Acute Hepatitis With Periportal Confluent Necrosis Associated With Human Herpesvirus 6 Infection in Liver Transplant Patients

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ABSTRACT

Objectives: To correlate human herpesvirus 6 (HHV-6) viral load with pathologic features in graft acute hepatitis of unknown origin.

Methods: Liver frozen samples from 26 patients with graft hepatitis of unknown origin were available for HHV-6 DNA quantification.

Results: In 10 (38.5%) of 26 liver samples, HHV-6 DNA was detectable, with a median viral load of $3.84 \log_{10}$ copies/10$^6$ cells. Confluent periportal necrosis was observed in 4 of 10 patients and associated with high viral load. These 4 patients responded to antiviral therapy. Mild unspecific hepatitis was observed in 4 patients with low intragraft viral load and in 2 patients with high viral load in a context of deep immunosuppression. Patients with HHV-6–negative graft hepatitis disclosed lobular necrotico-inflammatory activity without periportal necrosis.

Conclusions: Our study provides data supporting the pathogenic role of HHV-6 for liver allografts. The presence of confluent periportal necrosis could be a clue for prompt diagnosis of HHV-6–induced graft hepatitis.

Human herpesvirus 6 (HHV-6) is a member of the Betaherpesvirinae subfamily as cytomegalovirus (CMV) and human herpesvirus 7. Primary infection occurs most often in early childhood and is responsible for exanthema subitum.1,2 HHV-6 may also cause neurologic disorders and hepatitis, occasionally leading to acute liver failure.2,3 It is a lymphotropic virus infecting mainly CD4+ T lymphocytes but also CD8+ T lymphocytes, natural killer cells, and other cells such as macrophages, bone marrow progenitors, liver sinusoidal cells, and possibly epithelial cells.1,4,5 Like all Herpesviridae family viruses, HHV-6 establishes a latent infection for the lifetime of the host and may reactivate later.6 Viral reactivation can occur in the setting of acquired immunodeficiency syndromes or under immunosuppressive therapy, as in organ transplantation. Clinical syndromes attributed to HHV-6 reactivation include fever, maculopapular rashes, bone marrow...
suppression, interstitial pneumonia, encephalitis, and hepatitis,\textsuperscript{1,6-10} and HHV-6 reactivation of a latent infection has also been involved in posttransplant liver graft dysfunction.\textsuperscript{11,12,13} However, since the current literature about liver transplantation and HHV-6 mainly provides early posttransplant virologic data without documented clinical and pathologic features, the clinical spectrum of HHV-6 posttransplant graft infection in liver transplant recipients remains poorly defined. In addition, the virologic diagnosis relying on quantitative polymerase chain reaction (PCR) assays or antigenemia is not yet clearly standardized\textsuperscript{1,4,10,12,14} or included in routine screening.

This retrospective study was undertaken to correlate virologic data with pathologic and clinical features in posttransplant graft hepatitis of unknown origin to determine whether HHV-6 reactivation could be responsible for graft acute hepatitis and, if this is the case, to provide the diagnostic criteria for HHV-6–related graft hepatitis.

Materials and Methods

Patients and Samples

From January 11, 2002, through January 11, 2006, 1,665 consecutive biopsies of liver allografts in adult recipients who underwent liver transplantation between 1986 and 2006 were performed in our university hospital. Biopsies were performed either as protocol biopsies or for liver test abnormalities. In total, 183 biopsy specimens were diagnosed as acute graft hepatitis, defined by lobular spotty and/or confluent necrosis with lobular and portal inflammatory infiltrates. All patients with acute graft hepatitis were screened for anti–hepatitis A virus immunoglobulin M, hepatitis B surface antigen, anti–hepatitis C virus (HCV) antibodies, and HCV RNA. CMV, Epstein-Barr virus (EBV), and adenovirus infection was assessed by viral blood cultures or quantitative PCR on whole-blood samples (limit of detection, 500 copies/mL). The viral load was expressed by log\textsubscript{10} copies per 10\textsuperscript{6} cells. The threshold of detection for the HHV-6 assay was 2 log\textsubscript{10} copies per reaction.

Immunosuppression and Antiviral Prophylaxis Regimens

Posttransplant immunosuppression included a calcineurin inhibitor (cyclosporine or tacrolimus) plus corticosteroids. In cases of renal dysfunction, azathioprine or mycophenolate mofetil was added to lower the amount of calcineurin inhibitors. After liver transplantation, all patients received 100 mg fluconazole and 600 mg co-trimoxazole during 2 weeks for prophylaxis of \textit{Pneumocystis jirovecii} infection. CMV prophylaxis (aciclovir, valganciclovir, or valaciclovir according to the period) was given orally for 3 months in patients at intermediate risk (recipient+/donor– or donor+) and for 6 months in patients at high risk (recipient+/donor+). The prophylaxis regimen for CMV infection at the time of liver biopsy is detailed in Table I for each patient.

