

RAPID DIAGNOSIS OF PNEUMOCOCCAL MENINGITIS AND PNEUMONIA WITH SYNPNEMONIC EFFUSION USING A NOVEL STREPTOCOCCUS PNEUMONIAE IMMUNOCHROMATOGRAPHIC ASSAY

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ABSTRACT: CONTEXT: Streptococcus pneumoniae (*S. pneumoniae*) infections are an important cause of mortality in young children. There are a number of problems associated with establishing the microbial etiology by conventional methods, thus highlighting the need for a rapid, improved and accurate test method. A rapid immunochromatographic assay has been developed for detecting a pneumococcal cell wall antigen common to all 92 serotypes. **AIM:** The aim of the study was to ascertain whether the Binax NOW test when performed on Cerebrospinal fluid (CSF) and pleural fluid can give any additional information vis a vis the culture and other methods of identification of *S. pneumoniae*. **MATERIAL & METHODS:** CSF and pleural fluid samples were collected from suspected cases of both meningitis and/or pneumonia with or without synpneumonic effusions in the age group of 28 days to 60 months. The samples were subjected to Gram's stain, culture and sensitivity, CRP, cell count, cell type, protein and sugar tests. In addition, the immunochromatographic test (ICT) (Binax NOW, Scarborough, ME) was performed on all samples to detect *S. pneumoniae* polysaccharide antigen. Polymerase chain reaction (PCR) was done in patients who had a positive ICT in the pleural fluid. **RESULTS:** A total of 104 children were recruited, of whom 90 were cases of suspected meningitis and 14 of pneumonia with synpneumonic effusions. The ICT result was positive in 19 patients, in 10 of 90 CSF and 9 of 14 pleural fluid samples. Culture for *S. pneumoniae* was positive in 6 cases (9 specimens). When ICT was compared to culture, the sensitivity of ICT was 100% (6/6) and the specificity was 85.97 (85/95). *Streptococcus mitis* (one), *Staphylococcus epidermidis* (one), *Pseudomonas* (one) isolated in culture gave false positive ICT results. Previous antibiotic exposure did not seem to alter the sensitivity of the test.

CONCLUSION: To the best of our knowledge this is the first Indian study on Binax NOW test on CSF and pleural fluid. The ICT test performed on CSF and pleural fluid samples not only augments the standard diagnostic methods of blood and fluid cultures, but is useful even in patients with prior antibiotic therapy.

KEYWORDS: Immunochromatography, *S. pneumoniae*, Pneumococcal meningitis, Pleural effusion, CSF.

INTRODUCTION: *Streptococcus pneumoniae* (*S. pneumoniae*) infections are a common cause of hospital admissions and mortality of infectious origin. Some common modes of presentations are community acquired pneumonia with or without synpneumonic effusion, bacterial meningitis, otitis media and septic arthritis.

There are a number of problems associated with demonstrating the microbial etiology of the infectious process by conventional methods. For instance, only one third of the patients produce sputum suitable for culture, and the results lack specificity due to nasopharyngeal carriage of Pneumococci in healthy individuals. ^(1, 2, 3) Although blood cultures are specific, they have low positivity rates of less than 10%. Pneumonia cases are associated with pleural effusion ⁽⁴⁾ and a pathogen is recovered in less than half of those patients who undergo diagnostic pleural tap. ⁽⁵⁾ Finally many patients are pretreated with an antibiotic which decreases the diagnostic yield by culture. ⁽⁶⁾

The need for improved speed and accuracy in etiologic diagnosis of *Streptococcus pneumoniae* infection has led to the development of a rapid urinary assay for detecting pneumococcal cell wall components common to all 92 serotypes namely the NOW assay, (BINAX; Scarborough, ME). ⁽⁷⁾ It has also been validated with cerebrospinal and pleural fluid. ⁽⁸⁾ The Binax NOW *S. pneumoniae* test is an in vitro rapid immuno chromatographic assay for the detection of *S. pneumoniae* antigen in the urine of adult patients with pneumonia and in the cerebrospinal fluid (CSF) and pleural fluid of patients of all ages, with meningitis and pleural effusion. In conjunction with culture and other methods, it is intended to aid in the diagnosis of both pneumococcal pneumonia and pneumococcal meningitis.

The aim of the study was to ascertain the usefulness of Binax NOW test when performed on CSF and pleural fluid, and provide information on the findings of semi-automated culture for the identification of *S. pneumoniae*.

METHODOLOGY: The study was carried out by the Department of Microbiology, over a period of 24 months from February 2009 to January 2011. Paediatric patients in the age group of 28 days to 60 months, presenting to the department of paediatrics of three different teaching hospitals, with synpneumonic effusions and suspected bacterial meningitis were prospectively included in the study. A detailed history and consent was obtained from the parent accompanying the child. Clearance was obtained from the institutional Ethics Committee before the start of the study.

