalovirus surveillance) from children who underwent allogeneic HCST during the period January 2006–June 2010 were retrospectively tested for the presence of HHV-6 DNA using standard real-time polymerase chain reaction (PCR) assay. Clinical records were reviewed for correlation between viremia and clinical manifestations.

Results: Samples from 39 children were tested. Twenty patients had viral loads above 1000 copies/ml (51%) in at least one sample. Higher viral loads were seen in patients with primary immunodeficiency and in those with cord blood transplant. Attributable symptoms were present in 12 patients (60%) concurrently with positive PCR. Clinical manifestations spontaneously resolved without treatment in most cases, concomitantly with a decrease in viral load.

Conclusions: HHV-6 reactivation during allogeneic HSCT is common. HHV-6 reactivation should be considered in patients with graft-vs-host disease-like rash, onset of CNS symptoms, delay in engraftment, and in patients after cord blood transplantation.

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KEY WORDS: human herpes virus-6 (HHV-6), pediatric, bone marrow transplant (BMT), reactivation

Human herpes virus-6 (HHV-6), a member of the beta herpes virus subfamily, usually affects children in their first 2–3 years of life. The virus is the causative agent of roseola infantum (exanthema subitum) in young children, usually a mild febrile illness without complications. Most patients become infected with HHV-6 by the age of 2 years, and almost all adults have antibodies against it [1]. After recovery from the febrile illness, the virus becomes latent in the peripheral blood cells, mainly in mononuclear cells and in other tissue cells (e.g., kidney, lungs, central nervous system) [1]. Like other members in the herpes family, the virus can reactivate, mainly among immunocompromised patients, particularly recipients of bone marrow transplant (BMT) [2-7]. Two HHV-6 variants, HHV-6A and HHV-6B, are known.

The vast majority of documented primary infections and reactivations are due to HHV-6B [2]. Reactivation occurs in about 30–50% of transplant recipients, typically after 2–4 weeks [2]. Clinical manifestations of HHV-6 reactivation are varied, including mild febrile illness, rash, pneumonitis, hepatitis, and severe encephalitis [1-7]. A recent study of more than 300 allogeneic hematopoietic stem cell transplantation (HCT) recipients explored the association between HHV-6 reactivation and central nervous system (CNS) dysfunction, and demonstrated that reactivation was associated with delirium in the first 3 months following transplantation [8]. Viremia was claimed to be associated with graft-vs-host disease (GVHD) or delayed engraftment of the transplant [2,9]. The contribution of the virus to these clinical manifestations is controversial. HHV-6 may interfere with normal function of the immune system in both suppressive and pro-inflammatory mechanisms [10]. Detection of HHV-6 is mainly available through the polymerase chain reaction (PCR) technique. Detection of HHV-6 DNA in plasma or serum correlates well with active replication, and detection in whole blood has been associated with HHV-6-related manifestations [2].

There are no standard cutoff points for copies of the virus to discriminate infection from reactivation [2]. There is no licensed antiviral drug for the treatment of HHV-6 infection, but in vitro studies support the evidence that foscarnet, ganciclovir and cidofovir have antiviral activity [1,2,11]. Some studies have shown that ganciclovir when given for cytomegalovirus (CMV) prophylaxis may help lower the HHV-6 reactivation rate [12]. While data in the adult population are well-established (although controversies do exist), data for the pediatric population with BMT and HHV-6 reactivation are lacking [4,13-18]. Current guidelines for the management of HHV-6 in children following BMT do not support active surveillance and monitoring [19]. The aim of the present study was to retrospectively characterize HHV-6 reactivation in pediatric patients undergoing viral surveillance for CMV during BMT.

MATERIALS AND METHODS

Blood samples, obtained weekly for CMV surveillance from children who underwent allogeneic HCST from January 2006 to June 2010, were retrospectively tested for the presence of HHV-6 DNA. Patients with a surveillance period shorter than 2 months or who had fewer than six samples were excluded. Clinical records of children with viral loads exceeding 1000 copies/ml were considered positive and reviewed for correlation between viremia and clinical manifestations. The collected data from the patients' files included demographics, primary illness and type of transplantation. Clinical data included fever, presentation (encephalitis, rash, pneumonia, fever, hepatitis, GVHD) and any relevant laboratory abnormality (elevated liver function tests, thrombocytopenia). Co-infections were documented (bacteremia, other known viral infection or other infectious disease) as were all antiviral agents used in the same period.

IDENTIFICATION OF HHV-6 DNA

DNA extraction: The DNA from patient's samples (200 µl of whole blood) was extracted using the Magna Pure LC apparatus and Magna Pure LC total Nucleic Acid Isolation Kit Reagents (Roche Diagnostics, Germany).

DNA detection: Quantitative results were obtained by TaqMan real time PCR [20].

The primers and probe were targeted - U6 gene.

