Comparison of counter-current immunoelectrophoresis, latex agglutination, and radioimmunoassay in detection of soluble capsular polysaccharide antigens of *Haemophilus influenzae* type b and *Neisseria meningitidis* of groups A or C

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SUMMARY Three serological methods, radioimmunoassay (RIA), latex agglutination (LX), and counter-current immunoelectrophoresis (CIEP), for sensitivity in the detection of the capsular polysaccharide antigen of *Haemophilus influenzae* type b or *Neisseria meningitidis* groups A and C were compared. RIA was consistently the most sensitive, LX the next, and CIEP the least sensitive. When RIA and LX were used to test cerebrospinal fluid (CSF) samples of patients with meningitis, they gave very similar results. In only two out of 47 samples, in which RIA detected one of the three antigens, was the amount of the specific polysaccharide too low to be detected by LX. By the serological methods we could detect evidence of specific pathogen in 49 samples, including nine from patients who had received intensive antimicrobial treatment for up to three days and from whom specimens yielded no bacteria on culture. The reactions were specific in all cases except two out of 47 tests positive by LX. From these two CSF samples *N. meningitidis* group B could be cultivated, whereas the LX was recorded positive for *N. meningitidis* of group A in one case, and of group C in the other. The nonspecific reactions could be due to antibodies to bacterial components other than the capsular polysaccharide.

Bacterial meningitis is usually caused by bacteria with acidic polysaccharide capsules. These bacteria shed large amounts of the capsular material in their growth medium, including body fluids, during infection. The recent development of sensitive methods to detect these polysaccharides has led to the realisation that determination of the bacterial antigen could be used as an aid to rapid diagnosis of these diseases. Counter-current immunoelectrophoresis (CIEP) (Greenwood *et al.*, 1971), latex agglutination (LX) (Severin, 1972; Leinonen and Herva, 1977), and radioimmunoassay (RIA) (Siskind *et al.*, 1967; O'Reilly *et al.*, 1975; Käyhty *et al.*, 1977) have been used for this purpose. The sensitivities of each assay have been reported but not compared in the same laboratory. We therefore compared the three methods using solutions of

the pure polysaccharides in respect of *Haemophilus influenzae* type b and *Neisseria meningitidis* groups A and C, for which all three assays have been standardised. We also compared the two most sensitive assays for their ability to aid diagnosis using 86 samples of cerebrospinal fluid (CSF) from patients with meningitis.

Material and methods

CEREBROSPINAL FLUID
From 86 patients with purulent meningitis 86 samples were sent to the Central Public Health Laboratory for antigen determination in 1974-76. Because of an epidemic of group A meningococcal meningitis in Finland in 1973-75, there was a preponderance of cases caused by *N. meningitidis* group A. Bacterial cultures of CSF, blood, and swabs from the nasopharynx were performed in local laboratories, and the identity of the isolates

was confirmed as previously described by Peltola *et al.* (1977a and b). In 71 patients a positive CSF culture was obtained. The finding of *H. influenzae* type b (1 case) or *N. meningitidis* group A (5 cases) in the nasopharynx in patients with clinically typical meningitis was considered indicative of aetiology because the carrier rates for both these organisms were very low in the population at this time (A. Sivonen, personal communication). In three of these cases paired sera were available for antibody determination and showed a specific antibody rise (RIA) in each case (Peltola *et al.*, 1977b). In one case, diagnosis was based only on a specific rise in anti-MenC antibodies (RIA). In eight cases the disease was considered to be of viral origin.

Capsular polysaccharides for the calibration of the assays were the same preparations that we used previously (Käyhty *et al.*, 1977). The radio-iodinated polysaccharide antigens for RIA were also the same as before (Käyhty *et al.*, 1977; Mäkelä *et al.*, 1977).

ANTISERA
Antisera for *N. meningitidis* groups A and C were purchased from Difco Laboratories (Detroit, Michigan). The antiserum for *H. influenzae* type b used in RIA was purchased from Difco and that used in CIEP and LX from Hyland (Division of Travenol Laboratories, Inc, Costa Mesa, California, USA).

SEROLOGICAL METHODS
Counter-current immunoelectrophoresis (Greenwood *et al.*, 1971) was carried out in 1% agarose gel using barbital buffer, pH 8·6, ionic strength 0·05. The electrophoresis was run for one hour at room temperature using Hyland Counterelectrophoresis Power Supply with a constant current of 30 mA. The latex reagents were prepared and the LX assay was performed as described earlier (Severin, 1972; Leinonen and Herva, 1977). RIA was likewise performed as previously described (Käyhty *et al.*, 1977).

