Diagnosis of Cytomegalovirus (CMV) Polyradiculopathy and Documentation of In Vivo Anti-CMV Activity in Cerebrospinal Fluid by Using Branched DNA Signal Amplification and Antigen Assays

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Branched chain DNA assay (bDNA), cytomegalovirus (CMV) antigen assay, and cerebrospinal fluid (CSF) viral culture were studied for their utility in the diagnosis of CMV polyradiculopathy and for documenting in vivo antiviral effects. CMV was demonstrated in 15 of 16 patients by bDNA assay, 15 of 16 by CMV antigen assay, and 11 of 15 by CSF culture. When clinical criteria and results of the other two assays were used as reference standards, the sensitivity of bDNA was 94% and 100% and the specificity 95.2% and 100%; the CMV antigen assay sensitivity was 94% and 100% and specificity was 85.7% and 100%. Nine (90%) of 10 patients with polyradiculopathy and follow-up CSF culture showed a drop in CMV DNA after treatment; however, only 2 (20%) improved clinically. These results suggest that bDNA and antigen assays may be useful methods for the diagnosis of CMV polyradiculopathy, but treatment failures may not be due to inadequate antiviral activity.

Cytomegalovirus (CMV) infection is a frequent cause of morbidity and mortality among patients with human immuno-deficiency virus (HIV) infection. It is the most common opportunistic viral pathogen in patients with AIDS and was found in the central nervous system (CNS) of 33% of AIDS patients in one autopsy report [1]. Neurologic disease caused by CMV includes polyradiculopathy, encephalopathy, myelopathy, mononeuritis multiplex, and multifocal neuropathy [2]. While these clinical syndromes are responsible for severe neurologic sequelae and death in patients with AIDS, it is often difficult to definitively diagnose these conditions and differentiate them from other infections of the CNS. HIV encephalopathy, lymphomatous meningitis, tuberculous meningitis, toxoplasmosis, cryptococcal meningitis, and ascending demyelinating neuropathy are among other CNS conditions that may present in a manner similar to neurologic infection with CMV. The prognosis of CMV neurologic disease, particularly polyradiculopathy, is very frequently poor unless the syndrome is recognized and therapy is started rapidly [3–5].

A major obstacle to early diagnosis has been the absence of a sensitive and specific diagnostic assay. Culture of the cerebrospinal fluid (CSF) for CMV by use of tissue culture systems has been the reference standard for diagnosis, but it lacks adequate sensitivity. It is estimated that CSF culture for CMV is only 50% sensitive for the detection of CMV neurologic diseases when all other diagnoses are excluded and diagnosis is confirmed by autopsy [6]. In addition, 1–4 weeks may be required for CMV to grow in culture, since the rapid centrifuged shell vial cultures may be negative. Although empirical treatment may be initiated, current therapies for CMV have serious and frequent adverse effects. For these reasons, assays that can rapidly detect CMV with a high level of sensitivity and specificity are needed.

Recently, polymerase chain reaction (PCR), which detects nucleic acid, has been shown to be both sensitive and specific in detecting CMV in CSF but has not been evaluated as a diagnostic test for CMV neurologic disease [7–10]. Moreover, PCR does not always provide quantitation of virus in CSF, and thus a quantitative response to therapy cannot be readily measured. CMV antigen in CSF has been detected in a small number of patients with polyradiculopathy [11].

Branched DNA (bDNA) technology provides a sensitive assay that has the advantage of being quantitative and therefore could potentially be used to monitor severity of infection and assess response to therapy [12]. The CMV bDNA assay (Chiron, Emeryville, CA) is an overnight test consisting of primary hybridization of target DNA by specific CMV DNA probes. Branched DNA molecules are then added to provide multiple binding sites for an enzyme-labeled DNA probe, which in turn produces a chemiluminescent signal.

In contrast to PCR, the original target DNA is not amplified, but the detector DNA hybrids are. The signal produced by the luminometer is then reported as a digital number on a printout...
generated by a computer, thus minimizing operator error. This technique has been used successfully for the detection and quantitation of other viruses, including hepatitis B and C virus and HIV [12].

The purpose of this study was to determine the sensitivity and specificity of the branched DNA amplification assay, CMV antigen assays, and culture in CSF as diagnostic tests for CMV polyradiculopathy and to assess their utility as measures of in vivo antiviral activity in patients with follow-up CSF analyses. Results from this initial investigation would then provide a basis for future prospective studies.

Methods

Patient Population

CSF specimens from patients were obtained from six acute-care hospitals in San Francisco. Physicians from those hospitals submitted CSF for testing if CMV was considered a potential cause for a patient’s neurologic condition. CSF from consecutive patients was studied, and patients were excluded only if the CSF sample submitted was insufficient (defined as <1 mL) or if medical records were not retrievable for review.

