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Use of the NOW *Streptococcus pneumoniae* urinary antigen test in cerebrospinal fluid for rapid diagnosis of pneumococcal meningitis

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Abstract

Streptococcus pneumoniae is one of the most common pathogens in bacterial meningitis. Rapid diagnosis is critical for effective treatment. The aim of this study was to assess the accuracy of the NOW *S. pneumoniae* Urinary Antigen Test, (Binax, Portland, ME, USA) originally developed for urine testing, in detecting the *S. pneumoniae* antigen in cerebrospinal fluid (CSF). The study included 519 patients with suspected meningitis. CSF, blood and urine samples were cultured according to standard methods. CSF viral culture was also performed. CSF and urine specimens were tested for pneumococcal antigen with the NOW *S. pneumoniae* test.

S. pneumoniae was isolated from the CSF of 22 patients. The direct antigen test was positive in CSF in 21/22 patients (95.4% sensitivity), and in urine, in 12/21 (57.1% sensitivity). Direct CSF smear was positive in 15/22 (68% sensitivity). CSF samples that cultured negative for *S. pneumoniae* ($n = 470$) or positive for other bacteria ($n = 27$) were also negative on the NOW test (100% specificity). By contrast, urine samples of 63/470 of patients with negative CSF culture were positive on the NOW test, as were 5/27 urine samples of patients with CSF culture positive for other bacteria ($p = 0.45$).

The NOW *S. pneumoniae* antigen test in CSF yields a rapid and very reliable diagnosis of pneumococcal meningitis, enabling prompt and adequate treatment. Its low sensitivity in urine indicates that this mode of testing is not useful for the diagnosis of pneumococcal meningitis.

These data have been included in the FDA application for approval of the NOW test for use in the CSF for the diagnosis of pneumococcal meningitis.

1. Introduction

Streptococcus pneumoniae is currently the leading cause of bacterial meningitis in children and adults. The estimated mortality of pneumococcal meningitis is approximately 20%, higher than for any other meningitis of bacterial etiology (Aronin & Quagliarello, 2001).

Empiric treatment before culture results are obtained is complicated by the increasing bacterial resistance to penicillin and third generation cephalosporins (Paris et al., 1995) and should be guided by Gram staining and/or bacterial antigen tests in the cerebrospinal fluid (CSF). However, with Gram stain, the microorganism is likely to be seen and recognized in only about 75% of specimens from which it is eventually cultured. The sensitivity is decreased further to 50% in patients who have been given antimicrobial therapy (Tunkel & Scheld, 1995). Additionally, the sensitivities of current bacterial antigen tests in CSF samples vary from 50% to 100%, depending on the commercial assays used and the organisms studied (Camargos et al., 1995; Gray & Fedorko, 1992). Polymerase chain reaction (PCR) techniques for detecting pneumococcal antigen in CSF (Cherian et al., 1998; Kearns et al., 2002), though considered very sensitive, cannot be used for rapid diagnosis of sporadic cases and are not available in many countries. This has created an urgent need for a rapid and reliable laboratory method for the diagnosis of pneumococcal meningitis.

Researchers have recently developed a new immunochromato-graphic membrane assay, the NOW *S. pneumoniae* Urinary Antigen Test (Binax, Potland, ME, USA) (Dominiguex et al., 2001), which detects the C polysaccharide cell wall antigen common to all *S. pneumoniae* sero-

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types. The test is simple to perform and provides results within 15 min. It was approved by the US Food and Drug Administration (FDA) in 1999 for the diagnosis of pneumococcal pneumonia.

Several studies have investigated the value of the NOW test for detecting the *S. pneumoniae* antigen in urine specimens of patients with community acquired pneumonia (Burel et al., 2001; Dowell et al., 2001; Murdoch et al., 2001). One study, in which 8 of 25 patients with meninigitis were positive for *S. pneumoniae,* reported a 100% correlation between the results of the NOW test in urine and CSF in this subgroup (Marcos et al., 2001).

The aim of the present study was to assess the accuracy of the NOW test, originally developed for urine testing, in detecting the *S. pneumoniae* antigen in the CSF, in a large group of patients with suspected meningitis.

2. Materials and methods

The study was conducted at the Rabin Medical Center (900 beds) and the Schneider Children's Medical Center of Israel (250 beds), both major university-affiliated, tertiary-care facilities in central Israel. The study included 519 patients (218 female, 301 male, aged 1 day to 85 years) with suspected meningitis, admitted from January 2000 to February 2001. CSF and blood samples were taken concomitantly.

