Improved Detection of Bacterial Central Nervous System Infections by Use of a Broad-Range PCR Assay

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A universal PCR assay for bacteria and fungi detected meningitis pathogens in 65% of 20 cerebrospinal fluid (CSF) samples from patients with suspected central nervous system (CNS) infections compared to a 35% detection rate when culture and/or microscopy methods. Thus, the PCR assay can improve the diagnosis rate of infective meningitis when standard methods provide a negative result.

Bacterial meningitis is a life-threatening disease associated with significant morbidity and mortality that requires immediate medical assessment and treatment (1). As evaluations of clinical signs and symptoms lack both sensitivity and specificity (2), an analysis of cerebrospinal fluid (CSF) samples by laboratory tests is usually required for confirmation. Gram staining is a rapid and specific method for detecting bacteria in CSF but lacks sensitivity (3–5). Culture is more sensitive, with detection rates of >80% in the CSF samples of patients with acute disease before the initiation of treatment (6), but it depends on the number of viable bacteria. Nucleic acid amplification tests, such as PCR, act independently of the growth of etiological agents and can detect small amounts of pathogen DNA. Universal PCR assays that are based on the amplification of conserved regions of rRNA genes are capable of detecting and differentiating a broad range of bacteria and fungi (7). Bacterial pathogens were detected by 16S rRNA PCR in CSF samples of patients with bacterial meningitis with excellent sensitivity and specificity (8). Furthermore, about 30% of the culture-negative presumed bacterial meningitis cases were PCR positive, indicating superior sensitivity over that obtained with culture (8). However, the performance of 16S PCR varies in different studies (8), probably due to different methodologies, including PCR design and DNA preparation.

CSF samples from 40 patients with clinical symptoms of CNS infection were analyzed by UMD-Liquid broad-range PCR. Twenty samples were from group 1 patients presenting with white blood cell (WBC) counts of >500/µl CSF, who were suspected to have a bacterial infection, and 20 samples were from group 2 patients with WBC counts of <500/µl CSF who were unlikely to be associated with bacterial CNS infection. Various WBC counts were used as a threshold in studies where pleocytosis was included as a cutoff, because in viral meningitis, WBC counts typically are <500 cells/µl (10). Microbial DNA was isolated from 0.5 to 1 ml CSF, and 16S and 18S DNA sequences were amplified according to the manufacturer’s instructions. DNA was isolated using a two-step procedure that allowed for the enrichment of bacteria and fungi by prior removal of human DNA through selective lysis of human cells. Amplification was performed by real-time PCR on a Roche LightCycler 480. PCR signals that appeared before cycle 35 were considered positive and analyzed further by sequence analysis. Pathogens were identified by analyzing DNA sequences with SepsiTest BLAST (http://www.sepsitest-blast.de/en/index.html) and the BLAST tool of NCBI (www.ncbi.nlm.nih.gov/blast). All CSF samples were also analyzed by microscopy, culture on agar plates, and liquid culture (see Supplemental Methods in the supplemental material for methodological details).

In the group 1 CSF samples, PCR and culture/microscopy were positive in 13/20 (65%) and 7/20 (35%) samples, respectively (Table 1). The positivity rates of PCR, culture, and/or microscopy are congruent with those of previous studies (8, 9, 11). In our study, the PCR result matched that of culture/microscopy in 5 samples, and the bacteria were also detected in a blood culture taken 1 day before. In group 2 CSF specimens, neither PCR nor culture/microscopy detected bacterial pathogens in one sample taken from an HIV-infected patient, Cryptococcus neoformans, which was identified by culture. Except in patients with ventriculoperitoneal shunts, S. epidermidis is not considered a CNS pathogen, and WBC counts in the respective CSF samples were low (667 cells/µl), with only 60% neutrophils. The detection of K. pneumoniae (patient no. 15 in Table 1) is more likely a true positive result, as the patient had a ventriculoperitoneal shunt, and the bacteria were also detected in a blood culture taken 1 day before. Overall, 8/40 CSF samples were positive by culture and/or microscopy, 6 of which were also positive by PCR, resulting in a sensitivity of 75% compared to conventional diagnosis (86% when excluding the specimen positive for S. epidermidis by culture). Furthermore, 8/32 CSF samples negative by culture/microscopy were positive by PCR, most likely representing true positive results.

