COUNTERIMMUNOELECTROPHORESIS IN THE RAPID DIAGNOSIS OF BACTERIAL MENINGITIS

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INTRODUCTION

Acute bacterial meningitis is a medical emergency that requires the very best in diagnostic and therapeutic skills. The causative agent of infection must be identified quickly and correctly so that appropriate antimicrobial therapy can be instituted.

The conventional methods of laboratory diagnosis of bacterial meningitis are still the gram stain and culture of cerebrospinal fluid (CSF). Unfortunately, the gram stain is frequently unreliable and imprecise, and the culture may take days to provide a definitive identification of the causative agent. The sensitivity of both techniques also suffers from the all too frequent practice of treating patients with antibiotics before the collection of CSF for investigation. Very often meningitis is not suspected at the time antibiotics are given.

During the last two decades many rapid diagnostic methods, such as, counterimmunoelectrophoresis (CIE), latex agglutination, radioimmunoassay and enzyme linked immunosorbent assay have been developed (Rytel, 1979; Jones, 1979). Of these many rapid methods, the usefulness of CIE in the aetiologic diagnosis of bacterial meningitis has been established by many workers (Coonrod and Rytel, 1972; Colding and Lind, 1977; Anhalt *et al.*, 1978; Baker *et al.*, 1980).

The main advantages of the CIE are that it detects soluble antigen in the CSF and often within an hour of sample collection. Presence of viable bacteria is not essential and so antigen can be detected even in the CSF of patients who may have had antibiotics prior to collection of CSF. Antigen has been detected for as long as 18 days in the CSF in one case of pneumococcal meningitis (Fossieck *et al.*, 1973).

The present study reports our investigation of 198 specimens of CSF for bacterial agents by both CIE and culture.

MATERIALS AND METHODS

Cerebrospinal fluid specimens from clinically suspected cases of meningitis submitted to the Department of Pathology, Singapore General Hospital, were examined for bacterial agents by counter-immunoelectrophoresis and culture.

The CSF specimens were inoculated onto both blood and chocolate agars and incubated at 35°C in an atmosphere of 5% CO_2. The plates were examined daily for bacterial growth for a period of 3 days.

Antisera against Streptococcus Group B, *Haemophilus influenzae* type b and *Neisseria meningitides* (polyvalent groups A-D and XYZ-W135) were purchased from Wellcome Diagnostics, England. Antisera to *Streptococcus pneumoniae* (OMNI and Group A to I) were obtained from Statens Seruminstitut, Denmark.

Glass slides (81 mm × 81 mm) were each coated with 0.9% agarose (Sigma type 4) in barbitone/sodium barbitone buffer at pH 8.6 (Oxoid). 8 ml of agarose was used per slide to

This study was supported by Research Grant 125/82 of the National University of Singapore.

give a uniform thickness of approximately 1 mm. Pairs of wells placed at 5 mm apart were punched along the electrophoretic axis. Each well was 3 mm in diameter. 18 pairs of wells could be accommodated on each slide. The test sample was placed in the cathodal wells while the anodal wells were filled with the different antisera. Counterimmunoelectrophoresis was carried out at room temperature with a Pharmacia FBE-Immuno-EPS 500/400 machine for 30 minutes using a constant current of 5 mA/cm. After electrophoresis the gel was washed in 5% sodium citrate for 15 minutes. Precipitation lines were visualised in reflected light with the aid of a hand lens.

RESULTS

A total of 198 specimens of CSF from 191 patients were evaluated during this study. The number of specimens from which bacteria were isolated and the types of isolates are shown in Table 1. Antigens for the five organisms, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Candida* sp., *Klebsiella* sp. and *Bacillus subtilis*, were not looked for by CIE as suitable antisera against these organism were not available.

Table 2 shows an analysis of CSF findings by both CIE and culture for the 4 organisms

Table 1

CSF specimens which were culture positive and the type of organisms isolated.

