PCR Diagnosis of *Pneumocystis* Pneumonia: a Bivariate Meta-Analysis

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We undertook a bivariate meta-analysis to assess the overall accuracy of respiratory specimen PCR assays for diagnosing *Pneumocystis* pneumonia. The summary sensitivity and specificity were 0.99 (95% confidence interval, 0.96 to 1.00) and 0.90 (0.87 to 0.93). Subgroup analyses showed that quantitative PCR analysis and the major surface glycoprotein gene target had the highest specificity value (0.93). Respiratory specimen PCR results are sufficient to confirm or exclude the disease for at-risk patients suspected of having *Pneumocystis* pneumonia.

*Pneumocystis* pneumonia (PCP) remains a frequent opportunistic infection among immunocompromised patients, especially AIDS patients (5, 14, 15, 24). The gold standard for diagnosing PCP is the detection of cysts and/or trophic forms by staining (5, 15). Many studies have assessed the diagnostic yield of PCR techniques for diagnosing PCP. However, the true potential role of PCR assays remains controversial. Thus, we pooled prospective cohort studies with consecutive patients and undertook a bivariate meta-analysis to assess the diagnostic accuracy of PCR on the basis of sensitivity (SEN), specificity (SPE), and positive and negative likelihood ratios (PLR and NLR, respectively) (19).

We searched the MEDLINE and EMBASE databases for the English-language literature published up to May 2011. Full-text publications were included if (i) they used PCR on respiratory samples, such as bronchoalveolar lavage fluid (BALF), induced sputum (IS), or oropharyngeal wash (OW), for immunocompromised patients with pulmonary diseases or requiring bronchoscopy for suspected PCP and (ii) the prospective cohort studies were performed with consecutive patients. Studies with fewer than 10 patients with PCP were excluded. We explored potential heterogeneity by subgroup analyses (16). All analyses were performed using the Midas program (6).

Thirteen reports, including 20 eligible studies, met our inclusion criteria (1–3, 7–12, 18, 21, 25, 26) (Table 1). Twelve articles reported patients with false-positive results, and follow-up was complete for most of these patients. When probable PCP (clinical and radiographic findings consistent with the diagnosis and consequent recovery with anti-PCP treatment) was included, 31.8% (65/204) of the patients had PCP. We could not extract the exact data for AIDS and non-AIDS patients from two articles (7, 8), which led to discrepancies between the whole and subset populations. We found significant heterogeneity for all test performances.

The test performances for the whole and subset groups are shown in Table 2. For the whole population, the area under the summary receiver operating characteristic curve and 95% confidence intervals were 0.98 (0.96 to 0.99), indicating that the PCR assay has an excellent diagnostic value for PCP. For the whole population, the percentage of heterogeneity likely due to a threshold effect was 38%, indicating a moderate influence of a diagnostic threshold effect on the performance of the PCR assay. In subgroup analyses, the test performances varied by study design, reference standard, and type of assay (Fig. 1).

The internal transcribed spacer (ITS) PCR had the highest SEN at 1.00 (1.00 to 1.00) and the lowest SPE at 0.86 (0.76 to 0.95). The SEN and SPE of the major surface glycoprotein (MSG) PCR were 0.98 (0.94 to 1.00) and 0.93 (0.89 to 0.98), and those of the large-subunit mitochondrial rRNA (mtRNA) PCR were 0.98 (0.96 to 1.00) and 0.90 (0.87 to 0.94), respectively. The SEN of the test was 1.00 (0.99 to 1.00) and the SPE was 0.87 (0.83 to 0.91) for studies limited to using invasive specimens for staining. When the staining specimens included IS, the SEN and SPE were 0.97 (0.92 to 1.00) and 0.93 (0.90 to 0.95). Compared with nonquantitative PCR, quantitative PCR had a higher SPE of 0.93 (0.89 to 0.96) (*P* < 0.001). For subset groups, eight and four articles provided data for BALF PCR and OW PCR, respectively (Table 2). Compared with BALF PCR, OW PCR had a lower SEN (*P* < 0.001) and a higher SPE (*P* < 0.001).

