Special Article

Detection of Bacterial Antigen in Cerebrospinal Fluid in Patients with Baterial Meningitis: A Literature Review

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Rapid detection of bacterial pathogen causing meningitis is very important to guide antimicrobial therapy before the standard culture result is available. Other than gram stain, one of the most useful rapid methods is the detection of bacterial antigen in cerebrospinal fluid. This article reviewed the methods of bacterial antigen detection for diagnosis of meningitis as well as a microbiology aspect of this life-threatening disease.

***Keywords**: Detection, Bacterial antigen, CSF, Bacterial meningitis*

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Bacterial meningitis is the infection of leptomeninges mainly caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis* serogroups A, B, C, Y/W135, and *Streptococcus* group B (*S. agalactiae* or group B streptococci). Meningitis is an acute and serious disease and is potentially associated with a high rate of acute complications, risk of long term disability in survivors, and death[1-3]. Early implementation of appropriate antimicrobial therapy requires prompt identification of the etiologic pathogens. The non-specific nature of clinical presentation, especially in young children, and lack of laboratory facilities can delay or obscure the diagnosis[2,4]. The conventional technique for identification by culture, even though essential for the antibiogram and confirmation of the diagnosis, is slow, time consuming, and can give false-negative results. The sensitivity of culture decreased if cerebrospinal fluid (CSF) samples were transported or stored under inappropriate condition, or if antibiotic therapy has been initiated before CSF was obtained[2,5]. Gram stain of CSF may provide immediate information of the causative pathogen. Unfortunately, the yield of pathogens on Gram stain varies depending on many factors such as the amount of bacteria, prior use of antibacterial agents, technical skill of preparing slide, and observer's expertise. Previous studies have suggested that the sensitivity of this technique ranges from 60-90% and the specificity approaches 100%[6].

Immunological techniques for detecting soluble antigens released by bacteria into body fluid (CSF, urine, serum) during the infection permitted a more rapid diagnosis than routine culture. The detection of bacterial antigen in CSF can be an important diagnostic test for meningitis. Moreover, it has many distinct advantages such as high sensitivity and specificity, easy to perform, not requiring complicate or expensive instruments, simple for interpretation, and not affected by prior antibiotic therapy. However, some experts felt that bacterial antigen detection in CSF did not enhance the diagnostic accuracy over culture method as there are false-positive reactions and negative test does not rule out bacterial meningitis[7].

Principle[1]

Soluble bacterial antigen in CSF samples is identified using latex particles coated with specific homologous antibodies. In the presence of the homologous antigen, latex particles agglutinate. In the absence of antigen, they remain in a homogeneous suspension. The bacterial antigens that can be detected by Pastorex meningitis kit are the serogroups or serotypes-specific polysaccharides of the bacteria, *e.g.*, *S. pneumoniae* (83 types), *H. influenzae* serotype b, *N.*

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meningitidis serogroups A, B, C, Y/W135, and *Streptococcus* group B.

The polysaccharide antigen specific for *N. meningitidis* serogroup B is poorly immunogenic and very difficult to obtain specific antibodies that reproducible in rabbit. However, the monoclonal antibody technology has overcome this problem. The reproducible monoclonal mouse antibodies that recognize the polysaccharide antigen of *N. meningitidis* serogroup B is currently available. Moreover, the polysaccharide antigen of *N. meningitidis* serogroup B is identical to that found in *E. coli* K1. The antigenic homology enables the test to use for diagnosis of *E. coli* meningitis in newborns, of which about 80% are of the K1 strain. The interpretation was based on the fact that *E. coli* and *Streptococcus* group B are the principal bacteria responsible for meningitis in the neonates, and that the meningococcal infection is very rare in this age group.

Evaluation of the bacterial antigen detection test

The bacterial antigen test (BAT) screens CSF, or other body fluids, for antigens of classic bacterial meningitis pathogens (*i.e.*, *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis* serogroups A, B, C, Y/W135, *Streptococcus* group B and *E. coli* K1)[8]. The utility of BAT has been questioned in some published reports[9-13]. Furthermore, changes in the epidemiology of bacterial meningitis due to widespread use of vaccines have likely affected the positive predictive values of BAT[14-18]. Consequently, many laboratories have discontinued BAT. To date, there is no recommendations for the lab to perform BAT due to conflicting evidence in the literatures[2,10,19]. It is also proposed that BAT may only be valuable in cases of culture negative bacterial meningitis[12].

