The relationship between HHV-6 and HHV-7 reactivation and development of post-autologous peripheral stem cell transplantation complications was examined. The presence of viral genomic sequences in whole peripheral blood and cell free plasma was determined by nested PCR, HHV-6 and HHV-7 load by real-time PCR, virus specific antibodies and cytokines in serum by ELISA, and HHV-6 variants by restriction endonuclease analysis. Clinical features, reactivation of viruses and serum TNF-α, and IL-6 concentrations were determined in seventy-six patients with Roseolovirus infection before and after transplantation. Anti-HHV-6 antibodies were found in 62 of 76 (81.6%) patients before transplantation. A significantly higher rate of single HHV-7 infection was found in patients with viral infection in comparison with single HHV-6 infection (p = 0.0003) and concurrent (HHV-6 and HHV-7) infection (p = 0.0017). Complications after transplantation developed in 30.3% of patients and reactivation of viruses was detected in all of these patients. Significant increase of HHV-6 and HHV-7 reactivation with simultaneous increase of pro-inflammatory cytokines serum levels suggests that both viruses may be involved in the development of complications after autologous peripheral blood stem cell transplantation via their immunomodulatory ability. The kinetics of the Roseolovirus reactivation may reflect the potential role of HHV-7 as a co-factor for HHV-6 activation.

Key words: haematopoietic stem cell transplantation, kinetics of virus reactivation, human herpesviruses-6 and 7, post-transplantation complication.
HHV-6 and HHV-7 belong to the family Herpesviridae, subfamily Betaherpesvirinae, genus Roseolovirus (Berneman et al., 1992). The viruses are ubiquitous (seroprevalence rate in adults is 60–90%) lymphotropic and immunomodulating and can be reactivated in immunocompromised and immunosuppressed hosts (Dockrell and Paya, 2001; Yamanishi et al., 2001; Lusso et al., 2006). After primary infection, these viruses establish a state of life-long subclinical persistence in the human host and are controlled by a functioning immune system (Griffiths et al., 2000; Ljungman et al., 2008). Roseolovirus displays primary tropism to CD4+ T cells, where they efficiently replicate, possibly causing immune dysregulation (Dockrell and Paya, 2001). CD34+ cells, which are a major source of haematopoietic progenitor cells for transplantation, can be infected by HHV-6B and HHV-7 in vivo and in vitro, which may cause myelosuppression in immunocompromised patients (Movassaghi et al., 1996; Mirandola et al., 2000; Isomura et al., 2003). In 2012, the International Committee on Taxonomy of Viruses re-classified HHV-6 as separate viruses HHV-6A and HHV-6B based on their biological characteristics regarding cell tropism and pathological implications (Ablashi et al., 2014). The specific pathogenicity of HHV-6A remains poorly understood. HHV-6B reactivation is a common event in HSCT recipients (40–50%) and moreover, there is a positive correlation between HHV-6B infection and CMV reactivation (Zerr et al., 2005; Boutolleau et al., 2006; Wang et al., 2008). HHV-6 is reactivated early after HSCT (3–4 weeks) and is associated with a range of clinical symptoms (unexplained fever, skin rash, delay of monocyte and platelet engraftment, early and late graft failure, bone marrow suppression) and diseases including interstitial pneumonitis, encephalitis and encephalomyelitis, particularly in patients after peripheral hemopoietic HSCT and bone marrow HSCT (Carrigan et al., 1991; Carrigan and Knox, 1994; Imbert-Martille et al., 2000; Yohikava et al., 2001; Zerr, 2006). The clear association of HHV-7 with human disease and development of complications after HSCT has not been recognised. Beta-herpesviruses possess immunomodulating properties, including the ability to alter expression of immune activation molecules, modulation of expression of several cytokines and chemokines and induction of apoptosis in lymphocytes, which may contribute to immunosuppression (Lusso, 2006). The ubiquitous nature of beta-herpesviruses creates conditions for the development of concurrent infection and interaction between these viruses. The kinetics of the activation of these viruses during the post-transplantation period suggests that HHV-7 can act as a co-factor for HHV-6 and CMV reactivation, while both HHV-6 and HHV-7 can act as co-factors in CMV disease development (Miyoshi et al., 2001; Boutolleau et al., 2003). The prevalence of human herpesvirus reactivation ranges from 40 to 60% in auto-HSCT patients and usually occurs within 2–4 weeks (Zerr et al., 2005). HHV-6 and HHV-7 have been frequently detected in allo-HSCT recipients. Asymptomatic HHV-6 reactivations predominate in the post-HSCT setting. Previously, we investigated the role of HHV-6 and HHV-7 in development of complications after renal transplantation and showed that HHV-7 acts as a co-factor for HHV-6 and CMV reactivation, while both HHV-6 and HHV-7 act as co-factors in the pathogenesis of CMV disease, acute rejection and chronic allograft nephropathy (Chapenko et al., 2001, Folkmane et al., 2001, Chapenko et al., 2009). In the present study we examined presence of beta-herpesviruses and EBV infection in patients with auto- and allo-transplantation of peripheral blood stem cells, which allowed to estimate significance of infection activation of each virus and its relationship with development of complications.

