Latex agglutination, counterimmunoelectrophoresis, and protein A co-agglutination in diagnosis of bacterial meningitis

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SUMMARY Specimens of cerebrospinal fluid from 201 patients with meningitis caused by *Neisseria meningitidis* groups A/B/C/135, *Streptococcus pneumoniae* (23 types), and *Haemophilus influenzae* type b were tested for the presence of specific bacterial antigens by latex agglutination, counterimmunoelectrophoresis (CIE), and protein A co-agglutination. Specific antigens were found in 75% of the specimens by latex agglutination and CIE, and in 60% of the specimens by protein A co-agglutination. Non-specific reactions in protein A co-agglutination were prevented by heating the specimens to 100°C for a few minutes. The three methods are simple and quick to perform. The smallest amount of antiserum was used in protein A co-agglutination, but we found this method less sensitive. Latex agglutination and CIE proved to be equally sensitive, but the first method was easier to adopt in practice.

The treatment of bacterial meningitis requires a rapid aetiological diagnosis. Gram stain and culture are routine methods but the presence of microorganisms in the Gram stain will not prove the exact cause, and a culture takes 18 hours at least. In the last few years several methods to determine the cause of bacterial meningitis within the shortest possible time have been described (Greenwood *et al.*, 1971; Edwards *et al.*, 1972; Severin, 1972; Whittle *et al.*, 1974; Suksanong and Dajani, 1977). They are all based on the fact that specific bacterial antigens are liberated in the cerebrospinal fluid during the infection, as has been described by Dochez and Avery (1917). In testing 201 specimens from patients with suppurative meningitis caused by meningococci, pneumococci, and *Haemophilus influenzae*, we compared the three methods: latex agglutination, counterimmunoelectrophoresis (CIE), and protein A co-agglutination.

Material and methods

SPECIMENS

We examined 201 specimens of cerebrospinal fluids (CSF) taken from patients suffering from meningitis caused by *Neisseria meningitidis* (68), *Streptococcus pneumoniae* (73), or *H. influenzae* type

b (60). The meningococci belonged to group A (17), group B (33), group C (16), and group 135 (2), and there were 23 types of pneumococci. The specimens were sent to us by a number of bacteriological laboratories in the Netherlands in the course of 1976. Most specimens were sent with isolated microorganisms. When they could not be examined immediately, the specimens were stored at a temperature of -20°C. When microorganisms were brought in, they were inoculated again, identified, grouped, and/or typed and freeze-dried, and kept at -70°C.

CONTROLS

The 201 CSF specimens were examined with all antisera that were used to check on specificity. Additionally, we tested one specimen from a patient probably suffering from viral meningitis, two specimens from patients with suppurative meningitis from which microorganisms had not been isolated, and four samples from patients with meningitis caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *H. influenzae* type f.

ANTISERA

Latex agglutination: *N. meningitidis* groups A/B/C/135 (GZL)¹, *Strep. pneumoniae* omniserum

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Received for publication 25 July 1978

(Statens Seruminstitut), *H. influenzae* type b (Burro 132-Robbins).

Counterimmunoelectrophoresis: *N. meningitidis* groups A/B/C/135 (GZL), *Strep. pneumoniae* omni/pool/type-sera (Statens Seruminstitut), *H. influenzae* type b (Burro 132-Robbins), *H. influenzae* type b (Statens Seruminstitut), *H. influenzae* types a-f (Wellcome).

Protein A co-agglutination: *N. meningitidis* groups A/B/C/135 (GZL), *Strep. pneumoniae* omni/pool-sera (Statens Seruminstitut), *H. influenzae* type b (Statens Seruminstitut).

We prepared antisera of *N. meningitidis* groups A/B/C/135 (GZL) in our laboratory by the immunisation of rabbits (Severin, 1972). The antisera were tested for specificity by CIE and double gel diffusion (Slaterus, 1961). Nonspecific antisera were absorbed by cross-reacting antigens (Danielsson and Kronvall, 1974). The antiserum *H. influenzae* type b (Burro 132) was a gift from John E. Robbins (Department of Health, Education and Welfare, Bethesda, Maryland, USA).

