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Case Report

Genomic Integration of HHV-6 Mimicking Viral Reactivation after Autologous Stem Cell Transplantation

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Abstract. The monitoring of Human Herpesvirus 6 (HHV-6) after allogeneic stem cell transplantation has proven to be useful in preventing life-threatening complications; however, the pathogenic role of HHV-6 after autologous transplantation is not well-characterized, although viral reactivation might be responsible for significant complications even after this type of transplant. Here we report, for the first time to our knowledge, the case of a patient with chromosomally integrated HHV-6 (ciHHV-6), presenting with high titers of HHV-6 DNA copies after autologous transplantation, mimicking HHV-6 reactivation. The presence of viral DNA in the follicle bulb confirmed the ciHHV-6 and allowed for the discontinuation of the antiviral treatment. Due to the increasing awareness of HHV-6 potential pathogenicity and the fact that ciHHV-6 is expected in 1-2% of the population, such a case might be helpful in recognizing ci HHV-6, thus avoiding unnecessary and potentially toxic antiviral therapy once the viral genomic integration is confirmed.

Keywords: Bone marrow transplantation, Infectious disease, HHV-6 integration.

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Introduction. Human herpesvirus 6 (HHV-6) reactivation may be responsible for severe side effects after allogeneic hematopoietic stem cell transplantation (allo-HSCT) with early post-transplant mortality.[1-3] HHV-6 reactivation is typically detected 2 to 4 weeks after allo-HSCT and is associated with hepatitis, pneumonitis, CMV reactivation, fever, skin rash, myelosuppression, encephalitis, acute graft-versus-host disease; therefore, its regular monitoring is recommended after those allo-HSCTs with a higher risk of reactivation, such as cord blood or haploidentical HSCT.[4-5] At our center, the control of HHV-6 is routinely performed after cord or haploidentical HSCT, whereas for all the other transplant settings (i.e., allo-HSCT from HLA-identical donor, or autologous stem cell transplantation, ASCT) the search for HHV-6 is made only in the presence of clinical conditions evoking a potential role of the

virus, such as prolonged aplasia or cutaneous rash[6]. Recently, it has been discovered that 1% to 2% of the population have chromosomally integrated HHV-6 (ciHHV-6), that is the presence of HHV-6 in every somatic cell leading to high copy numbers of the virus in the absence of any viral reactivation or disease.[7-10]

Methods. Here we present a case of a patient affected by non-Hodgkin's lymphoma undergoing high-dose chemotherapy followed by ASCT, with ciHHV-6 (detected through CMV HHV6,7,8 R-gene®, bioMérieux) mimicking viral reactivation early after transplant.

Results. A 66-years old man was diagnosed with stage IVA diffuse large B-cell lymphoma in December 2015 following the appearance of hepatomegaly, leading to the detection of a 7-cm hepatic mass by abdominal ultrasound. Complete staging showed sub- and supradiaphragmatic nodal and extranodal disease (liver, lung), with a Revised-International Prognostic Index (R-IPI) = 4 (stage, LDH higher than the average range, age> 60 years old and extranodal disease) and an NCCN IPI=5. The patient's history disclosed myocardial infarction 25 years before and concurrent HCV positivity. He received six cycles of full-dose R-CHOP with concomitant central nervous system prophylaxis, from April to July 2016. A new disease assessment in August 2016 showed metabolic complete remission. Due to the high-risk features at diagnosis, consolidation therapy with high-dose chemotherapy followed by ASCT had been planned, and the patient was admitted to the transplantation unit at the end of August to receive ASCT, after conditioning chemotherapy with FEAM (fotemustine, etoposide, cytarabine, melphalan) regimen.[11] The aplastic phase was complicated by NCI-CTC grade 3 mucositis and Aspergillus pneumonia, classified as *probable*, according to EORTC criteria,[12] successfully treated with voriconazole (the patient has been receiving prophylaxis with fluconazole). On day +15 after ASCT, a determination of HHV-6 viral load was made because of persistent aplasia, showing 9.7 x 10e5 copies/mL, with a total leucocytes count of 1,380 x 10e6/L. Molecular analysis by PCR was negative for CMV and EBV. Then, in the hypothesis of HHV-6 reactivation, known to have an incidence of 8-15% after ASCT,[13-15] antiviral therapy with foscarnet was started, and subsequent shift to ganciclovir was realized, after hematological recovery. However, the constant increase of HHV-6 load, observed in conjunction of the augmentation of the leucocyte counts (see **Figure 1**), led to the alternative hypothesis of ciHHV-6, soon confirmed by the analysis of patient's follicle bulb at the beginning of October.

