Counterimmunoelectrophoresis in the Diagnosis of Bacterial Meningitis

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The aim of the present study was to investigate whether counterimmunoelectrophoresis (CIE) would facilitate the rapid, etiological diagnosis of bacterial meningitis when used in parallel with other routine methods in a medical bacteriological laboratory. Of 3,674 consecutive specimens of cerebrospinal fluid (CSF) received at the Department of Diagnostic Bacteriology, Statens Seruminstitut, 283 specimens (each representing one patient) were selected for examination by CIE on the basis of the following criteria: bacteria or pleocytosis or both by microscopy or positive culture or both. CIE was performed with antisera to *Neisseria meningitidis* (groups A, B, and C), *Streptococcus pneumoniae* (omniserum and pools A to I), and *Haemophilus influenzae* type b. Antigen was detected in 57% (72/126) of specimens in which cultures revealed these three kinds of microorganisms in CSF and in 12% (17/139) of the culture-negative specimens. CSF specimens from 21 patients with bacterial meningitis caused by other species were all negative in CIE, except four, three of which contained *Escherichia coli* antigen reacting with antiserum to *N. meningitidis* group B and one *E. coli* antigen reacting with antiserum to *H. influenzae* type b. Specific diagnosis was achieved in 60% (170/283) of the specimens studied and could be established within 1 h in 85% (145/170) by the combined results of microscopy and CIE. Ten specimens, nine of which showed a reaction with antiserum to *N. meningitidis* group A, were positive by CIE only.

Counterimmunoelectrophoresis (CIE) was developed for use in forensic medicine as a rapid method requiring only small amounts of reactants (6). The technique was further elaborated for the detection of Au antigen (9, 15) and has also proved useful in the detection of bacterial antigens in cerebrospinal fluid (CSF) or serum (3, 5, 7, 8, 11). The aim of the present study was to investigate whether CIE would make any contribution to the rapid, etiological diagnosis of bacterial meningitis when used in parallel with routine methods in a medical bacteriological laboratory.

MATERIALS AND METHODS

Specimens. During the period 1 September 1973 to 31 December 1974, a total of 3,674 specimens of CSF were received for bacteriological examination at the Department of Diagnostic Bacteriology, Statens Seruminstitut. All specimens in which an etiological diagnosis had been established by routine cultural procedures or which had shown bacteria or pleocytosis or both by microscopy were stored at -20°C for future examination by CIE. For specimens containing *Streptococcus pneumoniae* or *Haemophilus influenzae* type b, the specificity of micro-

copy findings was regularly confirmed by the performance of capsular reactions on the CSF specimens. Each of the 283 CSF specimens studied represented one patient. Storage for up to 2 years did not seem to cause any deterioration of the antigens involved, as judged by repeated examination of selected positive specimens.

CIE. The equipment employed for immunoelectrophoresis was from Dansk Laboratorieudstyr A/S, Copenhagen. In principle, the method used was that described by Coonrod and Rytel (4). Glass slides (10 by 10 cm) were coated with 15 ml of 1% agarose (Litex, batch number AGS, 105 AX) dissolved in Veronal buffer (pH 8.2, ionic strength 0.05) containing 0.001 M ethylenediaminetetraacetic acid. The same buffer was used in the buffer reservoir of the electrophoresis apparatus. Parallel rows of wells, 4 mm in diameter, were punched out on the slides at a distance of 3 mm from each other. Each well was filled with 10 μ l of the reactant. The wells containing the antisera were on the anodic side of the electrophoresis chamber. The slides were attached to the buffer reservoir by paper wicks (Whatman no. 17) and subjected to a constant voltage of 2.5 V/cm for 1 h at room temperature. The slides were examined for precipitation bands against a dark background in oblique transillumination and then stained for protein with Coomassie brilliant blue

(20). (Each experiment included known culture-positive CSF specimens in which the relevant antigen has been demonstrated previously by CIE [positive controls].)

Antisera. Rabbit antisera against *Neisseria meningitidis* groups A and C were produced as described previously for antisera to *N. gonorrhoeae* (13). Horse antiserum against *N. meningitidis* group B was produced by John B. Robbins, Food and Drug Administration, Bethesda, Md., and received via Frits Ørskov, Statens Seruminstitut. Rabbit antisera against *H. influenzae* type b and *S. pneumoniae* were provided by Erna Lund, Statens Seruminstitut. The set of pneumococcal antisera comprised omniserum, which is a pool of all 83 types, pools A to I inclusive, and relevant type/group antisera.