LATEX AGGLUTINATION TEST

Polystyrene latex particles (0.81 μ m, Difco) were sensitised with γ -globulin by the method described by Severin in 1972. With the exception of *Strep. pneumoniae* omniserum, γ -globulin was precipitated with a 50% saturated ammonium sulphate solution. A slide with hollows (Assistent) was used for the agglutination; 0.005 ml sensitised latex was added to 0.01 ml of specimen and rotated for three minutes at 150 rpm on the electric rotating apparatus (A. H. Thomas Co). The agglutination was then observed immediately with the naked eye.

The latex coated with pneumococcal omniserum showed granular agglutination (Whittle *et al.*, 1974), and we therefore checked each test with a physiological saline solution to prevent incorrect positive interpretation.

COUNTERIMMUNOELECTROPHORESIS

We used 7 $\times$ 7 cm glass slides with 1% agarose in a barbital buffer solution (pH 8.6—ionic strength 0.02) according to the method described by Edwards *et al.* (1972), Greenwood *et al.* (1971), and Fossieck *et al.* (1973). A barbital buffer solution with a pH of 8.6 and ionic strength of 0.1 was used in the buffer reservoir of the electrophoresis apparatus. The slides were subjected to a constant 80 volts at room temperature for 30 minutes. The precipitation lines were then observed immediately before the slides were put in a 0.02% thiomersal NaCl water bath for 18 hours. They were then dried and stained with Coomassie brilliant blue (R250 Merck).

PROTEIN A CO-AGGLUTINATION TEST

The staphylococcal protein A was prepared by the method described by Arvidson *et al.* (1970) and Kronvall (1973) with minor modifications. Columbia agar plates with a diameter of 14 cm were used for the culture of *Staph. aureus* (Cowan I NCTC 8530). The microorganisms were taken off with a glass rod and suspended in PBS pH 7.4. The suspension was treated with 0.5% formaldehyde and heated, rotating in a water bath at a temperature of 80°C. We used a 1% protein A suspension for all antisera at first, but co-agglutination was unsatisfactory with pneumococcal omniserum for which we then used a 2% protein A suspension. The staphylococcal protein A coated with antiserum was used unwashed (Edwards and Larson, 1974). The CSF specimens were heated to 100°C for two minutes and centrifuged. The clear liquid separated at the top was used for co-agglutination on a micro-slide with hollows (Assistent); 0.01 ml of the protein A coated with antiserum was added to 0.01 ml of the clear liquid and rotated for four minutes at 300 rpm on the electric rotating apparatus (A. H. Thomas Co). Co-agglutination was then observed immediately with the naked eye.

IDENTIFICATION OF MICROORGANISMS

N. meningitidis was inoculated on Mueller Hinton agar with 1% supplement B (Difco), incubated at 37°C in a candle jar, and then identified by Gram stain, oxidation, and fermentation of glucose and maltose on GC medium with indicator.

N. meningitidis was grouped by agglutination and microprecipitation of the microorganisms with the group-specific antisera (Slaterus, 1961). For the culture of *Strep. pneumoniae*, identified by Gram stain and sensitivity to ethylhydrocuprein (Optochin), 5% horse blood agar was used (Columbia agar base BBL). Pneumococci were grouped by the membrane swelling reaction according to Neufeld.

H. influenzae was inoculated on to chocolate agar and identified by Gram stain and growth dependency on X and V factors on NAD plates (Evans *et al.*, 1975). *H. influenzae* was typed by co-agglutination with staphylococcal protein A coated with specific antiserum (Kronvall, 1973; Christensen *et al.*, 1973).

Results

Of 201 CSF specimens from patients with meningitis caused by *N. meningitidis*, *Strep. pneumoniae*, and *H. influenzae* type b, we found specific antigens with latex agglutination in 151 (75.1%), with CIE in 152 (75.6%), and with protein A co-agglutination in 121 (60.2%) specimens.

