

Evaluation of a Rapid Immunochromatographic Test for Detection of *Streptococcus pneumoniae* Antigen in Urine Samples from Adults with Community-Acquired Pneumonia

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Streptococcus pneumoniae is the most common cause of community-acquired pneumonia but is undoubtedly underdiagnosed. Isolation of *S. pneumoniae* from blood is specific but lacks sensitivity, while isolation of *S. pneumoniae* from sputum may represent colonization. We evaluated a new immunochromatographic test (NOW *S. pneumoniae* urinary antigen test; Binax, Portland, Maine) that is simple to perform and that can detect *S. pneumoniae* antigen in urine within 15 min. Urine samples from 420 adults with community-acquired pneumonia and 169 control patients who did not have pneumonia were tested. Urine from 315 (75%) of the pneumonia patients and all controls was tested both before and after 25-fold concentration, while the remaining 105 samples were only tested without concentration. *S. pneumoniae* urinary antigen tests were positive for 120 (29%) patients with pneumonia and for none of the controls. Of the urine samples tested with and without concentration, 96 were positive, of which 6 were positive only after concentration. *S. pneumoniae* antigen was detected in the urine from 16 of the 20 (80%) patients with blood cultures positive for *S. pneumoniae* and from 28 of the 54 (52%) patients with sputum cultures positive for *S. pneumoniae*. The absence of *S. pneumoniae* antigen in the urine from controls suggests that the specificity is high. Concentration of urine prior to testing resulted in a small increase in yield. The NOW *S. pneumoniae* urinary antigen test should be a useful adjunct to culture for determining the etiology of community-acquired pneumonia in adults.

Streptococcus pneumoniae has consistently been shown to be the most common cause of community-acquired pneumonia (CAP) in both adults and children. *S. pneumoniae* accounts for about two-thirds of cases where an etiologic diagnosis is made (12) and is likely to be the leading cause of pneumonia of otherwise unknown etiology (19). Despite being the single most important pathogen causing CAP, *S. pneumoniae* is undoubtedly underdiagnosed due to limitations of conventional diagnostic tests. Isolation of *S. pneumoniae* from blood lacks sensitivity, isolation of *S. pneumoniae* from sputum may represent colonization, and lung aspirates are rarely performed. In an effort to improve the diagnostic yield for patients with suspected pneumonia, there has been a considerable interest in alternative techniques, such as PCR and antigen detection.

Detection of *S. pneumoniae* antigens in the urine of patients with pneumonia was first described in 1917 (8). Over the intervening years the detection of *S. pneumoniae* antigens (usually capsular polysaccharides) in urine has been extensively studied using a variety of techniques, including counterimmunoelectrophoresis, latex agglutination, coagglutination, and enzyme-linked immunosorbent assay (1, 2, 5, 6, 14, 23). To date, the performance of these tests has been variable, such that they have never received general acceptance.

Recently, an immunochromatographic test, the NOW *S.*

pneumoniae urinary antigen test (Binax, Inc., Portland, Maine), has been developed; the test is simple to perform, detects the C polysaccharide cell wall antigen common to all *S. pneumoniae* strains (21), and provides results within 15 min. We evaluated this test using concentrated and unconcentrated urine samples from adults admitted to hospital with and without pneumonia.

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MATERIALS AND METHODS

Urine samples from 420 adults (age range, 18 to 95 years; median age, 68 years; 51% male) admitted to hospital with CAP were tested for pneumococcal antigen using the NOW *S. pneumoniae* urinary antigen test. The patients were recruited as part of a prospective study of all adult CAP admissions to two large tertiary hospitals (Christchurch Hospital, Christchurch, New Zealand, and Waikato Hospital, Hamilton, New Zealand) over a 1-year period. All patients had an acute illness with clinical features of pneumonia and radiographic pulmonary shadowing that was at least segmental or present in one lobe and was neither preexisting nor due to some other known cause. Patients were excluded when pneumonia was not the principal reason for admission or was an expected terminal event or when the pneumonia was associated with bronchial obstruction, bronchiectasis, or known tuberculosis. We also tested urine samples from 169 hospitalized patients who did not have pneumonia (age range, 20 to 91 years; median age, 69 years; 53% male) to serve as controls. These patients had been admitted to one of the study hospitals (Christchurch Hospital) at the same time as the patients with CAP, with whom they were matched for age and sex. Control patients were excluded if their presenting problem was a respiratory or infectious disease, although 6 of the 169 were identified as having an infection by the time of discharge (3 with cellulitis, 1 with diverticulitis, 1 with a foot abscess, and 1 with cholecystitis). The urine samples were collected soon after admission from both patients with pneumonia and controls.