The following commercially available antisera were used in preliminary experiments: rabbit antisera to *N. meningitidis* group A (Difco, batch no. 604563), *N. meningitidis* group B (batch no. 604564), *N. meningitidis* group C (batch no. 604565), and *H. influenzae* type b (batch no. 596879).

Serological grouping of isolated strains of *N. meningitidis* and *S. pneumoniae*. Strains of *N. meningitidis* were examined by means of a coagglutination method (12) adapted for serological grouping of meningococci. So far, this test has only been established for groups A and C. All other strains were designated nongroupable (NG). Strains of *S. pneumoniae* were examined by Erna Lund by the Neufeld capsular reaction method.

RESULTS

CIE technique. The setup used for examination of the total material was chosen after a series of preliminary experiments in which all specimens previously found positive by culture were investigated by various modifications of CIE. Each experiment consisted of four slides, the wells of which were filled according to the same diagram. Electrophoresis was run for 1 h (two slides) and 2 h (two slides). One set of slides was examined immediately after CIE and then stained. The second set of slides was stored for 18 h at 10°C, reexamined, and then stained.

As compared with the results obtained after 1 h, no further specimens from patients with *N. meningitidis* or *H. influenzae* type b meningitis became positive after electrophoresis for 2 h and/or storage of the slides for 18 h.

For patients with *S. pneumoniae* meningitis, the number of CSF specimens in which antigen could be detected by CIE after 1 h, with or without subsequent storage of the slides, is shown in Table 1. With all the antisera used, particularly the omniserum, the number of positive findings was higher after storage for 18 h. The number of specimens that became positive increased further when pools A to I were used in addition. By extending the electrophoresis by 2 h, two of these specimens also became

positive with omniserum. For practical reasons, all specimens were examined primarily with omniserum and electrophoresis for 1 h. Subsequently, all CIE-positive specimens and all specimens from which *S. pneumoniae* had been isolated by culture were examined by means of pools A to I and relevant group/type antisera.

The sensitivity of CIE was slightly enhanced by staining for protein (Table 2). All precipitates seen before staining were recognized after staining.

Antisera. Antisera against *N. meningitidis* Groups A and C gave distinct precipitation lines with homologous group-specific polysaccharide antigens (received via Alice Reyn as a gift from Emil Gotschlich, The Rockefeller University, New York). They showed no cross-reactions with the other group-specific antigens, and in examination of culture-positive specimens, they gave single distinct precipitation lines only with those from which a strain of the homologous group was isolated.

Of all antisera against *N. meningitidis* group B tested, only that provided by J. B. Robbins gave a distinct precipitation line with group B

TABLE 1. Antigen detection in specimens of CSF from patients with *S. pneumoniae* meningitis^a

CIE performed for (h)	Total no.	No. positive with antiserum against <i>S. pneumoniae</i> : ^b					
		Omniserum		Pools		Group/type	
		S	U	S	U	S	U
1	32	14	9	19	18	20	17
18	32	18	16	21	20	21	21

^a Results of CIE performed for 1 h compared with those obtained by CIE for 1 h and subsequent storage of the slides for 18 h at 10°C.

^b S, Stained; U, unstained.

TABLE 2. Demonstration of bacterial antigens in 283 specimens of CSF by culture, microscopy, and CIE

Culture result	No. of specimens examined	No. of specimens positive by		
		Microscopy	CIE	
			Stained	Unstained
Culture positive				
<i>N. meningitidis</i>	64	50	35	33
<i>S. pneumoniae</i>	32	29	14	9
<i>H. influenzae</i>	30	25	23	20
<i>E. coli</i>	5	5	4	4
Other bacteria	13	6	0	0
Culture negative	139	16	17	16

polysaccharide. This antiserum precipitated group-specific polysaccharides B and C and gave blurred unspecific precipitates in a great number of specimens. Furthermore, three out of five specimens from patients with *E. coli* meningitis gave strong, distinct precipitation lines. Therefore, the interpretation of a positive result obtained by means of antiserum to *N. meningitidis* group B was based on the microscopy reading available at the same time.

With the routine setup, the pneumococcal antisera gave precipitates only with specimens from patients with *S. pneumoniae* meningitis. Antiserum to *H. influenzae* type b gave a single distinct precipitate, with the majority of CSF specimens containing the homologous antigen. One out of five specimens from patients with *E. coli* meningitis gave a distinct precipitate with this antiserum. Blurred unspecific precipitates occurred occasionally after storage of the slides for 18 h.