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TABLE 1  Laboratory findings in patients with WBC counts of >500 cells/μl CSF

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr), gendera</th>
<th>WBC counts (per μl)</th>
<th>Cell typesb</th>
<th>Results with:</th>
<th>Antibiotic pretreatmentc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microscopy</td>
<td>Culture</td>
</tr>
<tr>
<td>1</td>
<td>61, f</td>
<td>6,312</td>
<td>68, 1</td>
<td>Gram-positive cocci</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>2</td>
<td>15, f</td>
<td>1,083</td>
<td>79, 13</td>
<td>Gram-negative cocci</td>
<td>Neisseria meningitidis</td>
</tr>
<tr>
<td>3</td>
<td>69, m</td>
<td>1,601</td>
<td>&gt;90 N</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>4</td>
<td>59, f</td>
<td>803</td>
<td>&gt;90 N</td>
<td>Negative</td>
<td>Haemophilus Influenzae</td>
</tr>
<tr>
<td>5</td>
<td>18, m</td>
<td>3,667</td>
<td>ND</td>
<td>Gram-negative cocci</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>79, m</td>
<td>10,402</td>
<td>96, 1</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>69, f</td>
<td>10,200</td>
<td>92, 2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>29, f</td>
<td>1,496</td>
<td>90, 4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>&lt;1, m</td>
<td>&gt;1,000</td>
<td>ND</td>
<td>Negative</td>
<td>Streptococcus agalactiae</td>
</tr>
<tr>
<td>10</td>
<td>81, f</td>
<td>1,272</td>
<td>89, 3</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>11</td>
<td>71, f</td>
<td>3,445</td>
<td>81, 6</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>40, m</td>
<td>731</td>
<td>2, 75</td>
<td>Negative</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>13</td>
<td>40, m</td>
<td>667</td>
<td>60, 27</td>
<td>Negative</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>14</td>
<td>76, m</td>
<td>676</td>
<td>66, 13</td>
<td>Negative</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>15</td>
<td>28, f</td>
<td>667</td>
<td>60, 27</td>
<td>Negative</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>16</td>
<td>74, f</td>
<td>539</td>
<td>44, 50</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>17</td>
<td>50, m</td>
<td>691</td>
<td>54, 14</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>18</td>
<td>65, m</td>
<td>652</td>
<td>49, 32</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>19</td>
<td>29, m</td>
<td>8,053</td>
<td>79, 5</td>
<td>Negative</td>
<td>S. pneumoniae</td>
</tr>
</tbody>
</table>

a  m, male; f, female.
b Values are % neutrophils (N), % lymphocytes (L) unless otherwise indicated. ND, not determined.
c MER, meropenem; VAN, vancomycin; AMS, ampicillin-sulbactam; CRO, ceftriaxone; AMP, ampicillin; CTX, cefotaxime; PEN, penicillin; CIP, ciprofloxacin; FOS, fosfomycin; PIP, pipercillin.
d CSF sample came from a patient from another hospital with no available documentation of administration of antibiotics before lumbar puncture.

results because (i) these samples had WBC counts of >500 cells/μl, mainly consisting of neutrophils, (ii) these patients presented with clinical features characteristic of CNS infection (Table 2), and (iii) the PCR results represented typical CNS pathogens. The specificity of PCR might be calculated as 75% (see Table 1); however, regarding the obviously higher sensitivity of PCR, it appears inadequate to determine the specificity of PCR with culture/microscopy as the reference method.