Diagnosis of CMV Infection and Disease

Medical records were retrospectively reviewed and data were abstracted regarding HIV status and stage, prior CMV disease, and therapy. Presenting neurologic complaints and physical examination abnormalities that prompted CSF analysis and the course and response following therapy were also noted. Laboratory data, imaging studies, and electromyelography or electroencephalography results for each patient were recorded. Cases were then categorized into groups based on a set of preformulated clinical criteria. The categories were as follows: CMV polyradiculopathy, probable CMV encephalitis, other diagnosis (another pathogen was identified), or unknown diagnosis (where the cause of neurologic symptoms remained uncertain). The case definitions for the CMV clinical diagnoses are provided below.

A neurologist reviewer blinded to bDNA and CMV antigen assay results independently categorized patients on the basis of data obtained from medical charts. The sensitivity and specificity of the bDNA assay and CMV antigen assay were calculated in comparison to the clinical diagnosis of polyradiculopathy. Polyradiculopathy was used because it is a diagnosis that is more clearly defined clinically, easier to document in medical records, has fewer competing differential diagnoses than (e.g.) encephalopathy, and thus is subject to less misclassification error than is encephalopathy. The test performance of CMV bDNA was also assessed by comparing it to an alternative reference standard: the concordant diagnoses are provided below.

Assay Procedures

Branched DNA amplification assay. CSF (1–1.5 mL) was prepared for bDNA assay by ultracentrifuging (TL-100 centrifuge; Beckman Instruments, Palo Alto, CA) at 100,000 g for 1 h. The resulting pellet was resuspended in 40 μL of medium and stored at −35°C. To the ultracentrifuged pellet was added 50 μL of protein kinase-K. The mixture was incubated at 65°C for 30 min. To each tube was added 50 μL of denaturation buffer containing label and capture extender probes. The mixture was incubated at 65°C for 30 min. Following this incubation, 50 μL of neutralization buffer was added. In replicates of three, 40 μL of each tube was added to microwells that contained covalently bound capture probes. The microwell plates were incubated overnight at 65°C. The next day, 40 μL of amplifier probe was added to each well. The plates were incubated at 53°C for 30 min; 40 μL of alkaline phosphatase–labeled probe was then added to each well and the plates were incubated for 15 min at 37°C. The chromogen, dioxetane, was added to each well, and plates were incubated at 37°C for 30 min. Enzyme activity was then determined by luminometer measurement. A standard curve was run using known amounts of CMV DNA to quantify positive signal. A signal of >1 luminometer unit/mL was considered positive.

CMV antigen assay. The CMV antigen assay was performed by using the CMV-VUE immunoperoxidase kit (INCSTAR, Stillwater, MN). This assay detects the early structural protein (PP65) of CMV in CSF leukocytes by use of immunocytochemical methods. One milliliter of CSF was centrifuged in a microfuge at 14,000 g for 5 min to pellet cells. The cells were suspended in 40 μL of medium. Next, 15 μL of the cell suspension was spotted on slides, air dried, and fixed in acetone. The fixed cells were then incubated with a murine monoclonal antibody directed against PP65, followed by immunoperoxidase staining with peroxidase-labeled antiamouse IgG. Positively stained cells were then visualized by light microscopy. Results were reported qualitatively as either positive or negative; quantitation of the number of positive cells was not attempted.
Table 1. Results of CMV CSF assays by diagnostic category among 49 patients with neurologic syndromes.

<table>
<thead>
<tr>
<th>Diagnosis (n)</th>
<th>CMV bDNA assay</th>
<th>CMV antigen assay</th>
<th>CMV culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyradiculopathy (16)</td>
<td>15/16</td>
<td>15/16*</td>
<td>11/15</td>
</tr>
<tr>
<td>Probable CMV encephalitis (2)</td>
<td>2/2</td>
<td>2/2</td>
<td>0/1</td>
</tr>
<tr>
<td>Unknown (10)</td>
<td>2/10</td>
<td>3/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Other diagnosis (21)</td>
<td>1/21</td>
<td>3/21</td>
<td>0/21</td>
</tr>
</tbody>
</table>

NOTE. Denominators less than total n indicates test not done. * One test was indeterminate because too few cells were present in CSF to detect CMV antigen.

Culture. CSF (0.5–1 mL) was inoculated into human diploid fibroblast cultures and examined daily for cytopathogenic effect. Suspected isolates were confirmed by immunofluorescence by using a monoclonal antibody to CMV antigen.

The bDNA assay, CMV antigen assay, and CMV culture were performed on each specimen if the volume of CSF was sufficient. One milliliter of CSF was required for bDNA assay, 0.5 mL for CMV antigen assay, and 0.5 mL for CMV culture. In some cases, only bDNA and CMV antigen tests were performed.