Type of Organism	No. Culture Positive
Streptococcus pneumoniae	4
Neisseria meningiditis	1
Haemophilus influenzae	1
Streptococcus Group B	3
Klebsiella sp.	2
Pseudomonas aeruginosa	3
Candida sp.	3
Staphylococcus epidermidis	5
Bacillus subtilis	15
Total	37

- *Haemophilus influenzae*, *Neisseria meningitides*, Streptococcus Group B and *Streptococcus pneumoniae*, for which suitable antisera were available. In all those CSF specimens where *N. meningitidis*, *H. influenzae* and Streptococcus Group B were isolated corresponding antigens were detected by CIE. Only 13 specimens received from 10 patients were positive for pneumococcal antigen by CIE but *S. pneumoniae* was isolated only from 4.

Table 2

Analysis of CSF findings by CIE and culture.

Organism	No. Patients	No. CSF Specimens	Positive Cultures	Positive CIE
Haemophilus influenzae	1	1	1	1
Streptococcus Group B	3	3	3	3
Neisseria meningiditis	1	1	1	1
Streptococcus pneumoniae	10	13	4	12

DISCUSSION

CIE as a technique in the laboratory diagnosis of bacterial meningitis is now widely accepted. However, before any method can be effectively applied in routine diagnosis in a particular laboratory, the method has to be suitably standardised so as to give consistently reliable and reproducible results. It was with this aim the present study was undertaken.

CIE was performed for the detection of the antigens of the following - *S. pneumoniae*, *H. influenzae*, *N. meningitidis* and Streptococcus Group B - reputed to be among the common causes of bacterial meningitis. In 9 specimens where the four organisms were cultured corresponding antigens were detected only in 8 by CIE. This finding was similar to that of Coonrod and Rytel (1972). In our study there was only one specimen each, positive by both CIE and culture, for *H. influenzae* and *N. meningitidis*. *H. influenzae* isolated was type b while *N. meningitidis* was Group C. The culture positive CSF for *N. meningitidis* not only gave a positive result with Wellcome *Meningococcus* antiserum Groups A-D but was also positive with a locally raised *Meningococcus* Group C antiserum.

A total of 12 specimens of CSF were positive for *S. pneumoniae* by CIE but the organisms were cultured from only three of these. Another specimen was culture positive but CIE negative. This specimen of CSF was run at various dilutions to rule out the possibility of prozone phenomenon. Antigens of pneumococcus type VII and XIV, being neutral, tend to give a negative result when the pH of the buffer system is 8.6 (Anhalt and Yu, 1975). This may be the reason for not detecting the pneumococcal antigen in this particular specimen. Four specimens of CSF were received from one patient - a four month old baby. All were positive by CIE for pneumococcal antigen but were consistently culture negative. This patient was already on antibiotics before lumbar puncture. This illustrates the usefulness of CIE in the diagnosis of bacterial meningitis in those already on antibiotics.

Cross reactions are frequent pitfalls in immunological procedures (Bradshaw *et al.*, 1971; Coonrod and Rytel, 1973; Shackelford *et al.*, 1974). It is significant that in our study the antisera used in CIE did not show any cross reactions with the other six isolates namely, *B. subtilis*, *Ps. aeruginosa*, *S. epidermidis*, *Klebsiella* sp. and *Candida* sp. Unusually high isolations of *B. subtilis* were probably due to contamination of the corks that were used frequently as stoppers for the specimen containers. Necessary steps have been taken to eliminate this source of contamination.

Our study shows that CIE is suitable for routine use for the rapid diagnosis of bacterial meningitis. For it to be more comprehensive it is recommended that suitable antisera to other organisms causing meningitis also be included in the CIE system. Work is in progress towards this end. However, CIE as a rapid method should be used in combination with the conventional methods of gram staining and culture.

SUMMARY

Cerebrospinal fluid from patients with clinically diagnosed meningitis was tested for meningococcal, pneumococcal, Streptococcal Group B and *Haemophilus influenzae* antigens by counterimmunoelectrophoresis. Antigens were rapidly identified and the results compared favourably with that of bacteriological culture. In the case of pneumococcal meningitis counterimmunoelectrophoresis proved to be more sensitive than culture. The procedure was shown to be sensitive, specific, rapid and easily performed.

ACKNOWLEDGEMENTS

The authors are indebted to Miss Gurupatham Caroline for typing the manuscript.

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