JC Virus Load in Progressive Multifocal Leukoencephalopathy: Analysis of the Correlation between the Viral Burden in Cerebrospinal Fluid, Patient Survival, and the Volume of Neurological Lesions

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JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a demyelinating central nervous system infection that mainly affects patients with acquired immunodeficiency syndrome. The diagnostic value of the detection of JCV DNA in cerebrospinal fluid (CSF) has been proved. A correlation between the JCV burden in CSF and the PML prognosis has been proposed. To our knowledge, the present study is the first to examine JCV burden in CSF in relation to the magnitude of neurological damage. An in-house quantitative polymerase chain reaction assay was used for measurement of the JCV burden in CSF samples from 12 patients with PML. A wide variation in JCV load (6.4 log) was found among the patient CSF samples, a finding that makes JCV load measurements worthwhile. Virus load values of \(1^{4.68}\) log were associated with shorter patient survival time. No correlation was found between the virus load values and the global volume of brain tissue damaged. Our data suggest that factors other than the volume of neurological lesions influence the shedding of JCV in the CSF.
tween JCV load and the degree of neurological damage are still lacking. To our knowledge, the present study is the first to search for potential correlations between the JCV titer in CSF and the magnitude of demyelinated lesions in the CNS white matter, a parameter that is closely associated with the pathological effect of JCV.

PATIENTS AND METHODS

Patients. At our institution (Hospital General Universitario Gregorio Marañón, Madrid, Spain), PML was diagnosed in 36 patients during a 4-year period (May 1996–May 2000). Diagnosis was based on clinical findings (diverse neurological symptoms, such as monos- or hemiparesis, speech or visual disorders, or sensorial deficits) and neuroimaging findings, and it was confirmed by the detection of JCV DNA in CSF samples by means of an in-house nested qualitative PCR assay [4] or neuropathological analysis. For all cases, no other likely etiology for the neurological symptoms was found after complete biochemical analysis (cell counts, differential counts, and protein and glucose level determinations) and microbiological examination of the CSF (stains and cultures to detect the presence of viruses, bacteria, and fungi) had been performed. For 12 patients, the amount of CSF obtained was sufficient enough to quantify JCV burden after the diagnostic qualitative PCR assay [4] or neuronal pathological analysis. For all cases, no other likely etiology for the neurological symptoms was found after complete biochemical analysis (cell counts, differential counts, and protein and glucose level determinations) and microbiological examination of the CSF (stains and cultures to detect the presence of viruses, bacteria, and fungi) had been performed. For 12 patients, the amount of CSF obtained was sufficient enough to quantify JCV burden after the diagnostic qualitative PCR assay was performed. All 12 patients were HIV positive, and their clinical features are compiled in table 1.

Quantitative PCR. Eighteen CSF samples obtained from the 12 patients selected for the study were available for JCV quantification. The DNA purification procedures and the quantitative competitive nested PCR used to measure the JCV load in CSF samples were performed as described elsewhere [4]. A fixed amount of the problem JCV DNA was coamplified with a set of increasing and known amounts of competitive control. Competition was induced in both the first and second amplifications of the nested PCR. The final products of the quantitative competitive PCR were detected in agarose gels after electrophoresis. Bands that corresponded to amplified products were digitally imported by an image analysis system (Gel Station; T.D.I.). Products that corresponded to JCV and control amplicons were measured densitometrically (Intelligent Quanti- fier; BioImage). These densitometric values were represented against the known increasing virus load values of the competitive control (figure 1, bottom). Because of competition, the amplification signal for JCV increased when the amount of coamplified control was reduced. The crossing point between these 2 functions corresponded to a ratio of 1:1 for the amplification of JCV and control. The amount of control was known for all reactions, and we were therefore able to deduce the amount of JCV in the sample.

To obtain the coefficient of variation of the technique, which was calculated as \[ \frac{\text{SD}}{\text{mean}} \times 100 \], five independent extractions of DNA from each of 2 different CSF samples were performed. We used the logarithmic JCV load values to make this calculation.

