Effective Use of Polymerase Chain Reaction for Diagnosis of Central Nervous System Infections

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Polymerase chain reaction (PCR)–based testing of cerebrospinal fluid (CSF) specimens has become standard for confirmatory diagnosis of central nervous system (CNS) infections; however, these tests increase health care costs. We reviewed 3-year data from 974 consecutive CSF specimens submitted for detection of seven pathogens by PCR. In 1997, 237 of 367 specimens (64.6%) were submitted for multiple tests, compared with 203 of 522 (38.9%) in 1996 and 18 of 85 (21.2%) in 1995. In each year the arrival of new house officers coincided with a peak in multiple testing. Among 732 specimens submitted for herpesvirus detection, results were positive for 24 (4.6%) of 523 specimens with increased leukocyte counts or protein levels. None of 209 specimens with normal leukocyte and protein levels were positive for herpesviruses. None of 471 CSF specimens submitted for Borrelia burgdorferi detection were PCR-positive. Use of protein and leukocytes to screen CSF specimens before employing PCR for herpesvirus detection would save almost one-third of costs without reducing sensitivity.

Conventional methods remain inadequate for the laboratory diagnosis of many CNS infections. PCR performed on CSF is recognized as the new “gold standard” for some of these infections [1–5]. However, with implementation of these new assays, there is a tendency to request multiple tests in an effort to obtain at least one positive result.

Decreasing costs while maintaining quality has become an essential role of the clinical laboratory and physician services alike. This requires continuous evaluation of the value of all “routine” diagnostic tests [6, 7]. Physicians control >70% of health care expenditures; therefore, efficient selection of critical laboratory tests by physicians has significant impact on total medical costs [6, 8]. Clinicians at the Mayo Clinic can request PCR tests of CSF on a standard laboratory request form that lists seven pathogens, including herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), JC virus (JCV), Borrelia burgdorferi, and Tropheryma whippeli. The cumulative fee for these assays, several of which are usually ordered simultaneously, is $1,200.00 (United States currency), of which 9%–15% goes directly toward PCR patent royalties. The vast majority of these tests yield negative results.

CSF leukocyte counts and total protein levels are predictive of CNS infections [2, 9, 10]. In the present study, we evaluated these inexpensive, routine determinations for their ability to predict the outcome of more expensive PCR-based testing.

Methods

All CSF specimens from Mayo Clinic patients submitted for molecular testing between 1 January 1995 and 31 December 1997 were analyzed for this study. Specimens were tested in accordance with clinician requests; no tests were performed except those ordered by the clinical service submitting the specimen. Clinical information on these patients, including indications for testing, clinical diagnoses, other testing performed, treatment, and response, was not available for this analysis. An abnormal CSF total protein level was defined as >45 mg/dL, while an abnormal CSF leukocyte count was defined as >5 nucleated cells/mm³.

PCR-based methods for detection of HSV, CMV, EBV, VZV, JCV, B. burgdorferi, and T. whippeli have been published elsewhere and were not altered during the study period [4, 11–15]. The $x^2$ values for trends, odds ratios, and confidence limits were calculated with use of Epi Info, version 6.04b (Centers for Disease Control and Prevention, Atlanta).

Results

During a period of 3 years, a total of 16,125 CSF specimens were processed in the Molecular Microbiology Diagnostic Laboratory for PCR testing. We identified 974 specimens (6.0%) that were collected from Mayo Clinic patients, of which 24 (2.5%) were positive for one pathogen and 2 (0.2%) were positive for two pathogens. Table 1 shows that 458 specimens

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were submitted for two or more tests. Among the multiple-testing specimens, 271 (59.2%) were tested for HSV and B. burgdorferi. The prevalence of positive results was not associated with the number of tests performed per specimen.

Multiple testing on the same sample increased from 18 of 85 specimens (21.2%) in 1995 to 203 of 522 (38.9%) in 1996 and 239 of 367 (65.1%) in 1997 ($\chi^2 = 82.1; P < 10^{-5}$) (figure 1). Figure 2 shows that in the third quarter, when new house officers arrived, 167 of 303 specimens (55.1%) were submitted for multiple tests, compared with 291 of 671 (43.4%) submitted the rest of the year (OR, 6.7; 95% CI, 1.2–2.1). This tendency remained the same each year (data not shown).