The aim of this study was to examine: 1) the presence of HHV-6 and/or HHV-7 infection in patients before auto-HSCT; 2) frequency of reactivation of these viruses in early period after auto-HSCT; 3) the potential interactions between reactivation of the viruses and development of post-transplant complications; 4) potential relationship between reactivation of these viruses by co-infection.

MATERIALS AND METHODS

Patients. During the period from 2006 until 2010 and from 2013 until 2015, Roseolovirus infection was studied in 76 patients (44 females, 32 males; mean age 34.1 ± 10.7) who were placed in the Chemotherapy and Hematology Clinic of Riga East University Hospital and underwent auto-HSCT. The patients group was composed of 37 patients with Hodgkin’s disease, 16 with non-Hodgkin’s lymphoma, and 23 patients with myeloma. A summary of their characteristics including age, underlying disease, pre-transplant chemotherapy, the average number of cycles of chemotherapy before HSCT for each patient, serology CMV and EBV, conditioning regimens, amount of CD34+ cells in grafts, duration of severe neutropenia (absolute neutrophil count < 0.5 × 10^9/l), and post-transplant complications, are shown in Table 1. Stem cells for grafts were harvested before HSCT from peripheral blood in the period during treatment with non-myoeloblastic chemotherapy and mobilisation with granulocyte-colony-stimulating factor (G-CSF) (5 µg/kg on days 4–14). Patients with lymphoma before auto-HSCT had received myeloablative/conditioning therapy by BEAM protocol (carmustin, cytosar, etoposide, melphalan); myeloma patients received myeloablative/conditioning therapy with melphalan 200 mg/m². All patients for prophylaxis of Gram-positive and Gram-negative infections received (sulfametoxazol/trimephoprimum 960 mg b.i.d. a day orally 2 times per week for 3 months), for fungal (fluconazole 150 mg, orally or 200 mg i/v, 3 weeks) and viral infections (valacyclovir 500 mg/m² 2× day, orally or aciclovir 400 mg 2× day, orally, for one month) after HSCT. Blood samples from the patients were collected before auto-HSCT as the baseline and for 3 months after HSCT (day 7 to 10 and on days 14, 28, 56, 84). Aliquots were stored at –80 °C before use. Clinical events examined in association with active viral infection were fever, skin rash, pneumonitis, partial bone marrow suppression, central nervous system dysfunction, and gastroenteritis (diarrhea).

The cohort was established with the approval of the Ethics Committee of the Riga Stradiņš University and all participants were informed and gave consent prior to the examination.

HHV-6, CMV, EBV serology. Serum samples from patients before and after HSCT were tested for HHV-6 IgG class antibodies using HHV-6 IgG ELISA kits (Panbio, Sinnamon Park, Australia), according to the manufacturer’s recommendations. The serum samples before HSCT were tested for CMV IgM, IgG class antibodies using ELISA anti-CMV IgM or IgG/Enzygnost Anti-CMV IgM or IgG (Dade Behring, Germany) and EBV IgM, IgG class antibodies using ELISA IgM or IgG LIAISON EBV IgM, LIAISON VCA IgG (DiaSorin, Italy), according to the manufacturer’s recommendations.