Measurement of demyelinated CNS white matter lesions. On CT scans, lesions that were suggestive of PML appeared in the white matter as low-attenuation zones without contrast enhancement and with no mass effect; on MRI, such lesions appeared as areas of increased signal intensity in T2 and as areas that were isointense or hypointense in relation to the cortex in T1. T1 was not selected for quantitative assessments because the lesions of patients with acute-phase PML were frequently only detected in T2 as a result of the low degree of demyelin- ation associated with early lesions. For 10 patients, MRI and the JCV load measurement were performed chronologically close to each other (interval between MRI and JCV load measurement, 0–18 days; mean interval, 7 days), which thus allowed us to search for a correlation between both parameters.

Volumetric measurements were done by use of an Easy-Vision 4.2 workstation (Phillips). Axial T2-weighted sections were selected for analysis. The slice thickness was 5 mm, and the gap was 0.5 mm. The pathological areas were delimited for each of the lesions in each section. By considering these areas and the slice thickness, we obtained a volume of interest (VOI). The global volume of lesions in each patient was calculated by totaling the VOIs for all images. The methodological variation coefficient of the technique was calculated as 9.46%.

Definition of JC viral subtypes. For those samples for which CSF was available after JCV quantification, the viral subtype was assigned. A 494-bp fragment within the VPI gene was...
amplified as described elsewhere [22]. This fragment was fully sequenced, and subtypes were assigned according to the nucleotides found in 17 different positions [22].

**Statistical analysis.** For patients who had >1 virus load measurement, the baseline determination was selected for statistical analysis. Logarithmic values were used for the JCV load measurements. Survival time was defined as the time from diagnosis of PML to the last follow-up visit (i.e., the censoring date) for patients who survived. The time of diagnosis of PML was coincident with the time of virus load measurements. Spearman’s rank correlation test was used to analyze correlations. The Kaplan-Meier method was used to perform survival analysis, and the log-rank test was used to assess the significance of the comparisons. For multivariate analysis, a multiple linear regression model was used. The statistical package SPSS, version 6.0 (SAS Institute), was used in the statistical analysis.

**RESULTS**

**Precision of quantitative competitive PCR analysis.** Our initial purpose was to define the range of the JCV load in CSF samples obtained from selected study patients with PML. To
Table 2. JC virus (JCV) load values, survival, and corresponding volumetric measurements of CNS lesions attributed to progressive multifocal leukoencephalopathy, for each patient.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Survival,a days</th>
<th>JC viral subtype</th>
<th>Date assessed</th>
<th>Copies/mL b</th>
<th>Log b</th>
<th>Date volume was measured</th>
<th>Volume, mm³</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140</td>
<td>1b</td>
<td>14 May 1998</td>
<td>843</td>
<td>2.92</td>
<td>2 Jun 1998</td>
<td>1117.2</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 Jul 1998</td>
<td>12,648</td>
<td>4.1</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|         |                 |                 | 7 Aug 1998    | 575,919      | 5.7   | 21 Aug 1998             | 3054.5      | I
| 2c      | 240             | NA              | 5 Jun 1998    | 231          | 2.36  | NA                      |             | S        |
| 3       | 424             | 1a              | 26 May 1998   | 17,258       | 4.24  | 14 May 1998             | 14,887.3    | I + S    |
|         |                 |                 | 5 Jun 1998    | 35,638       | 4.55  | NA                      |             |          |
| 4       | 120             | NA              | 29 Jul 1998   | 150          | 2.17  | 28 Jul 1998             | 6279        | S        |
|         |                 |                 | 21 Aug 1998   | <140 (<2.14) |       | 3 Sep 1998              | 12,258.3    | S        |
|         |                 |                 | 9 Jul 1998    | 604,479      | 5.78  | NA                      |             |          |
| 6       | 82              | 1b              | 31 Mar 1999   | 10,004,255   | 7     | 9 Apr 1999              | 30,563.6    | I + S    |
| 7       | 59              | 1b              | 15 Sep 1999   | >1,350,000   | >6.13 | 21 Sep 1999             | 31,277.6    | S        |
| 8       | 60              | 1b              | 6 Nov 1999    | 324,591,200  | 8.51  | 11 Nov 1999             | 5624.6      | I        |
| 9       | 993             | NA              | 12 Jul 1999   | 140          | 2.15  | 20 Jul 1999             | 4493.1      | S        |
| 10      | 80              | New             | 15 Feb 2000   | 210,188      | 5.32  | 15 Feb 2000             | 44,215.3    | S        |
| 11      | 37              | 1b              | 28 Jan 2000   | 231,499      | 5.36  | 28 Jan 2000             | 33,298      | I + S    |
| 12c     | 1065            | NA              | 15 Apr 1999   | 408          | 2.61  | NA                      |             |          |

