Minimally Invasive Diagnosis of Acquired Immunodeficiency Syndrome-Related Primary Central Nervous System Lymphoma

Antonella Cingolani, Andrea De Luca, Luigi M. Larocca, Adriana Ammassari, Massimo Scerrati, Andrea Antinori, Luigi Ortona*

Background: The detection of Epstein-Barr virus (EBV)-DNA in cerebrospinal fluid (CSF) by means of the polymerase chain reaction (PCR) has been revealed, in retrospective studies, to be a good marker of primary central nervous system lymphoma (PCNSL) related to acquired immunodeficiency syndrome (AIDS); however, the technique’s usefulness in the management of AIDS patients with focal brain lesions is still unknown. We studied the clinical usefulness of testing CSF obtained by lumbar puncture for the presence of EBV–DNA as a minimally invasive approach to the diagnosis of AIDS–PCNSL in patients with focal brain lesions. Methods: Human immunodeficiency virus (HIV)-infected patients with focal brain lesions, observed prospectively during a 30-month period, underwent lumbar puncture if not contraindicated; otherwise, ventricular CSF was obtained at brain biopsy. The presence of EBV–DNA was determined by means of PCR. Results: We evaluated 122 patients: 42 diagnosed with brain lymphoma and the remaining 80 diagnosed with other brain disorders. Cerebrospinal fluid was collected from 101 patients—by lumbar puncture in 95, including 40 patients with AIDS–PCNSL. The sensitivity and specificity of PCR for EBV–DNA detection in lumbar CSF were 80% (95% confidence interval [CI] = 60.9%–91.6%) and 100% (95% CI = 92.6%–100%), respectively. Lumbar puncture and subsequent assessment of EBV–DNA would have allowed a correct diagnosis in 63.2% (95% CI = 46.0%–77.7%) of patients with AIDS–PCNSL and excluded this diagnosis in 76.3% (95% CI = 65.2%–84.8%) of patients without lymphoma (because EBV–DNA was not detected). Conclusions: The presence of EBV–DNA in lumbar CSF is a sensitive and highly specific diagnostic marker of AIDS–PCNSL, and EBV–DNA detection in this fluid may allow a minimally invasive diagnosis in a large percentage of patients with brain lymphomas. [J Natl Cancer Inst 1998;90:364–9]

The incidence of primary central nervous system lymphoma (PCNSL) in patients with acquired immunodeficiency syndrome (AIDS) is increasing (1–3), probably due to prolonged survival of the patients and to improved diagnosis, although there may be other, unknown causes. It is likely to become increasingly important among AIDS-related disorders that cause focal brain lesions as the incidence of toxoplasmic encephalitis (4,5) falls in response to widespread primary prophylaxis (6,7).

Brain biopsy is the method of choice for the definitive diagnosis of brain lymphoma in vivo (8), but it is an invasive procedure with morbidity and mortality and with considerable costs in terms of patient management and quality of life (9–11). Factors, such as low performance status, time from the first AIDS-defining event, the occurrence of opportunistic infections after brain biopsy, tumor burden, and rapid progression of the neoplastic disorder, worsen the prognosis and shorten patients’ survival (8,12,13). Furthermore, the time from clinical onset to the diagnosis of PCNSL is probably pivotal for the prognosis (14). Therefore, it is of prime importance to establish new strategies for making an early and preferably noninvasive diagnosis of brain lymphoma to select patients with favorable prognostic factors for an aggressive therapeutic approach, such as multimodal therapy (14), as well as to select patients with an unfavorable prognosis for palliative treatment (15).

The strict association of AIDS-related PCNSL with Epstein-Barr virus (EBV) (16) led to the suggestion that EBV–DNA in CSF might serve as a tumor marker. This approach was evaluated with favorable results in several retrospective analyses using polymerase chain reaction (PCR) techniques (17–19). Nevertheless, the clinical usefulness of this assay remains to be established for the management of AIDS-related focal brain lesions.