Specification of the positive reactions of each type of microorganism is found in Tables 1, 2, and 3.

Table 1 Numbers of specific polysaccharides found in CSF specimens

Isolated strains	Number of specimens	Specific polysaccharides found by:		
		Latex agglutination	CIE	Protein A co-agglutination
<i>N. meningitidis</i> groups A/B/C/135	68 (100%)	48 (70.6%)	50 (73.5%)	33 (48.5%)
<i>Strep. pneumoniae</i>	73 (100%)	57 (78.1%)	47 (64.4%)	54 (74.0%)
<i>H. influenzae</i> type b	60 (100%)	46 (76.7%)	55 (91.7%)	34 (56.7%)
Total	201 (100%)	151 (75.1%)	152 (75.6%)	121 (60.2%)

Table 2 Numbers of specific meningococcal polysaccharides found in CSF specimens

Isolated strains	Number of specimens	Specific polysaccharides found by:		
		Latex agglutination	CIE	Protein A co-agglutination
<i>N. meningitidis</i> group A	17 (100%)	16 (94.1%)	16 (94.1%)	11 (64.7%)
B	33 (100%)	17 (51.5%)	20 (60.6%)	9 (27.3%)
C	16 (100%)	13 (81.3%)	12 (75.0%)	11 (68.8%)
135	2	2	2	2
Total	68 (100%)	48 (70.6%)	50 (73.5%)	33 (48.5%)

Table 3 Numbers of specific pneumococcal polysaccharides found in CSF specimens

Isolated strains	Number of specimens	Specific polysaccharides found by:		
		Latex agglutination	CIE	Protein A co-agglutination
<i>Strep. pneumoniae</i> Pool A	19	16	16	17
B	18	15	11	13
C	9	8	4*	8
D	3	2	2	1
E	8	4	3	4
F	1	—	1	—
G	3	3	3	3
H	12	9	7	8
Total	73 (100%)	57 (78.1%)	47 (64.4%)	54 (74.0%)

*Three *Strep. pneumoniae* type 7 positive after 18 hours water bath.

Latex agglutination is just as sensitive as CIE in the detection of polysaccharides of *N. meningitidis* groups A, C, and 135 (Table 2). Polysaccharides were detected in only 50-60% of the specimens when meningitis had been caused by meningococci group B.

The results of protein A co-agglutination in the detection of polysaccharides in the case of meningococcal meningitis was only 48.5%. Protein A co-

agglutination gave better results than CIE in the detection of *Strep. pneumoniae* polysaccharides.

When pool-sera or type-sera were used in these two methods, the reaction did not increase in sensitivity (El-Refaie and Dulake, 1975). Polysaccharides of *Strep. pneumoniae* types 7 and 14 were not detected by CIE; three out of eight *Strep. pneumoniae* type 7 specimens showed precipitation lines with the antiserum after 18 hours' diffusion in a water-bath. We restricted research into polysaccharides of *H. influenzae* to type b because this type causes *H. influenzae* infections in 95% of cases (Weinstein, 1970). We used three different antisera (Robbins, Statens Seruminstitut, and Wellcome) to detect the antigens of this microorganism with CIE.

The *H. influenzae* type b antiserum Burro 132 (Robbins) showed a positive reaction in 55 out of 60 specimens (91.7%); the same types of antisera from the Statens Seruminstitut and Wellcome showed a similar reaction in 45 out of 60 (75.0%) and in 23 out of 60 (38.3%) specimens respectively. We could not use the Burro 132 antiserum in the co-agglutination test because staphylococcal protein A does not react to the γ G-globulins of this species (Grov *et al.*, 1970; Lind *et al.*, 1970).

Four CSF specimens from patients with pneumococcal meningitis showed nonspecific reactions: two specimens in latex agglutination with meningococci group B and two with meningococcal groups A and C antiserum in CIE.

