

Suspected chromosomally integrated human herpes virus 6 in hematopoietic stem cell transplantation

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Summary

Background and aims: We report a case of a 27-year-old male affected by acute myeloid leukaemia *MLL-PTD* positive. After autologous stem cell transplantation, he was monitored based on cytomegalovirus, Epstein-Barr virus and human herpes virus 6 (HHV-6) DNA quantification in blood. Relapse occurred one year after transplantation; then the patient underwent to allogenic bone marrow transplantation using genotypically HLA-identical donor (sister). HHV-6 DNAemia was positive and persistently elevated, either after autologous either after allogenic transplant suggesting the occurrence of HHV-6 chromosomally integration. The work aim is to prove the occurrence of chromosomally integrated-HHV-6 (ci-HHV-6).

Materials and Methods: HHV-6 DNA extraction was performed by automated extractor and DNA was amplified-quantified by Real Time polymerase chain reaction. Species identification was performed by sequencing HHV6-U100 glycoprotein using automated sequencer and sequencing products were analysed using the Blast program.

Results: After autologous transplantation HHV6-DNAemia was 5.4 log copies/mL setting to 3.9 log copies/mL for a long period post allogenic transplantation. The patient's hair follicles were tested for HHV-6 DNA having positive results. Sequences of both strains of HHV6 extracts from blood and hair follicles resulted species B. HHV6 viral load decreased significantly after Lymphocyte Infusion by ci-HHV6 negative donor (sister), having steady viral load during the following six months of monitoring. One year later, patient is in complete haematological remission.

Conclusions: Detection of HHV-6 in hair follicles and HHV-6 DNAemia persistently elevated before allogenic transplant, confirm the occurrence of ci-HHV-6. The observed important decreasing viral load is potentially due to the successful engraftment of ci-HHV-6-negative donor marrow after allogeneic transplant.

Introduction

Human herpes virus 6 (HHV-6) is a ubiquitous β -herpes virus recently classified into two different species: HHV-6A and HHV-6B (1). In immunocompetent individuals, HHV-6B is the cause of the common childhood illness (*exanthema subitum*); HHV-6 infections, especially from HHV-6B species, have been associated with morbidity after hematopoietic stem cell transplantation (HSCT), especially with delayed monocyte and platelet engraftment (5), encephalitis, fever (12) and rash (10). In most of infected people, HHV-6 has been found to persist throughout life and subsequently reactivate; HHV-6 is the only human herpes virus found integrated into host chromosomes persisting in latent state, a condition called chromosomally integrated human herpes virus 6 (ci-HHV-6) (7). Complete HHV-6 genome is integrated into the telomere of human chromosomes (6). Since HHV-6 can infect and integrate into germ cells, ci-HHV-6 can be inherited in a Mendelian manner, with a 50% chance of being passed to a child. Moreover, because of its presence into DNA of germ cells, at least one copies of HHV-6 is present in every nucleated cell of the body, from PBMCs to hair follicles (5). The mean HHV-6 DNA concentration in blood of individuals with ci-HHV-6 is 5.5 log copies/mL of whole blood and the DNAemia is persistently high (11). The demonstration of the presence of integrated HHV-6 can be achieved by DNA PCR testing of hair follicles or nails for the presence of >1 copies/hair follicle (2). Several studies suggest that ci-HHV-6 transplant patients may be more likely to experience GVHD and bacterial infections (4).

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Case Report

We report a case of a 27 years old male affected by acute myeloid leukaemia *MLL-PTD* positive (mixed-lineage leukaemia-partial tandem duplications). In 2009 he underwent autologous stem cell transplantation, showing delayed engraftment. Relapse occurred one year after transplant; the patient was treated by allogeneic bone marrow transplantation after high-dose chemotherapy using genotypically HLA-identical donor (sister). 10 months later a second relapse was treated by chemotherapy followed by Donor lymphocyte infusion (DLI). The present study was carried out during hematologic remission.

The patient was monitored based on cytomegalovirus (CMV), Epstein-Barr virus (EBV) and HHV-6 DNA quantifications in blood after allogeneic transplantation. We never observed reactivation of CMV neither of EBV. The patient had persistently high levels of circulating HHV-6 DNAemia, 5.4 log copies/mL, after autologous stem cell transplantation; the suspect of ci-HHV-6 led us to perform hair follicles analysis. DNA PCR testing of hair follicles resulted positive for the presence of HHV-6 DNA. It was performed gene sequencing U100 strains of HHV-6 extracts from blood and hair follicles. Sequences resulted perfectly stackable and relieved HHV-6 species B.

Results and Discussion

HHV-6 viral load was performed by QIAcube automated DNA extraction, using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Isolated DNA was amplified and quantified by Real Time PCR using Q-HHV6 Real Time Alert Kit (Elitechgroup Spa, Turin, Italy) based on TaqMan® chemistry, using 7500 Fast Dx Instrument (Life Technologies Carlsbad, CA, USA). Genomic DNA extraction was performed incubating overnight 10 hair follicles at 56°C with 180 µL ATL Buffer (QIAGEN) and 20 µL proteinase K, followed by QIAcube automated DNA extraction, using QIAamp DNA miniKit selecting protocol *Tissue*. Of a final volume = 200 µL, 5 µL were amplified by real time PCR. Nested RT-PCR was used to amplify glycoprotein U100 of HHV-6. To identify species after DNA extraction from whole blood and hair follicles, two rounds of PCR were performed using the primers sets from Norton *et al.* (8). Amplified products were separated by agarose gel electrophoresis,

stained with ethidium bromide and visualized using UV light in the Gel Doc 2000 (Bio-Rad, Hercules, CA, USA). BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) was used to sequencing PCR products by using ABI3130x/ Genetic Analyser automated sequencer (Applied Biosystems). 898 nucleotides sequencing products were analysed using the Blast program (<http://blast.ncbi.nlm.nih.gov>).

