RESEARCH PAPER

Diagnostic Yield of Pneumococcal Antigen Detection in Cerebrospinal Fluid for Diagnosis of Pneumococcal Meningitis Among Children in China

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Objective: To determine the diagnostic accuracy of pneumococcal antigen detection in diagnosis of pneumococcal meningitis in children. **Methods:** Purulent meningitis was diagnosed according to European Society for Clinical Microbiology and Infectious Diseases (ESCMID) guideline between July 2014 and June 2016. Along with a cerebrospinal fluid (CSF) culture, pneumococcal antigen detection in cerebrospinal fluid (CSF) was performed, and further identification of pathogens was done with 16S rDNA-PCR and high-throughput sequencing. **Results:** CSF samples collected from 184 children (median age of 1.92 mo). CSF culture was used as the gold standard. 46 (25%) had positive results for culture and 10 (5.4%) were pneumococci; 34 (18.5%) were pneumococcal antigen positive. The sensitivity and specificity of pneumococcal antigen detection were 100% (95% CI: 89.4%–100%) and 86.2% (95% CI: 96.4%–99.9%), respectively. 92.3% (12/13) were confirmed by nucleic acid detection to be pneumococci. **Conclusions:** Pneumococcal antigen detection in CSF has adequate sensitivity and specificity in diagnosing pneumococcal meningitis.

Keywords: *Etiology, Rapid diagnosis, Sensitivity, Specificity*.

Pneumococcal meningitis is a life-threatening disease with high incidence and case fatality rate (CFR) [1-2]. During 2000-15, the global incidence rate of PnM was 13/100,000 and CFR was 44%, and the burden was more in developing countries [1]. Early use of sensitive antibiotics is extremely important for improving its prognosis [3], which depends greatly on rapid etiological diagnosis. Usually, clinicians depend mainly on the cerebrospinal fluid (CSF) culture, which is time-consuming and can only detect the live bacteria in the specimen. The detection of the pneumococcal antigen or nucleic acid can improve the diagnosis of PnM [4-7]. Testing for pneumococcal urinary antigen helped identifying pneumococci as pathogen in patients with invasive pneumococcal diseases [4,6]; Immunochromatographic antigen test for the detection of pneumococci had high sensitivity and specificity in CSF samples from children with suspected bacterial meningitis [5]. However, till recently, testing for pneumococcal antigen in CSF was not available in China. The objective of this study was to determine the diagnostic accuracy of pneumococcal antigen detection in diagnosis of pneumococcal meningitis in children.

METHODS

We enrolled patients with purulent meningitis and hospitalized at our hospital between July 31, 2014 and June 30, 2016 after approval from institutional ethics committee. The inclusion criteria included: (*i*) The clinical characteristics including irritability, poor feeding, respiratory distress, marbling of skin and hyper- or hypotonia in neonates or very young babies [8]; fever, seizures, fontanell bulge, neck stiffness and vomiting in infants; and headache accompanied by fever in old children [8]; and (*ii*) mainly polymorphic leukocytes in CSF, elevated protein level, low glucose concentration, low CSF to blood glucose ratio [8]. Patients who had blood-tinged CSF that may affect the test results were excluded. From each patients, 4-5 mL CSF specimens were collected and divided in three portions: 1.0-1.5 mL for culture, 1-1.5 mL for cytology, and 1.5-2 mL for biochemistry, pneumococcal antigen detection and PCR. Microorganism identification and antimicrobial susceptibility test were performed by using the Vitek system (Mérieux, France). Pneumococcal antigen was detected by using the BinaxNOW Streptococcus pneumonia antigen detection kit (Alere, ME, USA).

Bacterial DNA was extracted from CSF, and bacterial 16S rDNA V3-V4 region was amplified by PCR using primer pairs: 341F: CCT AYG GGR BGC ASC AG and 806R: GGA CTA CNN GGG TAT CTA AT. The PCR products with sufficient quantity were

collected and purified. An OTU clustering analysis was carried out after high-throughput sequencing. The results on culture and pneumococcal antigen detection in CSF in patients with or without previous antibiotics were compared.

Statistical analysis: The collected data were compared using the chi-square test. $P<0.05$ was considered to be of statistical significance. Diagnostic accuracy testing was described by calculating sensitivity and specificity.