Quantitative PCR Assay for HHV-6

DNA was extracted from frozen liver biopsy specimens, frozen samples of native livers, and PBMCs with the QIAmp DNA mini kit (QIAGEN, Les Ulis, France). The HHV-6 viral load (variants A and B) was determined by TaqMan real-time PCR on the LightCycler instrument 1.0 (Roche, Meylan, France) using previously described primers and a probe targeting \textit{U65} to \textit{U66} genes.\textsuperscript{15} The amplification was performed from 9.5 µL of extracted DNA in a final volume of 20 µL containing 10 µL of QuantiTec probe PCR Master Mix (QIAGEN, Courtaboeuf, France), 200 nmol/L of each primer, and 100 nmol/L of probe. Serial 10-fold dilutions of a plasmid containing the sequence of interest were used for the construction of the standard curve. Real-time quantitative PCR for the \beta-globin housekeeping gene was carried out simultaneously to estimate the amount of cellular DNA and to calculate the number of cells as previously described.\textsuperscript{16} The HHV-6 viral load was expressed by log\textsubscript{10} copies per 10\textsuperscript{6} cells. The threshold of detection for the HHV-6 assay was 2 log\textsubscript{10} copies per reaction.

Liver Histology

Liver graft biopsy specimens were fixed in alcohol–formal–acetic acid and partly snap-frozen when the total length of the sample was 2.5 cm or higher. After fixation and paraffin embedding, 3-µm-thick sections were stained with H&E, Masson’s trichrome, picrosirius, and Perls. Biopsy specimens with hepatitis were systematically reviewed by an expert pathologist (C.G.) for the semiquantitative assessment of the following criteria: lobular confluent necrosis (percent of area), lobular spotty necrosis (0-2), portal inflammation (0-3), plasma cell infiltration (0-2), periportal necrosis (0-3 according to the METAVIR scoring system), portal fibrosis (0-4 according to the METAVIR score),\textsuperscript{17} steatosis, features of acute cellular rejection, and ductopenia.\textsuperscript{18}
Statistical Analysis

Nonparametric tests (Mann-Whitney and Fisher exact tests) were performed with Statistica version 6 software (StatSoft, Maisons-Alfort, France).

Results

Quantitative PCR Assays for HHV-6

HHV-6 was detected in the liver graft of 10 (38.5%) of 26 patients with acute hepatitis of unknown origin (Table 1). The median value of the HHV-6 viral load in liver samples was $3.84 \text{ log}_{10}$ copies/10$^6$ cells (range, 2.94-5.30). For 15 of 26 patients, PBMCs were available: HHV-6 was detectable in 2 of 7 patients with HHV-6–positive graft hepatitis (2.97 and 3.20 $\text{log}_{10}$ copies/10$^6$ PBMCs) and in 0 of 8 patients with HHV-6–negative hepatitis. In addition, HHV-6 was detected in 6 of the 9 available native livers, with a median viral load of $4.51 \text{ log}_{10}$ copies/10$^6$ cells (range, 3.58-4.60) and in 4 of 4 native livers of patients with HHV-6–positive graft hepatitis, favoring the hypothesis of viral reactivation instead of primary infection.

Morphologic Features of Liver Grafts According to HHV-6 Status

The morphologic features of liver grafts in 4 of 10 patients with HHV-6–positive hepatitis (patients 26, 27, 30, and 47) were characterized by confluent periporal hepatocellular loss leading to an unusual target picture centered by portal tracts (Image 1A) and Table 2. This periporal confluent necrosis was associated with abundant portal inflammatory infiltrates and severe periporal activity (Image 1B). Inflammatory cells consisted of lymphocytes associated with a few plasmocytes. The necrotic areas did not contain a ductular reaction. There were no viral inclusions in hepatocytes or lymphocytes. The HHV-6 viral load in these 4 patients ranged from 3.85 to 4.18 $\text{log}_{10}$ copies/10$^6$ cells.

For the 6 other patients with HHV-6–positive hepatitis, the histologic pattern was unspecific, with mild to moderate lobular activity and no or mild periportal activity. The HHV-6 liver load ranged from 2.94 to 5.30 $\text{log}_{10}$ copies/10$^6$ cells.

HHV-6–negative posttransplant hepatitis did not disclose severe periporal activity with hepatocellular loss. The main feature was lobular necroinflammatory activity with centrilobular confluent necrosis in 6 of 16 cases. Histologic features of mild acute rejection were associated with hepatitis features in 12 (46%) of 26 patients: 5 with HHV-6–positive liver samples, including those with the particular histologic pattern described above, and 7 with HHV-6–negative liver samples.

