Journal of Clinical Virology 87 (2017) 67–72

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Infections due to alphaherpesviruses in early post-transplant period after allogeneic haematopoietic stem cell transplantation: Results of a 5-year survey

Maciej Przybylski[a,b], Anna Majewska[b,*], Tomasz Dzieciatkowski[a,b], Patrycja Rusicka[d], Grzegorz W. Basak[d], Barbara Nasilowska-Adamska[c], Jaroslaw Bilinski[d], Wieslaw W. Jedrzejczak[d], Marta Wroblewska[a,e,f], Kazimierz Halaburda[c], Grazyna Mlynarczyk[b], Agnieszka Tomaszewska[c]

[a] Department of Microbiology, Independent Public Central Clinical Hospital in Warsaw, 1A Banacha Str., 02-097 Warsaw, Poland
[b] Chair and Department of Medical Microbiology, Medical University of Warsaw, 5 Chalubinskiego Str., 02-004 Warsaw, Poland
[c] Department of Haematopoietic Stem Cell Transplantation, Institute of Haematology and Transfusion Medicine, 14 Gandhi Str., 02-776 Warsaw, Poland
[d] Department of Haematology, Oncology and Internal Medicine, Medical University of Warsaw, 1A Banacha Str., 02-097 Warsaw, Poland
[e] Department of Dental Microbiology, Medical University of Warsaw, 1A Banacha Str., 02-097 Warsaw, Poland
[f] Infection Control Unit, Institute of Haematology and Transfusion Medicine, 14 Gandhi Str., 02-776 Warsaw, Poland

ARTICLE INFO

Article history:
Received 18 June 2016
Received in revised form
30 September 2016
Accepted 13 December 2016

Keywords:
HSV
VZV
HSCT
Post-transplant infectious complications
Real-time PCR

ABSTRACT

Background: Infections caused by human α-herpesviruses usually have a benign course with recurrencies. However, they may become dangerous in immunocompromised hosts. In this case, molecular methods constitute a reliable diagnostic tool enabling rapid assessment of the efficacy of antiviral treatment strategies.
Objectives: We estimated the frequency of alphaherpesviral DNAemia and the viral load during early post-transplantation period after alloHSCT; we also analyzed association of the DNAemia and chosen parameters of the patients.
Study design: A cohort of 190 alloHSCT recipients from two hospitals in Warsaw, Poland, was examined weekly during 100-day early post-transplantation period using quantitative real time PCR assays. A total of 2475 sera samples were evaluated for the presence of α-herpesviral DNA in patients, of whom 117 (62%) received unrelated grafts, while the remaining 73 (38%) received grafts from sibling donors. All patients received standard antiviral prophylaxis with acyclovir. In the examined group, anti-HSV-1, anti-HSV-2 and anti-VZV IgGs were examined prior to transplantation,
Results: Within the study period, DNA of α-herpesviruses was detected in 44 patients (23.2%). Most patients tested positive for HSV-1 DNA (43 patients, 22.6%), single patient for HSV-2, and no patient positive for VZV. Clinical symptoms such as pneumonia, skin changes, elevated levels of aminotransferases were observed in five patients, four of these patients presented symptoms of GvHD at the same time. (2,6%). Statistics shows that GvHD ($P < 0.001$) and matched unrelated donor as a source of HSCT ($P = 0.048$) are associated with the development of HSV-1 DNAemia.
Conclusions: Although our data demonstrate frequent reactivation of HSV-1 in the early post-transplant period, the rate of symptomatic infections was low. We did not find association between HSV-1 viremia and mortality, but significant association with GvHD and donor source was observed.

1. Background

Infections caused by α-herpesviruses (Herpes Simplex Virus type 1; HSV-1, Herpes Simplex Virus type 2; HSV-2 and Varicella Zoster Virus; VZV) are among the most common viral diseases after haematopoietic stem cell transplantation (HSCT) [1–3]. Herpes simplex viruses 1 and 2 are often the first herpesviruses which reac-

* Corresponding author.
E-mail address: amajewska@wum.edu.pl (A. Majewska).

http://dx.doi.org/10.1016/j.jcv.2016.12.008
1386-6532/

tivate in about 25–40% of transplant recipients, usually between the 2nd and 4th week after transplantation [2]. A disseminated infection of HSV-1 or HSV-2 etiology in immunosuppressed patients is usually fatal, despite antiviral therapy [2,3]. Before the use of acyclovir (ACV) as prophylaxis, VZV was a classic opportunistic infection in patients with lymphoma or acute leukaemia or after HSCT, sometimes becoming disseminated to the lungs, liver or the central nervous system. The studies of bone marrow transplant recipients, show that the risk of symptomatic infection amounts to 63% [4]. In the case of recipients of peripheral blood haematopoietic cells, the probability is assessed at 37%, but when the disease was eligible for transplant-related lymphoproliferative disorders, the incidence of reactivation could reach 72% [4].

Detection of viral DNA with use of quantitative real-time PCR (qPCR) is of particular value in patients who are at the greatest risk of illness and death [5–7] and for evaluation of antiviral treatment efficacy [2,8], but molecular monitoring of α-herpesviruses based on quantification of the virus load (VL) is not a routine procedure in most diagnostic laboratories.

2. Objectives

During the early post-transplant period, in a group of 190 Polish consecutive haematological patients subjected to allogeneic HSCT, we estimated the frequency of α-herpesviral DNAemia and the corresponding viral load, using qPCR assays; we also analyzed association of the DNAemia and chosen parameters of the patients (Table 1).

