Prognostic Significance of JC Virus DNA Levels in Cerebrospinal Fluid of Patients with HIV-Associated Progressive Multifocal Leukoencephalopathy

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Background. Progressive multifocal leukoencephalopathy (PML) remains a frequent and life-threatening complication of human immunodeficiency virus (HIV) infection in the era of highly active antiretroviral therapy (HAART). Although one-half of patients with this disease will survive, the outcome is unpredictable at diagnosis, and prognostic markers are needed.

Methods. JC virus (JCV) DNA levels were measured in cerebrospinal fluid (CSF) samples obtained from 61 HIV-infected patients with PML, including 38 patients who were treated with HAART and 23 patients who did not receive HAART, with use of real-time polymerase chain reaction. The diagnostic reliability of the assay was evaluated by comparing CSF findings with histopathological findings in patients with PML or other HIV-related diseases of the central nervous system. The prognostic value was assessed by comparing JCV DNA levels with survival and other patient variables.

Results. The assay had a diagnostic sensitivity of 76% and specificity of 100%. In the first CSF sample obtained after onset of PML symptoms, JCV DNA values ranged from undetectable to 7.71 log copies/mL (median, 3.64 log copies/mL). JCV DNA levels >3.64 log copies/mL correlated significantly with shorter survival and lower CD4+ cell counts in patients not receiving HAART. However, neither relationship was found in patients who were treated with HAART. The analysis of sequential CSF samples obtained from 24 patients demonstrated a marked decrease in JCV DNA levels over time in HAART-treated patients showing PML stabilization, but not in untreated or HAART-treated patients with progressively fatal disease.

Conclusions. Measurement of JCV DNA levels in CSF samples may be a useful virological marker for management of PML in patients receiving HAART.

The introduction of HAART for the treatment of HIV infection has been followed by a decrease in the incidence of HIV-related progressive multifocal leukoencephalopathy (PML) [1, 2] and by prolonged survival of patients with this disease [3–6]. However, the decrease in the incidence of PML has been less marked than the decrease in other HIV-related opportunistic diseases of the CNS, resulting in an increased prevalence of PML relative to these other diseases [2, 7]. Furthermore, the disease stabilizes or improves clinically in approximately one-half of patients receiving HAART, whereas the outcome is still progressively fatal in patients who do not receive HAART. In addition, patients who survive are usually left with severe neurological sequelae [7–9].

The outcome of PML in patients receiving HAART is unpredictable at disease onset; therefore, prognostic markers are urgently needed. The most promising virological marker of PML is JC virus (JCV) DNA detection in CSF by nucleic acid amplification techniques. JCV DNA amplification in CSF is a well-established tool for noninvasive diagnosis of PML [10–13]. Moreover, JCV DNA may be cleared from the CSF of HAART-treated patients in whom PML stabilizes.
[14–16], and it has been proposed as an indicator of disease activity in patients receiving HAART, in association with clinical and radiological criteria [17]. We performed a real-time quantitative PCR assay to evaluate the diagnostic and prognostic significance of JCV DNA levels in CSF.

PATIENTS AND METHODS

Patients and samples. The study involved 61 patients with HIV-related PML who were observed during the period of 1992–2001 in 5 Italian clinical centers and for whom CSF samples and clinical data were available. PML was diagnosed either histologically (in 5 patients), by detection of JCV DNA in CSF in the presence of suggestive clinical and radiological presentation [44], or both (12) [17]. Overall, 38 patients received HAART, defined as the association of ≥3 anti-HIV drugs. Twenty-three of these patients had been treated for ≥2 weeks before CSF sampling (median duration, 61 days; range, 17–589 days), and 13 of them were receiving HAART at the time of PML onset. All 38 patients received HAART afterwards. Five patients who were not treated with HAART and who were observed between 1993 and 1995 received intrathecal and intravenous cytarabine; 21 patients, including 19 patients who were treated with HAART and 2 who were not, received cidofovir at standard dosages.