The commercial antisera tested gave either fewer positive results than those found by means of the antisera described above or none at all. Therefore, these antisera were not used in this study.

Comparison of the results obtained by culture, microscopy, and CIE. Used as the only method, culture (Table 2) revealed the pathogen in the highest proportion of cases of meningitis in which the diagnosis could be established, viz., 85% (144/170). Fifty-seven percent (72/126) of the culture-positive cases of meningitis due to *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type b contained demonstrable group- or type-specific antigens. The frequency of CIE-positive results within these three groups differed significantly: *N. meningitidis*, 35/64; *S. pneumoniae*, 14/32; *H. influenzae* type b, 23/30 ($\chi^2 = 7.17$, $F = 2$, $P < 0.05$). Microscopy offered a diagnosis in more cases (104/126) than did CIE (72/126). Put together, these two methods yielded a correct diagnosis within 1 h in 85% (145/170) of the cases or 51% (145/283) of the specimens examined.

The results obtained for the culture-positive CSF specimens are shown in detail in Table 3. Four specimens negative by microscopy were positive by CIE. For all CIE-negative specimens that had shown large numbers of bacteria by microscopy, CIE was repeated with twofold dilutions of CSF. The negative results were confirmed, and thus they could not be due to excess of antigen. Four out of five specimens from which *E. coli* was isolated showed positive CIE reactions. Three gave strong, distinct precipitates with antiserum to *N. meningitidis* group B, and one gave a precipitate with antiserum to *H. influenzae* type b. These results

are referred to in Discussion. Specimens from 13 patients with infections due to other bacterial species were negative by CIE (*Streptococcus* spp. [8], *Staphylococcus aureus* [2], *Listeria monocytogenes* [1], *Klebsiella oxytoca*, [1], and *Acinetobacter calcoaceticus* [1]).

The results obtained for specimens positive by CIE and/or microscopy and negative by culture are shown in Table 4. Fourteen of seventeen CIE-positive specimens in this group gave a positive reaction with antiserum to *N. meningitidis*. Another two specimens gave precipitates with both pneumococcal omniserum, pool A, and antiserum against group 18. One specimen gave a positive reaction with antiserum against *H. influenzae* type b. A total of 10 specimens negative by both microscopy and culture were positive by CIE.

Detection of group/type-specific antigens by CIE. For culture-positive specimens, complete agreement was found between the serological grouping of the isolated strains and the CIE grouping. The majority of cases of meningococcal meningitis were caused by *N. meningitidis* group A. Five of nineteen specimens from which NG strains were isolated were

TABLE 3. Comparison of the results obtained by culture, microscopy, and CIE in the diagnosis of bacterial meningitis: culture-positive CSF specimens

Etiology	Total no.	No. culture + ^a			
		Microscopy +		Microscopy -	
		CIE +	CIE -	CIE +	CIE -
<i>N. meningitidis</i>	64	33	17	2	12
<i>S. pneumoniae</i>	32	13	16	1	2
<i>H. influenzae</i>	30	22	3	1	4
<i>E. coli</i>	5	4		0	0
Other bacteria	13	0	6	0	7

^a +, Positive; -, negative.

TABLE 4. Comparison of the results obtained by culture, microscopy, and CIE in the diagnosis of bacterial meningitis: Culture-negative CSF specimens

Etiology	Total no.	No. culture - ^a		
		Microscopy +		Microscopy -
		CIE +	CIE -	CIE +
<i>N. meningitidis</i>	19	5	5	9
<i>S. pneumoniae</i>	3	1	1	1
<i>H. influenzae</i>	1	1	0	0
Other bacteria	3	0	3	0

^a -, Negative; +, positive.

found to contain group B antigen (Table 5). The correlation between the CIE data for CSF specimens and the results of serotyping of isolated strains of *S. pneumoniae* is shown in Table 6. Strains of type 7F were isolated from five, and a type 14 strain was isolated from one, of the CIE-negative specimens. Since it has been reported that 7F and 14 are positively charged when the pH varies between 5 and 8 (11), CIE was repeated with CSF applied to the anodic well. The results remained negative.