Clinical Features of Liver Graft Hepatitis According to HHV-6 Status

The 10 patients with HHV-6–positive liver grafts were 6 men and 4 women aged 16 to 63 years (mean age, 44 years) at Table 1.
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The 16 patients negative for HHV-6 were 11 women and 5 men aged 35 to 62 years (mean age, 50.5 years) at the time of the biopsy. The median time elapsed from liver transplantation to the histologic diagnosis of hepatitis was 2.75 months (range, 0.3–180 months) for the HHV-6–positive hepatitis and 1.75 months (range, 0.5–144) for the HHV-6–negative hepatitis (P value not significant).

CMV prophylaxis was given to 12 of 26 patients (4 patients with HHV-6–positive hepatitis and 8 patients with HHV-6–negative hepatitis) at the time of graft biopsy. All had a negative CMV viremia as assessed by blood cultures or quantitative PCR. Among the 4 patients (25, 26, 27, and 28) who developed HHV-6–positive hepatitis within the time of the prophylactic treatment, only 1 (patient 28) with a 2.94

**Table 2** Morphologic Data of Graft Hepatitis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Liver HHV-6 Viral Load</th>
<th>Portal Inflammation, No.</th>
<th>PP Necrosis, No.</th>
<th>Confluent Necrosis, %</th>
<th>Lobular Necrosis, No.</th>
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HHV-6, human herpesvirus 6; PP, periportal.

![Image](https://academic.oup.com/ajcp/article-abstract/140/3/403/1767299/48)
The clinical, virologic, and pathologic features of HHV-6 graft reactivation in liver transplant recipients are not clearly defined, leading to overdiagnosis or underdiagnosis of a potentially treatable graft pathology. The current study shows that HHV-6 reactivation, detected by real-time quantitative PCR on liver samples, could be associated with morphologic features of graft hepatitis characterized by severe periportal activity and hepatocellular loss.

In this study, HHV-6 DNA was detected by a real-time quantitative PCR assay in liver samples from 38.5% of the 26 patients with graft hepatitis of undetermined origin. The reported incidence of HHV-6 reactivation in liver transplant recipients varies greatly according to the virologic assays used: 6.7% by serology and liver immunohistochemistry,11 22% through antigenemia detection in PBMCs by immunocytochemistry,19 49% by PCR on plasma samples,14 39% by viral isolation from culture,20 28% by quantitative PCR on whole-blood samples,21 and 38.8% by combining viral isolation, serology, and whole-blood PCR.7 The lack of standardized methods for the demonstration of active HHV-6 replication hampers the comparison of data. Indeed, because most humans harbor latent HHV-6 infection, the main point when dealing with virologic tests for the detection of HHV-6 is to differentiate active replication and latent infection. Quantitative PCR methods improve interpretability since cutoff points for active replication can be established. Our approach in the present study was to perform quantitative PCR for HHV-6 both on liver samples and PBMCs. Interestingly, PBMCs were positive in a range indicative for active replication21 for only 2 of the 7 tested patients with an HHV-6–positive liver graft. These results may indicate that for at least 5 patients, the viral reactivation was restricted to the liver, and the diagnosis of HHV-6 reactivation would have been missed if the virologic tests had been performed only on PBMCs. The detection of the HHV-6 genome in liver tissue specimens with concomitantly negative PBMCs has already been reported in immunocompetent children with acute liver failure.3 This discrepancy between liver tissue and PBMCs is an indirect argument for HHV-6 infection of hepatocytes. Indeed, using an in situ hybridization probe, HHV-6 was shown to be more prominent in hepatocytes than in intrahepatic mononuclear cells.22,23

The median time from transplantation to the histologic diagnosis of HHV-6–positive hepatitis was 2.75 months. However, 1 patient developed graft hepatitis with HHV-6 reactivation as late as 15 years posttransplantation. Comparatively,
most of the previously described HHV-6 reactivations diagnosed from PBMCs in liver transplant recipients occurred earlier, with a peak incidence at 2 to 4 weeks posttransplantation.\textsuperscript{24} In the past, HHV-6 reactivations in liver graft recipients were reported to be frequently associated with CMV reactivation, which followed HHV-6 reactivation in most cases.\textsuperscript{24} In current practice, the systematic anti-CMV prophylaxis by ganciclovir, valganciclovir, or valaciclovir prevents CMV reactivation. Ganciclovir or valganciclovir is effective in vitro against HHV-6, whereas valaciclovir is not.\textsuperscript{1} These drugs are without effect on the overall incidence of HHV-6 reactivation during the first 3 months posttransplantation in renal transplant recipients\textsuperscript{25} but are associated with delayed appearance and shorter duration of detectable HHV-6 DNA in PBMCs. In our series, the only patient who developed HHV-6–positive graft hepatitis under valganciclovir had mild hepatitis with a low viral load.