NOTE. I, infratentorial; NA, not available; New, a subtype that has not been previously described; S, supratentorial.

a Survival was calculated from the time of onset of progressive multifocal leukoencephalopathy.
b Virus load values that include a less than (<) or greater than (>) symbol are values for which a higher precision of measurement could not be obtained.
c Findings from MRI performed chronologically close to the time that JCV quantification was done were not available.

check the reproducibility of our technique and to guarantee that variations in JCV load values were due to differences in the virologic dynamics of JCV, we first defined the range of variation in JCV load measurements that could be the result of methodological deviations.

We therefore selected 2 CSF samples (A and B) that were known to be JCV positive by qualitative PCR. The JCV load measurements, as determined by quantitative PCR, were 2694 and 47,088 copies/mL for samples A and B, respectively. Five aliquots of each of these samples were reprocessed as if they were independent samples. JCV load values for all measurements are shown in figure 1. The ranges of the values obtained were 2828–4300 and 22,180–81,491 copies/mL for samples A and B, respectively. For both samples, the successive measurements were randomly distributed around the mean. The technique showed good reproducibility, as was indicated by the low coefficient of variation (1.92% for sample A and 4.45% for sample B) associated with methodological bias.

Range of JCV load values in the CSF of patients with PML. When we used quantitative competitive PCR to quantify the JCV load in the CSF samples obtained from our study patients (table 1), we found wide interpatient and intrapatient variations in JCV load values. The viral burden was distributed over a 6.37-log interval (2.14–8.51 log copies/mL; median, 5.22 log; table 2), a value much higher than the interval expected only on the basis of the methodological deviations (<0.56 log; figure 1). Longitudinal measurements could only be performed for 3 patients (2–3 samples were assessed at an interval of 10–62 days), and intrapatient variations were measured for all patients (table 2). The range of intrapatient variation was 0.32–1.66 log when successively obtained samples were compared. If we consider all data within the follow-up, the highest intrapatient variation was found in patient 1, in whom differences of up to 2.84 log were measured during an 83-day period.

Correlation between JCV load and patient survival. For the patients whose CSF samples were analyzed, the correlation coefficient for the association of JCV load values with survival times was −0.73 (P = .007), which suggests that there is an inverse association between these 2 parameters (table 2). When the median value of baseline virus loads (4.68 log) was considered a cutoff value, Kaplan-Meier survival estimates showed significant differences between patients with virus load mea-
measurements that were >4.68 log and patients with virus load measurements that were <4.68 log (by log-rank test, \( P < .05 \)) (figure 2).

**Correlation between JCV load and volume of CNS lesions.** The wide variation in the JCV loads measured in the patient CSF samples led us to search for the neurological significance of a high or low JCV burden in CSF. We hypothesized that a higher JCV load in the CSF would correlate with a higher extension of demyelinated lesions in the CNS white matter. To test this hypothesis, volumetric measurements on MRI were performed to determine the global volume of the brain lesions for each patient.