Nonspecific reactions did not occur when the specimens were heated. The CSF specimens from patients with meningitis caused by *H. influenzae* type f reacted only with the type-specific antiserum.

Discussion

We found scarcely any difference in sensitivity between latex agglutination and CIE for the detection of antigens in the cerebrospinal fluid of patients with meningitis caused by meningococci, pneumococci, and *H. influenzae* type b. This confirmed the findings of Whittle *et al.* (1974) and Leinonen and Herva (1977).

Protein A co-agglutination—used for grouping and typing microorganisms (Christensen *et al.*, 1973; Kronvall, 1973; Olcén *et al.*, 1975)—now proved to be a method for the detection of antigens in cerebrospinal fluid. Contrary to the findings of Suksanong and Dajani (1977), we found that most specimens showed nonspecific reactions with staphylococcal protein A. Olcén *et al.* (1975) eliminated nonspecific reactions by absorbing the CSF specimens with a pure solution of protein A. When we treated the specimens with a pure solution of protein A, they still showed nonspecific reactions, but these

disappeared when the specimens were heated to 100°C for 1-2 minutes before the test, probably by the coagulation of proteins that had caused the cross reactions. This method proved to be less sensitive for the detection of antigens in the specimens than latex agglutination and CIE, with the exception of pneumococci. At first we believed that heating the specimens caused the reduction in polysaccharide level, but the results of CIE were not influenced by heating. The cause may be found in the antisera that we used. These probably contained insufficient amounts of γ G-globulin that could be combined with protein A.

The lowest levels of polysaccharides were found in the specimens of patients with meningococcal group B meningitis, although over 50% of meningococcal meningitis in the Netherlands is caused by this group (Annual Reports, 1975 and 1976).

Severin (1972) and Leinonen and Herva (1977) found polysaccharides of meningococci groups A and C in the CSF specimens by latex agglutination but could not find polysaccharides in any of their meningococci group B specimens.

Cheever (1965) believed that the absence of a soluble polysaccharide in this group might be the reason. Maloney *et al.* (1972) said that after the exponential growing time the meningococci group B polysaccharide quickly decreases in the culture medium, groups A and C polysaccharides remaining stable. This is caused by the acid instability of the group B polysaccharide molecule.

When we started work in our laboratory we could not prepare a meningococcal group B antiserum that could be used in latex agglutination, CIE, and protein A co-agglutination. By using strain M2092 (Sara Branham) for the immunisation of rabbits and taking into account the time of growth (8 hours) and the freshness of the culture used as a vaccine, we obtained an excellent precipitating and agglutinating antiserum that was suitable for the detection of group B polysaccharide by the three methods.

Polysaccharides of *Strep. pneumoniae* types 7 and 14 were not found by CIE when the current buffers were used because these polysaccharides are neutral (Anhalt and Yu, 1975; Kenny *et al.*, 1972). This is the reason why test results by CIE were lower than those by the other two methods.

The results of protein A co-agglutination (56.7% positive) of *H. influenzae* type b cannot be compared to the results of latex agglutination (76.7% positive) and CIE (91.7% positive) because a different antiserum was used for protein A co-agglutination. When the same antiserum was used for CIE and protein A co-agglutination, CIE results were 75% positive.

For the purpose of the investigation, the three

methods compared here proved to be quick and simple to perform. Protein A co-agglutination required a very small quantity of antiserum (1/100th of the quantity used in CIE). The sensitivity of protein A co-agglutination was fairly low but would probably be increased by the use of a suitable antiserum. The antiserum combined with protein A will keep for only six months, and this remains a difficulty. Although latex agglutination and CIE are equally sensitive, the former would be preferable as a routine method as it does not require extensive and expensive apparatus.

Other advantages of this method are easy execution of the test and the good keeping qualities, three years at least, of the antiserum combined with latex.

We thank Miss C. Th. P. Hopman for preparing the meningococcal antisera, especially of group B.

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