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Different expression of human herpesvirus-6 (HHV-6) load in whole blood may have a significant impact on the diagnosis of active infection

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ABSTRACT

Background: Viral load in whole blood is the main virological marker for assessing HHV-6 infection and is used as an indication to begin antiviral therapy. Results are usually expressed as the number of genomic equivalent copies (gec) per mL of blood, although HHV-6 DNA in blood is mainly localized in lymphocytes and polymorphonuclear leukocytes.
Objectives: Since leukocyte counts vary in immunocompromised patients, especially in stem cell transplant recipients, the aim of this study was to compare HHV-6 load expressed as gec per mL with load expressed as gec per million cells (mc), using quantitative real-time PCR for HHV-6 and cell DNA.
Study design: 194 blood samples from 101 patients were analyzed. Leukocyte count was obtained for 142 samples.
Results: The two modes of expression were incompletely correlated ($p < 0.0001$; $R^2 = 0.732$). To understand this relative discrepancy, samples were classified according to hematological criteria (normal leukocyte count, leukopenia, agranulocytosis, lymphopenia). The expression modes were correlated in all cases except for agranulocytosis ($p = 0.21$; $R^2 = 0.087$). Moreover, the median of ratio between gec per mc and gec per mL ranged from 0.5 when leukocyte count was normal, to 8.2 in cases of agranulocytosis. HHV-6 load follow-up suggested that in agranulocytosis expressing results as gec per mc tended to provide a more representative result.
Conclusions: The different expression of HHV-6 load in whole blood, as either gec per mL or gec per mc resulted in different estimations of infection in the case of agranulocytosis. In this situation, the latter mode of expression is preferred.

1. Background

Human herpesvirus-6 (HHV-6) is a widespread betaherpesvirus that is closely related to human cytomegalovirus (HCMV). After primary infection, HHV-6 remains latent in different cells and tissues. In organ or stem cell transplant recipients (SCTRs), reactivation of HHV-6 frequently occurs and causes active infection.[1] As observed for HCMV, the pathogenic expression of HHV-6 infection is active replication of the virus. Different virological markers have been developed to assess an active infection; viral isolation in cell cultures,[2,3] detection of viral antigens in blood cells,[4] analysis of viral transcripts in blood,[5] and detection or quantitation of viral genomic DNA in whole blood, plasma or serum.[6–9] However, we showed previously that HHV-6 DNA in plasma reflected the presence of infected blood cells, rather than cell-free circulating viral particles, and was inadequate for estimating the amount of virus produced by active infection of distant lymphoid tissue and organs.[10] Moreover, the occurrence of HHV-6-related pathological manifestations was associated with a viral load of $>10^3$ genomic equivalent copies (gec)/10^6 peripheral blood mononuclear cells (PBMCs).[6] As with HCMV and Epstein-Barr virus infections, HHV-6 viral load in whole blood is an important virological marker for assessing and diagnosing HHV-6 infection, and is used as an indication to begin antiviral therapy. Results are usually expressed as the number of viral genomic DNA copies per mL of blood.[8,9] However,

Abbreviations: HHV-6, Human herpesvirus-6; Gec, Genomic equivalent copies; PMNLs, Polymorphonuclear leukocytes; HCMV, Human cytomegalovirus; SCTR, Stem cell transplant recipient.

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we showed that polymorphonuclear leukocytes (PMNLs) harbor the majority of HHV-6 DNA in blood.[11] Thus, variations in leukocyte count, especially PMNLs, would be expected to profoundly influence quantitation of HHV-6 viral load in whole blood, especially in SCTRs. In order to confirm this, we prospectively determined HHV-6 viral load in whole blood samples collected from patients suspected of having an HHV-6 infection, and expressed the results as both number of gec per mL and per million cells (mc). The study population included patients with either normal or disrupted leukocyte counts, with or without active HHV-6 infection.

2. Study design

2.1. Patients and samples

This prospective study was performed on 194 whole blood samples from 101 patients sent to the Pitié-Salpêtrière Hospital to be tested for the diagnosis of HHV-6 infection.

2.2. Extraction of DNA

Total DNA (viral and cell) was extracted from 400 μL of EDTA-treated whole blood using a MagNA Pure Compact Extractor (Roche Diagnostics, Mannheim, Germany) with MagNA Pure Compact Nucleic Acid Isolation kit I (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. DNA was eluted in 100 μL of elution buffer.

2.3. Viral and cell DNA quantitation by real-time PCR

HHV-6 genomic DNA was quantified on the ABI Prism 7500 instrument (Applied Biosystems, Courtaboeuf, France), using a real-time PCR method previously described,[12] on 10 μL of the total DNA extract. Results were given as the number of HHV-6 gec per 10 μL of DNA extract.

