Vol. 41, No. 7

Rapid Diagnosis of Bacteremic Pneumococcal Infections in Adults by Using the Binax NOW *Streptococcus pneumoniae* Urinary Antigen Test: a Prospective, Controlled Clinical Evaluation

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The diagnosis of severe pneumococcal infections is inadequate, relying heavily on culture of *Streptococcus pneumoniae* from blood or other normally sterile fluids, and is severely limited by prior administration of antibiotics. We evaluated prospectively the Binax NOW *S. pneumoniae* urinary antigen test, a rapid immunochromatographic assay, for the diagnosis of bacteremic pneumococcal infections in hospitalized adult patients. Antigen was detected in 88 of 107 cases overall, resulting in a test sensitivity of 82% (95% confidence interval [95% CI], 74 to 89%). Antigen detection was greater in those with pneumonia (67 of 77 [87%]) than in those without pneumonia (21 of 30 [70%]) ($P = 0.04$). Urinary antigen was also detected in 3 of 106 adult patients with community-acquired septicemic infections caused by other organisms, giving a test specificity of 97% (95% CI, 92 to 99%). For 45 pneumococcal bacteremia patients with a positive test on treatment day 1, urinary antigen excretion was monitored for the first week of antibiotic treatment. Antigen was still detectable in 83% (29 of 35 tested; 95% CI, 66 to 93%) on treatment day 3. Detection of urinary antigen is a valuable, sensitive, and rapid test for the early diagnosis of bacteremic pneumococcal infections in adult patients, even after antibiotic treatment has commenced.

Invasive infections with *Streptococcus pneumoniae* are a major cause of morbidity and mortality worldwide. The incidence is highest in young children and the elderly but varies geographically (15). *S. pneumoniae* is the most common cause of community-acquired pneumonia, the second most frequent cause of bacterial meningitis, and a common cause of bacteremia in the United Kingdom (5). In the United States it is estimated that *S. pneumoniae* causes 50,000 cases of bacteremia and 3,000 cases of meningitis per year (2).

Epidemiological studies of severe pneumococcal infections rely heavily on culture of *S. pneumoniae* from blood or other normally sterile fluids. The sensitivity of blood culture is severely limited by prior administration of antibiotics. Apparent geographical variation in the incidence of invasive infection can be explained in part by variations in blood culture sampling rates (17) and possibly also by hospital referral patterns. These factors undermine studies of vaccine efficacy at a time when new pneumococcal conjugate vaccines are being introduced. Sensitive and specific nonculture methods of detecting pneumococcal infections are needed. Such tests will also be useful for diagnosis of an individual patient's infection so that appropriate antibiotics can be used.

Urinary antigen detection tests have proved to be very useful for diagnosis of other infections such as legionellosis (10). However, previous pneumococcal antigen tests using latex agglutination or counterimmunoelectrophoresis, including those that detect only individual serotypes, have not had sufficient sensitivity and specificity to gain wide acceptance as useful diagnostic techniques in routine clinical practice (3, 12, 13, 18).

Recently, an immunochromatographic test (Binax, Inc., Portland, Maine) has been developed to detect *S. pneumoniae* C polysaccharide antigen in urine. The present study was undertaken to evaluate prospectively the sensitivity and specificity of the Binax NOW test for the diagnosis of bacteremic pneumococcal infections in adult patients and to determine its utility following antibiotic treatment. The study formed part of a wider program designed to improve the diagnosis of pneumococcal infections prior to the evaluation of conjugated pneumococcal vaccines in England and Wales.

MATERIALS AND METHODS

This was a prospective, controlled clinical study conducted in South West England between July 1999 and February 2002. The study was approved by the South and West Multicentre Research Ethics Committee. Informed consent was obtained from patients or relatives. The study group comprised adult patients (>16 years), presenting with community-acquired pneumonia or another infection upon admission to hospital, from whose admission blood cultures *S. pneumoniae* was grown, as well as some patients with hospital-acquired bacteremic pneumococcal pneumonia. Controls were selected from adult patients presenting with other community-acquired septicemic infections without pneumonia, or with pneumonia if it could be attributed clinically to the blood culture isolate (e.g., group A streptococcus pneumonia). In each category, patients were included in the study only if a urine sample was obtained prior to, or within 24 h of, the start of appropriate antibiotic therapy (treatment day 1).