Nested polymerase chain reaction (nPCR). nPCR was used for the detection of viral genomic sequences in DNA isolated from whole peripheral blood (WPB) and plasma (markers of persistent and active infection, respectively). Total DNA was isolated from 0.5 ml fresh whole blood by phenol-chloroform extraction. DNA purification was made from 200 µl cell free blood plasma QIAamp Blood Kit (QIAGEN, Hilden, Germany) was used. The plasma samples were treated with Desoxyribonuclease I (Fermentas, Vilnius, Lithuania) before DNA purification. To assure quality of the WPB DNA and to exclude contamination of plasma DNA by cellular DNA, a globin PCR was performed. PCR amplification for the viruses was carried out using 1 µg DNA and 10 µl plasma DNA (corresponding to 100 µl of plasma). The detection of HHV-6 and HHV-7 DNA was performed according to Secchiero et al. (1995) and Berneman et al. (1992), respectively. Positive (virus genomic DNA, Advanced Biotechnologies Inc, Columbia, MD, USA) and negative (DNA without virus-specific sequences) as well as water controls were included in each experiment.

Quantitative real-time polymerase chain reaction. HHV-6 load before HSCT was determined in WPB samples positive for viral DNA (according to nPCR data) using a HHV-6 Real-TM Quant kit (Sacace, Biotechnologies, Italy) and Applied Biosystems 7500 Real-time PCR System (Applied Biosystems, USA, according to the manufacturer’s instruction. The WBL with ethylenediaminetetraacetic acid from patients after HSCT were tested for CMV DNA quantified by Real Time PCR (Artus CMV LC PCR Kit, Qiagen, Germany) and EBV DNA quantified by Real Time PCR (Artus EBV LC PCR Kit, Germany), according to the manufacturer’s recommendations.
Restriction endonuclease analysis. Restriction endonuclease analysis was carried out using enzyme HindIII (Fermentas, Vilnius, Lithuania), which cuts HHV-6B 163 bp amplimer into two fragments: 66 bp and 97 bp, and does not cut HHV-6A amplimer. This difference allows to distinguish HHV-6A and HHV-6B variants. HHV-6B was tested for in all WPB DNA samples from patients with HHV-6 infection.

Assays for cytokine determination. Platinum ELISA kits Human IL-6 and TNF-alfa (AviBion, Helsinki, Finland) were used for the detection of TNF-alfa, and IL-6 levels in serum samples of patients PCR-negative for viral infection, with persistent infection in latent phase, and active viral infection. The sensitivity of the ELISA assay was 2 pg/ml for TNF-alfa and for IL-6. All samples were tested in duplicate.

Statistical analysis. Significant differences in the prevalence of HHV-6 and HHV-7 infection before and after transplantation were determined using the Fisher’s exact test. The non-parametric Wilcoxon’s matched-pairs signed test was performed using GraphPad Prism Version 6.0 (GraphPad Software Inc., La Jolla, CA, USA) for analysis of HHV-6 load in WPB of patients with persistent infection in latent phase and active phase. The serum cytokine level was expressed as mean ± SD. SPSS software was used to assess the continuous variables (cytokine levels) with a value of p < 0.05 considered as significant.

RESULTS

Specific anti-HHV-6 IgG class antibodies were found in serum samples of 62 of 76 (81.6%) pre-transplant patients. CMV and EBV IgM antibodies were not found in any of the patients. After HSCT, CMV, and EBV DNA were determined quantitatively weekly. Active CMV and EBV infection was not recorded in any of the patients.

HHV-6 and/or HHV-7 genomic sequences were found in samples isolated from WPB from 68 of 76 (89.5%) of the pre-transplant patients using nPCR (Table 2). In the patients with viral infection, a significantly higher rate of single HHV-7 infection was found in comparison with single HHV-6 infection (p = 0.0003) and concurrent (HHV-6+HHV-7) infection (p = 0.0017). HHV-6B was recorded in all 26 WPB DNA samples from patients with HHV-6 infection. Viral genomic sequences were found in plasma DNA samples from 5 out 68 (7.4%) patients before transplantation (Table 2). Of them, HHV-6 plasma viremia was detected in one patient, HHV-7 plasma viremia in 3 patients with single HHV-7 infection and simultaneous HHV-6 and HHV-7 plasma viremia in one patient with concurrent (HHV-6 and HHV-7) infection.

