RAPID DIAGNOSIS OF BACTERIAL MENINGITIS BY COAGGLUTINATION

M Nadarajah
S Sivakumaran
O K Chong

Department of Pathology
Singapore General Hospital
Outram Road
Singapore 0316

M Nadarajah, MBBS, Dip Bact
Senior Registrar

O K Chong
Technical Assistant

Department of Microbiology
Faculty of Medicine
National University of Singapore
Lower Kent Road
Singapore 0511

S Sivakumaran, MBBS, MRC Path
Lecturer.

SYNOPSIS

Seventy five specimens of cerebrospinal fluid from patients with a history of meningitis were cultured as well as tested for the presence of antigens of four micro-organisms, Streptococci pneumoniae, Haemophilus influenza type b, Neisseriae meningitidis, and group B Streptococci. Five specimens of CSF were culture positive, Streptococci pneumoniae was isolated from four specimens and group B Streptococci from one specimen. While coagglutination was positive in 16 specimens, 12 of the specimens were positive for S. pneumoniae and four specimens were positive for group B Streptococci antigens, showing a distinct advantage of the coagglutination technique over culture as the etiology was determined in more number of meningitis patients. There were no positive results by culture as well as coagglutination for H. influenzae and N. meningitidis.

INTRODUCTION

Several techniques are available for the detection of bacterial antigens in cerebrospinal fluid, enabling in the rapid diagnosis of the etiologic agent causing meningitis. Thus the clinician could administer the appropriate antibiotic without delay. Large proportion of the CSF specimens received are culture sterile. This may be due to the low numbers of viable organisms or due to antibiotic therapy prior to taking the CSF for culture. In such instances techniques used to detect bacterial antigens are useful in the diagnosis of meningitis. The tests available are Counterimmunoelectrophoresis (CIE), latex particle agglutination (LA) and staphylococci coagglutination (COA). CIE requires specialised equipment and takes about one hour to perform the test. Latex agglutination kits are available for detection of Haemophilus influenzae type b capsular antigen and group B streptococci antigen; while coagglutination test takes only about 10 minutes and is a simple test and can detect four bacterial antigens in a short period of time. It has been reported that coagglutination using Phadebact reagents was sensitive as counterimmunoelectrophoresis (1).

A commerical diagnostic kit known as Phadebact is available for the detection of four commonest organisms that give rise to meningitis: Streptococci pneumoniae, Haemophilus influenzae, Neisseria meningitidis, and group B beta haemolytic streptococci by coagglutination. Some of the feature documented are that the reagents in the kit can identify all 83 serotypes of Streptococci pneumoniae, N. meningitidis types A, B, C and Y, H influenza type b and group B streptococci. The test is rapid and sample size required to do the test is only 6 to 10 drops of CSF. It has been documented that the test has a 87% sensitivity and 99% specificity and positive results can be obtained with culture negative CSF samples (2).

The present study is to detect the presence of the above four bacterial antigens by coagglutination in the CSF from patients, who presented clinically with signs and symptoms of meningitis.

MATERIALS AND METHODS

A total of 75 CSF specimens from patients with a clinical history of meningitis were selected. These were centrifuged and a gram stain was done on each of the centrifuged deposits and was plated on a chocolate as well as a blood plate, and incubated at 35°C in an atmosphere of 7% CO_2. The plates were read after 24, 48 and 72 hours incubation and discarded.

The balance CSF was heated in a water bath at 80°C for 5 mins, this is to prevent non specific agglutination. The CSF was tested for bacterial antigens for the four organisms: H influenzae, S. pneumoniae, Group B streptococci and N. meningitidis using Phadebact coagglutination test reagents.

A drop of the CSF was mixed with a drop of the COA reagent on a disposable paper slide and rotated manualiy for 2 mins. The CSF containing the antigen produced agglutination with the appropriate reagent which contain the staphylococci particles coatetd with the specific antibodies. This being visible to the naked eye. In a negative reaction there was no agglutination. If agglutination occurred with two or more reagents these results are no interpretable and was not taken into account.

RESULTS

A total number of 75 specimens of CSF were evaluated. The table shows the comparison of detection of bacterial antigens by coagglutination with culture and gram stain.