We present here the results of a 30-month prospective study designed to assess the clinical use of EBV–DNA detection in CSF and the feasibility of a minimally invasive diagnostic approach to brain lymphoma in a cohort of human immunodeficiency virus (HIV)-infected patients with focal brain lesions.

Materials and Methods

Patients and Study Design

All HIV-infected patients with focal brain lesions detected by computed tomography scan or magnetic resonance imaging, who were observed between June 1994 and December 1996 at the Department of Infectious Diseases of the Catholic University in Rome, were consecutively enrolled in this prospective study. For each patient, demographic and epidemiologic features (the CD4+ T-lymphocyte count, the history of an AIDS-defining event, concurrent anti-Toxoplasma prophylaxis, and the interval since the onset of neurologic symptoms) were recorded. After neurosurgical evaluation of the risk of herniation, lumbar puncture was considered if not contraindicated. In all cases, the final decision to perform lumbar puncture was based on the physician’s judgment. All

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See “Notes” following “References.”
patients eligible for lumbar puncture provided written informed consent. The protocol was previously approved by the Institutional Ethical Committee.

Aliquots of cerebrospinal fluid (CSF) were collected and examined with standard microbiologic tests for cryptococcal antigen and HIV p24 antigen and with DNA amplification assays by PCR (EBV–DNA, JC virus-DNA, Toxoplasma gondii–DNA, cytomegalovirus–DNA, and herpes simplex virus–DNA). The diagnostic criteria for the different focal brain disorders were as follows: for toxoplasmic encephalitis, histology or a radiographic picture of brain lesions with mass effect (presence of indirect signs of increased intracranial pressure due to a space-occupying lesion, such as compression of the ventricular spaces, shift of the midline, or flattening of cerebral sulci) or contrast enhancement, combined with positive Toxoplasma serology and response to anti-Toxoplasma treatment; for progressive multifocal leukoencephalopathy, histology or compatible MRI findings; JC virus-DNA detected in the CSF; for cytomegalovirus- and herpes simplex virus-encephalitis, histology or compatible clinical and radiologic findings, combined with detection of viral DNA in the CSF; for HIV encephalopathy; and histology and positive cultures for cryptococcal and tuberculous infections. In addition to histologic findings on biopsy or autopsy specimens, a presumptive diagnosis of brain lymphoma was also based on the exclusion of other brain disorders and all of the following criteria: negative Toxoplasma serology, lack of response to anti-Toxoplasma treatment and presence at single photon emission computed tomography of an uptake ratio of 201 Tl consistent with a lymphomatous lesion (20).

Brain biopsy was performed in all patients with focal brain lesions if the patient failed to demonstrate clinical or radiologic response after 2 weeks of empiric therapy. Biopsy specimens were taken at an early stage from patients for whom toxoplasmic encephalitis was unlikely, such as those with negative Toxoplasma serology who were undergoing anti-Toxoplasma prophylaxis. The detection of EBV–DNA in patients with negative Toxoplasma serology was considered to be a positive selection criterion for early biopsy (21). In all cases, patients with contraindications to the surgical procedure were excluded and the remainder gave written informed consent. Stereotactic and ultrasound-guided biopsy was performed as previously described (22). Samples were sent for histopathologic examination and for microbiologic cultures. In patients in whom the procedure was not deemed hazardous, a 100-μL aliquot of ventricular CSF was collected during brain biopsy. To further test the specificity of EBV–DNA PCR, CSF specimens were collected as an external control from a concurrent subgroup of HIV-infected patients who underwent lumbar puncture for neurologic signs or symptoms without focal brain lesions.

Histopathology and In Situ Hybridization (ISH)

Tissue samples were stained with hematoxylin–eosin and Giemsa after being fixed in a 4% neutral-buffered formalin solution and being embedded in paraffin. Pathologic specimens were classified according to the Working Formulation for non-Hodgkin’s lymphoma (23) and the revised European–American classification of lymphoid neoplasms (REAL classification) (24).