The frequency of plasma viremia was significantly higher in patients after transplantation (25/68, 36.7%) in comparison with the frequency before transplantation (p = 0.00003) (Table 2). No significant difference in the rate of active concurrent HHV-6 and HHV-7 infection occurred in patients after transplantation compared to the rate of patients before transplantation (p = 0.180).

HHV-7 plasma viremia was found in 22 patients (17 with single HHV-7 infection and 5 with concurrent infection) (Table 2). HHV-6 reactivation was found in 8 patients (3 with single infection and 5 with concurrent infection).

The HHV-6B genomic sequence was identified in all plasma DNA samples from patients with HHV-6 plasma viremia.

The mean number of days before detecting HHV-7 and HHV-6 reactivation was 11 days (range: 7–14 days) for HHV-7 and 27 (range: 10–34 days) for HHV-6. Median HHV-6 load in WPB DNA of the patients examined in the period of persistent infection in latent phase before transplantation was 5657 copies/µg DNA [range interquartile range (IQR): 4514–9723 copies/µg DNA] and in the period of active phase infection after transplantation 59439 copies/µg DNA (IQR: 40309–63168 copies/µg DNA). There is no standard threshold for clinically significant HHV-6 viral load and development of post-transplant complications. CMV and EBV load ≤ 195 copies/ml and ≤ 240 copies/ml, respectively, are considered to indicate persistent infection in latent phase, and larger loads occur in cases of active infection. However, virus activation is not always linked to development of complications.

To explore the effect of HHV-6 and/or HHV-7 reactivation on cytokine levels, serum pro-inflammatory TNF-alfa, and IL-6 cytokine levels were determined in 25 patients (Table 3). TNF-alfa and IL-6 levels were significantly higher in patients with viruses reactivation than in patients with HHV-7 and HHV-6 persistent infection in latent phase (p < 0.0001, p = 0.01, respectively). The level of TNF-alfa but not IL-6 was significantly higher in patients with simultaneous HHV-6 and HHV-7 reactivation (p < 0.0001, p < 0.0001, respectively). Although the level of IL-6 was higher in patients with simultaneous HHV-6 and HHV-7 reactivation, in comparison with the level in patients with latent concurrent infection, the difference was not significant (p = 0.054).

Clinical complications (febrile neutropenia, gastroenteritis (diarrhea), pneumonitis) were detected in 23 of 76 (30.3%) transplant patients (Table 4). Febrile neutropenia was diag-
nosed in most patients (14/23, 60.86%) in the early post-transplant period (7–14 days), and febrile neutropenia combined with gastroenteritis (diarrhea) and/or pneumonitis development (7/23, 30.43%). HHV-7 reactivation was detected in 8 of these 23 patients (5/8 with febrile neutropenia and 3/8 with gastroenteritis). Pneumonitis was detected in 10 of 23 patients (43.47%) and gastroenteritis in 11 of 23 patients (47.82%). On average HHV-6 and HHV-7 reactivation was detected 15.8 and 10.2 days after transplantation, respectively, in the early post-transplant period and a longer neutropenic period was observed in patients who had complications.

Table 3

<table>
<thead>
<tr>
<th>Viral infections</th>
<th>TNF-alpha (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n = 8)</td>
<td>2.05 ± 1.24</td>
<td>3.25 ± 1.89</td>
</tr>
<tr>
<td>Latent (n = 43)</td>
<td>12.38 ± 2.96</td>
<td>4.39 ± 0.48</td>
</tr>
<tr>
<td>Active (n = 25)</td>
<td>62.30 ± 8.27</td>
<td>10.57 ± 1.74</td>
</tr>
<tr>
<td>HHV-6 (n = 3)</td>
<td>60.7 ± 9.23</td>
<td>11.5 ± 1.23</td>
</tr>
<tr>
<td>HHV-7 (n = 17)</td>
<td>58.50 ± 10.36</td>
<td>12.63 ± 1.42</td>
</tr>
<tr>
<td>HHV-6+HHV-7 (n = 5)</td>
<td>66.10 ± 6.17</td>
<td>8.50 ± 2.05</td>
</tr>
</tbody>
</table>

