Prognostic Value of JC Virus Load in Cerebrospinal Fluid of Patients with Progressive Multifocal Leukoencephalopathy

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The human polyomavirus JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating neurodegenerative disease associated with impaired cellular immunity [1]. The prevalence of PML in AIDS patients is ~4% [1]. PML results from lytic infection of oligodendrocytes, leading to foci of demyelination in the cerebral white matter [1]. JCV DNA detection in cerebrospinal fluid (CSF) is a reliable diagnostic marker of PML, particularly when combined with a typical magnetic resonance imaging pattern [1–5]. However, most available JCV polymerase chain reaction (PCR) methods provide no information on virus load. Here, by using quantitative PCR, we investigated JCV load in CSF of AIDS-associated PML patients.

Patients and Methods

Patients. CSF samples were collected for JCV quantitation from 11 men and 1 woman with AIDS-associated PML. PML was diagnosed on the basis of the following criteria: focal neurologic disease with subacute progression; white matter lesions on neuroimaging studies (computed tomography or magnetic resonance imaging) consistent with PML (no mass effect or contrast enhancement); JCV detection by PCR in CSF; no other likely etiology. Patients E and C underwent brain biopsy and necropsy, respectively, and the neuropathologic findings were consistent with PML.

Two CSF samples were taken after death in patients K and L, while the remainder were obtained at various times before death. CSF samples were aliquoted and frozen at −80°C until use.

Patient A received cidofovir, (S)-1-[3-hydroxy-2-(phosphonyl)methoxy]propyl)cytosine, starting 2 months after the onset of PML symptoms. A total of 11 infusions were given: 3.0 mg/kg/week for the first 2 weeks, then 9 infusions, once every 2 weeks, in a pattern of 2 infusions of 3 mg/kg, 6 infusions of 5 mg/kg, and 1 infusion of 3 mg/kg. He also received concomitant saline prehydration and oral probenecid, 4 g [6].

DNA extraction from CSF. CSF (100 μL) was mixed with 25 μL of salmon sperm DNA (100 μg/mL) and heated to 94°C for 5 min. Nucleic acids were precipitated overnight at −20°C by adding 3 vol of ethanol and 0.1 vol of 3 M sodium acetate. After centrifugation at 13,000 g for 20 min, the pellet was resuspended in 30 μL of diethyl pyrocarbonate–treated water.

Primers and probes. Primers and probes were synthesized by Genset (Paris). We used the PEP-1/PEP-2 primers described by Arthur et al. [7], which amplify the T antigen genes from both BK virus (BKV) and JCV. Amplicons were detected with a specific JCV probe. To rule out the simultaneous presence of JCV and BKV in CSF (coamplification could interfere with JCV quantitation), PEP-1/PEP-2 primer–generated amplicons were routinely tested with a BKV-specific probe; all samples were negative. Primers and probes were as follows: PEP-1, biotin-AGTCTTT-AGGGTCTTTCTACC; PEP-2, GTGCAACCTATGGAAACAG; JCV-specific probe, CCAACACTCAACCCACCT-digoxigenin; internal standard (IS) probe, ACCATCCCAACCTCCATC-digoxigenin; IS backward construction primer, ACCATCCCAACCTCCATC-digoxigenin; IS backward construction primer, ACCATCCCAACCTCCATC-digoxigenin;
AAG; IS forward construction primer, GATGGGAGGTTGG-GATGGTGATCCTGGTGTTCATCATCAGCGAAAACAT-TTCTT; and BKV-specific probe, GATGAAGACACGACAG-CAGATT-digoxigenin.

Construction of the JCV homologous IS. The JCV homologous IS was constructed from extracted JCV DNA by replacing the 19-nt JCV probing region by another IS-specific 19-nt sequence by using the IS backward and forward construction primers as described [8].

JCV quantitative PCR. IS (3 fg) was added to 100 μL of CSF, and DNA was extracted as described above. PCR was done with 10 μL of extracted DNA as described [8]. Amplions were quantitated at PCR plateau (42 cycles) by an ELISA-based procedure including a hybridization step with the JCV and the IS probes and luminometric revelation as described [8–10]. For each sample, the ratio of the luminescence signals obtained with the JCV and IS probes was compared with the ratios obtained by amplifying a JCV DNA scale (0.001–100 fg of JCV PCR products) together with 1 fg of IS [8]. Absolute values (femtograms per milliliter) were computed by linear regression analysis [8, 9]. Final results were expressed as JCV equivalents per milliliter of CSF. One femtogram of JCV PCR product (174 bp) corresponds to 5300 JCV copies (5300 JCV equivalents). The assay was linear over a 4 log range (0.01–100 fg of JCV amplions per PCR, corresponding to 53–533,000 JCV equivalents per PCR and 1590–15,990,000 JCV equivalents/mL of CSF).

