Diagnosis of *Streptococcus pneumoniae* Infections in Adults with Bacteremia and Community-Acquired Pneumonia: Clinical Comparison of Pneumococcal PCR and Urinary Antigen Detection

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The diagnosis of severe *Streptococcus pneumoniae* infection relies heavily on insensitive culture techniques. To improve the usefulness of PCR assays, we developed a dual-PCR protocol (targeted at pneumolysin and autolysin) for EDTA blood samples. This was compared to the Binax NOW *S. pneumoniae* urinary antigen test in patients with bacteremic pneumococcal infections. Patients with nonbacteremic community-acquired pneumonia also were tested by these methods to determine what proportion could be confirmed as pneumococcal infections. A direct comparison was made in a group of patients who each had both tests performed. The Binax NOW *S. pneumoniae* urine antigen test was positive in 51 of 58 bacteremic pneumococcal cases (sensitivity, 88%; 95% confidence interval [CI], 77 to 95%), whereas the dual PCR was positive in 31 cases (sensitivity, 53.5%; 95% CI, 40 to 67%; P = 0.0001), and all of these had detectable urinary antigens. Both tests gave positive results in 2 of 51 control patients (referred to as other-organism septicemia), giving a specificity of 96% (95% CI, 86.5 to 99.5%). In 77 patients with nonbacteremic community-acquired pneumonia, urinary antigen was detected significantly more often (in 21 patients [27%]) than a positive result by the dual-PCR protocol (6 [8%]) (P = 0.002). The development of a dual-PCR protocol enhanced the sensitivity compared to that of the individual assays, but it is still significantly less sensitive than the Binax NOW urine antigen test, as well as being more time-consuming and expensive. Urinary antigen detection is the nonculture diagnostic method of choice for patients with possible severe pneumococcal infection.

Making a specific diagnosis of pneumococcal infection is a major challenge, and until recently it has relied heavily on culture methods. In recent years various nonculture tests, using nucleic acid amplification or antigen detection, have been developed for the diagnosis of pneumococcal infections.

A relatively new, rapid immunochromatographic test that detects C polysaccharide antigen in urine (Binax NOW *Streptococcus pneumoniae*; Binax, Inc., Portland, ME) has good sensitivity and is very specific in adult patients with invasive pneumococcal infection (4, 11, 16).

Several PCR methods for the detection of *Streptococcus pneumoniae* have been developed and evaluated. In general, when performed on sputum samples they have good sensitivity but poor specificity (12, 17). In order to overcome the latter problem, PCR assays have been evaluated on blood samples (5, 8, 14, 15), giving excellent specificity but relatively poor sensitivity. Consequently, in an attempt to improve sensitivity but maintain good specificity, we have devised a dual-PCR testing protocol that tests for two pneumococcal targets (*lytA* and *ply*) in duplicate samples.

In the study reported here, we directly compare the Binax NOW urinary antigen test to the dual-PCR testing protocol on EDTA blood samples for the diagnosis of serious *S. pneumoniae* infections (pneumonia and/or bacteremia). The tests then were applied to identifying *S. pneumoniae* as the cause of nonbacteremic community-acquired pneumonia.

**MATERIALS AND METHODS**

This study was approved by the South and West England Multicenter Research Ethics Committee. Informed consent was obtained from patients or relatives. Samples of blood and urine were obtained from adult patients (>16 years old) who had been admitted to the hospital between January 1999 and April 2002.

Patients formed three broad groups: those with pneumococcal bacteremia, mostly presenting with community-acquired pneumonia; those who presented with community-acquired pneumonia but had negative blood cultures; and control patients with other community-acquired septicemic infections without pneumo-
monia (or with pneumonia if it could be attributed clinically to the blood culture isolate [e.g., Streptococcus pyogenes]).

Pneumonia was defined as an acute illness with the presence of new or progressive infiltrates on chest radiograph, plus at least two of the following: fever, cough, dyspnea, or pleuritic chest pain. Blood culture-negative pneumonias were divided, according to chest radiograph results, the white blood cell (WBC) count, and the amount of C-reactive protein (CRP), into two subgroups.

<table>
<thead>
<tr>
<th>Initial results</th>
<th>PCR resulta</th>
<th>Internal process control</th>
<th>Further action</th>
<th>Result interpretationb</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>+/+</td>
<td>NA</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>+/−</td>
<td>+/+</td>
<td>NA</td>
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<td>+/−</td>
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<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>−/−</td>
<td>−/−</td>
<td>+</td>
<td>Repeat hytA PCR</td>
<td>See below</td>
</tr>
<tr>
<td>−/+</td>
<td>−/+</td>
<td>≥1 +</td>
<td>Repeat hytA and phy PCRs</td>
<td>See below</td>
</tr>
<tr>
<td>−/−</td>
<td>−/+</td>
<td>≥2 +</td>
<td>Repeat hytA and phy PCRs</td>
<td>See below</td>
</tr>
</tbody>
</table>