Table 4

CHARACTERISTICS OF PATIENTS WITH COMPLICATIONS AND ACTIVE BETA-HERPESVIRUSES

<table>
<thead>
<tr>
<th>Patients No (n = 23)</th>
<th>Underlying disease</th>
<th>Pre-transplant /Conditioning regimens</th>
<th>Neutropenia &lt; 0.5x10^9/l (days)</th>
<th>Plasma viremia (days)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before auto-PBSCT</td>
<td>After auto-PBSCT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HHV-7</td>
<td>HHV-6</td>
</tr>
<tr>
<td>1 HD</td>
<td>DexaBEAM/BEAM</td>
<td>5</td>
<td>-</td>
<td>+ (7)</td>
<td>-</td>
</tr>
<tr>
<td>2 HD</td>
<td>DexaBEAM/BEAM</td>
<td>9</td>
<td>-</td>
<td>+ (7)</td>
<td>+ (21)</td>
</tr>
<tr>
<td>3 HD</td>
<td>DexaBEAM/BEAM</td>
<td>8</td>
<td>-</td>
<td>+ (7)</td>
<td>-</td>
</tr>
<tr>
<td>4 HD</td>
<td>DexaBEAM/BEAM</td>
<td>10</td>
<td>+</td>
<td>+ (10)</td>
<td>-</td>
</tr>
<tr>
<td>5 HD</td>
<td>MINE/BEAM</td>
<td>10</td>
<td>-</td>
<td>+ (10)</td>
<td>+ (10)</td>
</tr>
<tr>
<td>6 HD</td>
<td>IVE/BEAM</td>
<td>10</td>
<td>-</td>
<td>+ (7)</td>
<td>+ (14)</td>
</tr>
<tr>
<td>7 HD</td>
<td>IVE/BEAM</td>
<td>13</td>
<td>-</td>
<td>+ (14)</td>
<td>+ (14)</td>
</tr>
<tr>
<td>8 HD</td>
<td>IVE/BEAM</td>
<td>8</td>
<td>-</td>
<td>+ (10)</td>
<td>+ (21)</td>
</tr>
<tr>
<td>9 HD</td>
<td>IVE/BEAM</td>
<td>14</td>
<td>+</td>
<td>+ (9)</td>
<td>-</td>
</tr>
<tr>
<td>10 HD</td>
<td>IVE/BEAM</td>
<td>8</td>
<td>+</td>
<td>+ (10)</td>
<td>-</td>
</tr>
<tr>
<td>11 HD</td>
<td>IVE/BEAM</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>+ (10)</td>
</tr>
<tr>
<td>12 HD</td>
<td>IVE/BEAM</td>
<td>10</td>
<td>+</td>
<td>+ (9)</td>
<td>-</td>
</tr>
<tr>
<td>13 NHL</td>
<td>IVE/BEAM</td>
<td>13</td>
<td>-</td>
<td>+ (7)</td>
<td>+ (14)</td>
</tr>
<tr>
<td>14 NHL</td>
<td>IVE/BEAM</td>
<td>14</td>
<td>+</td>
<td>+ (12)</td>
<td>-</td>
</tr>
<tr>
<td>15 NHL</td>
<td>IVE/BEAM</td>
<td>11</td>
<td>+</td>
<td>+ (10)</td>
<td>+ (10)</td>
</tr>
<tr>
<td>16 NHL</td>
<td>IVE/BEAM</td>
<td>10</td>
<td>-</td>
<td>+(13)</td>
<td>-</td>
</tr>
<tr>
<td>17 MM</td>
<td>Endoxan/Melphalan</td>
<td>6</td>
<td>-</td>
<td>+ (10)</td>
<td>+ (28)</td>
</tr>
<tr>
<td>18 MM</td>
<td>Endoxan/Melphalan</td>
<td>11</td>
<td>-</td>
<td>+ (8)</td>
<td>+ (21)</td>
</tr>
<tr>
<td>19 MM</td>
<td>Endoxan/Melphalan</td>
<td>10</td>
<td>-</td>
<td>+ (21)</td>
<td>-</td>
</tr>
<tr>
<td>20 MM</td>
<td>Endoxan/Melphalan</td>
<td>12</td>
<td>+</td>
<td>+ (8)</td>
<td>+ (21)</td>
</tr>
<tr>
<td>21 MM</td>
<td>Endoxan/Melphalan</td>
<td>9</td>
<td>+</td>
<td>+ (10)</td>
<td>+ (14)</td>
</tr>
<tr>
<td>22 MM</td>
<td>Endoxan/Melphalan</td>
<td>10</td>
<td>-</td>
<td>+ (13)</td>
<td>+ (14)</td>
</tr>
<tr>
<td>23 MM</td>
<td>Endoxan/Melphalan</td>
<td>7</td>
<td>+</td>
<td>+ (14)</td>
<td>+ (10)</td>
</tr>
</tbody>
</table>