Human immunodeficiency virus type 1 (HIV-1) loads and CD4 cell count. HIV-1 plasma and CSF loads were determined with the Amplicor HIV Monitor kit (Roche Diagnostic Systems, Neuilly, France). Absolute CD4 lymphocyte counts were determined by flow cytometry (Becton Dickinson, San Jose, CA), according to the manufacturer’s instructions.

Statistical analysis. Spearman’s rank correlation was used for statistical analysis.

Results

JCV load in CSF of AIDS patients with PML. As shown in table 1, the CSF JCV count varied widely among the 12 patients tested. JCV loads ranged from 3.28 to 6.58 log equivalents/mL of CSF. In patient L, JCV was quantified in 2 additional CSF samples collected 10 weeks after the onset of PML and postmortem; virus load was high and stable (6.75 ± 5.7 and 6.56 ± 6.46 log JCV equivalents/mL, respectively).

To investigate potential mechanisms of JCV load heterogeneity between PML patients, we analyzed CD4 T cell counts and plasma and CSF HIV-1 RNA levels. CSF was drawn from patients between 2 and 30 months after initiation of highly active antiretroviral treatment (HAART) with the exception of patient 1, who only received two nucleoside analogues because of hepatic cirrhosis (table 1). It should be noted that patient K discontinued HAART a few weeks before his death (JCV was quantified in patient K in a postmortem sample).

At the onset of PML, all patients had <200 CD4 T cells/mm³ (median, 89; range, 2–195). At the time of CSF sampling, the median CD4 T cell count was 109/mm³ (range, 30–229). No significant correlation was found between CSF JCV load and CD4 T cell number either at PML onset (P = .73) or at CSF sampling (P = .14). However, it is noteworthy that patients A and B, who had the lowest CSF JCV loads, had >200 CD4 T cells/mm³ at the CSF sampling (table 1).

At the time of JCV quantitation, plasma HIV-1 RNA was below the quantification limit in 5 patients and ranged from 3 to >5.87 log copies/mL in the others. The highest HIV-1 plasma loads (>5 log copies/mL) were observed in patients I and K. No correlation was found between HIV-1 plasma and CSF JCV loads (P = .9; table 1). HIV-1 could not be measured in CSF in 3 patients because of limited sample volume. In the patients tested, no correlation was observed between CSF JCV and HIV-1 loads (P = .44; table 1).

Furthermore, the topographic location of brain lesions did not seem to influence CSF JCV load (table 1).

Prognostic value of CSF JCV load. There was a significant negative correlation in this group of 12 PML patients between CSF JCV load and survival time, either after CSF sampling (Spearman’s rank correlation, −0.83; P < .01) or after PML onset (Spearman’s rank correlation, −0.77; P = .011). Patients A–E, who had relatively low JCV loads (3.28–4.48 log equivalents/mL), were still alive at the cutoff date for this analysis, with survival times exceeding 19 months after the onset of PML (median, 21 months) and 13 months after JCV quantitation (median, 16 months). These patients had stable neurologic status, with the exception of patient C. In contrast, patients F–L (JCV loads from 4.96 to 6.55 log10 equivalents/mL) died within 8 months after the beginning of PML (median, 4.5 months) and within 3 months after the sampling of CSF (median, 2.5 months) (table 1).

Outcome of cidofovir treatment. As shown in figure 1, CSF virus load was measured in patient A 1 month after PML onset (4.07 log10 JCV equivalents). At that time, ritonavir was added to ongoing nucleoside analogue bitherapy (stavudine plus lamivudine), which reduced HIV-1 plasma load to below the detection limit. Similarly, the CD4 T cell count rose from 24 to 254 cells/mm³. JCV load was not modified 2 months after initiation of HAART. At that time, the patient was started on cidofovir (see Patients and Methods). Despite concomitant saline prehydration and oral probenecid [6], serum creatinine values rose and proteinuria occurred, leading to cidofovir withdrawal after 11 infusions. JCV load fell 2 months after the beginning of cidofovir treatment and became undetectable at the end of the treatment in both quantitative and standard PCR. The patient also showed significant neurologic improvement.

Discussion

This is the first report of JCV loads in CSF of patients with AIDS-associated progressive multifocal leukoencephalopathy. We observed a wide range of CSF JCV loads (3 log10 to
Table 1. Clinical and biologic features of 12 AIDS-associated PML patients; prognostic value of JCV load in CSF.