Microbial growth failure may result from fastidious pathogens that are difficult to cultivate or from antibiotic pretreatment (6, 12). All 9 PCR-positive culture-negative specimens in our study are represented by bacteria that grow easily on culture medium, and 7 of them were collected from patients with documented administration of antibiotics prior to lumbar puncture, indicating that antibiotic pretreatment was most likely the reason for failure of culture, as has been shown in other studies (11, 13). Especially

TABLE 2  Clinical features of patients with discrepant findings of CSF analysis by PCR and culture/microscopy

<table>
<thead>
<tr>
<th>PCR/culture results and patient no.a</th>
<th>Pathogen detected</th>
<th>Prodromal clinical feature(s)b</th>
<th>Clinical features at admission/during hospitalizationc</th>
<th>Other laboratory findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive/culture negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>N. meningitidis</td>
<td>Flu-like illness, cough, headaches</td>
<td>Meningism</td>
<td>Gram-positive diplococci detected by microscopy</td>
</tr>
<tr>
<td>6</td>
<td>H. influenzae</td>
<td>Headsaches</td>
<td>Sopor</td>
<td>H. influenzae also detected in blood culture</td>
</tr>
<tr>
<td>7</td>
<td>S. pneumoniae</td>
<td>Headsaches, chronic sinusitis</td>
<td>Meningism, organic brain syndrome</td>
<td>S. pneumoniae detected in a former CSF sample collected in another hospital</td>
</tr>
<tr>
<td>8</td>
<td>S. pneumoniae</td>
<td>Flu-like illness, headaches</td>
<td>Meningism, somnolence</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>S. agalactiae</td>
<td>Headsaches, reduced general condition</td>
<td>Meningism</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>S. pneumoniae</td>
<td>Headsaches, reduced general condition</td>
<td>Sopor, meningism, septic venous sinus thrombosis, meningism, somnolence</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>S. pneumoniae</td>
<td>Coma (GCS3)</td>
<td>Sopor, meningism, coma</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>N. meningitidis</td>
<td>Fever, headaches, WF-syndrome</td>
<td>Meningism, acral necrosis, renal failure</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>L. monocytogenes</td>
<td>Subfebrile, reduced general condition</td>
<td>Meningism, somnolence, organic brain syndrome</td>
<td>L. monocytogenes detected in a former CSF sample collected in another hospital</td>
</tr>
</tbody>
</table>

| PCR negative/culture positive       |                   |                               |                                                      |                          |
| 14                                  | S. epidermidis    | Somnolence                    | Sopor, coma, ICP increased, MCS |                          |
| 15                                  | K. pneumoniae     | Atypical intracerebral hemorrhage, VPS infection, UTI, polymorbid | Organic brain syndrome, MCS | K. pneumoniae also detected in blood culture and VPS device |

a  Numbering corresponds to that for patients in Table 1.
b  GCSE, Glasgow coma scale grade 3; WF, Waterhouse-Friderichsen; VPS, ventriculoperitoneal shunt; UTI, urinary tract infection.
c  ICP, intracranial pressure; MCS, minimal conscious state.
in cases of delayed lumbar puncture after the initiation of antibiot-
ic treatment, as for instance, in patients with increased intracra-
mal pressure. PCR assays seem to be superior to standard micro-
biological methods.

Inefficient DNA preparation and PCR inhibition are known to
impair PCR amplification (9, 14, 15). The procedure of DNA re-
covered in UMD-Liquid generates purified DNA of microbial
organisms largely cleared of human DNA. No inhibition was ob-
erved in any of the 40 CSF specimens, indicating that any inhib-
itors were removed efficiently by the DNA extraction procedure.
Furthermore, the reduced burden of human DNA is likely to im-
prove the sensitivity and specificity of pathogen detection com-
pared to other tests that are based on the isolation of total DNA
from CSF samples. Using blood samples spiked with methicillin-
resistant Staphylococcus aureus (MRSA), bacterial DNA was de-
tected by PCR with higher sensitivity in DNA extracts obtained
with the preanalytical UMD-Liquid system than in total DNA
extracts (16). Improved sensitivity over conventional diagnostics
was also shown in clinical studies on patients with endocarditis,
sepsis, and joint infections (12, 17, 18).

Universal PCRs may reduce diagnostic uncertainty in patients
with suspected CNS infection, but currently, they represent ad-
junctive tests rather than those that would replace culture because
they do not provide information about antibiotic susceptibilities.
Nevertheless, PCR results may be used to optimize antibiotic
treatment for patients and antibiotic prophylaxis of contact per-
sons based on the identified pathogens.

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