Results

CSF was submitted for 58 patients from 6 hospitals from 1 June 1993 through 30 July 1994. Forty-nine patients had medical charts available for review and sufficient quantity of CSF for testing. All but 1 patient had documented AIDS. Of those diagnosed with CMV polyradiculopathy with documented CD4 cell counts (14/16), all had CD4 cell counts below 70 cells/mm³ (range, 0–67; median, 15).

The neurologic presentations of patients are as follows: 16 patients presented with polyradiculopathy, 2 with probable CMV encephalopathy, 10 with neurologic symptoms of unknown etiology, and 21 with other diagnoses. These other diagnoses included cryptococcal meningitis; CNS lymphoma; progressive multifocal leukoencephalopathy; normal pressure hydrocephalus; headache or altered mental status that was either very transient, responsive rapidly to non-CMV therapy, or longstanding dementia; HIV sensory peripheral neuropathy; and chronic inflammatory demyelinating polyneuropathy.

Test Results

Branched DNA and CMV antigen tests were performed on CSF of all 49 patients; 2 of 49 did not have culture. Results of these three assays by diagnostic category are shown in table 1.

Branched DNA. Of 16 patients with polyradiculopathy, 15 were positive by bDNA. Of 21 patients in the “other diagnosis” category, 20 (95%) tested negative by bDNA assay. These results indicate a sensitivity of 94% and specificity of 95.24% when the reference standard of clinical diagnosis of polyradiculopathy is used. The concordant results of CMV antigen and CSF culture provided a second method for evaluating bDNA performance. Of the 12 patients with CSF positive by both CMV antigen and CSF culture assays, all 12 were also positive by bDNA assay. All 22 patients with concordant negative CMV antigen and culture assays had negative CMV bDNA assay results. Thus, sensitivity and specificity estimations of bDNA based on a comparison to concordant laboratory testing by culture and CMV antigen assays were both 100%.

CMV antigen. Of 16 patients with polyradiculopathy, 15 were positive for CMV antigen in CSF (sensitivity = 94%). Of 21 patients in the “other diagnosis” category, 3 had a positive antigen test result (specificity = 85.7%). Of 12 patients with positive results in both the bDNA and culture assays, 12 were positive for antigen (sensitivity = 100%).

CMV culture. Compared to the diagnosis of polyradiculopathy and to the other two assays, the sensitivity of culture was 73.3% and the specificity was 100%.

Response to Therapy

Repeat lumbar puncture and CSF analyses following the initiation of therapy were performed within 3 weeks for 10 patients to assess response to therapy. Results of CMV antigen, bDNA, culture, cell count, glucose, and protein analyses of CSF prior to and following treatment are shown in table 2. All patients had been treated with ganciclovir, foscarnet, or both for their neurologic syndromes. The median time of follow-up was 13 days (range, 6–17).

The bDNA signal declined in 9 patients after a mean of 11.5 days of treatment and in as few as 6 days. The bDNA signal failed to decrease in 1 patient (patient 10) after 13 days of treatment. In contrast, the CMV antigen assay remained positive in 5 of 10 treated patients, although quantitative evaluation of the number of positive cells was not performed. Culture remained positive in 2 of 6 patients with repeat study.

Discussion

These data demonstrate the utility of CMV bDNA assay and the CMV antigen assay for the detection of CMV neurologic infections, specifically CMV polyradiculopathy. Of 49 patients, 16 had syndromes compatible with polyradiculopathy; 15 of these 16 had positive bDNA assays and CMV antigen in CSF but only 11 of 15 had a positive culture. To further validate the bDNA and antigen assays, they were each 100% sensitive in the 12 patients who were culture-positive and had a positive result by the other investigational test.