Forward primer: 5' AAAATTTCTCACGCCGGTATTC 3'

Reverse primer: 5' CCTGCAGACCGTTCGTCAA 3'

PROBE- 6-FAM-TCGGTCGACTGCCCGCTACCA-BHQ-1.

PCR reaction was performed on a total volume of 25 µl containing Absolute Blue QPCR mix (Thermo Scientific, UK) in the presence of 10 µl target DNA, 300 nM of each primer and 200 nM of the probe. PCR was performed on the rotor gene 3000 or 6000 instrument (Corbett Research/QIAGEN, Hilden, Germany) under the following conditions: 15 min at 95°C, and 45 cycles of 15 sec at 95°C and 60 sec at 60°C. The standard curve was constructed by quantified HHV-6 DNA (Advanced Biotechnology Industry, USA). Results were reported as the number of HHV-6 genome copies per 1 ml of blood.

RESULTS

During the study period (January 2006 to June 2010) 62 children underwent BMT in our pediatric hematology oncology department. A total of 1588 samples for PCR obtained for CMV surveillance were collected. Thirty-nine children were included in the study according to the inclusion criteria. Of these, 23 were boys and 16 were girls and the age ranged from 2 months to 18 years (mean 9 years).

The primary diagnosis for which BMT was performed was cancer in 15 patients (38.5%), primary immunodeficiency in 6 (15%), hematologic disorders in 15 (38.5%), and metabolic disorders in 3 (8%). The number of samples taken ranged from 8 to 72 (mean 36) during a period of 2 months up to 3 years.

Twenty patients (51%) had a positive PCR (viral load > 1000 cp/ml). For eight patients this occurred during the first month of BMT (40%), while 65% had positive samples by the second month and 70% were positive within the first 3 months of BMT. Comparisons between negative and positive cases are shown in Table 1.

Children with primary immune deficiency and those with cord blood transplant were more likely to be positive than those with hematological conditions. Among patients with malignancy and those with an unrelated donor the chance was equal. Among the 20 patients with positive samples 12 (60%) had clinical symptoms possibly related to the virus [Table 2].

Seven patients (58%) had skin rash, in three of whom the rash was presumed to be GVHD (but no biopsy was available). Two patients underwent biopsy with non-specific changes and a virology study was not done at the time.

Fever of unknown origin was found in 5 of 12 children (42%), one of whom also had skin rash. All these children received antibiotic treatment with no positive blood cultures.

One child had clinical encephalitis; he had fever and confusion and was defined by the pediatric neurologist as encephalopathic. Lumbar puncture was not performed. He was considered to have a drug reaction to tacrolimus (FK-506) although the drug level was within normal range, and the drug was discontinued. His viral load at that point was 50,000 cp/ml.

Other manifestations included pneumonitis or lung manifestations as part of GVHD (no respiratory secretions for viral load were taken), diarrhea (with negative stool cultures, adenovirus, and rotavirus or *Clostridium difficile* toxin). Three patients had abnormal liver function tests (one had a fatal adenovirus infection and the other two were suspected of having GVHD). Treatment with ganciclovir was documented in four patients who had concomitant CMV viremia and in one patient who was known to have HHV-6 viremia and was treated accordingly with ganciclovir.

No patient died of HHV-6-related symptoms. One patient from the study group died with severe adenovirus infection; he had HHV-6 viremia at the same time.

Table 1. Negative and positive cases of HHV-6

	Positive N=20	Negative N=19	Total N=39
Age (year, mean)	7	11.5	
Primary immune deficiency	5 (84%)	1 (16%)	6
Hematologic diseases	5 (33.4%)	10 (66.6%)	15
Malignancy	7 (47%)	8 (53%)	15
Cord blood transplant	6 (75%)	2 (25%)	8
Related donor	6 (50%)	6 (50%)	12
Duration (months)	15	12	

Table 2. Characteristics of patients with associated clinical manifestations

Age/Gender Diagnosis	Max. VL(x10³)	Rash	GVHD	Rejection	Pneumonia	Diarrhea	Abn. LFT	Fever	Encephalitis	Other	Antiviral Tx
4.3/F ALL	11.5	+	+	+	+	+	+			Pulmonary aspergillosis	
1.8/M SCID	25			+			+			Fatal adenovirus	
4.8/M APL	2.5	+									
9/M FHL	37	+				+		+			
10.2/F TM	13				+					CMV	+
5/M X-ALD	50							+	+	CMV	+
0.2/F SCID	320							+			
7.2/M XLP	22	+	+			+		+			
4.2/F LADIII	350			+	+			+		Pseudomas bacteremia	
4.4/F TM	12	+	+		+		+			Acinetobacter CMV	
16.5/F Aplastic anemia	2.3	+				+				STN bacteremia CMV	+
1.8/M CEL	5.2	+		+	+					MRSA bacteremia	+