Direct CSF smear was done from the sediment after centrifugation at 2500 × g for 15 min (Reisner et al., 1999), and cultured for bacterial and viral pathogens according to standard methods. The blood culture was processed with the Bactec 9240 microbial detection system (Becton Dickinson, USA). Urine specimens were collected during the next 10 h and cultured for bacterial pathogens. The CSF and urine specimens were tested for the pneumococcal antigen with the NOW *S. pneumoniae* Urinary Antigen Test (Bimax, Portland, USA), in accordance with the instructions of the manufacturer. The test consists of a hinged, book-shaped device containing a nitrocellulose membrane on which rabbit anti-*S. pneumoniae* antibody is absorbed (sample line). Goat anti-rabbit immunoglobulin G (IgG) is absorbed onto the same membrane as a second strip (control line). Rabbit anti-*S. pneumoniae* antibodies are conjugated to visualized particles which are dried onto an inert fibrous support. A swab is dipped into the tested sample and then inserted into the test device. A buffer solution is added and the device closed, bringing the sample into contact with the test strip. Pneumococcal antigen present in the sample binds to the anti-*S. pneumoniae* conjugated antibodies, and the resulting antigen-antibody complex is captured by the immobilized anti-*S. pneumoniae* antibodies, forming the sample line. The immobilized goat anti-rabbit IgG captures excess visualizing conjugate, forming the control line. The result is read at 15 min and is interpreted by the presence or absence of visually detected pink to purple lines. The test is considered positive if both control and sample lines are detected, and negative if only the control line is detected. χ^2 test was used for statistical analysis.

Table 1
Results of CSF bacterial culture and NOW *S. pneumoniae* antigen test in CSF and urine specimens of 519 patients with suspected meningitis

CSF culture	Direct Antigen Test		No. of patients
Group	CSF	Urine	
I. Positive for *S. pneumoniae* (n = 22)	Positive	Positive	12
	Positive	Negative	8
	Positive	NT*	1
	Negative	Negative	1
II. Positive for other bacteria (n=27)	Negative	Negative	22
	Negative	Positive	5
III. Negative	Negative	Negative	407
	Negative	Positive	63
Total			519

* NT — not tested (dialysis patient)

Group I: Sensitivity of the NOW, 95.4% in CSF, 57.1% in urine; specificity in CSF, 100%.

3. Results

The results of the bacterial culture and the direct antigen test are given in Table 1. *S. pneumoniae* was isolated from the CSF of 22 patients (group I), and other bacteria were isolated from the CSF of 27 patients (group II). The remaining 470 CSF samples tested negative on culture (group III); in 21 of them, Enteroviruses were isolated.

In group I, the *S. pneumoniae* antigen test in the CSF was positive in 21 patients and negative in one. In the latter patient, only 2 colonies of *S. pneumoniae* were detected on blood agar, and 1 colony on chocolate agar after 48 h. On brain-heart infusion broth, growth was observed after incubation for 24 h. Urine specimens were obtained from 21 patients (the 22nd was on dialysis), and 12 (57.1%) were positive on the direct antigen test. All 21 urine specimens were negative on bacterial culture.

In group II, the other microorganisms isolated from the CSF included *Neisseria meningitis* (2), *Hemophilus influenza* (3), *Listeria monocytogenes* (1), *Streptococcus* group B (2) *Klebsiella pneumoniae* (2), *Escherichia coli* (2), *Acinetobacter baumannii* (2), *Enterobacter* (1), *Morganella morganii* (1), *Staphylococcus coagulase* positive (3), *Staphylococcus coagulase* negative (2), *Streptococcus viridans* (4), *Enterococcus* (1), *Cryptococcus neoformens* (1). The CSF direct antigen test was negative in all 27 patients, and the urine antigen test was positive in 5 (18.5%).

In group III, the CSF direct antigen test was negative in all 470 specimens, whereas the urine test was positive in 63 (13.4%). No significant difference was found in the prevalence of *S. pneumoniae* antigen in urine between groups II and III ($p = 0.45$). The 21 CSF specimens that cultured positive for Enterovirus were negative on the direct antigen test.

Table 2
Characteristics and laboratory results of the 22 patients with CSF culture-positive for *Streptococcus pneumoniae*