A proportion of patients with bacteremic pneumococcal infections had further urine samples collected after the start of antibiotic treatment, during the following week. The aim was to collect urine samples on treatment days 2, 3, 5, and 7, although this goal was not always achieved in full.

The Binax NOW *S. pneumoniae* urinary antigen test is an immunochromatographic assay that uses a rabbit anti-*S. pneumoniae* antibody, conjugated to visualizing particles, to bind any soluble pneumococcal antigen (C polysaccharide) present in the urine sample. The resulting complex is immobilized by a

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TABLE 1. Blood culture isolates of 106 control patients

Blood culture isolate	No. of patients
Escherichia coli	39
Klebsiella spp.	8
Enterobacter spp.	5
Other *Enterobacteriaceae*	5
Neisseria meningitidis	5
Staphylococcus aureus	16
Group A streptococcus	7
Group B streptococcus	4
Group G streptococcus	6
Enterococcus spp.	4
Other *Streptococcus* spp.	5
Other organisms	2

band of rabbit anti-*S. pneumoniae* antibodies adsorbed onto a nitrocellulose membrane (sample line). A second band of goat anti-rabbit immunoglobulin G (control line) captures excess visualizing complex. A swab is dipped into the urine and inserted into the test device; a buffer solution is added, and the device is closed. The result is read by eye, after 15 min. A pink to purple color on both the sample and control lines indicates a positive antigen test. Color on the control line alone indicates a negative test. Absence of color on the control line indicates an invalid test. Each box of tests includes positive- and negative-control swabs. In this study the test was performed according to the manufacturer's instructions, although results were also read after 1 h.

Normally distributed continuous data from cases and controls were compared by Student's *t* test or analysis of variance. Data not conforming to a normal distribution were analyzed by the Mann-Whitney or Wilcoxon two-sample test. Proportions were compared by the chi-square test with Yates' correction or by Fisher's exact test (Epi Info public-domain software; Centers for Disease Control and Prevention, Atlanta, Ga.).

RESULTS

A total of 107 cases (representing patients with pneumococcal bacteremia) and 106 controls were included in this study. The median ages of the patients and controls were 66 (range, 18 to 100) and 73 (range, 18 to 97) years, respectively. There was no significant difference between the two groups with respect to age, sex, or blood leukocyte and neutrophil counts upon admission. Patients with pneumococcal cases had higher C-reactive protein levels, but the difference was not significant. The pneumococcal bacteremia cases included 77 patients with pneumonia (67 community-acquired and 10 hospital-acquired cases) and 30 patients without pneumonia (24 with primary or nonfocal septicemia, 5 with meningitis, and 1 with peritonitis). The bacterial isolates and clinical presentations of the control bacteremia patients are shown in Tables 1 and 2, respectively.

Pneumococcal urinary antigen was detected, at 15 min, for

TABLE 2. Clinical presentations of 106 control patients

Clinical presentation	No. of patients
Nonfocal bacteremia	71
Urinary tract infection	10
Skin or soft tissue infection	8
Biliary tract infection	6
Infective endocarditis	4
Meningitis	2
Pneumonia[a]	2
Other focus	3

[a] One group A streptococcus infection and one *N. meningitidis* infection.

TABLE 3. Results of serial urine tests, following the start of antibiotic treatment, for patients with bacteremic pneumococcal infections and pneumococcal antigen detected in initial urine test

Treatment day	No. of samples tested	No. antigen positive	% Positive
1	45	45	100
2	32	27	84
3	35	29	83
4	8	6	
5	21	17	81
6	2	1	
7	20	18	90

88 of 107 pneumococcal cases and for 3 of 106 controls, giving a test sensitivity of 82% (95% CI, 74 to 89%) and a test specificity of 97% (95% CI, 92 to 99%) overall. However, the frequency of antigen detection was greater for pneumococcal pneumonia (67 of 77 [87%]) than for other pneumococcal infections without pneumonia (21 of 30 [70%]) (*P* = 0.04). There was no significant difference in test sensitivity between community-acquired and hospital-acquired pneumococcal infections (82 versus 80%).