Indeed, it is known that ciHHV-6 corresponds to the presence of one or more HHV-6 copies per white blood cell, with high HHV-6 titers persisting over time in blood, and of virus in tissues, allowing for a diagnosis made through the analysis of follicle bulb or nails.[16] Nonetheless, a confirmatory test is needed in the diagnostic process, as we did in the present case. The patient

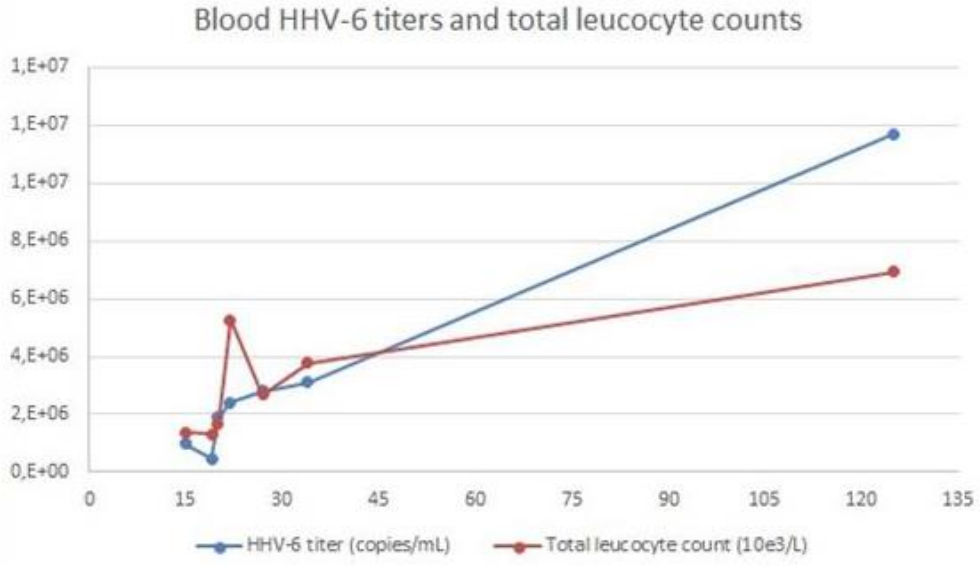

Figure 1. Whole blood HHV-6 titers and total leucocyte counts are shown here. On the X axis, the days after autologous transplant are shown.

was discharged in good clinical conditions at day +28 from ASCT, and the antiviral treatment was discontinued at the moment of hospital discharge. Finally, the cause of the prolonged aplasia was not identified. Three months later the patient was healthy, and a CT scan confirmed the complete remission and a favorable evolution of the fungal infection. As expected, leukocyte, neutrophil and platelet counts were normalized, while HHV-6 blood titer was 1.14 x 10e7 copies/mL with a total leucocyte count of 6,920 x 10e6/L in early January 2017.

Discussion. The present clinical case shows a genomic integration of HHV-6 mimicking reactivation early after autologous stem cell transplantation. Similar cases of transmission of integrated HHV-6 were described in recipients of allogeneic transplant from HLA matched or mismatched related donors;[17-18] more recently, it has been described after allogeneic, combined cord blood/haplo transplantation.[19] However, to our knowledge, our case is the first described after autologous stem cell transplantation. It is known from the literature that approximately 95% of the healthy adults experienced the HHV-6 infection in childhood. After primary infection, HHV-6 persists in the host and is detectable in multiple tissues, such as salivary glands, brain cells, monocytes, and early bone marrow progenitor cells in a similar way to other herpesviruses as CMV. However, ciHHV-6 represents an alternative form of viral persistence, occurring in a subgroup of individuals and characterized by very high viral loads in the blood (>1x10^6 HHV-6 copies/ml), in other body fluids or tissue samples. Growing evidence suggests that the integrated viral sequences are inherited through the germline and are therefore present in every nucleated cell. This phenomenon may confound the laboratory diagnosis of active HHV-6 infection because whole blood and spinal fluid from individuals with viral integration are persistently positive for HHV-6 DNA even in the absence of independent viral replication. Therefore, ciHHV-6 must be suspected every time whole blood HHV-6 DNA is detected at high load at the time of engraftment and persists at a high level after transplant, without any adverse influence on patients outcome.

Although the frequency of HHV-6 infection is quite different between allo-HSCT and ASCT, being higher after allo-HSCT,[20] the recognition of these cases may lead to the avoidance of unnecessary and potentially toxic antiviral treatments, particularly during the delicate phase of hematological recovery in the early post-transplant period. Currently, the screening for ciHHV-6 is not recommended, but we believe that the awareness of this phenomenon after ASCT is of interest for clinicians working in the field, since over 20,000 ASCTs are reported by the European Blood and Marrow Transplantation,[21] with an estimated number of 200-400 such new cases every year only in Europe.

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