In patients with synpneumonic effusions, thoracentesis was performed and pleural fluid was taken for estimation of protein, sugar, cell count, cell type, C - reactive protein (CRP), Gram's stain and culture and antimicrobial susceptibility. In patients with meningitis lumbar puncture was done and CSF used for estimation of the same parameters as with pleural fluid. Both pleural fluid and CSF were stored at - 70° C for pneumococcal antigen detection. Pleural fluid samples with positive ICT were also subjected to Polymerase chain reaction (PCR). Cultures were performed using semi-automated BACTEC 9050 from BD, while identification and sensitivity (Minimum Inhibitory Concentration- MIC) was done using automated walkaway 40 system from Siemens.

Streptococcus pneumoniae antigen detection in pleural fluid and CSF was performed using the Binax NOW® for *S. pneumoniae*, Scarborough, ME, USA. The frozen samples were thawed to room temperature. In Binax NOW® *Streptococcus pneumoniae* test ⁽²⁾, rabbit anti-pneumococcal antibody is adsorbed onto a nitro cellulose membrane (the sample line), and goat anti-rabbit IgG is adsorbed onto the same membrane as a second stripe (the control line). A

second set of rabbit anti pneumococcal antibodies are conjugated to gold particles and dried onto an inert fibrous support. A swab is dipped into the specimen (CSF/ pleural fluid) and inserted into the test device and a citrate buffer is added to facilitate antigen flow and the device is closed. If pneumococcal antigen is present in the specimen, it binds to the gold- conjugated rabbit antibodies and the resulting complex is captured by the immobilised rabbit IgG stripe, forming the sample line. In addition, immobilized goat anti-rabbit IgG captures excess conjugated rabbit antibody, forming the control line. Results are read visually (pink line) after 15 minutes, with the appearance of the control line only signifying a negative test and the appearance of both the control and sample lines signifying a positive test (Figure 1).

RESULTS: The study population included 104 paediatric patients (62 males and 42 females, aged between 28 days and 60 months) with suspicion of bacterial meningitis and bacterial pneumonia with pleural effusion. Diagnosis was based on clinical signs and symptoms, laboratory investigations (increased WBC count, elevated CRP levels in both blood and CSF /pleural fluids, cytology and biochemical tests of the fluids and Gram's stain of the fluid) and radiological diagnosis in case of pneumonia. There were 90 patients with suspected bacterial meningitis and 14 with bacterial pneumonia with pleural effusion. The ICT result was positive in 19 of 104 samples. Of the 90 CSF samples 10 were Binax- NOW positive and of the 14 pleural fluid samples, 9 were positive. The distribution of the different test results are shown in Table 1. Using culture and ICT alone - Of the total 19 cases which were positive, 6 patients were culture positive for *S. pneumoniae* either in blood or body fluid or both. Among these 6 patients, three patients grew *S. pneumoniae* both in the blood and body fluid. Of the 13 patients in whom *S. pneumoniae* was negative in culture, 3 patients were positive for non - pneumococcal organisms (*Streptococcus mitis*-1, *Staphylococcus epidermidis* -1 and *Pseudomonas spp* -1), of the remaining 10 patients , no organisms were grown either in the blood or body fluid. Of the 85 patients in whom the ICT was negative, none of these patients had *S. pneumoniae* grown either in blood, CSF or pleural fluid.

Using Gram's stain and ICT alone - of the 19 cases which were ICT positive, 9 patients showed pus cells and Gram positive cocci in pairs and short chains in Gram's smear of the fluid, while the 85 patients with ICT negative results showed neither pus cells nor any organisms. Of the 104 patients, 18 were on antibiotic treatment before hospital admission. 6 of them had a positive ICT result (33%), while of the remaining 86, who were not on prior antibiotic treatment, 13 had a positive ICT result (15%). There were 4 patients who had received antibiotic and were culture negative but positive for Binax NOW ICT assay.

DISCUSSION: The detection of pneumococcal antigen in cerebrospinal fluid,⁽¹⁰⁾ sputum⁽¹¹⁾ and broncho-alveolar lavage (BAL) fluid⁽¹²⁾ specimens as a method in diagnosis of pneumococcal infection has been previously documented. However, it is not performed commonly in pleural fluid samples^(13, 14, 8). Earlier studies have used a variety of techniques, such as counter immuno electrophoreses, agglutination tests and enzyme immunoassays to identify *S. pneumoniae* polysaccharide capsular antigen in pleural fluid samples.^(15,16,17) But these methods are laborious and time-consuming, when compared to the NOW method used in this study, which is a rapid and simple ICT test with a reported sensitivity of 50 to 80% and a high specificity for the detection of *S. pneumoniae* antigen up to 1 month after pneumonia onset with results available in just 15 minutes.^(18,19,20)

ORIGINAL ARTICLE

As is evident from this study, the use of ICT led to greater increases in pneumococcal positivity yields, compared to culture alone, in patients with prior antimicrobial treatment than in those without. Patients who had received antibiotics prior to the lumbar puncture / pleural tap were less likely to have viable organisms in their CSF/ pleural fluid for isolation on culture, thus enhancing the value of non culture based tools such as the Binax ICT in these patients.