To evaluate the diagnostic sensitivity of the real-time PCR assay, JCV DNA load was measured in CSF samples from the 17 patients with a definite histological diagnosis of PML determined either at postmortem examination (13 patients) or by brain biopsy (4 patients). In the case of those patients with postmortem diagnosis, CSF samples were obtained a median of 55 days (range, 19–263 days) before death. To assess the diagnostic specificity, CSF samples from 26 additional patients with other CNS diseases (4 patients), by brain biopsy (4 patients). In the case of those patients with postmortem diagnosis, CSF samples were obtained a median of 55 days (range, 19–263 days) before death. To assess the diagnostic specificity, CSF samples from 26 additional patients with other CNS diseases that were diagnosed at postmortem examination were examined as controls. These included samples from patients with HIV encephalitis (5 patients), cytomegalovirus encephalitis (5), tuberculosis (1), toxoplasmosis (3), cryptococcosis (1), lymphoma (6), or nonspecific lesions (5). In these cases, CSF samples were obtained a median of 30 days (range, 4–89 days) before death.

From each patient, the first CSF sample obtained after onset of PML symptoms was analyzed for corroborative purposes. Multiple CSF samples (range, 2–9 samples) were available from 24 patients with PML, including 16 patients who were treated with HAART and 8 who were not. In all cases, CSF samples were obtained for diagnostic or management purposes after obtaining informed consent from the patient.

Real-time PCR and other measurements. Nucleic acids were extracted from 50-μL samples of CSF using the QIAamp Blood Kit (Qiagen) and were eluted in a final volume of 50 μL. JCV DNA amplification and detection were performed using the ABI Prism 7700 Sequence Detection System (PE-Applied Biosystems). A standard curve was prepared, consisting of 10-fold dilutions of plasmidic DNA (range, 10^6–10^3 copies/mL) obtained by cloning the whole JCV genome into the plasmid pBR322 at the EcoRI site (kindly provided by Dr. Thomas Weber [Hamburg, Germany]). Each standard, each sample, and 1 negative control were analyzed in triplicate in each run. An internal control, consisting of 10^5 copies/mL of plasmid DNA, was added to 1 of the 3 aliquots for each sample. Primers and probe were chosen in the large T antigen region and consisted of 5′-GAG TGT TGG GAT CCT GTG TTT TC3′ (forward), 5′-GAG AAG TGG GAT GAA GAC CTG TTT3′ (reverse) and 5′-FAM-TCA TCA CTG GCA AAC ATT TCT TCA TGG C- TAMRA-3′ (probe). A 5-μL sample and 20-μL PCR mixture (12.5 μL Universal Mastermix, containing 900 nmol/L of forward primer, 300 nmol/L of reverse primer, and 200 nmol/L of probe) were added to each well. Cycling parameters were 50°C for 2 min, 95°C for 10 min, 50 cycles at 95°C for 15 sec, and 60°C for 1 min. A threshold cycle value (Ct) was calculated for each standard or sample tube by determining the point at which the fluorescence exceeded the threshold limit. The reference curve was obtained by calculating the mean of the Ct values obtained for each standard and by plotting these values against known JCV DNA concentrations. The mean of the Ct values obtained in the 2 sample aliquots without the internal control were calculated and plotted against the standard curve. The JCV DNA load was expressed as log of the number of copies per milliliter of sample.

To assess the analytical sensitivity and specificity of the assay, 10-fold dilutions of the JCV plasmid or of the plasmids pBR322 and H13 containing the whole BK virus or SV40 genomes, respectively, were tested. Intraassay and interassay reproducibility were defined by calculating the coefficients of variation (standard deviation:mean value) of the Ct values obtained by testing 9 (intraassay) or 6 (interassay) replicates of the standard curve dilutions. Plasma and CSF HIV loads were assessed at the time of CSF JCV DNA measurements using either the Roche Amplicor Cobas standard or ultrasensitive assay (with detection limits of 400 copies/mL and 20 copies/mL, respectively).

Statistical analysis. Spearman correlation and the χ² test were used to test associations between continuous and categorical variables, respectively. For continuous variables, medians and 25th–75th percentile values (quartile 1 [Q1] to quartile 3 [Q3]) are presented. The Cox proportional hazards regression model was used to evaluate the association between survival and continuous variables in univariate analysis. Hazard ratios (HRs) and 95% CIs are reported. The distribution of survival times was estimated by the Kaplan-Meier method, with comparisons among survival curves made by the log rank test. In subjects with ≥2 repeated JCV DNA load determinations, the slopes of JCV DNA, blood CD4+ cell counts, and plasma HIV RNA values over time (β coefficients) were investigated and com-
pared between patient groups using the Mann-Whitney U test. Disease stabilization or progression was defined in the presence of clinical and radiological criteria of inactive or active disease, respectively [17]. For statistical purposes, negative JCV DNA PCR values were defined as equal to 100 copies/mL, whereas undetectable levels of HIV RNA were defined as equal to 400 copies/mL.