HD, Hodgkin’s disease; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; DexaBEAM, carmustine, etoposide, cytarabine, melphalan, dexamethasone; BEAM, carmustine, etoposide, cytarabine, melphalan; IVE, ifosfamide, etoposide, doxorubicin; MINE, mitoxantrone, ifosfamide, etoposide; - negative; + positive; ( ) - days after transplantation

DISCUSSION

Autologous peripheral blood progenitor cell transplantation is used as a standard of care in patients with Hodgkin’s lymphoma, non-Hodgkin’s lymphoma and multiple myeloma in the Chemotherapy and Haematology Clinic. Evidence of a correlation between beta-herpesviruses reactivation and various clinical manifestations has accumulated mainly from studies on solid organs (Chapenko et al., 2001; Rasonable et al., 2010) and allogeneic bone marrow and hematopoietic stem cell transplantations (Rieger et al., 2009). In this study we examined incidence of HHV-6 and HHV-7 infection and frequency of virus reactivation before and after auto-HSCT, HHV-6 load and the relationship between virus reactivation and development of clinical complications in early period after auto-HSCT.

Our results show high frequency of HHV-6 and HHV-7 persistent infection in stem cell transplant patients in Latvia, which indicates a need to determine the risk of infection activation of these viruses after auto-HSCT and the validity of antiviral prophylaxis and pre-emptive therapy application. All HSCT recipients should be tested for the presence of serum anti-HHV-6 IgG class antibodies before HSCT, to determine the risk for primary herpesvirus infection and reactivation after HSCT. The study showed high frequency of HHV-6 seroprevalence (81.8%) and wide prevalence (89.5%) of persistent beta-herpesviruses infection among patients before HSCT, thus confirming the ubiquity of this virus. Considering that Roseoloviruses are ubiquitous, this study showed that single HHV-7 and concurrent HHV-6 and HHV-7 persistent infection in latent phase were prevalent among these patients. The obtained data allow to determine the risk of subsequent virus reactivation after transplantation and validity of antiviral prophylaxis and pre-emptive therapy application. Single HHV-7 and concurrent HHV-6 and HHV-7 persistent infections are highly prevalent also in Latvian blood donors (Kozireva et al., 2001).

The study showed that active single HHV-6 infection (1/68, 1.5%), single HHV-7 (3/68, 4.4%) and concurrent HHV-6 and HHV-7 (1/68, 1.5%) are presented in the patients before transplantation without clinical manifestations. This suggests that the virus reactivation can be a consequence of the application of pre-transplant chemotherapy. Active single HHV-7 infection only, but not active single HHV-6 or concurrent HHV-6 and HHV-7 infection, has been detected also in 10.6% of Latvian blood donors (Kozireva et al., 2001).

HHV-6B was detected in all WPB and plasma DNA samples from patients with HHV-6 infection. This suggests that either the HHV-6A variant occurs infrequently in these patients or it may be limited by sites other than the peripheral blood. Possibly the obtained results may be explained by geographical distributions of the variants (Čistjakovs et al., 2009).