X-ALD = X-linked adrenoleukodystrophy disease, ALL = acute lymphoblastic leukemia, SCID = severe combined immunodeficiency, APL = acute promyelocytic leukemia, FHL = familial hemophagocytic lymphohistiocytosis, XLP = X-linked lymphoproliferative disease, SCA = sickle cell anemia, LAD III = leukocyte adhesion deficiency type III, CEL = chronic eosinophilic leukemia, AML = acute myeloid leukemia, TM = thalassemia major, CMV = cytomegalovirus, STN = stenotrophomonas, MRSA = methicillin-resistant *Staphylococcus aureus*, Tx = treatment, Max. = maximal, Abn LFT = abnormal liver function tests

DISCUSSION

Reactivation of HHV-6 in allogeneic BMT patients is common. About 40–50% of patients experience reactivation, usually 2–6 weeks after transplantation [9]. The significance of reactivation and its consequences among pediatric patients is not well established [4,13-18]. The definition of reactivation (or a positive case) in the current study was defined as > 1000 cp/ml. In other studies, various cutoff points were used. Reactivation rates may be higher if "any detection" is considered positive. Differences in the PCR technique for detecting the virus, as well as the type of specimen, may influence the tests results (i.e., serum, plasma or whole blood). These differences may lead to different interpretations of the positive cases.

Our data show that reactivation is common, as demonstrated previously by others, especially in the first month and later in the first 3 months after BMT. Among the predisposing factors for reactivation, other studies cite age, where younger patients reactivate more commonly than older, underlying disease and HLA mismatch [unrelated donor and umbilical cord graft (UCG)]. Of the children who received UCG in our group, HHV-6 reactivation occurred in 6 (75%). The risk factor is well known in adults and pediatric BMT patients, and we found similar connections. UCG was associated with higher viral loads and longer viremia periods and encephalitis [21].

In the current study, 5 of the 6 patients (84%) with primary immunodeficiency had reactivation. This risk factor has not been adequately addressed so far, although for some of these patients the UCG may have been a contributing risk factor, and their age is usually younger. Only sparse studies have been conducted in this unique pediatric BMT group. More studies with higher numbers of patients are needed to address the relevance of HHV-6 testing in this subgroup. We believe systematic testing for viral infections including HHV-6 is warranted.

Patients with hematological diseases, such as thalassemia, in the current study were less likely to develop reactivation (only 33%). This might be related to the underlying normal immune system before BMT (in contrast to those with immunodeficiency), though the numbers are too small to draw conclusions.

The significance of reactivation and the associated viral load is a matter of debate. Dulery et al. [22] did not find any relation between viral load and clinical manifestations. Another study demonstrated the same occurrence of rash and fever among patients with 500–25,000 cp/ml and those with > 25,000 cp/ml [17]. Other studies stated that viral load was important, especially in patents with CNS symptoms [23]. Sustained viremia was claimed to affect the transplant engraftment and can contribute to rejection [4,17].

In the current study, clinical manifestations were similar to those described earlier in the literature but less common. Fever

and skin rash were common at the time of reactivation. This conclusion is limited by the retrospective data. Rash can be attributed to other causes such as GVHD, engraftment, other viruses or drugs. This confusion may be associated with unnecessary procedures and erroneous medical interventions.

A possible link between reactivation and GVHD exists, similar to CMV reactivation. This issue has not yet been clarified and more data are needed [24]. Demonstration of the virus in the skin biopsy was documented even without viremia [6].

Delayed engraftment and/or platelet engraftment was plausibly related to HHV-6 reactivation [23]. In the current study 3 of 12 patients with clinical manifestation had delayed engraftment as well, parallel with the HHV-6 viremia. One who was treated with ganciclovir finally had a successful engraftment.

Interestingly, encephalitis was more commonly documented in Japan [25]. One of our children had encephalitis but a cerebrospinal fluid sample was not available, and we can only presume that this case as well was attributed to HHV-6.

Other manifestations, such as diarrhea and abnormal liver function tests, are very common and non-specific. The relationship to HHV-6 may be of theoretical significance only.

Overall, most of the patients had a spontaneous decrease in viral load. All patients survived except for one who had a fatal adenovirus infection. There is uncertainty whether HHV-6 viremia increases mortality. Most studies found no association between mortality and HHV-6 infection [1,17]. Some studies emphasize the potential effect on mortality, mainly among patients with encephalitis [2,25].

In conclusion, the current study has demonstrated, similar to others [7,9,17], that HHV-6 reactivation is common among allogeneic BMT pediatric patients, manly those with congenital immunodeficiency and cord blood transplant. This subgroup may benefit from active surveillance for HHV-6 as is performed for CMV. We also believe that HHV-6 viremia should be tested when there is a clinical suspicion, for example in cases of rash and fever, abnormal liver function tests, or encephalitis, and possibly for children with GVHD. In light of the sparse data in the literature (especially among pediatric BMT patients), there is a need for prospective data regarding pediatric patients with HHV-6 reactivation.

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