Paired paraffin sections from each case were mounted on silanized slides and EBV small encoded RNAs (EBER) ISH studies were carried out by using a cocktail of fluorescein–isothiocyanate labeled oligonucleotides complementary to the nuclear EBER-1 and 2 RNAs (Dako, Glostrup, Denmark), according to the instruction of the supplier.

PCR in CSF

EBV–DNA was amplified by a nested PCR technique, as already described (19). Briefly, 10 μL of CSF, either untreated or as a supernatant after centrifugation at 15000 g at room temperature for 30 minutes if greater than 100 cells/mL were present, was placed directly in a 50-μL reaction volume, containing 50 mM KCl, 10 mM tris–HCl, 2.5 mM MgCl2, 400 μM of each deoxynucleoside triphosphate, 10% glycerol, 0.15–0.30 μM of each primer, and 2.5 U of AmpliTaq (The Perkin-Elmer Corp., Emeryville, CA). Oligonucleotides derived from the EBN1 gene were used as an outer (EB3/EB4) and as an inner (EB1/EB2) primer pair. Thirty cycles were performed in both amplification rounds consisting of 1 minute at 95°C, 1 minute at 50°C, and 1 minute at 72°C. After the first PCR round, 2.5 μL of the amplification product were transferred to the second PCR buffer. After the second round, 10 μL of the second amplification product was electrophoresed on a 2% agarose gel containing 0.5 μg/mL ethidium bromide and visualized by transillumination. The expected size of the nested PCR product was 209 base pairs. The detection limit of the nested PCR was tested using serial dilutions of DNA extracted from Daudi cells that are known to contain approximately 400 EBV–DNA copies per cell. Several negative and positive controls were included in each amplification run. Clinical samples were tested at least twice and only consistent results were considered. To assess whether CSF specimens with a negative EBV–DNA by PCR contained DNA suitable for amplification, the samples were retested using primers GH26 and GH27 (Perkin Elmer, Emeryville, CA, USA) from the HLA-DQA1 region.

Negative CSF samples at nested PCR were checked for the presence of Taq polymerase inhibitors. Ten EBV–DNA copies were put in the negative sample and amplified in parallel with 10 EBV-DNA copies in water: if the result with the tested CSF sample was again negative, the presence of PCR inhibitors could be assumed. If inhibitors were present, the CSF sample was serially diluted to detect a positive PCR result.

Statistical Analysis

The sensitivity, specificity, post-test probability of the target disorder following a positive test (positive predictive value) and the post-test probability of not having the target disorder following a negative test (negative predictive value) were all expressed as percentages and calculated by appropriate formulas based on 2 × 2 tables (25). The strength of the association between the presence of EBV–DNA in the CSF and several baseline characteristics of AIDS-related PCNSL was expressed as relative odds and its independent value was controlled using a logistic regression model. The feasibility of lumbar puncture and related variables was measured using appropriate contingency tables.

Results

Patient Characteristics

During the study period, we observed 122 HIV-infected patients with focal brain lesions. Demographic, epidemiologic, and clinical characteristics of the patients are shown in Table 1. Brain lymphoma was diagnosed in 42 patients (by brain biopsy in 27, by autopsy in 11, and by presumption in four). In the remaining 80 patients, the final diagnosis was as follows: toxoplasmic encephalitis in 40, progressive multifocal leukoencephalopathy in 24, HIV–encephalopathy with a focal pattern in four, cytomegalovirus- and herpes simplex virus-encephalitis in three and two, respectively, tuberculoma in three, and cryptococca and vasculitis in two patients each. Brain biopsy was performed a mean of 28 days after the first neuroradiologic lesion was found in EBV–DNA-negative patients and a mean of 17 days after a CSF sample was found to be EBV–DNA positive.