Overall, we concluded that PCR assay of respiratory specimens is very powerful for ruling PCP in or out for at-risk patients.
patients. Under most circumstances, PLRs of >10 and NLRs of <0.1 have been noted as providing convincing diagnostic evidence, respectively (13). According to our results, both PLRs and NLRs succeeded in passing the threshold index, irrespective of AIDS status.

Discrepancies between positive PCR and negative staining results have always been reported by many investigators (1–3, 7–12, 21, 25, 26). In fact, false-positive PCR and false-negative results have always been reported by many investigators (1–3, 7–12, 21, 25, 26). In fact, false-positive PCR and false-negative results have always been reported by many investigators (1–3, 7–12, 21, 25, 26).

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The marked variations in PCR techniques included DNA extraction procedures (standard or commercial kit), cell wall disruption (standard or commercial kit), choice of target gene (ITS, MSG, or mtRNA), and quantitative PCR (yes or no). We also found that the SPE of quantitative PCR was significantly higher than that of traditional PCR. It is particularly important for AIDS-unrelated patients who present low-burden PCP not diagnosed microscopically. According to subgroup analyses, all of the variations mentioned above were reasons for heterogeneity. Thus, the incorporation of PCR into the proven PCP definition might be hampered by the lack of PCR standardization and the limited availability of PCR. In light of the recently published quantitative real-time PCR experimental guidelines (4), further prospective cohort studies should focus on quantitative PCR standardization and determine the optimal cutoff of quantitative PCR results.

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TABLE 1. Characteristics of the 13 reports in our meta-analysis of the diagnosis of PCP using PCR

<table>
<thead>
<tr>
<th>Study (reference no.)</th>
<th>Patient population</th>
<th>Staining sample(s)</th>
<th>Staining method(s)</th>
<th>Blinded status</th>
<th>Proven PCP</th>
<th>Prior or later PCP/total possible false positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AIDS, 146 cases of suspected PCP</td>
<td>BALF</td>
<td>CS</td>
<td>NA</td>
<td>71</td>
<td>0/18</td>
</tr>
<tr>
<td>2</td>
<td>At risk, 238 cases (AIDS, 69) of suspected PCP</td>
<td>BALF, IS</td>
<td>CS, IFS</td>
<td>Yes</td>
<td>16</td>
<td>0/30</td>
</tr>
<tr>
<td>3</td>
<td>Non-AIDS, at-risk, 448 cases of suspected PCP</td>
<td>BALF, IS</td>
<td>CS, IFS</td>
<td>Yes</td>
<td>39</td>
<td>14 (5 proven, 9 probable)/21</td>
</tr>
<tr>
<td>4</td>
<td>At risk, 400 cases (AIDS, NA) of suspected PCP</td>
<td>BALF</td>
<td>IFS</td>
<td>NA</td>
<td>31</td>
<td>11 (proven)/35</td>
</tr>
<tr>
<td>5</td>
<td>At risk, 175 cases (AIDS, NA) of suspected PCP</td>
<td>BALF, IS</td>
<td>CS, IFS</td>
<td>Yes</td>
<td>32</td>
<td>0/11</td>
</tr>
<tr>
<td>6</td>
<td>At risk, 275 cases (AIDS, 105) of suspected PCP</td>
<td>BALF, IS</td>
<td>CS, IFS</td>
<td>No</td>
<td>16</td>
<td>26 (3 proven, 23 probable)/28</td>
</tr>
<tr>
<td>7</td>
<td>At risk, 110 cases (AIDS, 9) of suspected PCP</td>
<td>BALF, IS</td>
<td>CS, IFS</td>
<td>Yes</td>
<td>14</td>
<td>1/9</td>
</tr>
<tr>
<td>8</td>
<td>AIDS, 76 cases of suspected PCP</td>
<td>BALF</td>
<td>CS, IFS</td>
<td>Yes</td>
<td>28</td>
<td>1/3</td>
</tr>
<tr>
<td>9</td>
<td>AIDS, 132 cases of suspected PCP</td>
<td>BALF</td>
<td>CS</td>
<td>Yes</td>
<td>61</td>
<td>0/3</td>
</tr>
<tr>
<td>10</td>
<td>AIDS, 35 cases of suspected PCP</td>
<td>Biopsy specimen</td>
<td>CS</td>
<td>NA</td>
<td>16</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>AIDS, 250 cases (AIDS, 89) of suspected PCP</td>
<td>BALF, IS</td>
<td>CS</td>
<td>Yes</td>
<td>26</td>
<td>4 (probable)/23</td>
</tr>
<tr>
<td>12</td>
<td>AIDS, 47 cases of suspected PCP</td>
<td>BALF, biopsy specimen</td>
<td>CS</td>
<td>Yes</td>
<td>18</td>
<td>1 (proven)/4</td>
</tr>
<tr>
<td>13</td>
<td>AIDS, 173 cases of suspected PCP</td>
<td>BALF</td>
<td>CS</td>
<td>Yes</td>
<td>48</td>
<td>7 (probable)/19</td>
</tr>
</tbody>
</table>

