Human Herpesvirus 7 in Allogeneic Hemopoietic Stem Cell Transplant Recipients in the Central Clinical Hospital in Warsaw: A Three-Year Survey

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Key Words

Human herpesvirus 7 • Human herpesvirus 5 • Hemopoietic stem cell transplant • Real-time PCR

Abstract

Objectives: Human herpesvirus 7 (HHV-7) is spread worldwide and has been described as a potential pathogen in immunosuppressed patients. Different clinical manifestations have been described including fever and skin rash; HHV-7 may also be a possible cofactor for cytomegalovirus disease in transplant recipients. **Materials and Methods:** A retrospective review of a group of 58 adult recipients of allogeneic hemopoietic stem cell transplantation was made. Serum samples taken in the range of 0–180 days after transplant were examined for presence of specific HHV-7 sequences using the quantitative real-time PCR method. **Results:** HHV-7 DNA was detected in plasma samples in 26 (45%) of the 58 recipients between day 20 and day 65 of transplantation. All of them developed fever of unknown origin; also HHV-5 DNA was detected in plasma samples collected from 11 HHV-7-positive patients. None of the described individuals died during detectable HHV-7 or HHV-5 viremia periods. **Conclusions:** There is a high frequency of

detectable HHV-7 viral load in allogeneic stem cell transplant recipients in Poland. Limited availability and sensitivity of serological methods along with the necessity of rapid introduction of antiviral treatment has forced the development of molecular diagnostics. Furthermore, establishment of appropriate procedures for monitoring active HHV-7 infection is important to clarify the virus infection in transplant recipients.

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Introduction

Human herpesvirus type 7 (HHV-7) belongs to the β -Herpesvirinae subfamily and is very closely related to HHV-6 and also to HHV-5 (commonly known as cytomegalovirus) [1]. In most people, primary HHV-7 infection occurs between 6 months and 5 years of age, later than HHV-6 [2, 3]. After that, the virus remains latent in the body and reactivates upon host immunosuppression, similar to other HHVs.

The clear association of HHV-7 with human disease has not been recognized, although several cases of exanthema subitum have been linked to HHV-7 [4]. Few case

Table 1. Sequences of primers and the probe used in this work

Name	Sequence (5'–3')
HHV7-p1	CCC AAC TAT TTA CAG TAC GGT TGG T
HHV7-p2	TTT AGT TCC AGC ACT GCA ATC
HHV7-JOE	JOE-CTA TTT TCG GTC TTT CCA ATG CAC GCA-TAMRA

reports and small patient series have associated primary HHV-7 infection with hepatitis, upper respiratory tract infections and pancytopenia [2]. HHV-7 involvement has been also implicated in pityriasis rosea, based on the virus isolation from peripheral blood mononuclear cells (PBMC) of patients and molecular detection of HHV-7 DNA in plasma, PBMC and skin lesions [5].

All human β -herpesviruses – especially HHV-5 – have been recognized as important causes of morbidity and mortality in transplant recipients, but the number of studies of HHV-7 infection in immunosuppressed patients is very limited. One of the major problems is cross-reaction between HHV-6 and HHV-7 antibodies, which might confuse the results of serological analyses [6]. Some investigators have suggested that HHV-7 may be a possible cofactor for cytomegalovirus disease in transplant recipients. Chan et al. [7] monitored 61 bone marrow transplant recipients by PCR analysis, and found that patients with cytomegalovirus disease were more likely to have concurrent HHV-7 DNA in PBMC prior to onset of disease than were patients with asymptomatic HHV-5 infection. Similar findings have also been demonstrated in renal and liver transplant recipients [8, 9].

In a recent study, we tried to summarize retrospective results of the determination of HHV-7 infection status in allogeneic stem cell transplantation recipients. As the infections with HHV-7 are rarely accompanied by clinical symptoms, our first aim was to compare infection status, measured as viral DNA presence in the blood, in the post-transplantation period.