The frequency of Roseolovirus reactivation considerably increased after transplantation (36.8%), compared to that before transplantation (7.4%), and the rate of single HHV-7 infection was also prevalent in patients after HSCT. Our experience has shown that Valacyclovir, which is used for antiviral prophylaxis, is not sufficiently effective to prevent HHV-7 reactivation. Foscarnet, ganciclovir and cidofovir have been shown to inhibit HHV-6 replication in vitro while ganciclovir prophylaxis was associated with a significant decrease in frequency of HHV-6, but not of HHV-7 reactivation in stem cell transplant patients (Rapaport et al., 2002; Ongradi et al., 2010). Ganciclovir, cidofovir and foscarnet have variable activity against HHV-6 in vitro and may have a role in treatment of HHV-6-associated diseases. Presently, there is no data to support recommendations for monitoring of potential HHV-7-associated disease (Anonymous, 2008; Zaia et al., 2009; Anonymous, 2015). HHV-6 reactivation is confirmed by viral sequence detection in plasma DNA samples, and concomitant increase of HHV-6 load in WPB and HHV-7 reactivation by plasma viremia detection. Standardised quantitative PCR assays for Roseoloviruses are lacking, which limits the optimal use of this assay in the setting of transplantation. At present, the question of potential impact between beta-herpesviruses in cases of concurrent infection is pending. In this study, HHV-6 reactivation was found in patients with concurrent HHV-6 and HHV-7 infection and each virus had a definite temporal profile of detection. The reactivation of HHV-7 is observed before HHV-6 reactivation, which may suggest potential interaction between these viruses. These results are in accordance with those obtained in previous studies conducted in vitro (Katsafanas et al., 1996) and in vivo (Maeda et al., 2000; Boutolleau et al., 2003; Hall et al., 2006). However, Sassenscheidt et al. (2006), Humar et al. (2009), Tormo et al. (2010) did not find evidence for potential interaction between HHV-6, HHV-7, and CMV.

After auto-HSCT, the risk of bacterial infections and mortality rate are lower due to the usually less intensive mucositis and the shorter neutropenia. In the autologous setting, the infection risk almost completely disappears after neutrophil recovery. Only 30–35% of febrile episodes in neutropenic patients can be documented microbiologically; in the remaining cases, the cause of the fever cannot be demonstrated (Anonymous, 2012).

Although the precise mechanism by which beta-herpesviruses impair immunologic function is not completely clear, previous investigations have shown that these viruses are very effective modulators of immune response, mainly by modulating the production of pro-inflammatory cytokines, including TNF-α and IL-1β (Lusso, 2006). We found significantly higher expression levels of TNF-α, and IL-6 in serum samples from patients with virus reactivation than in those from patients with persistent infection in latent phase. Effect of HHV-6 and/or HHV-7 reactivation on IL-6 synthesis is measured unequally (Atezdoo et al., 1999; Fujita et al., 2008). The results of this study showed that HHV-7 reactivation, but not co-reactivation of both viruses, induces significant increase of IL-6 production. The impact of HHV-6 and/or HHV-7 reactivation on clinical episodes
REFERENCES


in the early period after auto-HSCT is not clear. The risk of viral infection activation is associated with severe neutropenia, which is induced by myeloablative chemotherapy before HSCT. Clinical complications were found in 33.8% of patients and in all of them severe neutropenia and Roseolovirus infection reactivation were found. Severe neutropenia was observed, however also in patients without beta-herpesviruses infection, which might be associated with specific pre-transplant therapy. The development of complications was detected only in patients with active beta-herpesvirus infection, suggesting possible influence of virus reactivation on strength of immunosuppression. These results are in accordance with those reported by Razonable et al. (2003). Carrigan et al. (1993) reported two cases of severe interstitial pneumonitis associated with HHV-6 infection in bone marrow transplant recipients. Zerr et al. (2012) reported that relatively mild immunosuppression associated with HSCT might become more profound in patients whose pretransplant treatment already resulted in immunodeficiency. The patients diagnosed with HHV-6 reactivation are presented with high unexplained fever. In most cases of active HHV-7 infection no side symptoms are observed; however, HHV-7 reactivation also leads to or is associated with a number of symptoms, including acute febrile respiratory disease, fever, rash, vomiting, diarrhoea, low lymphocyte counts and febrile seizures (Govern and Barbarà, 2016). High frequency of HHV-6 and HHV-7 infection activation with simultaneous increase of pro-inflammatory cytokine serum levels suggests that both viruses may be involved in the development of complications in the early period after auto-HSCT process via their immunomodulatory ability.