A culture and gram stain positive specimen of CSF for S. pneumoniae was negative by COA test.

To save reagents only CSF specimens from childrer under 3 years were tested for group B streptococci antigen, as meningitis due to group B streptococc affects usually neonates.

Thirty-seven specimens were tested for N. meningitidis antigen and 25 CSF specimens for H Influenzae. All proved to be negative for their respective antigens as well as culture and gram stain.

DISCUSSION

A large percentage of our CSF specimens were culture sterile and the etiology of the meningitis was unknown. This study was carried out to evaluate whether the agent causing the meningitis could be determined by coagglutination. The results show that the etiology was determined in 16 meningitis cases in comparison to 5 by culture. Thus showing a distinct advantage of the rapid agglutination technique over the bacteriological culture. On the other hand bacteriological culture is the most important test in the laboratory diagnosis of meningitis. It helps in the identification of the organism and also an antibiotic susceptibility of the pathogen could be done so that the appropriate antibiotic could be administered to the patient.

From the results it can be concluded that the incidence of S pneumoniae meningitis is high in comparison to other organisms tested for while the incidence of H. influenzae and N. meningitidis meningitis is low as proven by culture as well.

The incidence of group B streptococcal meningitis has been on the increase during the past few years. By doing only a culture on the CSF we may be missing a good proportion of the positive cases as the results show that only one case was culture positive and four

Comparison of Coagglutination Test for the Detection of Bacterial agents in CSF with culture and gram stain

Bacterial Agent	No. of Specimen	No. showing growth in culture	No. showing a positive gram stain	No. positive by COA
S. pneumoniae	75	4	4	12
Cp. B streptococci	21	1	1	4
N. meningitidis	37	—	—	—
H. influenzae	25	—	—	—

of the CSF were positive by coagglutination. The culture could have been negative due to reduced number of viable organisms as a result of prior administration of antibiotics. It has been documented bacterial antigens can be detected by coagglutination in the CSF even after 15 days of administrations of antimicrobial drugs (3).

We were unable to detect S. pneumoniae bacterial antigen in the CSF of one of the specimens which was culture positive. This type of negative reaction in bacteriological proven cases has been thought to be due to the absence of a capsule. Similar negative reactions have been documened in testing of the CSF by counterimmunoelectrophoresis and said to be due to poor reacting capsular antigen or failure of some of the antigens to migrate in commercial buffers (4).

The problems encountered in the coagglutination test is an occasional false positive like in all immunological tests. The false positives could be due to heat labile substances and to minimise false positive reactions the CSF is heated to 80°C for 5 minutes before testing. Cross reactions can occur. It has been reported that a specimen of CSF from a patient who had meningitis due to Klebsiella pneumoniae gave a false positive positive reaction with S. pneumoniae reagent (4). In our study no cross reactions were encountered. Other workers have reported to have no cross reactions in the tests carried out (5). The Phadebact reagents contain antibodies directed against the capsular antigens of the microorganisms. Therefore organisms which do not possess a capsule will give a negative reaction. The above are some of the limitation of the test. Nevertheless in spite of the tests limitations coagglutination is a useful rapid immunologicai test which can provide valuable rapid information in the diagnosis of meningitis though it is not a definite evidence as a positive bacterial culture of the cerebrospinal fluid.

REFERENCES

1. Tompkins DS: Comparison of Phadebact Coagglutination tests with Counterimmunoelectrophoresis for the detection of. Bacterial Antigen in cerebrospinal fluid. J Clin Pathol 1983; 36:819-22
2. Pharmacia Diagnostics Uppasala Sweden 1982.
3. Welch DF & Hensel D: Evaluation of Bactogen and Phadebact for detection of Haemophilis influenzae Type b Antigen in CSF fluid. J Clin Microbiol 1982; 16:905-8.
4. Benedict L Wasilauskas & Kenneth D Hampton: Determination of Bacterial meningitis — A Retrospective Study of 80 cerebrospinal fluid specimens. Evaluation by four vitro methods. J Clin Microbiol 1982; 16:531-5.
5. Burdash NM, Smith KA, Welborn AL: Rapid detection of Haemophilus influenzae Type b in cerebrospinal fluid by commercial coagglutination & latex Agglutination kits. J Clin Microbiol 1982; 1:131-3.