Among 732 specimens submitted for detection of herpesviruses (HSV, CMV, EBV, and/or VZV), results were positive for 24 of 523 (4.6%) with abnormal leukocyte counts or total protein levels (table 2). In contrast, none of 209 specimens with normal leukocyte and protein levels were positive for herpesviruses (OR, 0.0; 95% CI, 0.0–0.4). Furthermore, specimens in which both leukocyte counts and protein levels were abnormal had a higher diagnostic yield of herpesviruses than did samples in which only one of these indicators was positive (18 of 173 [10.4%] vs. 6 of 350 [1.7%]; OR, 6.7; 95% CI, 2.5–20.8).

There were too few positive results to demonstrate whether CSF leukocyte and protein levels were associated with positive test results for JCV or T. whippelii (table 2). None of 471 CSF specimens submitted for B. burgdorferi detection were PCR-positive, although 322 (68.4%) had abnormal leukocyte counts or protein levels (table 2). Among the 471 specimens submitted, only 143 (30.4%) were from patients who had undergone Lyme serological tests previously. Fifteen (3.2%) of 471 patients tested for neuroborreliosis had indeterminate serological status, and five (1.1%) were serologically reactive.

### Discussion

Our data indicate that the elimination of PCR testing of CSF specimens with normal leukocyte and protein levels would save almost one-third of costs associated with herpesvirus testing, without decreasing sensitivity. Significant improvement in patient care might result if expensive workups following false-positive results, common in PCR-based tests, can be avoided. Accordingly, we recommend that specimens with normal leukocyte and protein levels should be rejected for herpesvirus PCR testing, except in special circumstances (e.g., leukopenia or a recommendation by neurology or infectious disease consultants).

Our data demonstrated a significant trend toward multiple testing of specimens that provided little clinically useful information. An interesting finding was that multiple tests occurred more frequently in the third quarter of each year, when new residents and fellows start clinical training. Our results are similar to those of McGillivray et al., who showed increased laboratory usage in the third quarter and by less experienced physicians [16]. These data reinforce the importance of edu-

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### Table 1. Orders for multiple PCR tests, in relation to the diagnostic yield, of CSF specimens.

<table>
<thead>
<tr>
<th>No. of tests ordered</th>
<th>No. of specimens tested</th>
<th>No. of tests performed</th>
<th>Percentage of tests positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>516</td>
<td>516</td>
<td>2.13</td>
</tr>
<tr>
<td>2</td>
<td>230</td>
<td>460</td>
<td>1.74</td>
</tr>
<tr>
<td>3</td>
<td>114</td>
<td>342</td>
<td>0.58</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>244</td>
<td>1.23</td>
</tr>
<tr>
<td>≥5</td>
<td>53</td>
<td>300</td>
<td>1.33</td>
</tr>
</tbody>
</table>

* Included positivity for herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), JC virus (JCV), Borrelia burgdorferi, and Tropheryma whippelii.
† $\chi^2$ trend = 4.16; $P = .38$.
‡ Included one EBV/VZV coinfection.
§ Twenty-two, 27, and 4 specimens were subjected to 5, 6, and 7 tests, respectively.

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**Figure 1.** Yearly distribution of orders for multiple PCR tests. The number of CSF specimens tested was 85 for 1995, 522 for 1996, and 367 for 1997. Each specimen was tested for (bars, from left to right) 1, 2, 3, 4, or 5–7 pathogens.

**Figure 2.** Seasonal distribution of orders for multiple PCR tests. The number of CSF specimens tested was 183 for the period of January through March, 218 for April through June, 303 for July through September, and 270 for October through December. Each specimen was tested for (bars, from left to right) 1, 2, 3, 4, or 5–7 pathogens.
The use of PCR-test-request forms that list assays for only the most prevalent pathogens with the strongest indications for therapy (HSV, in our study) might reduce redundant testing frequently when order forms list the assays in a menu format. While multiple tests may be indicated for some patients, we suggest that menu-driven requests for multiple PCR assays engender low-yield secondary tests of limited diagnostic value. The use of PCR-test-request forms that list assays for only the most prevalent pathogens with the strongest indications for therapy (HSV, in our study) might reduce redundant testing while preserving the option to order tests for specific pathogens on an ad hoc basis.