Materials and Methods

This retrospective study involved patients who received an allogeneic hemopoietic stem cell transplantation (HSCT) and were hospitalized in the Hematology, Oncology and Internal Medicine Clinics, Medical University of Warsaw. In the considered period (from January 2006 to December 2008), there were 58 patients receiving allogeneic stem cell transplantations. Additional criteria for the study included appearance of at least one of the syndromes listed below in the period of 100 days after HSCT: appearance or intensification of graft versus host disease (GvHD), skin rash or neutropenic fever. Monitoring of clinical status of the pa-

tients and viral load in blood samples comprised the period of 180 days after HSCT.

Collection of plasma samples from all patients for PCR investigations began at a median of 3 days after transplantation (range 1–7 days) and lasted until a median of 107 days (range 28–180 days). Presence of viral DNA was tested by real-time PCR in sera samples obtained once a week until the 100th day after allogeneic HSCT, and thereafter once every 2 weeks. The median number of blood samples per patient was 13 (range 4–24 samples). A total of 574 samples obtained from 58 patients were examined using real-time PCR. Viral DNA was extracted from 200 μ l of plasma, using a High Pure Viral Nucleic Acid Kit® (Roche Diagnostics) in accordance with the manufacturer's instructions.

For the detection of HHV-7, a real-time PCR (qPCR) assay with fluorescent TaqMan probes, complementary for the sequence lying within the amplified product, was used. Tests were run on the LightCycler 2.0 instrument (Roche Diagnostics) with a modified in-house method described below [10]. A highly conservative region of HHV-7 genome encoding major capsid protein (U57) was chosen (GenBank, U43400), and a set of primers was developed, as well as the probe, labeled with the fluorophore reporter JOE on the 5' end and with TAMRA quencher on its 3' end (Oligo®; table 1).

Investigations were performed using the reaction mixture TaqMan Master Kit® (Roche Diagnostics). Besides chemicals supplied by the kit producer, the final reaction mixture contained 5 μ l of isolated viral DNA, 2.75 μ M of HHV7-p1 primer, 3.25 μ M HHV7-p2 primer and 1.50 μ M HHV7-JOE probe, in a total volume of 20 μ l. Amplification was performed with activation of the thermostable hot-start DNA polymerase for 10 min at 95°, followed by 45 cycles comprising denaturation (15 s at 95°), primer annealing (15 s at 60°) and strand elongation (15 s at 72°). After the end of cycling, the material was cooled down to 40° for 60 s. Fluorescence levels were read at 560-nm wavelength, specific for JOE fluorophore dye [10]. Every tested sample was amplified along with an internal control (positive control of DNA amplification process). For the assessment of reaction specificity, DNA isolated from the following viruses was used: HHV-1, HHV-5 and HHV-6. Each amplification reaction included, except tested samples, also positive HHV-7 controls (RK strain) in a range of 100–100,000 copies/ml and a negative control of DNA extraction and the amplification process [10].

HHV-5 DNA was detected using the commercially available quantitative CMV Quant Kit® (Roche Diagnostics) developed especially for monitoring cytomegalovirus disease with the LightCycler 2.0 instrument. The test uses SCORPIONS™ fluorescent probes. Analogically for HHV-7 detection, an internal control was added for every sample and amplification was performed in the presence of amplification-specific controls (positive, negative and extraction process control).

