Relationship between the Quantity of Kaposi Sarcoma–Associated Herpesvirus (KSHV) in Peripheral Blood and Effusion Fluid Samples and KSHV-Associated Disease

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The Kaposi sarcoma–associated herpesvirus (KSHV)-DNA level was determined in samples from 71 patients with Kaposi sarcoma (KS), 28 patients with multicentric Castleman disease (MCD), and 8 patients with primary effusion lymphoma (PEL). KSHV-DNA levels were higher in patients with active KS or MCD than in those with KS or MCD in remission. Among patients with active disease, the highest KSHV-DNA levels were observed in effusion fluid samples from patients with PEL (7.2 log10 copies/150,000 cells), followed by blood samples from patients with MCD and PEL (4.86 and 3.83 log10 copies/150,000 cells, respectively), and the lowest levels were observed in blood samples from patients with KS (2.63 log10 copies/150,000 cells). Determining the KSHV-DNA level may be useful in diagnosing KSHV-associated disease and for following up patients with KS when the development of MCD or PEL is suspected.

Kaposi sarcoma–associated herpesvirus (KSHV, also called human herpesvirus–8) is causally linked to Kaposi sarcoma (KS), the multicentric variant of Castleman disease (MCD), and a rare type of lymphoma called primary effusion lymphoma (PEL) [1, 2]. Although KSHV has clearly been associated with these 3 diseases, the level of KSHV DNA and the state of the virus in the target cells specific to these neoplasms are not the same. In patients with KS, KSHV-DNA sequences have been detected by polymerase chain reaction (PCR) in samples from KS lesions, and semiquantitative PCR has shown that the KSHV-DNA load is significantly higher in KS lesions, compared with normal skin [1]. In situ investigations have revealed that most spindle cells are latently infected, whereas a subset of cells (1%–5%) can support lytic infection [3]. The number of viral copies per spindle cell has been estimated at 1–5 [2, 4]. KSHV has also been detected in peripheral blood mononuclear cells (PBMCs) in up to 70% of patients with KS, and detection of KSHV in PBMCs is predictive of the development of KS in HIV-infected patients [5]. Moreover, it has been shown that the detection of KSHV-DNA sequences in PBMCs correlates with the clinical stage of KS [6]. In the context of HIV infection, MCD is always associated with KSHV infection, and a high KSHV viral load (or DNA level) in PBMCs is associated with the exacerbation of MCD-related clinical symptoms [7]. In the lymph nodes of patients with MCD, KSHV has been localized in plasmablast cells of the mantle zone, with 10%–20% of the cells harboring lytic infection, although the exact number of viral copies per cell has not been determined [3]. An earlier study using conventional PCR and Southern blot analysis demonstrated that a PEL cell contained approximately 50–150 copies of the KSHV genome [2]. Recently, real-time PCR was used to detect and quantify KSHV, and several reports have demonstrated that the number of viral copies in PBMCs varied depending on the disease and its stage [6, 8–10]. However, to date, only 1 report has used real-time PCR to compare the number of viral copies in samples from KS or MCD lesions, or from effusion fluid samples from patients with PEL, and this study was conducted on a limited number of samples [4].

We conducted a retrospective transversal study in patients with KS, patients with MCD, and patients with PEL to determine the relationship between the KSHV-DNA level in peripheral blood or effusion fluid samples and the clinical activity of KSHV-associated diseases.

Patients, materials, and methods. Between 2002 and 2005, a total of 107 patients were enrolled retrospectively in this study:

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Table 1. Characteristics of patients with Kaposi sarcoma herpesvirus–associated diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients</th>
<th>Male sex (range), years</th>
<th>Median CD4 count (range), cells/mm³</th>
<th>Disease status, no (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HIV+ (%) HIV− (%)</td>
</tr>
<tr>
<td>KS</td>
<td>71</td>
<td>48 (20–84)</td>
<td>180 (2–660)</td>
<td>Active</td>
</tr>
<tr>
<td>MCD</td>
<td>28</td>
<td>28 (100)</td>
<td>218 (23–866)</td>
<td>Remission</td>
</tr>
<tr>
<td>PEL</td>
<td>6</td>
<td>6 (75)</td>
<td>36 (5–43)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. KS, Kaposi sarcoma; MCD, multicentric Castleman disease; PEL, primary effusion lymphoma; NA, not applicable.