Three control patients had "false-positive" results. One was an alcoholic with an *Escherichia coli* urinary infection and bacteremia. Although chest radiography was not performed, there was no clinical evidence of a chest infection. The second was an elderly lady with *Klebsiella pneumoniae* bacteremia and a normal chest radiograph. Neither patient had received a pneumococcal vaccine before admission. The third patient was an alcoholic with *Enterobacter* sp. bacteremia and a normal chest radiograph. She died 5 days later, and a postmortem examination revealed nodular cirrhosis and mildly edematous lungs with evidence of early pneumonic changes within both lower lobes.

When the urinary antigen test was read again after 1 h, the result did not change for any of the controls, but one of the 19 antigen-negative pneumococcal cases became antigen positive, resulting in a minimal increase in test sensitivity (to 83%).

Forty-five patients with bacteremic pneumococcal infections, for whom antigen was detected in the initial urine samples, had repeat samples taken during the week after the start of appropriate antibiotic treatment. One to four (median, 3) further samples were collected from each patient. These test results are shown in Table 3. Antigen was still detectable in 83% of samples (29 of 35 tested) (95% CI, 66 to 93%) on treatment day 3. Forty-two patients were tested on day 2 and/or day 3, and 37 of these (88%) were antigen positive. Detectable urinary antigen persisted for at least 7 days in many patients (18 of 20 patients followed up to, and tested on, day 7).

Follow-up urine samples (one to three for each patient) were also collected from eight patients with pneumococcal infections whose initial urine samples were antigen negative. One patient, also antigen negative on treatment day 2, became antigen positive on days 3 and 7. The remaining seven patients remained antigen negative.

DISCUSSION

This study has shown that detection of urinary pneumococcal antigen by using the Binax NOW *S. pneumoniae* antigen

test is an extremely useful technique for the rapid diagnosis of bacteremic pneumococcal infections in adults. Our evaluation of this test was limited to adults because several other recent studies have shown it to have very poor specificity for young children, particularly where there is a high rate of pneumococcal carriage. Dowell et al. found that the test result was no more likely to be positive among 88 children (age, ≤5 years) with radiographically confirmed pneumonia than among 198 controls (frequencies of positive results, 35 and 34%, respectively) (7). In a study of 210 healthy children, in several age groups of ≤5 years, Hamer et al. found that the test gave positive results for 11 to 21%, with test positivity and pneumococcal carriage decreasing with increasing age (11). Other investigators reported that 28 to 50% of children had positive antigen tests (1, 8). In all of these studies, a positive result was very much more likely if concurrent pneumococcal carriage was demonstrated by nasopharyngeal culture.

When the Binax NOW *S. pneumoniae* urinary antigen test has been evaluated for adults, the results have been good, although the reported numbers of patients with pneumococcal bacteremia have been low. Murdoch et al. studied 420 adults with community-acquired pneumonia, including 20 patients with pneumococcal bacteremia, 16 of whom (80%) had detectable urinary antigen levels (14). Dominguez et al. detected pneumococcal antigen in urine specimens of 82% of 28 patients (precise ages not given) with bacteremic pneumococcal pneumonia (6). In another study, 75% of 16 adults with bacteremic pneumococcal pneumonia had positive urinary antigen test results (9).

In our evaluation, pneumococcal urinary antigen was detected in 82% (88 of 107) of bacteremic pneumococcal cases overall, a finding in agreement with those of the other smaller studies. However, we found that antigen detection was significantly greater for those with pneumonia (87%) than for those without pneumonia (70%). This may well result from the greater total bacterial load associated with pneumonic infections.

In determining the test specificity, Murdoch et al. used 169 adult control patients with an admission diagnosis other than a respiratory or infectious disease and found that none had detectable pneumococcal antigen (specificity, 100%) (14). It is highly unlikely that the test would ever be used for this type of patient, although it is reassuring that there were no false positives that might have been related to pneumococcal carriage. In our study we deliberately selected clinically relevant controls, i.e., adult patients with nonpneumococcal community-acquired bacteremic infections. Urinary pneumococcal antigen was detected in 3 of 106 patients, giving a specificity of 97%. The study by Dominguez et al. also used control patients with pneumonia or bacteremia due to other organisms and also reported a test specificity of 97% (2 of 71 tests positive) (6).