We did not find a significant correlation between single JCV load measurements and the volume of the lesions in the CNS (\( r = -.37 \); \( P = .225 \)). This was also the case for volumetric measurements from T1-weighted MRI. Furthermore, no correlation was found (\( P = .73 \)) when we applied a multiple linear regression model to control for the following potential confounding factors: CD4 count, receipt of highly active antiretroviral therapy (HAART), plasma HIV load, age, and location of the lesions.

For only 2 of the patients (patients 1 and 5) who had >1 JCV load measurement available was an association found between the longitudinal dynamics of JCV load and the evolution of the volume of the lesion (table 2). For the other patient who had >1 JCV load measurement available (patient 4), the increase in the virus load was inverse to the evolution of the lesions (table 2).

**Determination of JC viral subtypes.** To assess whether some clinical and/or neurological parameter in our PML study group was affected by the presence of different JC subtypes, the genotype of the virus isolated from CSF was defined for those cases for which a sufficient clinical sample was available. All but 2 patients who had genotyping performed were found to have JCV subtype 1b (table 2). This finding indicates the high prevalence of this subtype in our population and therefore limits the study of potential neurological differences associated with different JC subtypes.

**DISCUSSION**

If we assume the proven value of the detection of JCV DNA in CSF for the diagnosis of PML [2–7], it is worth analyzing the potential significance of measuring the JCV burden in this biological compartment. Different authors have found a correlation between the JCV load in CSF and the clinical outcome of patients with PML [18–21]. In our study group, we also found an inverse correlation between survival time and the JCV load in CSF. This correlation was supported by the significant differences in survival found for patients with a JCV load >4.68 log, compared with that found for patients with a JCV load <4.68 log. Our data are practically identical to those previously

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**Figure 2.** Kaplan-Meier survival curves for patients with JC virus load >4.68 log (dashed line) or <4.68 log (solid line). The log-rank test was used to assess the significance of the comparisons.
published by De Luca et al. [23], who reported a longer survival for patients who had a baseline JCV DNA load of <4.7 log.

The suggested correlation between virus load and PML prognosis would be strongly supported if JCV load values were found to correlate with the degree of neurological damage. Post et al. [10] showed no correlation between the location and volume of the lesions and the clinical outcome for 48 patients with PML. In our study, for the first time, we have searched for a correlation between virologic parameters and the magnitude of brain damage. In our opinion, this approach is more direct than one that uses parameters such as survival and/or prognosis, which may be influenced by many other factors.

We selected the volume of demyelinated CNS white matter observed on MRI as the criterion for evaluating the neurological damage in patients with PML. All neuroradiological analyses were performed chronologically close to JCV quantification (mean interval between MRI and JCV load measurements, 7 days). No correlation was found between these parameters, which suggests that there was no relationship between single measurements of the JCV load in CSF and the magnitude of the CNS lesions attributed to PML. A role for potential confounding factors in this lack of correlation was ruled out by a multiple linear regression model that controlled for CD4 count, age, HAART status, and plasma HIV load. A short longitudinal follow-up of 3 patients was performed, and for only 2 of these patients was a relationship found between the progression of viral shedding in the CSF and the evolution of the lesions.

We do not expect this lack of correlation between MRI and single JCV load measurements to be due to the short interval of time between the compilation of MRI and JCV load data. PML usually progresses over several months, and major virologic or neurological changes are not expected to occur within such short intervals.

At the time of the study, all patients showed neurological symptoms similar to those observed at the onset of PML. This fact is likely to correspond with “active” infection and thus rules out the possibility that the absence of correlation between neurological damage and JCV load was the result of patients having consolidated lesions and no viral replication.