71 had presented with KS (of which 48 were HIV-infected and 23 were not HIV-infected), 28 had presented with MCD (all of whom were HIV-infected), and 8 patients had presented with PEL (of which 6 were HIV-infected and 2 were not HIV-infected). Patients’ characteristics are shown in table 1. Patients with both AIDS and KS were classified in accordance with the AIDS Clinical Trial Group criteria by tumor extent, severity of immunosuppression, and systemic HIV-associated illness. Of the patients who had both AIDS and KS, 34 had active KS and 14 were in complete remission. In patients with KS who not infected with HIV, the KS stage was determined with clinical data in accordance with Kriegel’s classification: stage I, locally indolent cutaneous lesion; stage II, locally aggressive cutaneous lesion with or without regional lymph node involvement; stage III, multiple cutaneous lesions or generalized lymph node involvement, without palatal KS or radiographic evidence of pulmonary KS; stage IV, palatal or visceral KS [11]. Of the patients with KS not infected with HIV, 9 were in complete remission and 14 had active KS (stages I–IV).

One hundred five blood samples and 4 lymphomatous effusion fluid samples (pleural or peritoneal) were analyzed. In cases of active disease, samples were obtained before any treatment was administered. PBMCs were retrieved by use of Ficoll Hypaque from whole blood collected in EDTA tubes, and cells were pelleted and stored at −80°C until DNA was extracted. Lymphomatous effusion fluid was collected in dry tubes and centrifuged to form cell pellets. DNA was extracted from PBMCs or dry pellets using the QIAamp Blood Kit (Qiagen).

Extracted DNA was subjected to a real-time PCR assay that quantified both KSHV (ORF73) and albumin genes as described elsewhere [12]. The number of viral copies was calculated by dividing the number of ORF73 copies by half the number of albumin gene copies. The lower limit of quantification for the real-time PCR was 10 KSHV copies/150,000 cells.

In this transversal study, there was only 1 sample per person used in the analysis. Only 2 patients had 2 samples analyzed; for these patients, analysis included both a blood sample and a lymphomatous effusion fluid sample. Statview (Abacus Concepts) software was used to perform nonparametric tests, specifically, the Mann-Whitney U test and Spearman rank test.

Results. Overall, KSHV-DNA levels in blood were statistically significantly higher in the 48 patients with active KS (median, 2.63 log₁₀ copies/150,000 cells [range, 1.00–5.58 log₁₀ copies/150,000 cells]), than in the 23 patients with KS in remission (median, 1.00 log₁₀ copies/150,000 cells [range, 1.00–1.23 log₁₀ copies/150,000 cells]) (P < .0001) (table 2). Of the 48 patients with active KS, KSHV-DNA levels in blood were not statistically significantly different between men and women. Moreover, of the 31 patients with KS infected with HIV, the 10 patients in remission had statistically significantly higher CD4 cell counts.

Table 2. Measurement of the Kaposi sarcoma–associated herpesvirus (KSHV)-DNA level in peripheral blood from patients with KSHV-associated diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients, no.</th>
<th>Patients with detectable KSHV DNA, no. (%)</th>
<th>Median KSHV-DNA level, log₁₀ copies/150,000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS</td>
<td>71</td>
<td>45/48 (94)</td>
<td>2.63</td>
</tr>
<tr>
<td>MCD</td>
<td>28</td>
<td>24/24 (100)</td>
<td>4.86</td>
</tr>
<tr>
<td>PEL</td>
<td>6</td>
<td>6/6 (100)</td>
<td>3.83</td>
</tr>
</tbody>
</table>

NOTE. KS, Kaposi sarcoma; MCD, multicentric Castleman disease; PEL, primary effusion lymphoma; NA, not applicable.
KSHV-DNA levels in blood were statistically significantly higher (median, 7.20 log_{10} copies/150,000 cells) than in the 4 patients in remission (median, 1.96 log_{10} copies/150,000 cells) (table 2). KSHV-DNA levels in effusion samples were statistically significantly higher (median, 4.86 log_{10} copies/150,000 cells) than in the 9 patients with KS in remission (median, 1.00 log_{10} copies/150,000 cells) (table 2). KSHV-DNA levels in blood were statistically significantly different between men and women.

For the 8 patients with PEL, KSHV-DNA levels were measured in 6 peripheral blood samples and in 4 effusion fluid samples. KSHV-DNA levels in effusion samples were statistically significantly higher (median, 2.06 log_{10} copies/150,000 cells) than in the 4 patients in remission (median, 1.96 log_{10} copies/150,000 cells) (P < .005) (table 2). KSHV-DNA levels in blood were not statistically significantly different between men and women.

For 2 patients, KSHV-DNA levels were measured concomitantly in blood and in effusion fluid, and the results revealed that KSHV-DNA levels were approximately 100 times higher in effusion fluid than in blood.

