

A PRELIMINARY STUDY ON THE USE OF COUNTERIMMUNOELECTROPHORESIS AND COAGGLUTINATION METHODS IN THE DIAGNOSIS OF BACTERIAL MENINGITIS

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SUMMARY

The usefulness of counterimmunoelectrophoresis (CIEP) and coagglutination (COAG) methods in the diagnosis of bacterial meningitis was evaluated. Out of the 31 cerebrospinal fluid (CSF) specimens which had a cell count of $>5 \times 10^6$ wbc/l and were negative on gram stain and culture, pneumococcal antigens were detected in four specimens and Haemophilus influenzae type b antigen was detected in one specimen by both the methods. No false positives were detected in 10 specimens obtained from cases of febrile fits whose CSF showed no evidence of meningitis. One CSF sample, from which Klebsiella spp. was isolated, cross reacted with the meningococcal polyvalent group A-D antiserum in the CIEP test. From this study we found that these methods are rapid, simple and useful adjunctive tests in the diagnosis of bacterial meningitis, especially in the partially treated cases.

INTRODUCTION

Bacterial meningitis is a disease in which early definitive diagnosis is imperative in the management of the patient. The conventional bacteriological

techniques are normally too time-consuming to be of help in an acute situation. Moreover, certain hospitals in this country still do not have culture facilities available in their laboratories and much time is wasted in transporting the specimen to a reference laboratory. This also reduces the sensitivity of the culture technique which is dependent not only on the number of viable bacteria present, but also on the condition in which the specimen was transported and whether the patient was given any antibiotics previously. Therefore, there is a need for a simple, more rapid and sensitive method in the diagnosis of bacterial meningitis.

Recently, serological techniques have been developed to detect specific bacterial antigens in body fluid. They are the counterimmunoelectrophoresis (CIEP),^{1,2,3} latex agglutination (LA),^{4,5} coagglutination (COAG),^{6,7} radioimmunoassay (RIA)⁸ and enzyme-linked immunosorbent assay (ELISA)⁹ tests. The advantages of these methods are their rapidity and the ability to detect the specific antigen present, especially in partially treated meningitis cases. The reagents for the first three tests are commercially available for the diagnosis of bacterial meningitis. These 'meningeal kits usually include the three commonest bacterial causes of meningitis, namely, *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*.¹⁰ The RIA and ELISA methods have not been used routinely in bacteriological diagnostic work. The disadvantage with all these methods, however, is that only the common antigens can be detected routinely and it would be too laborious to

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prepare specific antisera to cover all possible causes of meningitis. The other drawback is the inability to provide any information about the antibiotic sensitivity of the causal organism.

In this preliminary study, we evaluated two of the simpler tests, CIEP and COAG, for their usefulness in routine usage in the diagnosis of bacterial meningitis in our laboratories. No attempts have been made to study the sensitivity and specificity of these tests as the samples available were too few for analysis.

METHODS AND MATERIALS

Specimens

61 cerebrospinal fluids (CSF) from our routine diagnostic section and three hospital laboratories were collected during the year 1981. They were evaluated under the following groups: (i) 31 specimens with more than 5×10^6 wbc/1 but microscopy and culture results were negative; (ii) 10 specimens from cases of febrile fits with normal CSF findings; (iii) 15 specimens from which bacteria other than *H. influenzae*, *S. pneumoniae* and *N. meningitidis* were isolated; (iv) five specimens where *H. influenzae* was isolated, one specimen each where *S. pneumoniae* and *N. meningitidis* was isolated, respectively. Group (ii) served as the negative control.

Microscopy and culture of the CSF were performed by the respective hospitals before being sent to us. All the specimens were collected and stored at -20°C until tested by the CIEP and COAG methods.

Counterimmunoelectrophoresis (CIEP)

Glass slides (2.5x7.5 cm) were covered with 3 ml of 1% agarose (Sea Kem ME; Marine Colloids, Inc., Springfield, N. J.) in Veronal buffer (pH 8.6; ionic strength, 0.05). Parallel rows of circular wells, 3 mm in diameter, were cut in the agarose gel, 4mm apart, edge to edge. Each anodal well was filled with 10 μl of antiserum and each cathodal well with 10 μl of uncentrifuged CSF. The antisera used were: pneumococcal omniserum containing antibody against 82 pneumococcal types (State Serum Institute, Copenhagen, Denmark); haemophilus

antiserum, type b and meningococcal polyvalent, groups A-D and groups X-Z, W135 (Wellcome Diagnostics, England). Crude antigen extracts were prepared according to Wasilauskas and Hampton⁷ and used as positive controls for the test. Slides were placed in an electrophoretic chamber (Apelab, France) containing Veronal buffer (pH 8.6; ionic strength, 0.05) and run at a voltage of 5 V/cm for 60 min at room temperature. The slides were examined by incident light for precipitation lines immediately after electrophoresis and again after overnight storage in a humid atmosphere at 4°C .