RESULTS

In this study, CSF samples were collected from 184 patients (36.4% neonates), aged from 1 day to 13 years and 8 months (median age of 1.92 months). Only 46 (25%) had positive culture results; with isolated bacteria being *Escherichia coli* (15 isolates), pneumococci (10 isolates), *Streptococcus agalactiae* (7 isolates), by *Staphylococcus aureus* (3 isolates), *Enterococcus faecium* (3 isolates, *Candida famata* was isolated in one of them), *Streptococcus mitis* (2 isolates), *Listeria monocytogenes* (2 isolates), *Streptococcus sanguis* (1 isolate), *Enterobacter cloacae* (1 isolate), *Haemophilus influenzae* (1 isolate), *Acinetobacter baumannii* (1 isolate), and *C. famata* (1 isolate). Fewer positive culture results were found in patients who had received previous antibiotics when compared with those who had not ($P<0.001$); but this difference was not seen for pneumococcal culture ($P=0.08$).

Pneumococcal antigen was tested positive in 34 specimens (18.5%), which included these 10 positive pneumococci cultures. No difference in the positivity rate of antigen detection was found between those with history of previous antibiotics and those without previous antibiotics ($P=0.09$) (***Table* I**). 46 (25%) had positive results for culture and 10 (5.4%) were pneumococci; 34 (18.5%) were pneumococcal antigen positive. The sensitivity and specificity of pneumococcal antigen detection were 100% (95% CI: 89.4%-100%) and 86.2% (95% CI: 96.4%-99.9%), respectively (***Table* II**).

Twenty-one CSF specimens were selected for 16s rDNA-PCR product sequence analysis. Among these were 13 positive and 8 negative for pneumococcal antigen testing. The distribution of the bacteria at the species level based on OTU is shown in ***Fig*. 1**. In one case with positive pneumococcal antigen, it was also positive for *E. faecium* and *C. famata* in the CSF culture and the abundance of pneumococcal OTU was low. As the sample with many species of OTU may be contaminated, pneumococcal infection could not be confirmed in this case.

Finally, thirty-three patients were diagnosed with PnM based on the combination result of pneumococcal antigen detection and PCR [median (range) age: (2 mo 16 d-9 y 11 mo) 11.1 mo]. Thirty (90.9%) were under the age of 5 years old, and one was a newborn (3%) 19 were boys. Twenty-eight were treated with β-lactams or β-lactams and other antibiotics in combination for 1-27 days (median: 3 days) before they received lumbar puncture. All of the 10 pneumococcal isolates were resistant to penicillin and erythromycin but were sensitive to ceftriaxone and vancomycin or linezolid. After admission to hospital, all 33 patients were treated with β-lactams antibiotics for 8-43 days (median: 18 days), including 81.8% (27/33) who received another antibiotic in combination (24 with vancomycin and 3 with linezolid). Thirty-one patients (90.9%) were cured and the incidence of complications was 27.3% (9/33). Two children did not survive (2/33, 6.1%).

DISCUSSION

The pneumococcal antigen test is a rapid diagnosis method in the diagnosis of pneumococcal meningitis [7,10-11]; Its advantage is the simplicity, rapidity, and usefulness in cases that have already received prior antibiotics. The sensitivity and specificity in our study were high, which was in accordance with the results from previous studies that have evaluated the diagnostic accuracy by detecting pneumococcal antigen in CSF specimens [7,9,10]. In the present study, 72.7% of all (24/

TABLE I PNEUMOCOCCAL ANTIGEN DETECTION IN CEREBROSPINAL FLUID OF PATIENTS WITH OR WITHOUT PREVIOUS ANTIBIOTICS (N=184)

	With previous antibiotics (n=136)	*Without previous antibiotics* (n=48)
All bacteria		
Culture positive	25 (18.4)	21 (43.8)
Culture negative	111 (81.6)	27 (56.2)
Pneumococcus		
Culture positive	5 (3.7)	5 (10.4)
Culture negative	131 (96.3)	43 (89.6)
Antigen positive	29 (21.3)	5 (10.4)
Antigen negative	107 (78.7)	43 (89.6)

TABLE II PNEUMOCOCCAL ANTIGEN DETECTION IN CEREBROSPINAL FLUID IN PATIENTS WITH SUSPECTED PNEUMOCOCCAL MENINGITIS

	Positive (n=10)	*Negative (n=174)*
Antigen positive, *n* (%)	10 (100)	24 (13.8)
Antigen negative, *n* (%)	0	150 (86.2)

WHAT THIS STUDY ADDS?