Of the three false-positive results in our study, two occurred with patients who might be considered at high risk for pneumococcal infection. One alcoholic patient had an *E. coli* urinary tract infection and bacteremia. Although radiography was not performed, there was no clinical evidence of a chest infection. Another patient with alcoholic cirrhosis had *Enterobacter* sp. bacteremia. Although the admission chest radiograph was clear, she died 5 days later, and evidence of early pneumonia was found upon postmortem examination. The third patient

was an elderly lady admitted with *K. pneumoniae* bacteremia and a normal chest radiograph. The first and third patients had not received pneumococcal vaccine recently; the vaccine status of the second was unknown. None of these patients had nasopharyngeal swabs taken to detect pneumococcal carriage. It is possible that these patients had coinfection with *S. pneumoniae*.

Selection bias may possibly have affected our sensitivity and specificity results. We did not include all adult pneumococcal cases and other-organism controls admitted to the participating units during the period of this study. Selection depended on the submission of a urine sample taken prior to, or within 24 h of, the start of antibiotic treatment. Other factors included the availability of a member of the study group and obtaining consent to participate.

In an attempt to improve the sensitivity of the NOW *S. pneumoniae* urinary antigen test, we tried a slight modification to the manufacturer's protocol. This was to read the test after 1 h, which resulted in just one additional positive result in the pneumococcal bacteremia cases but no additional false positives among the controls. Murdoch et al. found that urine concentration resulted in a slight increase in the sensitivity of pneumococcal antigen detection without any deleterious effect on test specificity (14). It should be noted that neither of these protocol modifications is recommended by the manufacturer and both result in delay to an otherwise very rapid test.

Studies of other pneumococcal antigen tests for bacteremic patients, using latex agglutination or counterimmunoelectrophoresis, have revealed highly variable detection rates from 0 to 88% (3, 4, 12, 13), and test specificities have often been poorly defined. A recent serotype-specific tube latex agglutination assay has been evaluated thoroughly in adults; it was found to have a sensitivity of 57% for the 10 serotypes included and a specificity of 98% (16). Despite the excellent specificity, this assay does not perform as well as the Binax NOW test: it has much lower sensitivity and is more labor-intensive.

We also wanted to find out how long urinary pneumococcal antigen was detectable in order to determine how useful the NOW *S. pneumoniae* test might be after the commencement of appropriate antibiotic treatment. This was studied with 45 patients selected at random from those who were antigen positive on initial testing. Detectable urinary antigen was still present in 83% of those retested on treatment day 3 and persisted for at least 7 days in many patients. Cerosaletti et al. described similar posttreatment results obtained by using latex agglutination (4).

We were unable to collect samples from all patients in the case group at the designated times in our original study protocol (i.e., treatment days 2, 3, 5, and 7). Some patients with severe infections died, while some with mild infections were discharged, before day 7. However, nearly all patients had a urine sample tested on treatment day 2 and/or treatment day 3. Patients were less likely to have further samples taken after the test had become negative on one or two occasions. This resulted in lower numbers of samples being tested on treatment days 5 and 7 but a higher-than-expected proportion of antigen-positive samples. However, for the 45 cases that were initially antigen positive, if some of the missing data could be assumed (i.e., a missing sample between two positive tests is positive; missing samples between a positive and a negative test have no

result; and once a sample tests negative, further samples remain negative), 86% would have detectable antigen on day 3 and 73% would have detectable antigen on day 7 (data not shown). In clinical practice it is quite possible that patients may receive antibiotics, perhaps at home, for a day or two before the urinary antigen test is performed, although it is unlikely that a patient would be tested after this time.

The Binax NOW *S. pneumoniae* antigen test is a valuable and rapid tool for the early diagnosis of bacteremic pneumococcal infections in adults, even after appropriate antibiotic treatment has commenced. Further studies are needed to compare the diagnostic utility of this immunochromatographic urinary antigen test with alternative nonculture techniques in bacteremic and nonbacteremic pneumococcal infections.

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