Case report:

HHV8 replication, in a HIV positive patient during disseminated Tuberculosis

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Running Head: HHV8 replication in HIV positive patient
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Abstract

We describe the case of an HIV positive patient, in whom significant HHV8 replication was detected during disseminated tuberculosis. The initial clinical presentation was highly suggestive of MCD, highlighting the fact that high HHV8 viral loads should be interpreted with caution, while awaiting the histological analysis of lymph nodes biopsy.
Introduction:

Human herpes virus 8 is associated with the development of Kaposi’s sarcoma (KS) and multicentric Castelman disease (MCD), mostly in untreated HIV positive and immunocompromised patients.

MCD is an atypical lymphoproliferative disease, characterised by systemic symptoms, which include fever, weakness, severe weight loss, generalised lymphadenopathy and hepatosplenomegaly. The pathological examination of lymph nodes reveals angio-follicular hyperplasia, atrophic germinal centers surrounded by concentric layers of small B cells with a typical « onion skin » feature, and intense inter-follicular plasma cell hyperplasia. Immunohistochemistry using antibodies against LANA, an HHV8 latent antigen, allows the detection of HHV8-infected B cells. These cells have undergone plasma cell differentiation and are mainly located in the
mantle zone. This staining is particularly useful when the typical features such as the «onion skin» lesion is lacking and when intense inter-follicular hyperplasia may be considered as non specific or secondary to HIV infection. MCD is clinically very aggressive and can progress to frank monoclonal lymphoma. The median survival is 14 to 48 months from diagnosis (1, 2).

In HIV positive patients, in addition to anti-retroviral treatment, MCD requires chemotherapy by etoposide, vinblastine, anti-CD20 (rituximab) or combined chemotherapy (CHOP), which may be associated with major side effects (3).

The clinical presentation of MCD resembles opportunistic infections (OI), for which chemotherapy would be emphatically contra-indicated. It is therefore crucial to rapidly establish the correct diagnosis.

HHV8 DNA can be detected in the blood, by gene amplification. Positive values are detected during KS and MCD, but levels rise by several orders of magnitude during active MCD (3, 4, 5).

We describe a patient with HIV infection and severe constitutional symptoms in whom an extensive search for an opportunistic disease was initially negative. HHV8 DNA was detected at high levels and chemotherapy, for presumed MCD, was to be initiated, just before a repeat lymph node biopsy finally established that the patient had tuberculosis.

**Methods**

**Detection and quantification of HHV8 viral load**
HHV8 viral load in the plasma, whole blood and PBMC were evaluated by qualitative and quantitative PCR. DNA was extracted using a EZ1-DNA extraction robot (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer’s instruction. For qualitative evaluation, a nested PCR for the HHV8 ORF 26 region was amplified as previously described (6). All reactions were performed in duplicates. For the calculation of molecule quantity, serial dilutions of ORF 26 containing HHV8 plasmid DNA were analyzed and a standard curve established as previously described (7). Results were expressed in copies/ml in whole blood and plasma; copies per 100’000 cells for PBMC.

**Case Report**

A 25-year-old man of West African origin was diagnosed HIV positive in August 2006. In January 2007, the patient started complained of several bouts of asthenia, weight loss, fever and abdominal pain. He was hospitalised in February 2007. On physical examination, he had fever (38.9° centigrade), multiple inguinal and axillary adenopathies and hepatosplenomegaly. The chest examination was clear. No skin or mucosal lesions were seen.

The blood count revealed haemoglobin of 113 g/L and low platelet count 70 G/L. There were mild liver test disturbances (ASAT 103 U/l (N: 14-50 U/L), ALAT 69 U/L (N: 12-50 U/L), Alcaline Phosphatase 350 U/L (N: 30-125 U/L), Gamma Glutamyl Transferase 271 umol/L (N: 9-40 U/L) and LDH 529 U/L (N: 125-240 U/L)). CD4 count was 224 /mm3 (13%) and HIV viremia 2.7E6 copies/ml (table1).

CT scan revealed multiple mediastinal, retroperitoneal and pelvic lymphadenopathies; hepatosplenomegaly and disseminated pulmonary
micronodules. The tuberculin skin test and the whole blood interferon gamma assay were both positive.

Cultures for mycobacteria in sputum, BAL, urine and blood were performed, but no acid-fast bacilli were seen. An axillary lymph node biopsy showed non-specific reactive lymphoid hyperplasia. A PCR search revealed neither DNA of Mycobacterium tuberculosis complex nor clonal B or T cells.

At this point, because of the absence of mycobacteria in all samples and because the patient’s presentation was also compatible with MCD, HHV8 DNA was measured. The results revealed a HHV8 viral load of 198,000 copies/ml in whole blood, 260 copies/10E5 cells, in peripheral blood mononuclear cells, and 39,400 copies/ml, in plasma. Physical examination did not reveal any mucocutaneous signs of KS.