DISCUSSION

The majority of investigators comparing different methods for the detection of the specific pathogen in bacterial meningitis have found bacteriological culture procedures to be the most sensitive (3, 7, 19, 21). CIE is a rapid method and is also a sensitive method, in that it is possible to detect the presence of 0.02 to 0.5 μ g of capsular polysaccharide antigens per ml (4, 7, 8). Another advantage is that results are independent of the presence of living microorganisms, which means that CIE can be used for specimens containing formalin or obtained

from patients already under treatment with antibiotics, and for specimens that have taken a long time to arrive by mail.

In this study, the results obtained by CIE for selected specimens of CSF were compared with those obtained by direct microscopy and culture. The pathogen was detected in 85% of confirmed cases of bacterial meningitis by means of bacteriological culture procedures, in 77% by direct microscopy, and in 55% by CIE. By using the combination microscopy and CIE, etiological diagnosis was achieved within 1 h in the same number of cases as was obtained by culture alone (85%). CIE was positive in 57% (72/126) of the culture-positive cases. CIE offered a diagnosis in 12% (17/139) of cases with negative culture. Nine out of ten specimens also found negative by direct microscopy showed a reaction with antiserum to *N. meningitidis* group A (Table 4). This means that, used as the only method, CIE gave the specific diagnosis in the same number of cases of group A meningococcal meningitis as culture and that an increase of about 30% in the number of confirmed cases could be obtained by using both methods.

Antiserum to *N. meningitidis* group B gave strong precipitation reactions with three out of five specimens from patients with *E. coli* meningitis. The group B antigen is known to be identical with *E. coli* capsular polysaccharide K1 (10), which frequently occurs in strains causing neonatal meningitis (16). In all three cases, direct microscopy revealed gram-negative rods, so the mistake of initiating treatment appropriate for meningococcal meningitis could be avoided. Serological cross-reactivity between *H. influenzae* and *E. coli* as shown with one specimen has been demonstrated previously by Ouchterlony analysis of bacterial extracts (18). Serological cross-reactivity between enteric bacteria and *N. meningitidis* groups A and C and *S. pneumoniae* types I and III has also been described (17), but cross-reactive antigens have not been detected in any of the CSF specimens studied.

There was a complete agreement in this study between serological grouping of the isolated strains of *N. meningitidis* and *S. pneumoniae* and that obtained by CIE performed on the corresponding CSF specimens. In cases of meningococcal meningitis, this information is obtained at an earlier stage than usual, and group B antigen was detected in 5 of 19 specimens from which NG strains had been isolated. CSF specimens from patients with infections due to *S. pneumoniae* types 7F and 14, which occur quite frequently in Denmark (14), were all negative by CIE. Since the present investigation was completed, a buffer which permits

TABLE 5. Detection of group-specific *N. meningitidis* antigen in CSF by CIE

Culture result	No. of specimens	No. CIE positive with antiserum against <i>N. meningitidis</i>		
		group A	group B	group C
Culture-positive group				
A	37	26	0	0
NG	19	0	5	0
C	8	0	0	4
Culture negative	14	12	1	1

TABLE 6. Correlation between CIE data obtained after 1 h and serotypes of *S. pneumoniae* strains isolated by culture from CSF of 32 patients

Antiserum pool	No. of specimens	Serotypes of <i>S. pneumoniae</i>	
		CIE + ^b	CIE - ^a
A	6	4, 18F, 18C(3)	4
B	11	6A(2), 6B, 8(3), 19A, 19F(2)	6B, 19A
C	6		7F(5), 31
D	0		
E	2	10A(2)	
F	1	41A	
G	1		29G
H	4	23F(3)	14
I	1		38

^a One strain of each type was found unless indicated by figure in parentheses.

^b +, Positive; -, negative.

the detection of type 7F antigen by means of CIE has been described (1).

In conclusion, CIE yielded an increase of 6% (10/170) in the number of confirmed cases of bacterial meningitis when used in parallel with routine methods in a well-established bacteriological laboratory. The average transport time for the specimens examined was less than 24 h. Under conditions less optimal for the cultural procedures, CIE can be an even more useful supplement, especially with respect to the diagnosis of group A meningococcal meningitis. Furthermore, the diagnostic value of CIE can be extended by examination of both CSF and serum for the presence of bacterial antigens (2). However, the value of CIE depends entirely on the quality of the antisera employed and, due to serological cross-reactivity between species causing meningitis, e.g., between *N. meningitidis* group B antigen and *E. coli* capsular polysaccharide K1, the interpretation of some positive findings requires that the result of direct microscopy be available at the same time.

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