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TABLE 1. Characteristics of the pneumonia patients with pneumococcal bacteremia

Urinary antigen test result	Patient no.	Age (yr)	Sex	Serotype of <i>S. pneumoniae</i> isolated from blood cultures	Time between onset of symptoms and urine collection (days)	Antibiotic therapy at time of urine collection ^a	Urine tested after concn	Sputum culture positive for <i>S. pneumoniae</i>
Positive	1	85	F	23	8	FLU	No	No
	2	85	M	22	2	AMC	No	NT ^b
	3	70	M	22	3	None	No	NT
	4	78	F	14	3	None	Yes	No
	5	76	M	14	4	AMX	Yes	No
	6	58	M	12	4	AMC	Yes	No
	7	40	M	9	3	AMX	Yes	NT
	8	87	F	4	6	AMC	Yes	NT
	9	48	M	1	7	None	No	Yes
	10	62	F	14	7	None	No	Yes
	11	88	F	9	3	None	Yes	NT
	12	88	F	9	4	None	Yes	No
	13	95	M	9	3	None	Yes	No
	14	65	F	3	6	None	Yes	No
	15	37	M	8	5	None	Yes	NT
	16	71	M	8	7	None	Yes	NT
Negative	17	86	F	22	14	None	Yes	No
	18	38	M	1	4	AMX	Yes	No
	19	28	M	1	3	AMX	No	No
	20	68	F	1	17	AMX	No	No

^a FLU, flucloxacillin; AMC, amoxicillin-clavulanic acid; AMX, amoxicillin.

^b NT, not tested.

Blood cultures were collected at the time of admission from the patients with pneumonia and processed using the BacT/Alert microbial detection system (Organon Teknika, Durham, N.C.). Sputum samples were examined by Gram stain microscopy for the presence of bacteria and the quantities of squamous epithelial cells and polymorphonuclear leukocytes and were cultured on sheep blood agar and chocolate agar.

Most antigen testing was performed at Duke University Medical Center after urine samples had been rapidly transported from New Zealand. On arrival in North Carolina, all samples remained in a completely or mostly frozen state. Urine samples were stored between -80 and -70°C in both New Zealand and North Carolina and were thawed immediately prior to testing. Samples from 315 pneumonia patients and all control patients were tested both before and after 25-fold concentration using Minicon-B15 concentrators (Millipore, Bedford, Mass.). An additional 105 samples were tested only without concentration. Testing was performed in a blinded fashion, without knowing whether samples were from cases or controls.

The NOW *S. pneumoniae* urinary antigen test consists of a hinged, book-shaped test device containing a nitrocellulose membrane on which the rabbit anti-*S. pneumoniae* antibody is adsorbed (the sample line). Goat anti-rabbit immunoglobulin G (IgG) is adsorbed onto the same membrane as a second stripe (control line). Rabbit anti-*S. pneumoniae* antibodies are conjugated to visualizing particles, which are dried onto an inert fibrous support. The test was performed according to the manufacturer's instructions. A swab was dipped into the urine sample and then inserted into the test device. A buffer solution was added, and the device was closed, bringing the sample into contact with the test strip. Pneumococcal antigen present in the urine sample binds to the anti-*S. pneumoniae*-conjugated antibodies, and the resulting antigen-antibody complexes are captured by immobilized anti-*S. pneumoniae* antibodies, forming the sample line. Immobilized goat anti-rabbit IgG captures excess visualizing conjugate, forming the control line. The test was read at 15 min and was interpreted by noting the presence or absence of visually detectable pink-to-purple lines. A positive test result was indicated by the detection of both sample and control lines. A negative test result was indicated by the detection of a control line only.

The Mann-Whitney U test was used to compare urine sample collection times from patients with positive and negative urinary antigen test results.