Table 2. Characteristics and clinical status of patients positive for HHV-7 after HSCT

Patient No.	Sex	Age years	Disease	SCT	HHV-7 DNA in plasma (days after SCT)	HHV-5 DNA in plasma (days after SCT)	Time and cause of death (days after HSCT)
1	male	44	AML	rPBSCT	++ (43–65)	–	sepsis, multiorgan failure (140)
2	male	43	AML	rPBSCT	+ (30)	–	ARDS, shock (35)
3	male	56	AML	rPBSCT	++ (48–56)	–	infection (>500)
4	male	50	MM	rPBSCT	+ (42)	–	infection (>600)
5	female	22	ALL	uPBSCT	++ (44–62)	–	N/A
6	female	19	ALL	rPBSCT	+ (48)	–	N/A
7	female	26	AML	rPBSCT	+ (40)	–	N/A
8	female	39	AML	rPBSCT	+ (52)	–	N/A
9	female	19	ALL	uPBSCT	+ (22)	–	infection, hemorrhage within CNS (25)
10	male	49	MM	rPBSCT	+ (20)	–	GvHD (25)
11	female	25	AML	rPBSCT	++ (38–55)	+ (55)	N/A
12	female	31	HD	rPBSCT	++ (42–58)	++ (55–68)	N/A
13	male	45	AML	uPBSCT	++ (32–50)	++ (58–74)	N/A
14	female	19	AML	uPBSCT	++ (42–60)	++ (56–70)	N/A
15	male	56	CLL	rPBSCT	+ (38)	++ (42–70)	N/A
16	male	30	AML	uBMT	++ (45–50)	++ (50–72)	sepsis (140)
17	male	48	AML	rPBSCT	+ (33)	–	N/A
18	female	33	AML	uPBSCT	++ (40–50)	+ (55)	N/A
19	female	51	HD	rPBSCT	+ (34)	–	N/A
20	male	19	AML	uPBSCT	+ (50)	++ (54–68)	pneumonia (220)
21	female	24	SAA	uPBSCT	++ (44–56)	++ (60–80)	N/A
22	male	27	CML	uPBSCT	++ (28–42)	–	N/A
23	female	25	HD	rPBSCT	+ (30)	–	N/A
24	female	42	AML	uPBSCT	++ (34–42)	++ (52–65)	N/A
25	female	56	AML	uPBSCT	++ (20–35)	–	N/A
26	male	26	AML	uPBSCT	++ (42–52)	+ (58)	pneumonia (200)

ALL = Acute lymphoblastic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphoblastic leukemia; CML = chronic myeloid leukemia; HD = Hodgkin's disease; MM = multiple myeloma; SAA = severe aplastic anemia; SCT = stem cell transplantation; rPBSCT = related peripheral blood stem cell trans-

plantation; uPBSCT = unrelated peripheral blood stem cell transplantation; uBMT = unrelated bone marrow transplantation; – = negative; + = positive in one serum sample; ++ = positive in two or more serum samples; ARDS = acute respiratory distress syndrome; CNS = central nervous system; N/A = not applicable.

Statistics was done with the Kruskal-Wallis rank test. We compared viral load in samples taken from patients with a single virus with that from patients who had β -herpesvirus coinfection.

Results

Positive results of HHV-7-specific in-house real-time PCR, expressed as exponential gain of fluorescence at the desired wavelength, were detected in samples taken from 26 patients (45%). Twelve of them (21%) had determined HHV-7 DNA presence only in single positive samples; 14 (24%) others had a positive result in two or more subsequent tests. Obtained quantitative results were usually at

low to medium level, placed between 1,000–5,000 copies/ml, and viremia was observed between 20 and 65 days after transplantation (table 2). In control samples containing DNA from an uninfected Vero cell line, DNA isolated from HHV-1, HHV-5 and HHV-6, fluorescence was not observed, which speaks for high specificity of the method used.