Cell DNA was quantified from 10 μL of total DNA extracted using a previously described real-time PCR method to amplify an albumin gene fragment.[13] Based on the presence of two copies of the albumin gene per cell, results were given as the cell equivalents per 10 μL of DNA extract.

HHV-6 viral load, expressed as the number of gec per million cells, was calculated from the results obtained for HHV-6 and cell DNA quantitations as follows: (number of HHV-6 gec per 10 μL of DNA extract/number of cell equivalents per 10 μL of DNA extract) × 10^6.

HHV-6 viral load expressed as the number of gec per mL of whole blood was calculated as follows: number of HHV-6 gec per 10 μL of DNA extract X25.

HHV-6 active infection was defined when the viral load expressed as cells was between 4 $\log_{10}$ gec/mc and 5.5 $\log_{10}$ gec/mc.

2.4. Differential leukocyte count determination

Differential leukocyte counts were obtained for 142 blood samples for which viral load had been determined in parallel. Differential leukocyte counts for patients from Pitié-Salpêtrière Hospital were determined from EDTA-treated whole blood using an XE2100 automatic blood cell analyzer (Sysmex Europe, Germany) or an ADVIA 2120 (Siemens HealthCare Diagnostics, France). Differential leukocyte counts for patients from Clermont-Ferrand Hospital were determined from EDTA-treated whole blood using an XE2100 automatic blood cell analyzer (Sysmex Europe, Germany).

Hematological disorders were defined as follows according to the guidelines used at the hospital and discussions with the clinicians: Leukopenia: leukocytes <4000/μL; Agranulocytosis: PMNLs <200/μL; Lymphopenia: lymphocytes <1000/μL; Severe lymphopenia: lymphocytes <200/μL.

2.5. Statistical analyses

Statistical analyses were performed using StatView 5.0. Descriptive statistics, regression analyzes or Mann–Whitney tests were used as appropriate. p values below 0.05 were considered significant.

3. Results

3.1. Comparison of HHV-6 load in blood expressed as gec/mL and gec/mc

The median and range values of HHV-6 load expressed as gec/mL or gec/mc for the 194 blood samples were 1103 (1.25–7.64 $\log_{10}$) gec/mL and 1274 (0.60–7.57 $\log_{10}$) gec/mc. The two modes of expression were well correlated ($R^2 = 0.732$; $p < 0.0001$) in median and range values.

3.2. Influence of differential leukocyte count disorders on HHV-6 load expression modes

Patients were divided into subgroups according to the differential leukocyte count. Statistical analyzes showed a very high correlation between the two modes of expression for normal as well as leukopenic (63 samples) and lymphopenic patients (91 samples), even in cases of severe lymphopenia (28 samples) (in this case, $R^2 = 0.976$; $p < 0.0001$). By contrast, this correlation was not observed in patients with agranulocytosis (10 samples) ($R^2 = 0.087$; $p = 0.21$) (Fig. 1). This lack of correlation was also observed for

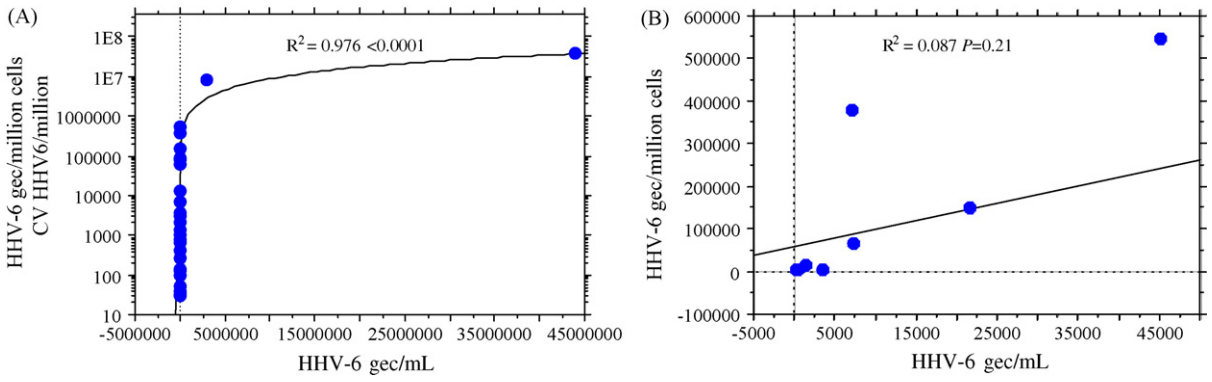

Fig. 1. Comparison of HHV-6 load in blood expressed as genomic equivalent copies per million cells and per mL for patients with perturbation of the differential leukocyte count. Patient with severe lymphopenia (28 samples)(A), patient with agranulocytosis (10 samples)(B).