RESULTS

Analytical sensitivity, specificity, and reproducibility. The analytical sensitivity of the assay was between 10^3 and 10^5 copies/mL. With use of this assay, the other human polyomavirus, BK virus, was detectable at concentrations ≥10^6 copies/mL, whereas SV40 was detectable at concentrations ≥10^11 copies/mL. The intraassay coefficient of variation was .01 (for standards of 10^6, 10^5, and 10^4 copies/mL) or .02 (for 10^3 copies/mL). The interassay coefficient of variation was .01 (for a standard of 10^6 copies/mL), .02 (for 10^5 and 10^4 copies/mL), or .08 (for 10^3 copies/mL).

Diagnostic reliability. JCV DNA was detected in CSF samples obtained from 13 of 17 patients with histologically confirmed PML (median value, 3.04 log copies/mL; range, <2.00–6.89 log copies/mL), resulting in a 76% diagnostic sensitivity (95% CI, 50%–93%). In none of the 26 control patients was JCV DNA detected in CSF samples, giving a specificity of 100% (95% CI, 87%–100%).

Correlations between JCV DNA load and patient variables. Characteristics of the 61 patients at the time of first CSF sampling are shown in table 1. At this time, JCV DNA was detected in 52 (85%) of 61 patients with PML, with values ranging from undetectable to 7.71 log copies/mL (median value, 3.64 log copies/mL). No different distribution of JCV DNA values was observed between patients who received HAART and those who did not at the time of sampling. In neither of the 2 groups did we find a correlation between JCV DNA levels and the number of days between onset of PML symptoms and CSF sampling.

In univariate analysis, CSF JCV DNA levels were correlated to survival, as were other variables, including use of HAART after CSF sampling, CD4+ cell counts, and plasma or CSF HIV RNA levels. Only receipt of HAART was significantly associated with longer survival, both from the time of CSF sampling (HR, 4.28; 95% CI, 2.24–8.18; P < .0001) and from onset of symptoms (HR, 3.78; 95% CI, 1.94–7.39; P < .0001). JCV DNA level was not a significant predictor of survival when assessed as either a continuous or a dichotomous variable and using a cut-off value of 3.64 log copies/mL (i.e., the median value of JCV DNA level in the first CSF samples obtained after PML onset) (figure 1A).

The possible prognostic value of JCV DNA load in CSF for survival was further analyzed separately in patients who received HAART and those who did not. In patients who were not treated with HAART, a JCV load in CSF of >3.64 log copies/mL was associated with a significantly shorter duration of survival both from the time of CSF sampling and from onset of PML. This correlation was not maintained in HAART-treated patients. However, this correlation was present if only patients

### Table 1. Demographic and clinical characteristics of patients at the time of the first CSF sample obtained after onset of progressive multifocal leukoencephalopathy (PML).

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 61)</th>
<th>Received HAART (n = 38)</th>
<th>Did not receive HAART (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of men/no. of women</td>
<td>45/16</td>
<td>27/11</td>
<td>18/5</td>
</tr>
<tr>
<td>Age, median years</td>
<td>37</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>Time between onset of symptoms and CSF sampling, median days (Q1–Q3)</td>
<td>34 (16–58)</td>
<td>32 (16–50)</td>
<td>40 (19–85)</td>
</tr>
<tr>
<td>No. (%) of patients with detectable JCV DNA in CSF sample</td>
<td>52 (85)</td>
<td>31 (82)</td>
<td>21 (91)</td>
</tr>
<tr>
<td>Level of JCV DNA in CSF sample, median log copies/mL (Q1–Q3)</td>
<td>3.64 (2.00–4.98)</td>
<td>3.85 (2.98–5.19)</td>
<td>3.44 (3.03–4.11)</td>
</tr>
<tr>
<td>CD4+ cell count, median cells/µL (Q1–Q3)</td>
<td>64.5 (28.5–190.5)</td>
<td>62 (26.5–190.5)</td>
<td>70 (31–162)</td>
</tr>
<tr>
<td>HIV RNA level in plasma, median log copies/mL (Q1–Q3)</td>
<td>4.01 (2.66–5.06)</td>
<td>4.21 (3.66–5.08)</td>
<td>3.34 (2.60–4.75)</td>
</tr>
<tr>
<td>HIV RNA level in CSF, median log copies/mL (Q1–Q3)</td>
<td>2.60 (2.60–3.59)</td>
<td>3.59 (2.60–4.54)</td>
<td>2.60 (2.60–2.93)</td>
</tr>
</tbody>
</table>