RESULTS

S. pneumoniae urinary antigen tests were positive for 120 (29%) patients with pneumonia and for none of the controls.

None of the tests were read as invalid due to the absence of a control band. Of the 484 urine samples tested with and without concentration, 96 were positive, of which 6 were positive only after concentration.

Of the 420 patients with pneumonia, 396 (94%) had blood cultures and 296 (70%) had sputum collected. Coincidentally, 9 of the 169 control patients had blood cultures collected, and all were negative. *S. pneumoniae* antigen was detected in the urine from 16 of the 20 patients with blood cultures positive for *S. pneumoniae*. Details of the patients with pneumococcal pneumonia and bacteremia are shown in Table 1. *S. pneumoniae* antigen was detected in the urine from 28 of the 54 patients with sputum cultures positive for *S. pneumoniae*. Of the 15 patients with pneumonia who had received a pneumococcal vaccine, 5 had positive *S. pneumoniae* urinary antigen tests. None of these patients had the vaccine administered within 5 days of testing; the manufacturer recommends that testing for *S. pneumoniae* urinary antigen not be performed within 5 days of receiving the pneumococcal vaccine because of the risk of false-positive results (product instructions, NOW *S. pneumoniae* urinary antigen test; Binax, Inc.).

Urine samples were collected a median of less than 1 day after admission (range, 0 to 3 days) from the patients with pneumonia and a median of 1 day after admission (range, 0 to 4 days) from controls. The median time from onset of symptoms to collection of urine was 5 days both for patients who had positive (range, 1 to 29 days; interquartile range, 3 to 7 days) and negative (range, 0 to 61 days; interquartile range, 3 to 9 days) *S. pneumoniae* urinary antigen test results (*P* value for the difference between distributions, 0.71). Of the patients with pneumonia, 76% were taking antibiotics at the time the urine samples were collected (79% of those with positive urinary antigen tests). Of the 120 patients with positive *S. pneu-*

moniae urinary antigen tests, 65 had no other etiological diagnoses, 33 had *S. pneumoniae* isolated from blood or sputum and had no evidence of infection with another pathogen, 14 had evidence of infection with only non-*S. pneumoniae* pathogens (*Haemophilus influenzae*, 3; respiratory syncytial virus, 3; *Legionella* spp., 2; *Chlamydia pneumoniae*, 2; parainfluenza virus, 2; *Staphylococcus aureus*, 1; influenza B virus, 1), and 8 had mixed infections with *S. pneumoniae* and other pathogens (*H. influenzae*, 3; respiratory syncytial virus, *Legionella* spp., influenza A virus, *Moraxella catarrhalis*, and *Mycoplasma pneumoniae*, 1 each).

DISCUSSION

The NOW *S. pneumoniae* urinary antigen test is easy to perform, provides results within a few minutes, and detects an antigen common to all *S. pneumoniae* strains (21). The manufacturer's own study confirmed that the test was able to detect 44 different strains of *S. pneumoniae*, representing the 23 serotypes responsible for at least 90% of pneumococcal infections (product instructions, NOW *S. pneumoniae* urinary antigen test; Binax, Inc.). Our findings indicate that the sensitivity of the test is 80% when positive blood cultures are used as the "gold standard." The absence of *S. pneumoniae* antigen in the urine from controls suggests that the specificity is high.

These findings are similar to those of other investigators who have used the NOW *S. pneumoniae* urinary antigen test. Domínguez et al. (9) tested urine samples from patients with pneumococcal pneumonia, and detected *S. pneumoniae* antigen in 23 of 28 bacteremic patients (82%) and in 18 of 23 nonbacteremic patients (78%). The specificity was 97%, based on two positive results among 71 patients with documented infections caused by microorganisms other than *S. pneumoniae*. Yu et al. detected *S. pneumoniae* antigen in the urine from 86% of bacteremic patients (V. L. Yu, J. A. Kellog, J. F. Plouff, J. A. Coladonato, J. Manzella, W. Alves Dos Santos, R. B. Kohler, A. Torres, T. M. File, and J. D. Rihs, Abstr. 38th IDSA Annu. Meet., abstr. 262, 2000). In another study, *S. pneumoniae* antigen was detected in urine from 24 of 45 patients with CAP and the diagnostic yield for pneumococcal pneumonia was increased by 60% (15).