<table>
<thead>
<tr>
<th>Patients</th>
<th>PML is first AIDS-defining event</th>
<th>CD4 cell count at onset of PML (cells/μL)</th>
<th>Main clinical symptoms</th>
<th>Localization of brain lesions by MRI</th>
<th>Antiretroviral therapy before PML</th>
<th>Antiretroviral therapy after PML</th>
<th>Time from PML onset to CSF sampling (months)</th>
<th>CD4 cell count at CSF sampling (cells/mm³)</th>
<th>HIV-1 plasma load at CSF sampling (log₁₀ copies/mL)</th>
<th>HIV-1 CSF load (log₁₀ copies/mL)</th>
<th>CSF JCV load (log₁₀ JCV equivalents/mL)</th>
<th>Time of survival from CSF sampling (months)</th>
<th>Time of survival from PML onset (months)</th>
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<tbody>
<tr>
<td>A No</td>
<td>24 Axial and left cerebellar ataxia</td>
<td>Posterior fossa</td>
<td>D4T/3TC</td>
<td>D4T/3TC/RTV</td>
<td>7</td>
<td>225</td>
<td>&lt;2.30</td>
<td>&lt;2.30</td>
<td>3.28 ± 2.63</td>
<td>&gt;14</td>
<td>&gt;21</td>
<td></td>
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<tr>
<td>B No</td>
<td>112 Bilateral cerebellar ataxia</td>
<td>Multifocal</td>
<td>AZT/ddI</td>
<td>D4T/3TC/RTV</td>
<td>9</td>
<td>213</td>
<td>4.12</td>
<td>3.21</td>
<td>3.30 ± 2.77</td>
<td>&gt;16</td>
<td>&gt;25</td>
<td></td>
<td></td>
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<tr>
<td>C Yes</td>
<td>35 Right hemiplegia, left cerebellar ataxia</td>
<td>Multifocal</td>
<td>AZT/3TC/IDV</td>
<td>D4T/3TC/IDV</td>
<td>30</td>
<td>46</td>
<td>4.75</td>
<td>3.32</td>
<td>3.65 ± 3.40</td>
<td>&gt;16</td>
<td>&gt;46</td>
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<td></td>
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<tr>
<td>D Yes</td>
<td>125 Right cerebellar ataxia, diplopia</td>
<td>Posterior fossa</td>
<td>None</td>
<td>AZT/3TC/ADV</td>
<td>3</td>
<td>109</td>
<td>3.03</td>
<td>2.47</td>
<td>4.41 ± 3.49</td>
<td>&gt;16</td>
<td>&gt;19</td>
<td></td>
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<tr>
<td>E Yes</td>
<td>195 Left homonymous hemianopsia, bilateral cerebellar ataxia</td>
<td>Multifocal</td>
<td>None</td>
<td>AZT/3TC/ADV</td>
<td>7</td>
<td>228</td>
<td>&lt;2.90</td>
<td>2.37</td>
<td>4.48 ± 4.0</td>
<td>&gt;13</td>
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<td>F Yes</td>
<td>134 Dysarthria, bilateral cerebellar ataxia</td>
<td>Multifocal</td>
<td>None</td>
<td>D4T/3TC/IDV</td>
<td>2.5</td>
<td>190</td>
<td>3.60</td>
<td>ND</td>
<td>4.96 ± 3.60</td>
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<tr>
<td>G Yes</td>
<td>141 Right homonymous hemi-anopia</td>
<td>Left posterior cerebral hemisphere</td>
<td>None</td>
<td>AZT/3TC/ADV</td>
<td>3</td>
<td>229</td>
<td>3.12</td>
<td>&lt;2.30</td>
<td>5.18 ± 4.63</td>
<td>1.5</td>
<td>4.5</td>
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<tr>
<td>H No</td>
<td>2 Right hemiparesia, aphasia</td>
<td>Left anterior cerebral hemisphere</td>
<td>ddI</td>
<td>D4T/ddI/IDV</td>
<td>5</td>
<td>88</td>
<td>&lt;2.30</td>
<td>ND</td>
<td>5.42 ± 4.90</td>
<td>3</td>
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<tr>
<td>I No</td>
<td>27 Left cerebellar ataxia, dysarthria</td>
<td>Multifocal</td>
<td>AZT/3TC</td>
<td>D4T/3TC</td>
<td>1</td>
<td>30</td>
<td>&gt;5.87</td>
<td>ND</td>
<td>5.72 ± 5.20</td>
<td>2.5</td>
<td>3.5</td>
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<tr>
<td>J Yes</td>
<td>44 Aphasia</td>
<td>Multifocal</td>
<td>None</td>
<td>AZT/3TC</td>
<td>2</td>
<td>75</td>
<td>&lt;2.30</td>
<td>2.30</td>
<td>5.75 ± 5.15</td>
<td>3</td>
<td>5</td>
<td></td>
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<tr>
<td>K Yes</td>
<td>151 Right hemiparesia</td>
<td>Posterior fossa</td>
<td>None</td>
<td>D4T/3TC/IDV</td>
<td>2.5</td>
<td>130</td>
<td>5.26</td>
<td>ND</td>
<td>6.55 ± 5.99</td>
<td>0</td>
<td>2.5</td>
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<td></td>
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<tr>
<td>L No</td>
<td>66 Aphasia, frontal syndrome</td>
<td>Bilateral anterior cerebral hemisphere</td>
<td>AZT/ddI</td>
<td>D4T/3TC/IDV</td>
<td>5</td>
<td>38</td>
<td>&lt;2.30</td>
<td>&lt;2.30</td>
<td>6.58 ± 5.84</td>
<td>2.5</td>
<td>5</td>
<td></td>
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</tbody>
</table>