It could be argued that our failure to find correlations is the result of imprecise measurements of the volume of the lesions caused by the inability to discriminate between demyelinated tissue and edema. We assume limitations, in this sense, for the methodology used. Nevertheless, in contrast to the findings of some authors [24], our experience indicates that the inflammatory component in white matter lesions attributed to PML is rare. When stereotaxic biopsy specimens were available, infiltrating inflammatory cells were not observed in the neuropathological analysis. Inflammatory response is considered in neuroimaging when the lesions show mass effect or contrast enhancement and/or when compressions of adjacent regions are observed. None of these radiological features were found in the lesions attributed to PML. This finding agrees with studies that have considered mass effect and contrast enhancement to be highly infrequent in PML lesions [10].

It could also be argued that the absence of correlation between JCV load and the volume of the lesions is the result of a lack of specificity in defining a PML-associated lesion. In this sense, the presence of different infectious agents in the CNS of the patients was ruled out by microbiological and biochemical analysis of CSF samples. When brain tissue was available, histopathological analysis of such tissue detected foci of myelin destruction and abnormalities in oligodendrocytes that were clearly indicative of PML infection. The CT and MRI parameters suggestive of PML lesions were all fulfilled for the patients analyzed (see the Patients and Methods section). The topography of the demyelinated regions was consistent with that of the multifocal and bilateral lesions expected for PML. These features, taken together, led us to be confident about the precision and specificity of our radiological measurements.

Our data suggest that the amount of virus in the CSF is not closely related to the magnitude of brain damage. This could reflect the fact that levels of viral replication within the tissue are not proportional to the amount of virus released to the CSF and would suggest that correlation may only be found if the JCV load is measured in situ. Higher interpatient variability in JCV load values has been found in CSF samples than in brain tissue [16], which suggests the role of certain parameters in modulating shedding of the virus in the CSF. In this sense, it would be convenient to evaluate the potential role of (1) the different JCV genotypes and subtypes [25–27], (2) the different levels of virologic response and/or immunologic restoration due to HAART, (3) the topographic location of the demyelination foci, and (4) the number and relative magnitude of lesions. In our population, most patients shared the same subtype (1b), including patients with high or low JC viral shedding in the CSF and those with a high or low volume of lesions. This therefore does not support a role for specific subtypes in specific neurological responses. Regarding topography, it is possible that deeply located lesions may shed fewer viruses in the CSF than may other lesions that are located adjacent to the ventricular spaces. In our patients, all lesions were located close to periventricular spaces, thus ruling out differential shedding of JCV.

Our hypothesis that the amount of JCV in CSF could be determined by parameters other than the volume of damaged tissue is consistent with the wide variation in JCV load found in our patients. All the patients analyzed were HIV positive and had an equivalent PML status, with neurological symptoms similar to those observed at the onset of PML. Nevertheless, JCV titers were distributed along a 6.37-log range, a much higher variability than has been proposed elsewhere (1–4 log) [16–18, 21]. This wide variation in virus load makes JCV quan-
tification worthwhile and encourages the search for the parameters that may lead to such a high variability in an apparently clinically homogeneous group.

Reduced statistical power, which was caused by the low number of patients in our study, could play a role in the lack of correlation found between the JCV load in CSF and the volume of CNS lesions. This reduced statistical power is an assumed limitation because of the low prevalence of PML. Nevertheless, our data suggest that the amount of JCV in isolated measurements of CSF should not be considered a general indicator of the degree of brain damage. It would be essential to analyze the ranges in variation of serial virus load measurements performed over time for each patient (1 of the patients in the present study showed differences of almost 2 log in a short period of time). Additional standardized studies of patients with PML, in which there is precise definition of controlled measurement points at diagnosis and follow-up, could help to clarify the factors involved in the neurological outcome of this infection.

On the other hand, single JCV load measurements in CSF could be useful to predict poor prognosis in patients with PML when high titers are found. In conclusion, before a neurological studies must be performed to define the factors that determine when high titers are found. In conclusion, before a neurological outcome of this PML, in which there is precise definition of controlled measurement points at diagnosis and follow-up, could help to clarify the factors involved in the neurological outcome of this infection.

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