The causal relationship between HHV-6 reactivation and pathologic conditions in liver transplant recipients is still unclear from the literature, even when assessed from liver tissue. A recent study reported the presence of HHV-6 DNA in 58% of patients with graft hepatitis of unknown origin and in 54% of patients without graft hepatitis,\textsuperscript{26} with no significant difference in the viral DNA concentration between both groups. Nevertheless, graft hepatitis or graft dysfunction is included in the reported clinical associations of HHV-6 reactivation besides unexplained fever, bone marrow suppression, interstitial pneumonitis, skin rash, and encephalitis.\textsuperscript{5,11,13,19,27,28} Histologic lesions of liver graft related to HHV-6 infection are reported to be infrequent, occurring in 0.5% to 6% of the patients with HHV-6 reactivation\textsuperscript{21,24} and associated with PBMC viral loads greater than $3 \log_{10}$ viral copies/μg DNA. The lesions hitherto described are not specific, characterized by lymphocytic portal infiltrates and spotty lobular necrosis.\textsuperscript{21,27}

In the present study, the morphologic analysis of liver graft biopsy specimens combined with virologic data suggests consideration of 3 different situations among patients with HHV-6–positive graft hepatitis: (1) high intragraft viral load associated with severe periportal hepatitis, (2) low intragraft viral load associated with unspecific mild hepatitis, and (3) high intragraft viral load with unspecific mild hepatitis.

The first situation was observed in 4 patients (patients 26, 27, 30, and 47). Liver graft hepatitis was associated with high liver viral loads (about $4 \log_{10}$ copies/10$^6$ cells) and characterized by unusual pathologic features of severe periportal activity with periportal confluent hepatocyte loss. Intense periportal activity has been described in some cases of acute hepatitis A but never in association with such hepatocellular confluent loss. The 4 patients benefited from the antiviral treatment by ganciclovir or valganciclovir. These data suggest that this peculiar histologic picture could be specific for HHV-6–related graft hepatitis. The presence of portal lymphocytic inflammation with periportal necrosis is more suggestive of an indirect immune mechanism as observed in chronic hepatitis B or C than of a direct cytopathogenic viral effect on hepatocytes. Indeed, HHV-6 reactivation after hematopoietic stem cell transplantation is significantly associated with a higher incidence of graft vs host disease,\textsuperscript{29} suggesting that HHV-6 reactivation could have an immunomodulation effect. Thus, HHV-6 could be able to modulate and maybe enhance both cellular and humoral immune response of the recipient against virus-infected cells of the graft. The graft immunologic background seems to be a sine qua non condition for a deleterious effect of HHV-6 reactivation in the liver since the presence of HHV-6 in native livers is not associated with histologic lesions of hepatitis; one explanation could be that only mononuclear cells but not hepatocytes harbor HHV-6 in the native livers. In these 4 patients, HHV-6 reactivation could have been favored by previous episodes of acute rejection responsible for enhancement of immunosuppression.

The second situation was observed in 4 other patients (4, 25, 28, and 33). The HHV-6 viral load was about $3 \log_{10}$ copies/10$^6$ cells, and the histologic pattern of hepatitis, essentially characterized by lobular activity and mild to moderate portal inflammatory infiltrates, was similar to that described in the literature.

The third situation was observed in 2 highly immunosuppressed patients who presented with concomitant human herpesvirus 8 primary infection (patients 32 and 35). The HHV-6 viral load was quite high. Nevertheless, portal inflammation and periportal activity were mild or absent. These multiple virus infections are probably indicative of a state of heavy immune suppression resulting in the incapacity of the immune system to develop any type of response.

In conclusion, our study provides additional data supporting the pathogenic role of HHV-6 for liver allografts: HHV-6 reactivation is restricted to the liver graft in recipients with undetermined graft hepatitis, as well as the association of the highest liver viral loads with unusual histologic features characterized by confluent periportal necrosis. To the best of our knowledge, this study is the first to demonstrate unusual pathologic features associated with HHV-6 intragraft reactivation.

From a practical point of view, 2 points are of interest. First, the occurrence of HHV-6 reactivation restricted to the liver graft incites performing PCR on liver samples. Second, the specific pathologic findings could be a key clue for prompt diagnosis of HHV-6–induced graft hepatitis, which is susceptible to antiviral agents effective against HHV-6 such as ganciclovir, foscarnet, or cidofovir.

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