A CSF specimen was collected from 101 (82.7%) patients, including 95 (77.9%) patients from whom it was obtained by lumbar puncture. None of the patients experienced a serious adverse event related to lumbar puncture. CSF was obtained from 40 (95%) of the 42 patients with brain lymphoma (36 with a histologic and four with a presumptive diagnosis). In 34 (80.9%) cases (30 with a histologic and four with a presumptive diagnosis), the specimen was obtained by lumbar puncture. In 17 cases of AIDS-related primary CNS lymphoma, a ventricular CSF specimen was collected during brain biopsy. In 22 (55%) of 40 patients with toxoplasmic encephalitis, in 23 (95.8%) of 24 patients with progressive multifocal leukoencephalopathy, and in all cases of HIV encephalopathy or cytomegalovirus- and herpes simplex virus-encephalitis, a CSF specimen was obtained by lumbar puncture. Among the control subjects without focal lesions, 73 had HIV encephalopathy, 33 had cryptococcal meningitis, and five had tuberculous meningitis. In all of these cases, CSF was collected by lumbar puncture.

Among patients with confirmed AIDS–PCNSL, histology showed that there are two varieties of brain lymphoma: 21 cases were characterized by medium- to large-sized immunoblasts,
with or without plasmocytic differentiation (immunoblastic plasmocytoid lymphoma), whereas 17 were characterized by diffuse, large noncleaved cells with a variable percentage of immunoblasts (large noncleaved cell lymphoma). All cases of PCNSL were classified as diffuse large B-cell lymphoma, according to the REAL classification.

### EBV–DNA Amplification

PCR testing of serial 10-fold dilutions of DNA extracted from DAUDI cells showed that the minimum detection level of the PCR assay was 10 EBV genomes. In brain lymphoma, CSF samples were drawn an average of 13 days before biopsy and 20 days before autopsy. Considering patients from whom lumbar CSF samples had been drawn, the sensitivity of the assay was 80% with a specificity of 100% and a positive and negative predictive value of 100% and 91%, respectively (Table 2). Combining the results of PCR assays performed on lumbar and ventricular CSF specimens, EBV–DNA was detected in 36 of 40 patients with AIDS-related PCNSL, demonstrating a sensitivity of 90%. All CSF specimens drawn by ventricular puncture harbored EBV–DNA. No false-positive result for EBV–DNA was found among the 111 patients without focal brain lesions.

### Feasibility of Lumbar Puncture

Lumbar puncture was not performed in 27 (22%) of 122 patients with a focal brain lesion because a neurosurg evaluation indicated that these patients were at increased risk of herniation. A correlation analysis between several clinical variables and the probability that the patient would undergo lumbar puncture are reported in Table 4. A multivariate analysis indicated...
that only the mass effect (odds ratio [OR] = 0.25; 95% confidence interval [CI] = 0.06–0.93; \( P = .039 \)) and anti-
*Toxoplasma* prophylaxis (OR = 3.31; 95% CI = 1.30–8.37; \( P = .012 \)) significantly influenced the probability of the patient undergoing lumbar puncture.

Finally, we analyzed the reliability of EBV–DNA detection in CSF collected by lumbar puncture. We found that brain lymphoma could have been diagnosed in 24 of 38 patients by this minimally invasive method, since EBV–DNA was detected in their lumbar CSF (sensitivity, 63.2% [95% CI = 46.0–77.7]), whereas, in 61 of 80 patients with other focal brain lesions, AIDS-related PCNSL could have been excluded, since EBV–DNA was undetectable in lumbar CSF (specificity, 76.3% [95% CI = 65.2–84.8]).

**PCR Inhibitors**

In three negative samples of CSF, all obtained by ventricular puncture, dilution to remove PCR inhibitors allowed the detection of EBV–DNA. None of the negative lumbar CSF samples in patients diagnosed with brain lymphoma were found to contain PCR inhibitors. In these samples, EBV–DNA was not detected following several attempts to concentrate DNA in these samples (data not shown).