Overall when compared to culture, the sensitivity of ICT in the present study was 100% (6/6) and specificity was 89.5% (85/95).

In our study, the ICT results on pleural fluid samples confirms its excellent sensitivity of 100% (2 of 2) and moderate specificity of 58% (7 of 12) for the diagnosis of pneumococcal pneumonia. The addition of the pleural ICT test allowed the detection in 9/14 (64%) cases of pneumococcal pneumonia as compared to pleural culture alone 2/14 (14%). All the 9 pleural fluid samples which were ICT positive were subjected to polymerase chain reaction (PCR) which was also positive for *S. pneumoniae*. Although PCR may be useful in this setting but it is time consuming, expensive and requires specially trained staff.

In the 90 patients with suspected bacterial meningitis, 10 samples of CSF were positive by ICT test. However, only in four of these patients could we culture *S. pneumoniae* from blood and CSF. Thus, there were 6 patients with a positive Binax Now test and a negative culture. At the same time *S. pneumoniae* was not cultured from any patient in whom Binax – Now was negative. The possible explanation for this could be that Binax – Now is more sensitive in detection of pneumococcal pneumonia from CSF, or that it could be having low specificity. To determine this, further studies and data may be required and probably also PCR to detect pneumococcal antigen in CSF to help determine the usefulness of this test in the diagnosis of pneumococcal meningitis.

We observed false – positive results in 3 patients due to micro organisms other than *S. pneumoniae*, namely *S. mitis*, *Staph. epidermidis* and *Pseudomonas* spp. This finding can be explained by the cross reactivity of cell wall antigen components among the genus *Streptococcus*. As for *Staph. epidermidis* and *Pseudomonas* spp., they may have been probable skin contaminants, as these patients recovered fully after a course of antibiotics and second sample of fluid could not be drawn. Another probability of so called false positive results of the ICT noted may have actually been caused by mixed infections. ⁽¹⁹⁾

In our study the ICT results in comparison to culture, showed 100% sensitivity and 89.5% specificity. Other studies have also shown excellent sensitivity and specificity with ICT results. A study by Jose M. Porcel et al showed 70.6% sensitivity of ICT results on pleural fluid samples and specificity of 93.3% (21). In another study, Ploton et al showed in a paediatric population, the NOW test result to be positive in all 15 pleural fluid samples yielding *S. pneumoniae* in culture (14). In yet another study by J C Moisi et al , compared to culture the Binax NOW *S. pneumoniae* ICT was >99% sensitive for the diagnosis of pneumococcal meningitis. ⁽⁹⁾

Based on our results, ICT was positive in 6/18 patients (33%) who were on prior antibiotic treatment as compared to 13/86 (15%) who had no prior exposure to antibiotics. These results are similar to what was reported by Marie Gisselssen Solen et al (22). In the study by J C Moise et al also, the prior use of antibiotics did not adversely affect the ICT results (24.2% with prior antibiotic use versus 12.2% without) (9). There are studies which do not concur with this as seen in the study by Gutierrez et al (19). Thus based on our study it may be inferred that prior exposure to antibiotics does not alter the results of ICT. This will have clinical implications

as it is not uncommon for patients to be started on antibiotic therapy before they present at a secondary or a tertiary care centre.

In conclusion, detection of the pneumococcal antigen by the Binax NOW *S. pneumoniae* ICT in pleural fluid/ CSF is easy, quick and makes treatment of complicated pneumonia and meningitis suitable and early. Some of its advantages are its high sensitivity and specificity in CSF and pleural fluid, which make it ideal as a point of care test, requiring minimal equipment and expertise. The test is rapid and portable, taking just 15 minutes for test results. The kit is stable at 15 –25° C and its sensitivity is less affected by prior antibiotic use when compared to culture. Some of the disadvantages of the test are its high costs, false positivity due to cross reactions with other related bacteria, persistence of positive results up to one month after an acute infection may create confusion in cases of recurrent multiple infections and in children where nasopharyngeal carriage of *S. pneumoniae* is common urine samples cannot be used as test samples because these organisms are excreted in the urine. More data is required to determine its sensitivity in detecting pneumococcal infections to decide whether this would be a cost – effective tool or not.

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TABLE 1: Distribution of different test results

Samples	CSF	Pleural Fluid
Total	90	14
Increased WBC	51	09
Blood CRP positive	60	14
Fluid CRP positive	nil	14
Blood culture positive	04	01
Fluid culture positive	02	02
Gram positive cocci in pairs or chains	03	06
Increased protein and decreased sugar	07	10
Binax NOW positive	10	09

Figure 1: Binax NOW *S. pneumoniae* test