**NOTE.** JCV, JC virus; Q1, quartile 1; Q3, quartile 3.

* In 4 patients, JCV DNA was undetectable in the first CSF sample obtained but was detectable in subsequent samples.

* Data are for 52 patients overall (31 patients who received HAART and 21 patients who did not).

* Data are for 49 patients overall (28 patients who received HAART and 21 patients who did not).

* Data are for 34 patients overall (14 patients who received HAART and 20 patients who did not).
Figure 1. Kaplan-Meyer survival curves according to JC virus (JCV) DNA levels in the first CSF sample. Survival is calculated from the time at which the CSF sample was obtained. Filled line, a JCV DNA level of >3.64 log copies/mL; dotted line, a JCV DNA level of ≤3.64 log copies/mL. A, All patients (P value was not significant according to the log-rank test); B, Patients not treated with HAART (P = .016); C, Patients treated with HAART (P value was not significant according to the log-rank test); D, Only patients who died of progressive multifocal leukoencephalopathy, including both patients treated with HAART and patients who were not (P = .006).

who died of PML were considered, irrespective of HAART intake (figure 1B–D). Among patients who died of PML, higher JCV DNA levels in CSF were observed in patients whose CSF samples were obtained at a shorter interval before death (ρ, −.321; P = .036).

JCV DNA levels >3.64 log copies/mL were observed in 24 (59%) of 41 patients with CD4+ cell counts of ≤200 cells/μL but in only 3 (27%) of 11 patients with CD4+ cell counts of >200 cells/μL (P = .062). The correlation between elevated JCV DNA levels and low CD4+ cell counts became significant in patients who were not treated with HAART (17 [68%] of 25 vs. 1 [17%] of 6; P = .020), but not in those receiving HAART at the time of CSF sampling (7 [44%] 16 vs. 2 [40%] of 5).

No significant association was observed between JCV DNA level

Figure 2. Trend lines of JC virus (JCV) DNA levels from the date on which the first CSF sample was obtained. A, Patients who did not receive HAART (n = 8; median β value, 0; Q1–Q3, −0.004 to 0.021). Five patients received cytarabine. B, HAART-treated patients showing disease progression (n = 5; median β value, −0.0002; Q1–Q3, −0.002 to −0.0002). C, HAART-treated patients showing disease stabilization (n = 11; median β value, −0.007; Q1–Q3, −0.013 to −0.004). The β values were significantly lower for patients shown in panels B and C considered as a whole, compared with those for patients shown in panel A (P = .046); β values were also significantly lower for patients shown in panel C, compared those for patients shown in panel B (P = .011).
Table 2. JC virus (JCV) DNA level in CSF, CD4+ cell counts in blood, and HIV RNA levels in plasma at the time of the first and last CSF samples obtained from patients with progressive multifocal leukoencephalopathy (PML) after onset of disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients not receiving HAART (n = 8)</th>
<th>Patients receiving HAART with PML progression (n = 5)</th>
<th>Patients receiving HAART with PML stabilization (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCV DNA level in CSF, log copies/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀</td>
<td>2.00 (2.00–2.80)</td>
<td>3.02 (3.00–3.44)</td>
<td>3.63 (3.30–3.63)</td>
</tr>
<tr>
<td>Tₙ</td>
<td>2.00 (2.00–4.31)</td>
<td>3.34 (2.71–3.94)</td>
<td>2.00 (2.00–2.79)</td>
</tr>
<tr>
<td>CD4+ cell count, cells/μL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀</td>
<td>NA</td>
<td>242 (144–395)</td>
<td>53 (27–160)</td>
</tr>
<tr>
<td>Tₙ</td>
<td>NA</td>
<td>342 (207–500)</td>
<td>137 (77–248)</td>
</tr>
<tr>
<td>HIV RNA level in plasma, log copies/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀</td>
<td>NA</td>
<td>4.48 (3.93–5.32)</td>
<td>3.39 (2.91–4.44)</td>
</tr>
<tr>
<td>Tₙ</td>
<td>NA</td>
<td>2.60 (2.60–2.95)</td>
<td>2.60 (2.60–4.43)</td>
</tr>
<tr>
<td>Interval between T₀ and Tₙ, days</td>
<td>48.5 (21–102)</td>
<td>61 (40–274)</td>
<td>153 (132–275)</td>
</tr>
</tbody>
</table>