The two assays under investigation appeared to be not only more sensitive than culture but also more rapid, especially when the shell vial centrifugation cultures were negative. More rapid assays may be especially important to facilitate early
Table 2. Clinical course and serial CSF test results of patients with CMV polyradiculopathy \((n = 10)\).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>bDNA (LU/mL)</th>
<th>CMV antigen</th>
<th>CSF culture</th>
<th>CSF WBC</th>
<th>CSF protein</th>
<th>CSF glucose</th>
<th>Therapy</th>
<th>Clinical course</th>
<th>Prior diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/18/94</td>
<td>150.5</td>
<td>+</td>
<td>+</td>
<td>1500</td>
<td>253</td>
<td>32</td>
<td>FSC, GCV</td>
<td>Worsened</td>
<td>Retinitis</td>
</tr>
<tr>
<td>2</td>
<td>3/31/94</td>
<td>46.2</td>
<td>NA</td>
<td>ND</td>
<td>2</td>
<td>369</td>
<td>ND</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>3/01/94</td>
<td>87.72</td>
<td>+</td>
<td>+</td>
<td>3190</td>
<td>786</td>
<td>15</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>3/07/94</td>
<td>1.27</td>
<td>+</td>
<td>+</td>
<td>290</td>
<td>290</td>
<td>55</td>
<td>GCV</td>
<td>No change</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>3/01/94</td>
<td>8.46</td>
<td>+</td>
<td>ND</td>
<td>459</td>
<td>226</td>
<td>34</td>
<td>GCV</td>
<td>No change</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>3/07/94</td>
<td>4.31</td>
<td>+</td>
<td>ND</td>
<td>21</td>
<td>99</td>
<td>44</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>3/25/94</td>
<td>43.84</td>
<td>+</td>
<td>+</td>
<td>206</td>
<td>220</td>
<td>44</td>
<td>GCV, FSC</td>
<td>Improved</td>
<td>Esophagitis, colitis, no therapy</td>
</tr>
<tr>
<td>8</td>
<td>3/07/94</td>
<td>0.944</td>
<td>+</td>
<td>ND</td>
<td>2</td>
<td>96</td>
<td>95</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>3/07/94</td>
<td>4.31</td>
<td>+</td>
<td>+</td>
<td>290</td>
<td>220</td>
<td>44</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>3/07/94</td>
<td>0.944</td>
<td>+</td>
<td>+</td>
<td>21</td>
<td>99</td>
<td>44</td>
<td>GCV</td>
<td>Improved</td>
<td>None</td>
</tr>
</tbody>
</table>

NOTE. Dates are given as month/day/year. WBC = white blood cell count; NA = insufficient cells to permit antigen assay; FSC = foscarnet; GCV = ganciclovir; ND = not done; ± = indeterminate result; LU = luminometer units.

initiation of anti-CMV therapy, since this may be crucial in determining the clinical outcome.

Several recent studies have indicated excellent sensitivity results with PCR for detection of CMV in CSF, but they have not assessed the sensitivity for diagnosing a specific CMV neurologic syndrome (e.g., polyradiculopathy), nor have they simultaneously compared results with those of at least two other assays \([7–10]\). Prior to this study, detection of CMV antigen in CSF was evaluated only in patients with polyradiculopathy; however, it appeared useful in that study \([11]\).

PCR is not usually quantitative, and therefore, response to therapy cannot be precisely measured and monitored over time. Our results show that CMV bDNA signal may decline as few as 6 days after initiating therapy, thereby providing a rapid in vivo assay for antiviral susceptibility. Persistence of an elevated bDNA signal despite therapy may indicate the presence of resistant virus. In this study, CMV antigen assay was not quantitated by counting the number of positive cells in serially collected samples. It is possible that the number of positive cells might have declined with treatment of susceptible CMV strains. Alternatively, quantitating the decline of antigen-positive cells in response to antiviral therapy may have lagged behind the decline of viral DNA, since the available antivirals inhibit viral DNA synthesis as their primary mode of action.

The bDNA assay also provides evidence that monotherapy with ganciclovir or foscarnet, as well as the combination, does achieve adequate antiviral effect in CSF; therefore, this assay could be used to determine the appropriate treatment for individual patients.

Since 8 of 10 patients whose bDNA signal declined had worsening of their clinical course or did not improve, it is likely that factors other than antiviral susceptibility are important determinants of the outcome of polyradiculopathy. Among the most important of these may be early induction of therapy before an irreversible course is underway. Other studies suggest that a significant response to therapy in some patients may be achieved only after a substantial period of follow-up \([13]\). Our results underscore the need for rapid diagnosis, which may be achieved by using the bDNA or antigen assay.

Several limitations of this study must be considered in the interpretation of these results: The retrospective nature of this investigation and relatively small number of CSF samples studied preclude definitive conclusions regarding test accuracy. However, this study does provide sufficient evidence that the assays under study may be useful as diagnostic tools and fill a critical need, suggesting that further investigation is warranted.

There is as yet no commercially available PCR assay for CMV, and we did not perform a head-to-head comparison with PCR technology. We therefore cannot determine the relative sensitivity and specificity of PCR in comparison to bDNA or CMV antigen assay. Such a study, with a prospective design, would permit further evaluation of these tests as diagnostic tools for CMV neurologic disease syndromes.

One patient with a clinical diagnosis of chronic inflammatory demyelinating polyneuropathy had positive bDNA and CMV antigen results. In this patient, dual disease and misdiagnosis must be considered but are difficult to prove, since culture was negative and clinical criteria for polyradiculopathy or probable
encephalopathy were not met. Clearly, the spectrum of CMV neurologic disease outside of these two diagnoses needs further investigation with these new assays.

In conclusion, our findings suggest that the bDNA assay and CMV antigen assay may enhance the diagnostic armamentarium as sensitive, rapid tools. The bDNA assay further can provide important information regarding in vivo drug effect when patients are followed over time and may indicate that monotherapy is appropriate. Prospective studies should be performed to further validate these observations.

References