In contrast, the performance of other methods for detection of *S. pneumoniae* antigen in urine has been inconsistent, with sensitivities ranging from 0 to >80%, usually <50% (2, 3, 6, 7, 14, 16, 18, 20). The improved sensitivity of the NOW *S. pneumoniae* urinary antigen test may be due, in part, to the detection of the cell wall C polysaccharide common to all *S. pneumoniae* strains, rather than type-specific capsular polysaccharides. This has been demonstrated in at least one comparative study using latex agglutination assays, where the C polysaccharide was detected in the urine of 23 of 33 patients with pneumococcal bacteremia, while type-specific polysaccharides were detected in only 17 of these patients (3).

The NOW *S. pneumoniae* urinary antigen test may be less useful for detecting pneumococcal pneumonia in children because of the high false-positive rate due to nasopharyngeal colonization with *S. pneumoniae*. In one series, the test was no more likely to be positive among 88 children with pneumonia than among 198 control subjects but was significantly more likely to be positive among children who were nasopharyngeal

carriers of *S. pneumoniae* (10). While we did not test for nasopharyngeal carriage in the present study, the absence of positive results in our control group suggests this may not be an issue in adults. These findings may reflect the lower rates of pneumococcal colonization in adults than in children.

It is unclear why *S. pneumoniae* antigen was not detected in urine from four patients with pneumococcal bacteremia. Although two of these patients had urine collected many days after the onset of symptoms, *S. pneumoniae* was isolated from blood cultures within 2 days of urine collection in both cases, suggesting recent or concurrent antigenemia. Three of the 4 bacteremic patients with negative urinary antigen tests were taking antibiotics at the time of urine collection, compared with 6 of the 16 with positive tests. However, the numbers are too small to determine whether this trend is a true association. It is possible that the relative dilutions of the urine samples may affect test results and that pneumococcal antigen was present in very low concentrations in the four negative samples. Unfortunately, we were unable to test concentrated urine from two of the four patients because of insufficient volume. The relatively low urinary antigen positivity rate among patients with sputum cultures positive for *S. pneumoniae* may partly reflect the inherent problems of interpreting sputum cultures. It is difficult to be certain that *S. pneumoniae* isolated from sputum represents infection rather than colonization, and, although we cytologically screened all samples, it is likely that some of our positive samples were due to contamination with oropharyngeal flora.

Concentrating urine samples before antigen testing is widely practiced, but few studies have determined the increase in yield by testing samples both before and after concentration. Twentyfold concentration of urine resulted in a 1.6-fold increase in yield of positive *S. pneumoniae* antigen results (from 14 to 22%) using counterimmunoelectrophoresis (13). Using the NOW *S. pneumoniae* urinary antigen test, Marcos et al. noted a 1.4-fold increase in yield following concentration of urine (from 38 to 53%) (15), which is at variance with the small increase in sensitivity that we documented (from 29 to 30%). They did not record the method they used for concentrating urine, but it is unlikely that using a concentrator with a lower-molecular-mass cutoff would increase sensitivity. The molecular mass of the C polysaccharide is 20 to 30 kDa (22), whereas the Minicon-B15 concentrator has a molecular mass cutoff of 15 kDa (product instructions, Minicon and Miniplus clinical sample concentrators; Millipore). While the identification of these additional cases may be important, the small increase in yield in our study would not justify routine concentration of samples by diagnostic laboratories, especially given the substantial increase in costs that this would entail.

Although new tests for diagnosing pneumococcal disease have traditionally been compared with blood cultures, bacteremia was documented in only 20 to 30% of patients with pneumococcal pneumonia (4, 11, 17). In the present study, of the pneumonia patients with positive urinary antigen tests who had blood cultures collected, only 18% were bacteremic. The NOW *S. pneumoniae* urinary antigen test will be especially useful for identifying the large number of patients with nonbacteremic pneumococcal pneumonia and for rapidly identifying a group of patients in whom narrow-spectrum antibiotics may be used.

We conclude that the NOW *S. pneumoniae* urinary antigen test is a useful adjunct to culture for determining the etiology of CAP in adults. Further research should focus on the time course of urinary antigen positivity and the use of this test in settings other than adult pneumonia.

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