3. Study design

This retrospective study utilized serum samples collected from a cohort of 190 adult patients, who underwent consecutive allogeneic HSCTs between January 2009 and December 2013 at the Medical University of Warsaw and at the Institute of Haematology and Transfusion Medicine. The viral load in serum samples was monitored weekly in the early post-transplant period of 100 days after HSCT, during routine CMV monitoring, concordant with EBMT guidelines. All HSCT recipients received standard antiviral prophylaxis with ACV in typical doses (3×500 mg iv./day or 4×400 mg p.o./day) for at least 100 days after transplantation. Among 190 patients, 68 received myeloablative conditioning and 122–reduced intensity (non-myeloablative) conditioning. The most common myeloablative conditoning was fludarabine/busulphan for 4 days and the most common non-myeloablative conditioning regimen for HSCT recipients from related donors was fludarabine/busulphan for 2 days, and for HSCT recipients from matched unrelated donors–fludarabine/busulphan/anti-thymocyte globulin for 4 days. Brief characteristics of the patients are shown in Table 1.

The presence of antibodies specific for HSV-1, HSV-2 and VZV, both in IgM and IgG classes, was measured in a panel of patients' serum specimens, using commercial qualitative EIA tests standardized for *in vitro* diagnostics (Bioactiva Diagnostica GmbH, Germany). The examination was performed once, in a serum sample collected prior to transplantation.

Detection of α-herpesviral DNA in 2475 sera samples was performed with two separate real-time PCR assays, detecting HSV-1/HSV-2 and VZV, respectively. Total DNA was extracted from 200 μl of serum, using a High Pure Viral Nucleic Acid Kit® (Roche Diagnostics, Germany), in accordance with the manufacturer's instructions. Real-time PCR tests were run on a LightCycler 480 instrument (Roche Diagnostics, Germany), using *in-house* quantitative methods described previously [9,10]. In each run, except for samples, HSV-1/HSV-2 and VZV DNA standards in the range of 100–100,000 copies/ml, were used, as well as a negative control of DNA extraction and amplification process. Efficacy of DNA extraction and amplification process was assessed during routine CMV monitoring, with use of internal control (Roche Diagnostics, Germany), added to each sample prior to DNA extraction. Viral DNA copy numbers was quantified on the basis of threshold cycle (*Ct*) values of viral calibrators. As a *cut-off* values, LODs (limit of detection) of used qPCR assays were adopted for this study.

Prior to comparisons, VL values were transformed to their decimal logarithms. One-way ANOVA was used for identification of significant differences in parametric values, and a *t*-test was used for pairwise comparisons. For the analysis of proportions, Chi-square test with Yates correction was used. To identify relationships, Pearson correlation was used for non-parametric variables and Spearman correlation for parametric variables. Survival curves were generated with the Kaplan-Meier method. *P*-value for significance was set at 0.05. All statistical analyses were performed using SigmaStat 3.1 software package (Systat Software, Inc.).

4. Results

Prior to transplantation, patients were examined for the presence of specific antiviral antibodies in both IgM and IgG classes. In the examined cohort of 190 adult HSCT recipients, 110 individuals had anti-HSV-1 IgG (57.9%), while anti-VZV IgG was detected in 163 patients (85.8%), anti-HSV-2 IgG in 19 patients (10%) and anti-HSV-1 IgM in a single patient. Antibodies against HSV-2 and VZV in IgM class were not detected in any patient.

The presence of viral DNA was monitored on a weekly basis for 100 days after transplantation, with a total number of 2475 serum samples obtained for examination (median 13 samples obtained from a single patient). In general, DNA of α-herpesviruses was detected in 142 serum samples collected from 44 patients (23.2%). Most patients tested positive for HSV-1 DNA (43 patients, 22.6%), with HSV-2 seen only in one patient and no patient positive for VZV. All patients who developed HSV-1 and HSV-2 DNAemia had specific IgGs against these viruses, what suggests reactivation of the latent virus rather than a primary infection. In a single patient with positive anti-HSV-1 IgM result, viral DNA was not detected during the assessed 100-day period.

The median time to the first detection of HSV-1 DNAemia was at 15 days post-HSCT (ranged from 6 to 93 days). The duration of HSV-1 viraemia ranged from 5 to 37 days, with the median of 12 days. The average viral load (VL), expressed as decimal logarithm of viral DNA copies number, was 2.183 $\log_{10}$/ml (SD = 0.175), and ranged from 2.0 to 2.65 $\log_{10}$/ml. In the single patient in whom HSV-2 DNA has been detected, the average VL from two consecutive positive samples of serum (collected at 20th and 26th day after transplantation) was 2.06 $\log_{10}$/ml. Because of very small variations in VLs of HSV-1 between patients, no significant differences were observed, regardless of the considered factor (Table 1). During assessed period, the most common symptom was fever of unknown origin (FUO), observed in 153 patients (80.5%). Rate of FUO episodes among the patients with and without HSV-1 DNAemia was similar (81.2% vs 80.3%). Moreover, in the group of patients with HSV-1 DNAemia, there was no clear correlation between FUO episodes and time when HSV-1 DNA was detected in serum samples. However, there was group of five patients which presented pneumonia during episodes of HSV-1 DNAemia; four of them had coexisting skin changes correlated with intensification of GvHD, and in three of them elevated levels of transaminases were also observed. Prospective assessment of CMV viral load was performed as a part of routine monitoring in HSCT recipients according EBMT guidelines [11], and we did not observed any correlation between HSV-1 DNAemia and results of CMV DNA testing (data not shown).