**Discussion**

The strong association between EBV and AIDS-related PCNSL was further confirmed in our study. Consistent with reported data (16), ISH allowed us to document EBV infection of the tumor clone in more than 90% of the lymphomatous tissues. After the examination of a large number of focal brain lesions, we found that the specificity of detection of EBV–DNA by PCR obtained in this study was almost absolute. Predictive values of the positive and negative results were higher than 90%: these values are influenced by the high prevalence of lymphoma among our patients with focal brain lesions. The predictive values of the assay may be lower when this is applied to populations with a lower prevalence of this disorder. This result, obtained by prospective evaluation, confirms the high diagnostic value of this assay and has important implications for the management of AIDS-related focal brain lesions.

Our results show the possibility of a direct and rapid selection of candidates for a brain biopsy if EBV–DNA is detected in the CSF, especially in those patients with negative *Toxoplasma* serology (26) or who are undergoing anti- *Toxoplasma* prophylaxis (4). Indeed, the survival benefit conferred by brain biopsy is higher in patients diagnosed and treated for PCNSL than in those with other focal disorders (27). Moreover, preliminary observations in patients with PCNSL who were treated with multimodal therapy suggest that treatment may be successful in producing clinical response and survival only if implemented early in the course of disease (14). On the other hand, several diagnostic algorithms proposed formerly considered the patient likely to have toxoplasmosis if an enhanced mass lesion was detected at computed tomography scan (28,29). Nevertheless, in a recent decision-making study, the diagnostic capacity of clinical variables alone was demonstrated to be low and the combination of clinical data with PCR tests was found to be necessary for an accurate diagnosis of focal brain lesions (21). Therefore, the confounding effect of toxoplasmic encephalitis may interfere with the optimal diagnosis of brain lymphoma, increasing the time required for a definitive diagnosis. In fact, a retrospective

**Table 3. Risk of having Epstein-Barr virus (EBV)–DNA detectable in lumbar cerebrospinal fluid according to different baseline variables, in patients with definitive primary central nervous system lymphoma**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>EBV detected/not detected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;35 y</td>
<td>17</td>
<td>13/4 (76)</td>
</tr>
<tr>
<td>Age ≤35 y</td>
<td>13</td>
<td>11/9 (85)</td>
</tr>
<tr>
<td>Sexual HIV transmission</td>
<td>20</td>
<td>18/2 (90)</td>
</tr>
<tr>
<td>No sexual HIV transmission</td>
<td>10</td>
<td>6/4 (60)</td>
</tr>
<tr>
<td>CD4 &lt;50/µL</td>
<td>30</td>
<td>24/6 (80)</td>
</tr>
<tr>
<td>CD4 ≥50/µL</td>
<td>—</td>
<td>8/2 (80)</td>
</tr>
<tr>
<td>No previous AIDS diagnosis</td>
<td>10</td>
<td>16/4 (80)</td>
</tr>
<tr>
<td>Previous AIDS diagnosis</td>
<td>10</td>
<td>9/1 (90)</td>
</tr>
<tr>
<td>KPS ≥50</td>
<td>20</td>
<td>15/5 (75)</td>
</tr>
<tr>
<td>KPS &gt;50</td>
<td>17</td>
<td>13/4 (76)</td>
</tr>
<tr>
<td>Time from onset &lt;14 days</td>
<td>13</td>
<td>11/2 (85)</td>
</tr>
<tr>
<td>Time from onset ≥14 days</td>
<td>14</td>
<td>12/2 (86)</td>
</tr>
<tr>
<td>Single lesion</td>
<td>16</td>
<td>12/4 (75)</td>
</tr>
<tr>
<td>Perventricular site</td>
<td>14</td>
<td>13/1 (93)</td>
</tr>
<tr>
<td>No periventricular site</td>
<td>16</td>
<td>11/5 (69)</td>
</tr>
</tbody>
</table>

*HIV = human immunodeficiency virus and KPS = Karnofsky performance status.