Combined results after repeated PCR tests

<table>
<thead>
<tr>
<th>PCR resultc</th>
<th>Internal process control</th>
<th>Further action</th>
<th>Result interpretationd</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 +</td>
<td>NA</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>≥2 +</td>
<td>−/−</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1 +</td>
<td>−/−</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>−/+</td>
<td>1 +</td>
<td>NA</td>
<td>Sample inhibitory</td>
</tr>
</tbody>
</table>

Proportions were compared by the chi-square test with Yates' correction or Fisher's exact test (Epi Info; Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

A total of 332 adult cases were used in this study, including 82 patients with pneumococcal bacteremia (23 without pneumonia, 59 with pneumonia), 149 patients with nonbacteremic community-acquired pneumonia, and 101 control patients (94 had other-organism sepsis and 7 had other-organism pneumonia). The median age (range) of these three groups was 63 (29 to 95), 67 (21 to 102), and 73 (18 to 97) years, respectively. These patients were tested by urine antigen detection and/or PCR.

Overall, pneumococcal urinary antigen was detected in 66 of 80 bacteremic pneumococcal cases, giving a test sensitivity of 82.5% (95% confidence interval [CI], 72.5 to 90%). The dual-PCR protocol was positive for 31 of 60 bacteremic pneumococcal cases, giving a test sensitivity of 52% (95% CI, 38.5 to 65%; P < 0.0001). These test results are shown in Table 2.

A subgroup of 186 patients were tested by both antigen and PCR methods, including 58 patients with pneumococcal bacteremia, 77 patients with nonbacteremic community-acquired pneumonia, and 51 control patients. In this subgroup for which a direct comparison could be made, the results were similar. Pneumococcal urinary antigen was detected in 51 of 58 bacteremic pneumococcal cases (sensitivity, 88%; 95% CI, 77 to 95%), whereas the dual-PCR protocol was positive for only 31 of 58 bacteremic pneumococcal cases (sensitivity, 53.5%; 95% CI, 40 to 67%; P < 0.0001). All PCR-positive cases had detectable urinary antigen. Both tests gave false-positive results in 2 of 51 control patients, giving a specificity of 96% (95% CI, 86.5 to 99.5%).

Both tests also were applied to identifying a cause for nonbacteremic community-acquired pneumonia. In 47 patients...
classified clinically as having likely pneumococcal pneumonia and who had both tests performed, urinary antigen was detected significantly more often than was a positive result by the dual-PCR protocol (15 [32%] cases and 4 [8.5%] cases, respectively [P = 0.005]). For 30 patients classified clinically as having possible pneumococcal pneumonia and who had both tests performed, urinary antigen also was detected more often than a positive result was achieved by the dual-PCR protocol (six [20%] cases and two [6.5%] cases, respectively), but this was not statistically significant (P = 0.13). Of the 21 PCR-positive cases, only 1 did not have detectable urinary antigen.

Similar results were seen in the larger groups of patients with nonbacteremic community-acquired pneumonia who had either antigen, PCR, or both tests performed (Table 2). However, in this larger group a slightly smaller proportion of patients with likely pneumococcal pneumonia had detectable urinary antigen.

Of the patients with a positive result by the PCR protocol, three patients with pneumococcal bacteremia, three patients with culture-negative pneumonia, and one control patient were positive for lytA alone. No patient in this study was positive for ply alone. There was no sample in which the autolysin PCR assay’s internal positive control indicated inhibition.

**DISCUSSION**

Many of the pneumococcal bacteremia patients and control patients used in this comparative study have been included in previous individual reports on urinary antigen detection (16), pneumolysin-targeted TaqMan PCR (14), or autolysin-targeted LightCycler PCR (15).

Several studies of the Binax NOW *S. pneumoniae* urine antigen test have demonstrated that it performs well in the diagnosis of bacteremic pneumococcal infections in adults. It has good sensitivity, ranging from 77 to 87% (1–4, 6, 9, 11, 16). Specificities of 97 to 100% are reported from studies using control groups with nonpneumococcal bacteremia or noninfectious disorders (1, 4, 11, 16). Briones et al. also included a control group of patients with chronic obstructive pulmonary disease or asthma and found that the specificity was lower (92%); the 12 patients with detectable antigen had exacerbations of chronic obstructive pulmonary disease and may have been colonized with *S. pneumoniae* (1).

PCR methods for the detection of *S. pneumoniae* DNA, when performed on sputum or other respiratory samples, have good rates of positivity (70 to 100%) but poor specificity (42 to 66%) (2, 12, 17). The latter problem may be overcome by performing PCR assays on blood samples, with several studies reporting excellent specificity (97 to 100%) but at the expense of relatively poor sensitivity (35 to 55%) (5, 8, 14, 15).

The development of our dual-PCR protocol, which was performed on blood, has increased test sensitivity (52%) in patients with bacteremic pneumococcal infections compared to that of the previous individual pneumolysin-targeted TaqMan assay (44%) or the autolysin-targeted LightCycler assay (43%). However, it still is significantly less sensitive than the Binax urinary antigen test (82%). The specificity of the dual-pneumococcal PCR protocol (96%) is similar to that of urinary antigen detection, and it also is similar to those of the individual PCR assays reported previously (14, 15).