- Pneumococcal antigen detection in the cerebrospinal fluid has adequate sensitivity and specificity in diagnosing pneumococcal meningitis.

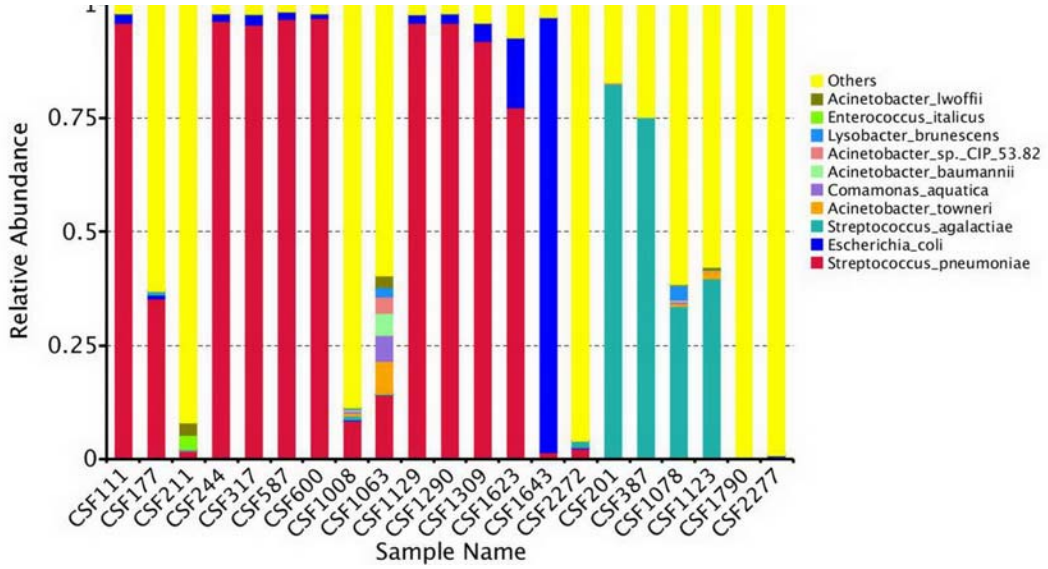

FIG. 1 *The distribution of bacterial species in 21 CSF samples by 16S rDNA-PCR high-throughput sequencing and OTU clustering.*

33) patients with pneumococcal meningitis were missed when the diagnosis was based on the CSF culture, which was mainly attributed to the fact that a majority of these patients had received antibiotics before the sampling. The introduction of the pneumococcal antigen test significantly improved the diagnosis of pneumococcal meningitis in our study. One advantage of pneumococcal antigen test is that the pneumococcal antigen might degrade slowly; it usually persists *in vivo* until 7 days (90%) to 4-6 weeks (40-48%) after recovery [11,12].

The pneumococcal antigen test was confirmed by PCR as a method with high accuracy. Bacterial DNAs are still detectable by PCR within several months after being killed by antibiotics; therefore, the diagnosis of pathogen based on the pneumococcal antigen and nucleic acid detection should be suggested in conjunction with clinical manifestations. However, both positive results of pneumococcal antigen detection and nucleic acid detection only provide the evidence of pneumococcal infection, rather than ongoing infection. There was one case positive for pneumococcal antigen testing but also positive for *E. faecium* and *C. famata* in the CSF culture. As contamination by *E. faecium* may lead to a false positive result of the pneumococcal antigen test [6] and the abundance of penumococcal DNA was not high, the exact pathogen in this case could not be determined and was not considered as PnM.

There are certain limitations in this study. The CSF specimen used for antigen detection and PCR were the same for biochemical tests and thus had a certain risk of contamination. The 16S rDNA sequencing could not be performed in all samples because of the inadequate CSF volume, which may cause biased results. Further studies are needed to confirm our conclusion with more patients.

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