The kinetics of the virus reactivation may reflect the potential role of HHV-7 as a co-factor of HHV-6 activation. Our data suggest that valacyclovir used in for antiviral prophylaxis and therapy is not sufficiently effective to prevent HHV-6 and HHV-7 infection activation after auto-HSCT. However, prophylaxis with acyclovir or valaciclovir in stem cell transplantation can be recommended to decrease the risk of herpesviruses infection reactivation during the early phase of transplant (Ljungman et al., 2011; Anonymous, 2008).
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Received 10 March 2016
HHV-6 UN HHV-7 INFEKCIJAS IETEKME UZ PĒCTRANSPLANTĀCIJAS KLĪNisko GAITU UN KOMPLIKĀCIJU ATTĪSTĪBU PACIENTIEM PĒC AUTOLOGAS CILMES ŠŪNU TRANSPLANTĀCIJAS

Autologā hemopoētisko cilmes šūnu transplantācija šobrid tiek izmantota kā standarta ārstēšanas metode pacientiem ar Hodžkina slimību, ne-Hodžkina limfomu un mielomas slimību. Šajā darbā tika pētīta HHV-6 un/vai HHV-7 infekcijas marķieru klātbūtnes pacientiem pirms autologās cilmes šūnu transplantācijas, šo virusu reaktivācijas biežums agrānajā pēctransplantācijas periodā un ietekme uz komplikāciju attīstību pēc autologās perifēro asīpu cilmes šūnu transplantācijas. Virusu genoma sekvenēšanu klātbūtnes DNS, kas izdala no pilnām perifērajām asinīm, tika noteikta ar polimerāzes ķēdes reakciju (polymerase chain reaction, PCR), HHV-6 slodze ar reālā laika PCR, virusspecifiskās antivielas un citokīni serumā ar ELISA, HHV-6 varianti ar endonukleāzes restrikcijas analīzi. Pētījumā iekļauti 76 pacienti, no kuriem 68 konstatēta Roseola virusu infekcija. Tika novērtēta virusu reaktivācija, mijiedarbība ar proinflamatorajiem jeb iekaisuma citokīniem TNF alfa un IL-6, kā arī pēctransplantācijas kliniskā gaita un komplikāciju attīstība. Anti-HHV-6 IgG klasses antivielas tika konstatētas 62 no 76 (81,6%) pacientiem pirms autologās cilmes šūnu transplantācijas. Visiem pacientiem pirms transplantācijas tika atrastas arī specifiskās anti-CMV un anti-EBV IgG klasses antivielas. Pacientiem pēc transplantācijas tika konstatēta nozīmīga biežāka HHV-7 aktivācija, salīdzinot ar HHV-6 (p = 0,0003) un vienlaicīgas HHV-6 + HHV-7 infekcijas aktivāciju (p = 0,0017). Komplikācijas pēc transplantācijas novēroja 30,3% pacientu, un visiem pacientiem ar komplikācijām tika konstatēta HHV-6 un /vai HHV-7 reaktivācija. Ievērojams HHV-6 un HHV-7 reaktivācijas paauglums ar vienlaicīgu iekaisuma citokīnu līmeņa palielināšanos serumā, ņemot vērā minēto virusu imūnmodulējošās spējas, liecina, ka abi virusi ir iesaistīti komplikāciju attīstībā pēc autologās perifēro asīpu cilmes šūnu transplantācijas. Šie dati ļauj domāt, ka HHV-7 darbojas kā HHV-6 aktivācijas kofaktors pēctransplantācijas pacientiem.