The patient underwent a second lymph node biopsy, which revealed areas of necrosis surrounded by giant cell granulomas. The Ziehl-Neelson staining was again negative, but the PCR search for Mycobacteria tuberculosis complex was positive enabling us to conclude to disseminated tuberculosis. There was no evidence for MCD or any other lymphoproliferative process.

Isoniazide, rifampicin, ethambuthol and pyrazinamide were started. The patient’s condition improved. HAART was started two months later, once the antiTB treatment had been simplified. Eventually, *M tuberculosis* grew in the cultures of the first bronchial aspirate and then in the second biopsy.

We analyzed the HHV8 DNA in two other plasma samples: one dating back to August 2006 and one taken 10 days after the positive sample, interval during which the patient had received methyprednisolone 250 mg tid for 2 days. Both samples
were negative. All the samples were double-checked in the same laboratory. Genotypic analyses of HIV in both the positive and negative samples were identical. A search for latent and lytic HHV8 antibodies was performed in the August 2006 sample. It confirmed that the patient was HHV8 seropositive before this episode. No variation in the titles between August 2006 and March 2007 was noted.

**Discussion**

The clinical presentation of fever, weight loss, generalised lymphadenopathy and enlargement of liver and spleen, in a young HIV positive patient of African origin, was suggestive of tuberculosis. Nevertheless, as the time went passed and the search for mycobacteria remained negative, the diagnosis was enlarged to include MCD. This was supported by the clinical presentation and the high HHV8 viral load measured in the plasma. Finally the histology and positive PCR assay of the second lymph node biopsy established the diagnosis of disseminated tuberculosis and the patient was apparently cured by antiTB treatment. This evolution, as well as two lymph node biopsies without evidence of MCD, make it unlikely that the patient had both TB and MCD. Physical examination never revealed any signs of KS.

This case reveals that high levels of HHV8 can be measured during an OI other than KS or MCD, suggesting that HHV8 infection can be transiently reactivated, in apparently an asymptomatic way.

HHV8 DNA can be detected in whole blood, plasma and most frequently in peripheral blood mononuclear cells (PBMCs). Positive values in PBMCs have been found in up to 73.2 % of patients with KS (8) and in all patients with MCD or primary effusion
lymphomas (9). Oksenhendler and al, in a prospective study on 23 HIV patients with MCD, measured a mean value of 4,77 log copies/ug DNA in the PBMCs of all his patients. This value was slightly higher in patients with MCD and KS. It is noteworthy that 2 of the 12 patients with asymptomatic HHV8 infection had a low detectable level of 2,91 log copies (5). In a retrospective study on 8 HIV positive patients with either KS or KS and MCD, Boivin and al measured HHV8 DNA levels as high as 47 210/10E5 cells in PBMCs and 256/10 ul (25 600/ml) in plasma, in the patients with MCD, whereas most of the patients with KS had undetectable values or at the most 135 copies/10E5 cells (4). HHV8 DNA detection predicts the clinical evolution and the response to treatment of both KS and MCD but its positive predictive value is still unknown. (4, 5). On the contrary, it must be noted that the negative predictive value of HHV8 DNA in PBMCs, when associated with clinical and biological symptoms of MCD, is quite high. In this case, the high viral concentration found in the plasma may suggest replication and non specific activation of a latent HHV8 infection in the context of an other infection.

There are few references on HHV8 reactivation without evidence of MCD or KS. Van der Kuyl and al detected HHV8 DNA in the PBMCs of 14% of asymptomatic HHV8 infected patients (median 2,0 log/10E6 cells Half of the patients with HHV8 reactivation had concomitant CMV disease (10). Lisco et al detected a positive HHV8 viral load in PBMCs of pregnant HIV-infected women, during the second and third trimester, suggesting that pregnancy may induce HHV8 replication (11). Finally, Hudnall et al detected active HHV8 replication in healthy HHV8 seropositive renal transplant patients (12).
In non HIV immunosupressed patients, for instance transplant recipients, other herpes viruses are known to reactivate, such as CMV or EBV. A regular follow-up of the DNA load is suggested to detect continuous replication and elevated values, in order to try to prevent the development of disease and adapt the immunosuppressive treatment (13).

This case report highlights the fact that the full clinical meaning and implication of a positive HHV8 viral load and its quantification, in patients with AIDS, remains to be clarified. Its potential for diagnosing or predicting MCD needs to be studied further and sensitivity and specificity determined in whole blood, PBMCs and plasma. Finally, when faced with the dilemma of urgently starting chemotherapy, in a deteriorating patient with a clinical presentation suggestive of MCD, high HHV8 viral loads should be interpreted with caution and histological analysis of lymph nodes obtained first, in order to establish the definitive diagnosis of MCD.

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**REFERENCES:**


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**Table 1.** Laboratory values.