After autologous stem-cell transplantation, HHV-6 DNAemia resulted 5.4 log copies/mL and was persistently >8000 copies/mL (log 3.9) (Figure 1), being between 8500 and 23000 copies/mL, for a long period post allogeneic transplantation (July 2010 to May 2011). During the fifth week he developed peak viraemia and was treated with ganciclovir, achieving just a moderate viraemia reduction. In April 2011 a relapse occurred and was treated with azacitidin in combination with DLI; at 46th week, in may he was treated with ganciclovir achieving again just a moderate HHV-6 viraemia reduction (median viral load 4550 copies/mL). In September, after the finding of bone marrow blast cell ratio >50%, salvage chemotherapy called FLANG was initiated obtaining complete haematological remission with a recovery of donor chimerism. In January 2012, and again in February, during haematological remission he received DLI after that occurred moderate graft-versus-host-disease with skin and liver involvement. HHV-6 viral load decreased to <556 copies/mL (lower limit of linearity) whole blood, having steady viral load during the following six months of monitoring.

A year later, patient is in complete haematological remission, showing in August HHV-6 steady viral load <556 copies/mL whole blood.

Viral load results suspicious for ci-HHV-6, lead to conduct DNA PCR testing of hair follicles. Qualitative Real Time PCR results tested positive for the presence of HHV-6 DNA, with comparable value of Beta-globin cycle threshold (26.9 and 27.88 respectively), meaning ≥1 HHV-6 copies/cell.

HHV6 DNAemia performed on donor was negative.

Nucleotides sequencing products analysed from whole blood and hair follicles revealed a 99% identity with the HHV-6B HST strain (AB021506.1) and HHV-6B Z29 strain (AF157706.1).

Conclusions

Detection of HHV-6B in hair follicles obtained from the patient and HHV-6 viral load persistently elevated (5.4 log copies/mL whole blood)

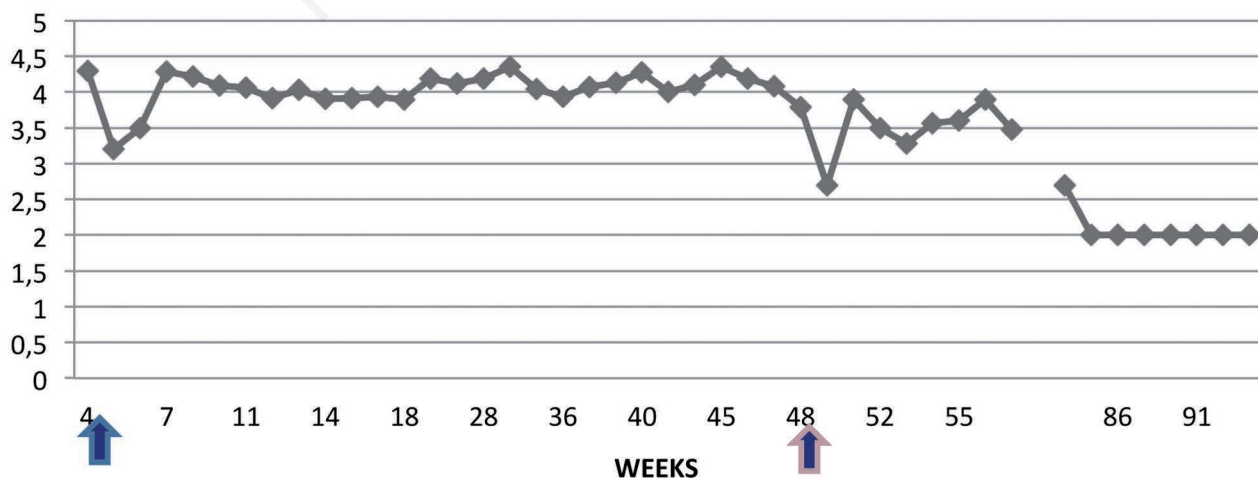


Figure 1. Monitoring the human herpes virus 6 load (weeks after hematopoietic stem cell transplantation) in whole blood (copies/mL).

before allogeneic transplant, confirm the occurrence of ci-HHV-6. Level of viral load after allogeneic bone marrow transplantation decreased consistently, 3.9 log/4.36 log copies/mL, and, even if case of viraemia peak, no longer reached high viral load suggestive of ci-HHV-6 (10,6,3). The observed further decreasing viral load is potentially due to the successful engraftment of ci-HHV-6-negative donor marrow after DLI administration performed in 2012.

Further studies are needed to clarify if a correlation does exist between virus reactivations and frequent relapses, and between ci-HHV-6 and GVHD.

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