**Table 4. Variables related to reduced or enhanced probability of undergoing lumbar puncture (LP)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>LP done/ not done</th>
<th>Univariate</th>
<th>Logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;35 y</td>
<td>62</td>
<td>48/14</td>
<td>0.95</td>
<td>0.37–2.44</td>
</tr>
<tr>
<td>CD4 &lt;50/µL</td>
<td>92</td>
<td>71/21</td>
<td>0.85</td>
<td>0.25–2.51</td>
</tr>
<tr>
<td>No previous AIDS diagnosis</td>
<td>53</td>
<td>38/15</td>
<td>0.54</td>
<td>0.20–1.38</td>
</tr>
<tr>
<td><em>Toxoplasma</em> prophylaxis</td>
<td>72</td>
<td>63/9</td>
<td>3.89</td>
<td>1.47–11.03</td>
</tr>
<tr>
<td>Positive <em>Toxoplasma</em> serology</td>
<td>70</td>
<td>49/21</td>
<td>0.31</td>
<td>0.09–0.88</td>
</tr>
<tr>
<td>KPS ≥50</td>
<td>50</td>
<td>50/20</td>
<td>0.39</td>
<td>0.13–1.08</td>
</tr>
<tr>
<td>Abnormal level of consciousness†</td>
<td>7</td>
<td>5/2</td>
<td>0.70</td>
<td>0.11–7.73</td>
</tr>
<tr>
<td>Multiple lesions</td>
<td>62</td>
<td>49/13</td>
<td>1.15</td>
<td>0.45–2.96</td>
</tr>
<tr>
<td>Perventricular site of lesion</td>
<td>21</td>
<td>18/3</td>
<td>1.86</td>
<td>0.48–10.70</td>
</tr>
<tr>
<td>Mass effect‡</td>
<td>83</td>
<td>59/24</td>
<td>0.21</td>
<td>0.04–0.76</td>
</tr>
</tbody>
</table>

*CI = confidence interval; AIDS = acquired immunodeficiency syndrome; and KPS = Karnofsky performance status.
†Abnormal level of consciousness assessed by slowing of mental processing and/or lethargy and neurocognitive impairment.
‡Mass effect is defined by the presence of indirect signs of enhanced intracranial pressure due to a space-occupying lesion, such as compression of the ventricular spaces, shift of the midline, or flattening of cerebral sulci.
multicenter analysis of a series of 158 brain biopsies performed in HIV-infected patients, recorded by the Italian Cooperative Group on AIDS and Tumors, showed that failure to react to anti-Toxoplasma treatment had been the selection criterion for brain biopsy in 72% cases, with median delay from the first neuroradiologic finding to biopsy of 42 days (Antinori A: unpublished data). In this study, the delay in brain biopsy for patients with EBV–DNA detected in their CSF was only 17 days, even though a direct selection to biopsy based on the PCR results was not part of the study protocol.

A second attractive aspect of screening CSF for EBV–DNA is to use it as a minimally invasive diagnostic approach for AIDS-related PCNSL. For AIDS patients, a brain biopsy may not be accepted either by the patients or by the neurosurgeons (11). This has a negative impact on the ability of clinicians to improve current therapeutic strategies as it decreases the number of patients potentially eligible for new treatment protocols (30).

Researchers have reported using empiric radiotherapy for treat presumed AIDS-related PCNSL (31), but a presumptive diagnosis based exclusively on the failure of anti-Toxoplasma treatment may not be suitable in assigning patients to this therapy. Our findings indicate that the minimally invasive approach accurately diagnosed PCNSL in 63% of the case patients and excluded PCNSL in 76% of the focal control subjects. Lumbar puncture appears feasible for use in patients with focal brain lesions, especially if PCNSL is strongly suspected. The high efficiency of EBV–DNA detection by this test and its feasibility of use may permit a diagnosis of AIDS-related PCNSL without a brain biopsy. It seems best to combine the detection of EBV–DNA by PCR with radionuclide imaging (i.e., 201T1-Single Photon Emission Computed Tomography [SPECT] or 18F-fluorodeoxyglucose-Positron Emission Tomography [FDG-PET]). These techniques can help to distinguish between lymphoma and toxoplasmic encephalitis (20,32,33) but their sensitivity may be not adequate (Antinori A, et al.: manuscript in preparation). A combined minimally invasive approach could also obviate confounding factors, such as the presence of multiple lesions with different etiologies or the early detection of EBV–DNA before the radiologic detection of lymphomatous lesions (17) when toxoplasma or other infectious abscesses are present. Strategic studies may be required on cost-effectiveness to compare a minimally invasive approach based on EBV-DNA detection combined with 201T1-SPECT or FDG-PET with conventional diagnostic methods, measuring as end-points survival, quality of life, and hospitalization time.

