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Rapid diagnosis of bacterial meningitis by counter-immunoelectrophoresis

by

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Counterimmunoelectrophoresis is described as a rapid and specific method for the early detection of causative organisms in bacterial meningitis. This technique was applied to CFS samples from 213 patients in whom neonatal septicaemia and/or meningitis or bacterial meningitis was suspected. Out of 14 patients with meningitis all but one bacterial organism could be detected by CIE, long before results of routine microbiological cultures were available. CIE was especially helpful in children treated with antibiotics prior to admission. Despite some shortcomings of the method, CIE is a useful diagnostic tool for the early determination of the aetiology of bacterial meningitis.

The causative organism in bacterial meningitis is usually identified by examination of Gram-stained smears and by culture. Pitfalls of the former procedure are well-known, and the latter may take days to provide a definitive identification of the pathogen. In addition, both techniques suffer from the all too frequent practice of treating patients with antibiotics prior to collection of CSF for microbiological studies. Hence, there has been a real need for more reliable and rapid methods for the early detection of bacterial antigens. A promising approach to the rapid determination of the organism in bacterial meningitis has been the method of counterimmunoelectrophoresis [6, 3, 7, 4, 8]. The present paper reports on our experience with this technique in the prospective evaluation of patients admitted with suspected bacterial meningitis.

Material and Method

Patients. The study included 213 patients (newborns, infants and older children) admitted from July, 1979, to February, 1981. The clinical diagnosis on admission was suspected neonatal septicaemia and/or meningitis, and bacterial meningitis in children beyond the newborn period. Samples of CSF were obtained from all the children and sent for aerobic and anaerobic culture to the Department of Medical Microbiology and Virology, University of Düsseldorf (Chairman Prof. Dr. Naumann). In addition, analysis of the CSF was performed by counterimmunoelectrophoresis in the Unit's laboratory within 3 h of admission. A number of patients had received antibiotic therapy prior to admission.

Counterimmunoelectrophoresis (CIE). The technique of CIE depends upon the reaction of antibody and antigen molecules and buffer to an electric current. Under certain conditions of buffer, pH and diffusion media, antigens are negatively charged and migrate in an electric field toward the anode. The antibodies are less negatively charged and are swept toward

the cathode with positive buffer ions in the agar and thus migrate in the opposite ("counter") direction as a result of endosmotic flow. Where antigen and reacting antiserum meet at points of equilibrium, a visible precipitin band forms.

In our study CIE was performed with an immunoelectrophoresis kit (Instrumentation Laboratory Boskamp GmbH, D-5303 Hersel/Bonn) containing a universal electrophoresis chamber, a glass slide ($225 \times 75 \times 3$ mm), Michaelis-buffer solution pH 8.6 of 0.056 ionic strength, filter paper wicks, a power supply (Pherostat 273) and an agar gel puncher.

The glass slide was coated with 25 ml of 1% agarose (Agarose H; LKB) dissolved in Michaelis buffer solution. After cooling, parallel wells 3 mm in diameter were punched 7 mm apart (edge to edge), and the slide was placed in the centre of the chamber. Wells were filled with solution, using capillary pipettes. Antibody-containing wells were placed at the anodal side and body fluids (CSF) on the cathodal side. The agarose-coated slide was attached by filter paper wicks to reservoirs containing Michaelis buffer solution. The universal chamber was attached to the power source, and a constant current of 60 MA (at power source) was applied at room temperature for 80 min. Slides were inspected unstained for precipitin lines without any additional aids.

Antisera used for detection of bacterial antigens were: Streptococcus A—G, Listeria, E. coli, Neisseria meningitidis antisera (Difco Laboratories, Detroit, U.S.A.); Pneumococcal omniserum containing antibodies reactive with 82 pneumococcal types, and Haemophilus influenzae type b antisera (Statens Serumstitut, Copenhagen, Denmark); Serratia, Pseudomonas antisera (self-made antisera from the Institute of Medical Microbiology and Virology of the University of Düsseldorf provided by Prof. Dr. Brunner).

TABLE I

Results of conventional cultures and CIE from patients with bacterial meningitis

Patient	Organism	Results of conventional CSF cultures	Results of CIE in CSF	Antibiotics prior to admission
1	E. coli	—	+	—
2	Strept. B	—	+	+
3	Strept. B	+	+	—
4	Strept. B	+	+	—
5	Haemophilus influenzae	+	+	—
6	Haemophilus influenzae	—	+	+
7	Serratia	+	+	—
8	Serratia	+	+	—
9	Pseudomonas	+	+	—
10	Neisseria meningitidis	—	+	+
11	Neisseria meningitidis	+	—	—
12	Neisseria meningitidis	—	+	+
13	Pneumococcus	—	+ (diluted sample)	+
14	Pneumococcus	+	+	—

Results

Thirtythree of the 213 patients had septicaemia, 14 had meningitis (6 had both septicaemia and meningitis). Table I displays a comparison between the results of conventional cultures and those of counterimmunoelectrophoresis for the cases with meningitis.