NOTE. Data are median (quartile 1 to quartile 3) values. NA, not available; T₀, time of first CSF sample; Tₙ, time of last CSF sample.

Analysis of repeated CSF measures. The slope of the variation of JCV DNA levels in CSF over time (i.e., β coefficient) was measured in patients undergoing additional CSF sampling. Median β values were significantly lower in 16 patients who received HAART, compared with 8 patients who did not (P = .046). Among HAART-treated patients, JCV DNA levels decreased markedly in patients with disease stabilization but did not decrease in those with persistently active disease (P = .011) (figure 2).

Table 2 shows JCV DNA values in CSF for the times at which the first and last CSF samples were obtained, together with CD4+ cell counts and plasma HIV RNA values at the same time points. JCV DNA and plasma HIV RNA β values were strictly correlated in HAART-treated patients in whom PML stabilized (P = .0002), but not in those patients who showed disease progression. The latter patients showed a more marked decrease in plasma HIV RNA levels over time (a median β value of −0.020 [Q1–Q₃, −0.042 to −0.006], compared with a median β value of −0.003 [Q1–Q₃, −0.006 to 0]; P = .037), and a trend towards a more pronounced increase in CD4+ cell count (a median β value of 1.38 [Q1–Q₃, 0.40–1.48], compared with a median β value of 0.18 [Q1–Q₃, 0.08–0.70]; P = .072). CD4+ cells and plasma HIV RNA trends were not analyzed in patients who were not treated with HAART because of the small number of additional measurements available.

No significant differences were observed in the variation of JCV DNA levels between 6 patients receiving HAART and cidofovir (median β value, −0.006; Q₁–Q₃, −0.015 to 0) and 10 patients receiving HAART alone (median β value, −0.003; Q₁–Q₃, −0.004 to −0.001). In patients receiving HAART and cidofovir, median JCV DNA levels decreased from 3.30 log copies/mL (Q₁–Q₃, 3.02–3.50) in the first sample to 3.00 log copies/mL (Q₁–Q₃, 2.00–3.34) in the last sample; median values decreased from 3.88 log copies/mL (Q₁–Q₃, 3.02–4.53) to 2.68 log copies/mL (Q₁–Q₃, 2.00–3.87) in patients treated with HAART alone.

DISCUSSION

In this study, we showed that real-time PCR was a sensitive and robust method for measuring JCV DNA levels in CSF samples. This assay proved to be useful for establishing a diagnosis of PML and for monitoring the response to HAART, and it proved to be a valuable tool for studying the natural history of PML.

Sensitivity and specificity of PCR of CSF samples for diagnosis of HIV-related PML have largely been evaluated in the past 10 years. A number of studies comparing CSF findings with diagnoses obtained either histologically or by clinical and radiological criteria showed a diagnostic specificity of this method of 92%–100% and a sensitivity of 60%–80% [10–13]. Our figures for sensitivity and specificity (100% and 76%, respectively) are similar to or higher than those previously reported using histopathological findings as the diagnostic standard. Besides being sensitive and specific, real-time PCR offers the advantage of providing quantitative results, which may be useful for management purposes. In this regard, measurement
of JCV DNA levels proved to be highly reproducible over a clinically relevant dynamic range.