As an amplification result of DNA isolated from plasma using the HHV-5-specific qPCR method, products were detected in samples taken from 16 individuals (28%). HHV-5 DNA was also detected in the plasma samples collected from 11 HHV-7-positive patients. In all observed cases, HHV-5 DNAemia was found in the typical period of 40–80 days after transplantation, al-

Table 3. Viral load in the different groups of patients for HHV-5 and HHV-7

Patient group	Patients	HHV-5 DNA in plasma, days after SCT	Average HHV-5 load copies/ml	Maximum HHV-5 load copies/ml	HHV-7 DNA in plasma, days after SCT	Average HHV-7 load copies/ml	Maximum HHV-7 load copies/ml	Mortality %	Average time of death, days after SCT
HHV-5+/HHV-7+	11	34–80	2,900	7,500	32–53	2,200	5,000	18.2	187
HHV-5-/HHV-7+	16	N/A	N/A	N/A	20–65	1,400	3,500	37.5	221
HHV-5+/HHV-7–	5	43–71	2,500	6,800	N/A	N/A	N/A	20	164
HHV-5-/HHV-7–	26	N/A	N/A	N/A	N/A	N/A	N/A	25.4	218

ways placed in the range between 700–7,500 copies/ml (table 2).

Overall mortality in the entire group of HSCT recipients during the first 180 days after transplantation was 20.7% (12 patients from 58), and the most frequent direct death cause was: ARDS (4 patients), sepsis (4 patients) and pneumonia (2 patients). In the HHV-7-positive patients, mortality was 19.2% (5 patients from 26), but none of them died during detectable HHV-7 or HHV-5 viremia periods (table 2).

Results of statistical analysis of comparison of HHV-5 and HHV-7 load in sera samples revealed a statistically insignificant difference ($p \geq 0.005$, Kruskal-Wallis rank test) between patients with a single β -herpesvirus detected and patients who had HHV-5 and HHV-7 coinfection.

Discussion

After primary infection, herpesviruses persist throughout life. Reactivation occurs usually under conditions of immunosuppression and can lead to illnesses that differ in clinical presentation from disease associated with primary infection. Reactivating HHV-7 has been associated with hepatitis in liver transplant patients [9], encephalitis in bone marrow transplantation [7] and sometimes with graft rejection [7, 11]. Available data suggest that HHV-7 infections are common after transplantation and could implicate HHV-5 reactivation and cytomegalovirus disease [7, 12]. The evidence between these two viruses is not consistent, because of absence of standardized diagnostic tests for HHV-7. Other authors have demonstrated that HHV-7 reactivation occurs earlier than HHV-5, and HHV-7 has been implicated as a factor

for subsequent HHV-5 reactivation and disease [7, 13]. Our observations show that HHV-7 viremia may also occur simultaneously at the time of active infection caused by HHV-5 (table 2, 3).

In our study, we found that 45% (26 individuals) of allogeneic graft recipients developed HHV-7 infection. Fourteen patients (24%) had detectable viral DNA level in two or more samples in subsequent tests during the 10 weeks following allogeneic HSCT and other 12 (21%) had a single positive sample in the same period (table 2). HHV-7 was probably reactivated from the recipient's own body by factors such as a profound immune dysfunction or an allogeneic reaction after transplantation. This is only our hypothesis, due to the lack of commercial serological methods for measuring presence of anti-HHV-7 antibodies.

We did not find any correlation between HHV-7 viremia and used conditioning regimen or anti-GvHD prophylaxis. Most of HHV-7-positive graft recipients during 10 weeks after SCT had fever of unknown etiology, and cytomegalovirus DNA was found in plasma samples taken from 11 individuals during or shortly after HHV-7 onset. No association between HHV-5 and/or HHV-7 viremia and mortality was found in this study (table 2, 3).

HHV-7 could cause well-known clinical symptoms in allogeneic HSCT recipients, but has also indirect effects such as contributing to graft rejection and increasing the risk of additional cytomegalovirus infection [14, 15]. Nevertheless, the question of interactions between the β -herpesviruses is still pending. It seems likely that the impairment of the immune system by HHV-5 or other immunological events surrounding the transplantation might trigger an HHV-7 infection [15]. The availability of

the qPCR technique means that diagnosis is available in a clinically helpful time frame, which should assist with implementing timely therapeutic intervention and assessing response to further treatment [16].

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