Recently, a study reviewed the results of microscopic examination, routine culture, and antigen detection by latex agglutination, for establishing the etiological diagnosis of bacterial meningitis was reported[2]. The latex agglutination was performed in 65 clinically suspected meningitis cases, age ranged from 5 days to 60 years of age and compared with culture and Gram stain. Using latex agglutination, an etiologic agent was identified in 10 of 65 (15.4%) cases. In contrast, Gram stain and culture revealed 16.9% and 23.1% positives, respectively. From this study, it was concluded that the test can be recommended as an adjunct test for rapid etiological diagnosis of bacterial meningitis for prompt initiation of proper antibiotics therapy.

In 2010, a similar study was conducted with a larger sample size[20]. It was a retrospectively analysis of 918 CSF specimens of adults and children at Mayo Clinic, Rochester, USA between January 2000 and March 2009. Forty-two cases were identified by at least one of the following criteria: (i) positive BAT; (ii) positive Gram stain consistent with a classic bacterial meningitis pathogens; (iii) positive culture with a classic meningitis pathogens. This study reported that the abilities to detect organisms by BAT and Gram stain were not significantly different. In the cases with positive cultures from CSF, blood, or ear drainage, the results of BAT and Gram stain were shown in Table 1.

Other techniques

Pneumolyin, a toxin produced by *S. pneumoniae* is associated with virulence and is found in all invasive isolates. Its role for use as a diagnostic tool was reported[21]. Assay to detect of pneumolysin in CSF was found to be a simple, low cost, and suitable for rapid diagnosis of pneumococcal meningitis for routine use in the developing countries. This study did not use latex agglutination technique. The Cowan 1 staphylococcal protein A co-agglutination technique was used instead.

A rapid immunochromatographic membrane assay (*S. pneumoniae* urinary antigen test, Binax®, Portland, USA) showed a sensitivity and specificity of 100% for detection of *S. pneumoniae* antigen in CSF and urine specimens. However, these results should be regarded with caution with CSF specimens because of the small numbers of patients studied[22]. By contrast with other less sensitive bacterial antigen tests that detect capsular polysaccharide antigens, this commercial kit detects *S. pneumoniae* C-polysaccharide which is found on the cell wall, and is common to all serotypes. To use this test, a swab is dipped in the urine specimen and placed on a nitrocellulose membrane containing complexes consisting of rabbit antibody against *S. pneumoniae* conjugated with colloidal gold particles. Two lines of immobilized antibodies are adsorbed onto the membrane. The first line (specimen line) contains a rabbit antibody against *S. pneumoniae*, and the second (control line) contains goat antibody against rabbit antibodies. If *S. pneumoniae* antigen is present in the urine specimen, it binds to the conjugated antibody, and the resulting antigen-conjugate complexes are captured by the immobilized antibody, forming a visible line. The immobilized goat antibody against rabbit antibody captures excess visualizing conjugate, forming the control line. Recently, Binax®

Table 1. Cases with and without classic bacterial meningitis-causing bacteria and number of cases missed by BAT and Gram stain[20]

	Total	Missed by bacterial antigen test (%)	Missed by Gram stain (%)
Bacteria detected by culture[a]			
S. pneumoniae	22	6 (27%)	3 (14%)
Streptococcus group B	7	3 (43%)	0 (0%)
N. meningitidis	7	3 (43%)	1 (14%)
H. influenzae serotype b	2	0 (0%)	1 (50%)
Bacteria not detected by culture[b]	4	1 (25%)	0 (0%)
Total	42	13 (31%)	5 (12%)

[a]Growth from cerebrospinal fluid, blood, ear drainage

[b]Results of both bacterial antigen test and Gram stain were positive in three cases, with results indicating *N. meningitidis* in two, *S. pneumoniae* in one. In one case, the Gram stain showed Gram-positive cocci resembling streptococci, the bacterial antigen test was negative, and the urine pneumococcal antigen test was positive.

was used in blood culture specimens that was inoculated in the automate system with signal of growth but unable to grow out any organism. It was able to detect many additional cases of pneumococcal bacteremia. However, it is still a subject to debate whether these positive tests were real as the test was not performed in other samples that were positive or negative cultures[23].