In one study of pneumonia in adults, in 15 patients with definite pneumococcal pneumonia the apparent sensitivities of sputum PCR and urine antigen detection were comparable (approximately 75%), but the sputum PCR assays had poor specificity (2). Michelow et al. compared PCR on blood samples and urine antigen detection using the Binax NOW *S. pneumoniae* test in children with lower respiratory tract infection and, in the 12 children with culture-confirmed pneumococcal pneumonia, the two tests had comparable sensitivities (88 to 92%) (10). Our larger study of bacteremic adult patients gives a comparable sensitivity (82%) for the Binax NOW urine antigen test, but the sensitivity of the dual-PCR protocol (52%) is not as good. This could be due partly to the sample processing procedure used in the Michelow study, in which the blood samples were stabilized with guanidinium within 3 h of collection. The samples in our study were retrieved from hematology departments after blood counts had been completed and then stored (frozen) prior to processing.

Having evaluated the pneumococcal dual-PCR protocol in bacteremic patients and compared it to the Binax NOW urine antigen test, we directly compared the ability of both tests to give a specific etiological diagnosis for patients with nonbacteremic community-acquired pneumonia. These patients were categorized as having likely or possible pneumococcal pneumonia on the basis of the type of shadowing on the chest.

**TABLE 2. Comparison of results from pneumococcal urine antigen test and blood dual-PCR protocol in the overall study group**

<table>
<thead>
<tr>
<th>Patient category</th>
<th>Urine antigen test</th>
<th>Blood PCR protocol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested (n = 279)</td>
<td>No. (%) positive</td>
<td>No. tested (n = 239)</td>
</tr>
<tr>
<td>Nonpneumonic bacteremia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonbacteremic pneumonia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Likely pneumococcal</td>
<td>65</td>
<td>16 (24.5)</td>
<td>82</td>
</tr>
<tr>
<td>Possibly pneumococcal</td>
<td>35</td>
<td>7 (20)</td>
<td>44</td>
</tr>
<tr>
<td>Combined subtotal</td>
<td>100</td>
<td>23 (23)</td>
<td>126</td>
</tr>
<tr>
<td>Control</td>
<td>99</td>
<td>2 (2)</td>
<td>53</td>
</tr>
</tbody>
</table>

* The study group was comprised of 332 patients, of which 279 had antigen tests and 239 had PCR tests performed.
radiograph and laboratory markers of bacterial infection (WBC, neutrophils, and CRP). We did not use sputum Gram stains or culture results in our categorization, as they would not be available on admission for many patients. For 77 patients with likely or possible pneumococcal pneumonia who had both tests performed, the positivity of urine antigen (27%) was significantly greater than that of the dual-PCR protocol (8%). The dual-PCR protocol was positive in only one of the patients who did not have detectable urinary antigen, so it does not really contribute anything more to diagnosis. In our larger group of patients who had one or both tests performed (Table 2), the results were almost the same. Within the group of patients with nonbacteremic community-acquired pneumonia, our clinical distinction between the likely pneumococcal and possible pneumococcal groups does not indicate which types of patient are more likely to have a positive test result and so would not help to make the best use of these expensive diagnostic tests.

There have been several other diagnostic studies of community-acquired pneumonia in adults that include groups of patients for which the causative organism has not been definitively confirmed by the culture of blood or pleural fluid. Overall, immunochromatographic pneumococcal antigen detection in urine appears to be the most accurate predictor of pneumococcal infection. The Binax NOW S. pneumoniae urine antigen test is positive in 18 to 41% of such cases (1–4, 6, 7, 9, 11, 13).

Selection bias in the subgroups of nonbacteremic pneumonias makes it difficult to compare studies and to determine accurately what proportion of all community-acquired pneumonia is caused by the pneumococcus. For example, we did not collect all consecutive pneumonia patients admitted to our hospital units during the study period. In our study, selection was dependent on samples being taken within 24 h of starting antibiotic treatment, which was not always so in other studies.

Compared to PCR, even with a complex dual-target testing protocol, the Binax NOW S. pneumoniae urinary antigen test is a much better test for the early diagnosis of bacteremic pneumococcal infections in adults. The Binax NOW test also performs better in cases of nonbacteremic community-acquired pneumonia and will more accurately detect those cases caused by S. pneumoniae.

ACKNOWLEDGMENTS

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Other members of the South West Pneumococcus Study Group are Keith Cartwright, Rhonwen Morris, and James Stuart (Health Protection Agency South West, United Kingdom); Rob Heyderman, John Leeming, and Martin Williams (Health Protection Agency South West Regional Laboratory, Bristol, United Kingdom); and Adam Finn, Margaret Fletcher, and Caroline Trotter (University of Bristol, United Kingdom).

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