Patient No.	Sex	Age	CSF direct smear	Serotype of *S. pneumoniae*	NOW CSF	Test Urine	Blood culture	Antibiotic therapy at time of CSF collection	Time between onset of symptoms and CSF collection
1	M	57 y	Positive	19 F	Pos	Neg	*S. pneumoniae*	No	2 days
2	M	7 m	Positive	6 B	Pos	Pos	Neg	No	3 days
3	M	3 y	Positive	23 F	Pos	Pos	*S. pneumoniae*	No	1 day
4	F	85 y	Negative	8	Pos	Pos	*S. pneumoniae*	Augmentin (3 days)	2 days
5	M	14 m	Positive	19 F	Pos	Pos	*S. pneumoniae*	No	1 day
6	F	18 y	Positive	10	Pos	Neg	Negative	No	1 day
7	M	14 m	Negative	19 F	Pos	Pos	Negative	No	5 days
8	F	77 y	Positive	13	Pos	Pos	Negative	Ciprofloxacin (1 day)	1 day
9	M	41 y	Negative	19 F	Pos	Neg	Negative	No	1 day
10	M	6½ y	Negative	23 A	Neg	Neg	Negative	No	1 day
11	M	7½ m	Positive	14	Pos	Pos	Negative	No	1 day
12	M	46 y	Negative	NT*	Pos	Neg	Negative	No	1 day
13	F	69 y	Positive	7 F	Pos	Pos	Negative	No	1 day
14	M	62 y	Positive	23 B	Pos	Pos	*S. pneumoniae*	No	1 day
15	F	61 y	Negative	NT	Pos	Neg	Negative	Rocephin (1 day)	2 days
16	F	69 y	Positive	10	Pos	DP*	*S. pneumoniae*	No	1 day
17	M	45 y	Positive	8	Pos	Neg	Negative	No	1 day
18	F	6 m	Positive	6 B	Pos	Neg	Negative	No	2 days
19	F	4 y	Negative	5	Pos	Pos	Negative	No	1 day
20	M	2 m	Positive	2	Pos	Pos	Negative	No	1 day
21	M	3 m	Positive	14	Pos	Neg	Negative	No	1 day
22	F	5 m	Positive	9	Pos	Pos	*S. pneumoniae*	Rocephin (1 day)	2 days

* NT – not typable, DP – dialysis patient.

The characteristics of the 22 patients with pneumococcal meningitis are given in Table 2. The sensitivity of the NOW test in this subgroup was 95.4% in CSF and 57.1% in urine. Specificity was 100% in CSF. Direct smears were found to be positive in 15 patients (68.2% sensitivity). *S. pneumoniae* was isolated from blood cultures of 7 patients.

4. Discussion

The morbidity and mortality of pneumococcal meningitis remain unacceptably high (Lu et al., 2001; Schuchat et al., 1997), while the rate of antibiotic resistance of the pneumococcal strains has risen dramatically (Mufson, 1998; Shaloul et al., 2000; Tunkel & Scheld, 2002). Rapid and accurate diagnosis of pneumococcal meningitis is critical for adequate initial empiric treatment.

The sensitivity of the Gram stain in this study was 72.2% in untreated patients (13/18) and 50% in previously treated patients (2/4). The Gram stain is generally accepted to be most reliable for detecting $\geq 10^5$ bacteria per ml of body fluid. This has been demonstrated for CSF by La Scolea & Dryja (1984) who showed that 25%, 60% and 97% of CSF specimens with $<10^3$, 10^3-10^4 and $\geq 10^5$ bacteria/ml, respectively, were positive by Gram stain. These findings might explain the sensitivity of 68.2% observed in our study. In addition, the small group of our positive patients should be taken into consideration.

Initial studies of the NOW direct antigen test, which was originally developed for urine testing, found it to be a valuable tool for the diagnosis of pneumococcal pneumonia (Burel et al., 2001; Dominguex et al., 2001; Murdoch et al., 2001). However, Dowell et al. (2001) reported that it failed to distinguish patients with pneumonia from controls without pneumonia and was not entirely useful for distinguishing patients with pneumococcal pneumonia from those with pneumococcal colonization. In a recent study (Hamer et al., 2002) of healthy children with and without nasopharyngeal carriage of *S. pneumoniae*, the NOW antigen test yielded positive results in both groups. This finding might explain the positive findings in the present study on the direct antigen test in 63 out of the 470 culture-negative specimens and in 5 of the 27 culture-positive specimens for other bacteria.

Positive results in urine may also occur in patients with invasive pneumococcal infections. Therefore, the CSF is the preferred mode of testing, because the diagnosis of meningitis must always be confirmed in CSF samples.

Only one study of the NOW test has been performed in CSF samples from patients with meningitis (Marcos et al.,

2001), and they reported that sensitivity and specificity were 100%. However, these results should be regarded with caution because of the small sample size.

The present study was conducted in a much larger sample of 519 patients, and the results were more conclusive. We found that the NOW *S. pneumoniae* antigen test in the CSF provides a rapid and very reliable diagnosis of *S. pneumoniae* meningitis (95.4% sensitivity, 100% specificity), allowing prompt and adequate treatment. The low sensitivity of the test in urine (57.1%) indicates, that this mode of testing is not useful for the diagnosis of pneumococcal meningitis.

These data have been included as part of the US FDA approval for the CSF application of the NOW test.

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