A polymerase chain reaction (PCR) for diagnosing *S. pneumoniae* meningitis was developed by targeting a gene encoding for pneumolysin, so called "*ply*" gene[24]. By comparing the results in CSF specimens between PCR, bacterial culture, Gram stain, and bacterial antigen detection by latex agglutination test, the sensitivity of PCR was 96% (95% confidence interval [CI] 90-99%), compared to 59% of culture (95% CI 49-69%), 66% of Gram stain (95% CI 56-74%), and 78% of latex agglutination test (95% CI 69-86%). The specificity of PCR was 100% (95% CI 83-100%). The PCR results were available within 4 hr of the start of the assay[24].

In 2009, a novel multiplex PCR (a form of PCR in which two or more different DNA targets are amplified simultaneously in a single assay, and the reaction mixture usually contains two types of primer for each DNA target) by Luminex 100 suspension array system was reported[25]. The assay covered the nine most important bacterial and viral pathogens found in Danish meningitis patients. The pathogens included *S. pneumoniae, N. meningitidis, E. coli, Streptococcus* group B *Staphylococcus aureus, Listeria monocytogenes,* herpes simplex virus types 1 and 2, and varicella-zoster virus. The study was based on 1,187 specimens, of which 55 were found to be positive by PCR. The assay was found to have an excellent sensitivity and specificity compared to the "gold standard". The sensitivities and specificity for detecting *S. pneumoniae* was 95 and 99.1%, respectively, and *N. meningitidis* was 100 and 99.7%, respectively). The PCR results were available within 1 working day[25].

By two PCR assays, the detection of *N. meningitidis* in CSF followed by genogrouping into A, B, C, X, Y, W135 was possible[26]. Likewise, a PCR assay for detection of *H. influenzae* type b was also reported[27]. Although it seemed promising, PCR requires expensive equipments and settings which may not be feasible in poor settings.

Meningitis caused by *Mycobacterium tuberculosis* is difficult to diagnose. Rapid confirmation is important to start specific therapy. There are very few published reports about the diagnostic significance of 65 kD heat shock protein in these patients[28-30]. The method of indirect enzyme-linked immunosorbent assay (ELISA), using monoclinal antibodies against the 65 kD heat shock protein, indicated that this test was specific for *M. Tuberculosis* and could be a promising test for diagnosis of tuberculous meningitis[31].

Conclusion

Technology for detection of bacterial antigen in CSF by latex agglutination is still imperfect for widespread use. New antigens and methods recently developed such as the immunochromatographic membrane assay Binax® and multiplex PCR are promising. The ideal rapid etiologic diagnostic tool for routine use is still lacking and should be further

developed.

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การตรวจหาแอนติเจนของเชื้อแบคทีเรียในน้ำไขสันหลัง: บทความฟื้นวิชา

สมพร ศรีเฟื่องฟุ้ง, กุลกัญญา โชคไพบูลย์กิจ

การตรวจหาเชื้อก่อโรคของภาวะเยื่อหุ้มสมองอักเสบที่ได้ผลเร็ว ก่อนที่จะทราบผลเพาะเชื้อจุลชีพ มีความสำคัญต่อการรักษาผู้ป่วยนอกเหนือจากการย้อมสีกรัมแล้ว การตรวจหาแอนติเจนของเชื้อแบคทีเรียในน้ำไขสันหลังนับว่าเป็นวิธีการตรวจแบบได้ผลเร็วที่มีประโยชน์มากวิธีหนึ่ง บทความนี้ได้ทบทวนความรู้เกี่ยวกับการตรวจหาแอนติเจนของเชื้อก่อโรคเพื่อการวินิจฉัยโรคเยื่อหุ้มสมองอักเสบ