KSHV-DNA levels were statistically significantly different among the groups of patients with active disease, ie, patients with active KS (n = 48), active MCD (n = 24), or PEL (n = 8) (P < .0001) (figure 1). The lowest KSHV-DNA level in blood was observed for patients with KS (median, 2.63 log_{10} copies/150,000 cells), whereas the KSHV-DNA level in blood for patients with PEL was 1 log higher (median, 3.83 log_{10} copies/150,000 cells), and in blood from patients with MCD, the level was 2 logs higher (median, 4.86 log_{10} copies/150,000 cells). The highest KSHV-DNA level was observed in lymphomatous effusion fluid from patients with PEL (median, 7.20 log_{10} copies/150,000 cells).

Discussion. This study demonstrates that patients with active KS have higher KSHV-DNA levels in their PBMCs, compared with patients with KS in remission. Although this difference is significant, KSHV-DNA levels in patients with KS were not very high, suggesting that for such patients, the measurement of KSHV-DNA levels is not sufficient to monitor KS activity and guide therapy. The relatively low level of KSHV in the blood compartment may suggest that, in patients with KS lesions, most of the spindle cells are latently infected and production of viral particles is limited. The fact that patients with AIDS and active KS had higher KSHV-DNA levels than patients with active KS who were not infected with HIV may suggest that the KSHV-DNA level is indirectly related to the degree of immunosuppression. It could also indicate that the tumor burden is higher in patients with AIDS and KS than in patients with KS who are not infected with HIV. However, it is difficult to answer this question because the classification of KS in patients with AIDS is not the same as in patients not infected with HIV. However, in this study, among HIV-infected patients with KS, patients in remission had statistically significantly higher KSHV-DNA levels than those with active KS, suggesting that the level of KSHV DNA is related to immunosuppression.

With respect to MCD, our findings confirm previous studies that suggested that the KSHV-DNA level in PBMCs reflects disease activity [7]. Indeed, patients with active disease have significantly higher KSHV-DNA levels than patients in remission. In patients with MCD, KSHV-DNA levels in PBMCs can be very high, indicating that 10%-20% of the infected plasmablasts support lytic infection and that these cells may release viral particles in the blood compartment while at the same time exacerbating clinical symptoms. However, it is still unclear whether the increase of KSHV DNA in the blood compartment is the cause of clinical symptoms or the consequence of a cytokine environment favoring KSHV replication. The correlation between the KSHV-DNA level and disease activity suggests that antiviral drugs shown to be active against KSHV replication in vitro may have a positive effect on the evolution of MCD. This hypothesis is appealing, and in fact, a report on MCD remission during treatment with the antiviral drug ganciclovir has already been published [13]. However, this hypothesis needs to be confirmed by prospective studies, considering that a separate report did not find cidofovir to be effective in 5 other patients with MCD [14]. Unlike the situation with KS, monitoring the
KSHV-DNA level in PBMCs for patients with MCD has been shown to be very valuable when specific therapy for MCD is administered [7]. The clinical efficacy of drugs such as vincristine or, more recently, rituximab has been associated with decreased levels of KSHV DNA in PBMCs, whereas in cases of clinical failure, no decrease in KSHV-DNA levels has been observed [12]. In effusion fluid samples from patients with PEL, we showed that the KSHV-DNA levels were very high, even higher than in the PBMCs, and even for the 2 patients for whom the KSHV-DNA level was measured in both compartments at the same time. The high DNA level found in effusion fluid samples reflects the high number of KSHV copies per lymphoma cell reported in cell lines derived from specific effusion in these patients [15].

Because these 3 diseases can occur consecutively or simultaneously in a given patient, measurement of the KSHV-DNA level may have important clinical implications. For example, in a patient presenting with KS and pleural effusion, a high level of KSHV DNA in the pleural fluid (approximately 7 logs) could suggest a diagnosis of PEL. In a patient presenting with KS, fever, and lymph node enlargement, a high level of KSHV DNA in the PBMCs (approximately 4-5 logs) may suggest that the patient has MCD.

In conclusion, measuring the KSHV-DNA level in patients with KS has little impact on the follow-up of KS by itself, because of the low levels of KSHV DNA in PBMCs. However, measuring the KSHV-DNA level in PBMCs for patients with MCD is very useful because the KSHV-DNA level is a reliable marker of disease activity and therapy efficacy. This measurement could also be helpful in cases for which there are some difficulties in establishing a clinical diagnosis of KSHV-associated disease. Moreover, it has been shown that the real-time PCR assay used to determine KSHV-DNA levels in this study is highly specific [12]. Indeed, people with asymptomatic infection never have detectable KSHV, suggesting that use of this marker is appropriate for such patients. Finally, because these diseases are sometimes observed in the same patient and because the level of KSHV DNA seems to differ depending on the disease, measuring the KSHV-DNA level should be encouraged for those cases in which the physician suspects the occurrence of MCD or PEL in patients with KS.

References