NOTE. AZT, zidovudine; 3TC, lamivudine; D4T, stavudine; ddI, didanosine; IDV, indinavir; RTV, ritonavir. ND, not done. MRI, magnetic resonance imaging.

a Patient I received only 2 nucleoside analogues because of advanced cirrhosis.

b Limits of quantitation were 2.3 and 5.87 log₁₀ HIV-1 RNA copies.

c Each value represents mean ± SD of 2 or 3 determinations in independent experiments. Each determination was done in duplicate. Mean values of duplicates were used.

d JCV was quantified in postmortem sample from patient K. Date of last point is 30 May 1998 for surviving patients (A–E).
Figure 1. Changes in CSF JCV load in a PML patient receiving cidofovir. CD4 cell number, HIV-1 plasma load, and CSF JCV load were determined at different times during highly active antiretroviral therapy (HAART) and cidofovir treatment. Each CSF JCV load value was determined as described in table 1. Third JCV load point is also reported in table 1. Limits of quantitation were 1590 equivalent copies/mL for JCV and 200 copies/mL for HIV-1. CNS, central nervous system.

>7 log_{10}) and a negative correlation between CSF JCV load and survival time.

The level of immunodeficiency may influence JCV replication. In this study, all of the patients had <200 CD4 T cells/mm^3 at PML onset and <250 CD4 T cells/mm^3 at CSF sampling, but within this group of immunocompromised patients, no correlation was observed between CD4 T cell number and JCV load. However, the absolute CD4 T cell count does not necessarily reflect the JCV-specific immune response. In this regard, it would be interesting to investigate CD4 T cell reactivity against JCV antigens by using proliferation tests.

Differences in central nervous system JCV loads among PML patients may also be related to infection by different JCV strains with different intrinsic replicative capacities. Indeed, different rearrangement patterns by deletion and duplication in the archetypal regulatory region have been reported [11]. JCV has also been genotyped on the basis of sequence variations in the major capsid protein gene (VP1) and the regulatory protein gene (T antigen) [11]. Although no relationship was observed between CSF HIV-1 and JCV loads, it cannot be ruled out that HIV-1 brain infection might influence JCV replication. HIV-1 tat protein is known to transactivate the JCV late promoter in glial cells [12]; in addition, HIV-1 might act indirectly by modifying brain secretion of cellular factors that modulate JCV replication.

As regards the clinical value of CSF JCV load, patients with high JCV loads (>5 log_{10} JCV equivalents/mL of CSF) died rapidly. In contrast, patients with low virus loads were still alive at the end of the study, with prolonged survival times. Except for patients A and L, JCV load was measured at only one time point, 1–30 months after the onset of PML, and yet a significant correlation was nonetheless observed between JCV load and survival time, both after the onset of PML and after CSF sampling. This suggests that a single CSF JCV load determination during the PML course might be of prognostic value. In patients A and L, JCV load determined at different times appeared to be relatively stable (moderate and high, respectively).
Patients A–E had unusually long survival times. HAART, instituted at the onset of PML, may have improved the control of JCV replication, thereby slowing the progression of PML, by decreasing HIV-1 load and inducing some functional immune reconstitution [13]. In a series of 67 PML patients, we observed that HAART significantly improved survival relative to a combination of two nucleoside analogues (median survival of 12 months vs. 3.5 months) [14]. However, HAART was inefficient in slowing PML progression in patients F–H and J–L; these patients probably had severe forms of PML with an initially high JCV load that rapidly induced fatal brain lesions.

Cidofovir is an acyclic nucleoside phosphonate that exhibits activity against human herpesviruses [6]. Cidofovir inhibits in vitro replication of mouse polyomaviruses and the simian virus 40 [15]. Patient A received cidofovir in addition to HAART, and a drop in CSF JCV load was observed. This was accompanied by neurologic improvement. HAART may have contributed to this effect, although the fact that JCV also became undetectable in urine (data not shown) points to a specific effect of cidofovir.

Taken together, these data suggest that the assay of JCV load in CSF may be useful in determining the prognosis of PML patients and in monitoring the effectiveness of anti-JCV therapies.

References