a Other samples, such as tracheal and endotracheal aspirates.
b CS, colorimetric staining.
c IFS, immunofluorescent staining.
d NA, not available.

TABLE 2. Pooled test performance of the studies included in our meta-analysis of the diagnosis of PCP using PCR

<table>
<thead>
<tr>
<th>Study subjects or method</th>
<th>No. of articles</th>
<th>No. of studies</th>
<th>SEN</th>
<th>SPE</th>
<th>PLR</th>
<th>NLR</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td>13</td>
<td>20</td>
<td>0.99 (0.96–1.00)</td>
<td>0.90 (0.87–0.93)</td>
<td>10.2 (7.8–13.4)</td>
<td>0.01 (0.00–0.04)</td>
<td>0.98 (0.96–0.99)</td>
</tr>
<tr>
<td>AIDS population</td>
<td>10</td>
<td>16</td>
<td>0.99 (0.98–1.00)</td>
<td>0.91 (0.86–0.95)</td>
<td>11.5 (6.9–19.3)</td>
<td>0.01 (0.00–0.03)</td>
<td>0.99 (0.98–1.00)</td>
</tr>
<tr>
<td>Non-AIDS population</td>
<td>5</td>
<td>8</td>
<td>0.99 (0.98–1.00)</td>
<td>0.92 (0.91–1.00)</td>
<td>12.2 (9.6–15.6)</td>
<td>0.01 (0.00–0.02)</td>
<td>0.95 (0.93–0.97)</td>
</tr>
<tr>
<td>BALF PCR</td>
<td>8</td>
<td>11</td>
<td>1.00 (0.98–1.00)</td>
<td>0.88 (0.82–0.92)</td>
<td>8.0 (5.4–11.7)</td>
<td>0.01 (0.00–0.02)</td>
<td>1.00 (0.98–1.00)</td>
</tr>
<tr>
<td>OW PCR</td>
<td>4</td>
<td>5</td>
<td>0.76 (0.60–0.87)</td>
<td>0.93 (0.88–0.96)</td>
<td>10.4 (5.4–19.8)</td>
<td>0.26 (0.14–0.47)</td>
<td>0.94 (0.92–0.96)</td>
</tr>
</tbody>
</table>

a Shown are pooled values with 95% confidence intervals in parentheses.
b AUC, area under the summary receiver operating characteristic curve.
PCR assay of respiratory specimens is very powerful for ruling PCP in or out for at-risk individuals with pulmonary diseases, especially AIDS patients. To exclude Pneumocystis colonization, PCR results should be interpreted in parallel with clinical manifestations and radiological and laboratory findings.

We have no conflicts of interest to declare.

The work described here was performed at the First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People’s Republic of China.

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