Coagglutination (COAG)

The Phadebact CSF test (Pharmacia Diagnostics, Upsala, Sweden) was used. Briefly, the principle of the test is that antibodies raised in rabbit and specific against *S. pneumoniae* (83 serotypes), *N. meningitidis* (Groups A, B, C, Y and W135) or typable *H. influenzae* are bound to Protein A on the surface of non-viable staphylococci.¹¹ When a CSF sample containing microorganism or antigen belonging to one of these groups is mixed with the reagents, the specific antigens would bind to the corresponding specific antibodies. A coagglutination lattice is formed, which is visible to the naked eye.

Each CSF specimen was heated in a boiling water bath for 5 min. This was necessary to eliminate non specific agglutination reactions.⁷ A loopful of the CSF was placed on each of the marked sites in the card provided. One loopful of each reagent was then added and mixed thoroughly. The card was rocked for 1 min and the results were read within 2 min. If coagglutination occurred with more than one reagent, the results were considered as not interpretable. The crude antigens prepared for CIE were also used here as positive controls.

RESULTS

Of the 31 CSF specimens in group (i), pneumococcal antigens were detected in five specimens and *H. influenzae* type b antigen was detected in one specimen by both the CIEP and COAG methods. No false positives were obtained in the 10 specimens belonging to group (ii). Of 15 specimens with other organisms, including

Pseudomonas aeruginosa, *Escherichia coli*, *Moraxella* spp., *Cryptococcus neoformans*, *Salmonella* spp., *Klebsiella* spp., *Flavobacterium meningosepticum*, *Staphylococcus aureus* and *Streptococcus pyogenes*, only one specimen from which a *Klebsiella* spp. was isolated gave a positive reaction. This specimen cross reacted with the *N. meningitidis* polyvalent group A-D antiserum in the CIEP test. Of the five *H. influenzae* meningitis cases, only four were positive by the COAG and two by the CIEP methods. The negative samples were repeated using a ten-fold dilution to avoid the prozone effect, and they were still negative. Both the COAG and CIEP were positive for a case each of *N. meningitidis* and *S. pneumoniae* meningitis.

It was noted that the CSF for COAG test had to be properly inactivated, otherwise it would give rise to non-interpretable results.

DISCUSSION

Clinicians are frequently faced with cell count and biochemical results of CSF suggestive of either a viral, tuberculous, fungal or partially treated bacterial meningitis. In such a situation, decision on the line of management has to be made while waiting for a definitive diagnosis by culture. Before the availability of these immunological procedures, gram staining of CSF was the only rapid diagnostic tool available. Since only small numbers of organisms are usually present in infected CSF which may even be absent in partially treated cases, gram staining alone would not be able to detect the organisms. Hence, with the development of immunological procedures, more sensitive methods for the rapid diagnosis of meningitis are now available.

In this study, despite the small numbers of CSF tested, five out of 31 samples of CSF which had $> 5 \times 10^6$ wbc/l but were culture and gram stain negative, gave positive results with both the CIEP and COAG methods. Four were positive for pneumococcal antigens and one for *H. influenzae* type b antigen. These five specimens were from cases of suspected meningitis and two of them had had antibiotic treatment before. For the other three cases, the history of previous antibiotic treatment was not available. The results indicate that these two tests do

serve as a rapid adjunctive procedure in establishing the etiology of meningitis, especially in partially treated cases.

No false positive reactions occurred in both the tests with CSF from all the 10 normal controls. Other workers have also found these tests to be rather specific.^{1,2,6} However, in evaluating the 15 CSF specimens where other bacteria were isolated, there was one cross reaction by the CIEP test between a specimen where *Klebsiella* spp. was isolated and the meningococcal polyvalent group A-D. Serological cross-reactivity between enteric bacteria and *N. meningitidis* groups A and C has been described.¹² Cross reactions have also been reported by other workers^{6,7} and it was noted that they occurred when high quantities of the antigen was present.⁶ In this case, the gram stain showed numerous gram negative rods, therefore, when these tests are interpreted together with the gram stain, the problem of cross reaction would be recognised.

It is not possible to assess the sensitivity of the tests in this study, as the numbers of CSF with positive cultures were too few. However, in an analysis of 347 samples of CSF from patients with meningitis, the sensitivity of the COAG reagents used were 87% for *H. influenzae* type b, 50% for *H. influenzae* types a, c-f, 84% for *S. pneumoniae* and 60% for *N. meningitidis* (Data on file, Pharmacia Diagnostics). The sensitivity of CIEP varies according to the antisera used. Only antisera with high titres of precipitating antibodies should be utilised.¹³ Workers who have done a comparative study of the COAG and CIEP methods found that the COAG is more sensitive than the CIEP.^{6,7} Our results also supported this, as two of the culture positive cases were positive by the COAG method but negative by CIEP.

There was one specimen in which *H. influenzae* was isolated but both the COAG and CIEP were negative. The reason could be that the *H. influenzae* isolated was a non typable strain and therefore could not be detected by the antisera which was prepared against the capsular antigen.

From this study, we found that serological methods are useful adjunctive tests in the identification of bacteria causing meningitis,

especially in the partially treated cases. They should be used routinely together with gram stain and culture. The COAG method would be a better choice between the two methods as it is simpler to perform, more rapid, easily available commercially and more sensitive.

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