Unlike the preliminary observations (17), our prospective data show that nested PCR was unable to reveal the presence of EBV–DNA in the CSF of a certain number of patients with AIDS-related PCNSL. Since the detection limit of the assay did not differ significantly between these observations (10 versus four genome equivalents), we think that alternative explanations to test sensitivity should be considered. A first possibility is that the difference in sensitivity might be related to heterogeneous characteristics of the patients. Previous studies (17,18) mostly concerned autopsy-diagnosed AIDS-related PCNSL, with a different time interval between CSF sampling and histologic diagnosis. It has been hypothesized that autopsy findings might not reflect brain disease present at the time of CSF sampling (18). In our cohort, 27 (71%) of 38 patients with lymphoma had an in vivo diagnosis, and biopsy might have diagnosed the tumor mass at an early stage, perhaps before EBV–DNA was released in the CSF. Support for this hypothesis is the observation that in three of six lumbar CSF samples from six EBV–DNA negative/EBER-1-positive patients, EBV–DNA was detected later in the therapeutic follow-up after an average of 6.5 weeks (data not shown).

Even if we did not find a significant correlation between the detection of EBV–DNA and the extent and location of the tumor, the probability of having a positive amplification result seems to be higher when there are multiple lesions or when PCNSL is localized periventricularly. Indeed, it has been previously suggested that EBV–DNA is more likely to be detected when there is a widespread cerebral lymphomatous localization (18). However, the temporal relationship between EBV–DNA release in the CSF and the natural history of brain lymphoma is still unclear, as shown by preliminary observations of early EBV–DNA detection in CSF before the appearance of radiologically detectable mass lesions (17; Cingolani A, Murri R, De Luca A, Ammassari A, Tartaglione T, Larocca LM, et al.: manuscript submitted for publication).

Alternatively, the fact that the sensitivity of PCR in the present study is not absolute could be explained if EBV–DNA in the CSF is below the detection limit of nested PCR in a proportion of the patients with AIDS-related PCNSL. It has, in fact, been suggested (17,18) that the concentration of EBV–DNA may be very low in the CSF of some patients with AIDS-related PCNSL. In our experience, all CSF samples in which we detected EBV–DNA, yielded a positive PCR product in all five consecutive experiments. This excludes a viral load close to the detection limit of 10 genome equivalents in positive samples, as confirmed by semiquantitative experiments that revealed more than 500 EBV genomes in most samples of CSF tested (data not shown). Furthermore, in all CSF samples with undetectable EBV–DNA, the negative result was confirmed in all five consecutive experiments. Applying Poisson’s assumption to nested PCR analysis (34), this last finding seems to indicate that, in samples where EBV–DNA remains undetectable, its concentration should be far below the sensitivity threshold of the assay. However, the constant expression of viral transcripts in lymphomatous tissue specimens (16) suggests that a very low number of viral copies in lymphomatous tissue rather than a lack of EBV–DNA leakage into the CSF explains the negative PCR result.

In conclusion, the present prospective, in vivo study shows that the detection of EBV–DNA in CSF by PCR appears to be a simple and highly effective tool for the diagnosis of AIDS-related PCNSL. The test’s high specificity supports its use for rapid selection of patients for an early biopsy or as a minimally invasive surrogate diagnosis in the management of patients, particularly patients in clinical trials.

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Notes


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