To assess whether JCV DNA load in CSF samples could have prognostic implications, the levels of JCV DNA in the first CSF sample obtained after onset of PML symptoms were correlated to survival. Although previous studies have shown an association between high JCV DNA levels and survival in patients with PML [6, 14, 18], we could not confirm these findings in a preliminary evaluation [19]. The present study shows clearly that high JCV DNA levels are significantly associated with short survival in patients with PML, but only in patients who never received HAART. In contrast, the association between JCV DNA levels at diagnosis and survival was not present in patients treated with HAART. Notably, this effect appeared to be determined exclusively by the proportion of patients in whom PML subsided, because high JCV DNA levels remained associated with short survival in HAART-treated patients with fatal disease.

Our findings for patients who were not treated with HAART are similar to those obtained for the patients enrolled in the cytarabine trial, which began before HAART became available [20]; those findings show an association between JCV DNA levels of ≥10^5–10^6 genomes/mL, as estimated by a semiquantitative procedure, and short survival [21]. We also found higher JCV DNA levels in patients whose CSF samples were collected closer to death, which is consistent with the observation that it was common in the pre-HAART era for CSF samples that were initially negative for JCV DNA to have positive results on additional examinations [12, 13]. Overall, these observations suggest that, in the absence of HAART, constitutionally high JCV replication rates in CSF samples may reflect a more rapid progression of PML, although viral DNA levels may further increase through the course of the disease.

In addition, a significant association between high concentrations of JCV and low CD4+ cell counts (i.e., <200 cells/μL) was also observed in patients who were not treated with HAART. In this regard, several patients with cases of PML characterized by slow progression in the absence of antiretroviral treatments have previously been documented, and elevated CD4+ cell counts were almost invariably observed in such cases [22]. However, the correlation between JCV DNA level and CD4+ cell counts was also abolished by treatment. Finally, no correlation between JCV DNA level in CSF samples and HIV RNA levels in plasma or CSF samples was observed in patients regardless of whether they had received HAART, which argues against a direct effect of HIV on JCV replication.

Although the single-point determination of JCV DNA levels at the time of diagnosis did not appear to be prognostically useful among HAART-treated patients, the analysis of sequential specimens showed that levels decreased in treated patients in whom PML stabilized, in contrast to in patients who did not receive HAART or HAART-treated patients with progressive disease. Reduction or suppression of JCV replication in the CNS might thus be the prerequisite for PML to become inactive and represent a prognostic marker of favorable outcome. This effect also seems to provide an explanation for the previously reported association between low or undetectable JCV DNA levels in CSF samples and long survival in patients receiving HAART [6, 14, 18]. A relevant number of samples analyzed in these earlier studies were in fact obtained several months after onset of PML in patients showing disease stabilization, and thus low JCV DNA loads most likely reflected good virological outcome.

Taken together, our observations may contribute to characterize the impact of HAART on the natural history of PML. In the absence of treatment, host defences might exert a relative control on JCV replication in the CNS, thus determining the pace of disease progression. In contrast, the initial extent of JCV replication and the degree of immune system impairment seems to be no longer relevant once HAART is introduced and host ability to control the infection is regained. Such a model, however, would not apply to the proportion of HAART-treated patients in whom PML worsens despite optimal responses in terms of decreased HIV loads and increased CD4+ cell counts. It is possible that these patients do not restore the JCV-specific cell-mediated and/or humoral immunity [23–25]. On the other hand, inflammatory tissue reactions have been associated with PML worsening during receipt of HAART [26]. In this regard, we observed strong HIV RNA and CD4+ cell responses in patients who had persistently elevated JCV DNA levels and PML progression. Although this observation needs to be further confirmed in large patient groups, it supports the hypothesis that, in some patients, a strong response to HAART might be detrimental and even lead to paradoxical progression of PML [26–28].

In summary, high JCV DNA levels in CSF samples obtained early in the course of PML were shown to be a predictive marker of poor survival in patients with PML and were associated with low CD4+ cell counts only in patients who were not treated with HAART (and not in patients who were treated with HAART). However, a reduction or suppression of the JCV DNA burden in the CSF of patients receiving HAART was associated with stabilization of PML, irrespective of plasma HIV RNA level and CD4+ cell count responses to treatment. Measuring JCV DNA load in CSF samples with use of real-time PCR appears to be a reliable virological marker of disease activity in patients receiving HAART, and it appears to be of potential relevance for clinical trials investigating new treatment approaches.

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Potential conflicts of interest. All authors: no conflicts.
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