Discussion

CIE is an immunoprecipitin technique which has proved a simple and reliable method for the qualitative detection of numerous bacterial antigens [11, 12, 5, 13, 9]. The results (Table I) emphasize that a rapid and specific diagnosis of the causative organism in bacterial meningitis can be obtained by using a commercially available immunoelectrophoresis kit. CIE frequently can establish a specific aetiologic diagnosis in 80 min, long before results of routine microbiological cultures are available. Since the test does not rely on the presence of viable organisms, positive results may be obtained in patients given antibiotic therapy prior to admission, when routine cultures [14] usually remain sterile. In spite of these advantages there are, however, some shortcomings of the technique, which seem to limit its usefulness.

1. The lack of standardization among the various types of commercial antisera may lead to false negative results.

2. A further limitation is that the commercial antisera do not show all the agents responsible for bacterial meningitis. Although there are good antisera for Haemophilus influenzae type b and Pneumococcus, the quality of the antisera available for the detection of Neisseria meningitidis is rather variable. There is for example, no commercially available source of reliable antisera for N. meningitidis group B, which is responsible for approximately 50% of the cases of meningococcal meningitis.

3. Cross reactions and nonspecific precipitation may occur with some of the antisera. For instance, antisera for Haemophilus influenzae type b may crossreact with certain strains of E. coli K1 and with S. pneumoniae polysaccharides types 6, 15, 29 and 35 [9, 2].

4. False negative results due to prozone phenomenon may occur when fluid that contains a high concentration of antigen is tested. CSF specimens that are suspected to contain a high concentration of antigen, such as when many bacteria are observed on Gram staining or in case of an already turbid fluid during lumbar puncture (Patient No. 13 in Table I), should be tested both undiluted and diluted 1:20 with sterile saline or water [1].

With the development of new and more specific antisera by the way of inoculation of rabbits with various antigens, or by the technique of isolation of hybrid myeloma cell lines secreting homogeneous monoclonal antibodies of predetermined specificity [10], current restrictions of the method of CIE can be overcome

in our experience. But even with the tentative shortcomings of the technique, Table I reveals that CIE is a useful diagnostic tool for the early detection of various causative organisms in bacterial meningitis.

Literatur

1. Anhalt, J. P., Kenny, G. E., Rytel, M. W.: Detection of microbial antigens by counterimmunoelectrophoresis. Cumitech 8, American Society for Microbiology, December 1978.
2. Bradshaw, M. W., Schneerson, R., Parke, J. C., Robbins, J. B.: Bacterial antigens cross-reactive with the capsular polysaccharide of Haemophilus influenzae type b. Lancet **1**, 1095—1097 (1971).
3. Coonrod, J. D., Rytel, M. W.: Determination of aetiology of bacterial meningitis by counterimmunoelectrophoresis. Lancet **1**, 1154—1157 (1972).
4. Edwards, E. A., Muehl, P. M., Peckinpaugh, R. O.: Diagnosis of bacterial meningitis by counterimmunoelectrophoresis. J. Lab. clin. Med. **80**, 449—454 (1972).
5. Edwards, E. A.: Counterimmunoelectrophoresis and double immunodiffusion. In: Rapid diagnosis in infectious disease. M. W. Rytel (ed.), CRC Press, Inc., Boca Raton, Florida 1979.
6. Greenwood, B. M., Whittle, H. C., Dominic-Rajkovic, O.: Counter-current immunoelectrophoresis in the diagnosis of meningococcal infections. Lancet **2**, 519—521 (1971).
7. Ingram, D. L., Anderson, P., Smith, D. H.: Countercurrent immunoelectrophoresis in the diagnosis of systemic diseases caused by Hemophilus influenzae type b. J. Pediat. **81**, 1156—1159 (1972).
8. Kaplan, S. L., Feigin, R. D.: Rapid diagnosis of bacterial meningitis. In: Rapid diagnosis in infectious disease. M. W. Rytel (ed.), CRC Press, Inc., Boca Raton, Florida (1979).
9. Kaplan, S. L., Feigin, R. D.: Rapid identification of the invading microorganism. Pediat. Clin. N. Amer. **27**, 783—803 (1980).
10. Polin, R. A., Kennett, R.: Use of monoclonal antibodies in an enzyme-linked inhibition assay for rapid detection of streptococcal antigen. J. Pediat. **97**, 540—544 (1980).
11. Rytel, M. W.: Counterimmunoelectrophoresis in diagnosis of infectious disease. Hosp. Pract. **10**, 75—82 (1975).
12. Rytel, M. W.: Rapid diagnostic methods in infectious diseases. Ann. intern. Med. **20**, 37—60 (1975).
13. Storm, W., Lemburg, P.: Early detection of bacterial antigens by counterimmunoelectrophoresis. Eur. J. Pediat. **135**, 65—67 (1980).
14. Storm, W.: Nachweis bakterieller Antigene bei antibiotisch vorbehandelten Patienten durch Gegenstromimmunelectrophorese. Klin. Pädiat. **193**, 39—40 (1981).

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