Both serology and PCR tests have been used for diagnosis of Lyme neuroborreliosis [19–21]. Our data suggest that PCR of CSF is unsuitable as a screening test for the diagnosis of Lyme neuroborreliosis, even when CSF abnormalities are present. Studies have found that *B. burgdorferi* can invade the CNS early in the course of infection, but for patients with illness of >1 month’s duration, serological testing is almost always positive [19–22]. Since objective neurological deficits generally appear later in the course of *B. burgdorferi* infection, serological testing for Lyme disease should be used to screen appropriate candidates for PCR testing, which in our experience was highly specific but very expensive.

We feel that PCR testing of seronegative patients is not cost-effective, particularly for diagnosis of CNS infections. HSV is of special importance among organisms for which we offer PCR testing. PCR is proven to have high sensitivity for detection of HSV DNA in CSF specimens [4, 5]. It is the only one of these tests whose results may lead to proven lifesaving therapy [17, 18], although therapeutic protocols do exist for the other pathogens. HSV was also the most frequently detected pathogen in our study, and none of 13 HSV-positive patients were found to have any other CSF pathogens. We therefore suggest that when HSV PCR is ordered in combination with other CSF PCR tests, the HSV PCR should be performed first, before the other assays.

Our study indicated that multiple PCR tests are requested frequently when order forms list the assays in a menu format. While multiple tests may be indicated for some patients, we suggest that menu-driven requests for multiple PCR assays engender low-yield secondary tests of limited diagnostic value. The use of PCR-test-request forms that list assays for only the most prevalent pathogens with the strongest indications for therapy (HSV, in our study) might reduce redundant testing while preserving the option to order tests for specific pathogens on an ad hoc basis.

<table>
<thead>
<tr>
<th>Organism detected</th>
<th>Both protein level and leukocyte count normal</th>
<th>Protein level normal, leukocyte count abnormal</th>
<th>Leukocyte count normal, protein level abnormal</th>
<th>Both protein level and leukocyte count abnormal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>0/202 (0)</td>
<td>0/32 (0)</td>
<td>2/311 (0.6)</td>
<td>11/171 (6.4)</td>
<td>13/716 (1.8)</td>
</tr>
<tr>
<td>EBV</td>
<td>0/17 (0)</td>
<td>1/3 (33.3)</td>
<td>0/35 (0)</td>
<td>4/38 (10.5)</td>
<td>5/93 (5.4)</td>
</tr>
<tr>
<td>VHZ</td>
<td>0/20 (0)</td>
<td>0/2 (0)</td>
<td>3/54 (5.6)</td>
<td>6/42 (14.3)</td>
<td>9/118 (7.6)</td>
</tr>
<tr>
<td>CMV</td>
<td>0/27 (0)</td>
<td>0/3 (0)</td>
<td>0/53 (0)</td>
<td>0/47 (0)</td>
<td>0/130 (0)</td>
</tr>
<tr>
<td>Herpesvirus*</td>
<td>0/209 (0)</td>
<td>1/33 (3.0)</td>
<td>5/317 (1.6)</td>
<td>18/173 (10.4)</td>
<td>24/732 (3.3)</td>
</tr>
<tr>
<td>JCV</td>
<td>1/35 (2.9)</td>
<td>0/1 (0)</td>
<td>0/68 (0)</td>
<td>0/40 (0)</td>
<td>1/144 (0.7)</td>
</tr>
<tr>
<td>T. whippeli</td>
<td>0/56 (0)</td>
<td>0/3 (0)</td>
<td>1/101 (1.0)</td>
<td>0/30 (0)</td>
<td>1/190 (0.5)</td>
</tr>
<tr>
<td>B. burgdorferi</td>
<td>0/149 (0)</td>
<td>0/18 (0)</td>
<td>0/215 (0)</td>
<td>0/89 (0)</td>
<td>0/471 (0)</td>
</tr>
</tbody>
</table>

* Including HSV, EBV, VZV, and CMV.

- **Effective Use of PCR Tests**


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References