Results

Testing with the individual polysaccharides (Table 1) showed that RIA was the most sensitive, LX the next, and CIEP the least sensitive of the methods. All assays were most sensitive with the *H. influenzae* type b polysaccharide, which we could detect at a concentration of 0·5 ng/ml by RIA, of 5 ng/ml by LX, and of 20 ng/ml by CIEP. The detection limits of the meningococcal polysaccharides were two to 10 times higher. All methods gave positive results

Table 1 *Sensitivity of counter-current immunoelectrophoresis (CIEP), latex agglutination test (LX), and radioimmunoassay (RIA) for detection of capsular polysaccharides of* H. influenzae *type b or* N. meningitidis *group A or C*

Method	Capsular polysaccharide detected (ng/ml)		
	H. influenzae type b	N. meningitidis	
		Group A	Group C
CIEP	20	50	75
LX	5	10	25
RIA	0·5	2	5

up to the highest concentration of polysaccharide (10 µg/ml) tested. The general finding in earlier studies was similar: LX compared with CIEP was more sensitive (Severin, 1972; Ingram *et al.*, 1973; Leinonen and Herva, 1977) or equal (Coonrod and Rytel, 1972; Whittle *et al.*, 1974), whereas RIA showed the best sensitivity (O'Reilly *et al.*, 1975; Käyhty *et al.*, 1977). We therefore used only RIA and LX to study the clinical specimens.

The results of the examination of CSF samples from 86 meningitis patients are set out in three groups in Table 2. The first two groups are samples from patients whose meningitis was caused by one of the three organisms (*H. influenzae* type b, *N. meningitidis* groups A or C) that we could detect with the RIA and LX assays. The first group includes all 43 samples from which the specific organism could be grown, whereas the second group consists of 23 samples that were negative on culture, in most cases because they were collected from one to several days after the beginning of intensive antimicrobial treatment. The third group contains samples from patients whose meningitis was of viral origin or was caused by *N. meningitidis* groups B or Y, for which we had no specific RIA or LX assay.

In the first group, most samples (36 out of 43) were positive in both RIA and LX, while three (7%) were negative in both, and four were positive in one or other only. An examination of these four cases did not reveal the reason for the discrepant findings. In the samples that were positive by RIA only, the amount of polysaccharide in the sample (≥100 ng/ml of the *H. influenzae*, 50 ng/ml of the *N. meningitidis* group C polysaccharide) was well within the detection range of the LX assay. The LX-positive, RIA-negative results could reflect a reaction caused by other than antipolysaccharide antibodies adsorbed to the LX reagent (see Discussion).

Table 2 *Detection by radioimmunoassay (RIA) or latex agglutination (LX) of specific capsular polysaccharide in cerebrospinal fluid (CSF) of meningitis patients*

CSF samples	Meningitis caused by*	No. of patients	No. received antibiotic before sample	Specific polysaccharide† in CSF				Amount of polysaccharide in positive samples (ng/ml by RIA) (range)
				+ by RIA and LX	+ by RIA − by LX	− by RIA + by LX	− by RIA and LX	
Group I:								
specific disease,	*H. influenzae* type b	13	0	11	1	1	0	6·0 – ≥ 100
culture-positive	*N. meningitidis* group A	27	1	23	0	1	3	40 – ≥1000
samples	*N. meningitidis* group C	3	0	2	1	0	0	50 – ≥1000
	Total	43	1	36 = 84 %	2	2	3 = 7 %	
Group II:								
specific disease,	*H. influenzae* type b	6	6	2	2	0	2	3·5 – 43
culture-negative	*N. meningitidis* group A	12	9	2	0	0	10	16 and 40
samples	*N. meningitidis* group C	5	5	3	0	0	2	21 – 220
	Total	23	20	7 = 30%	2	0	14 = 60%	
Group III:								
other disease	*N. meningitidis* group B	11	4	0	0	2‡	9	—
	N. meningitidis group Y	1	0	0	0	0	1	—
	virus	8	0	0	0	0	8	—
	Total	20	4	0	0	2	18	

*Based on culture of CSF or blood or, in a few cases, nasopharyngeal samples, and specific antibody rise in paired sera, in conjunction with typical clinical picture.
†Specific polysaccharide = capsular polysaccharide of bacterial type or group of causative organism.
‡Nonspecific reactions: one positive for *N. meningitidis* group A, one positive for *N. meningitidis* group C.

Among the 23 culture-negative samples (group II, Table 2) the specific polysaccharide could still be detected in nine (40%) by RIA or in seven (30%) by LX. In the two CSF samples from patients with *H. influenzae* type b meningitis that were positive by RIA only, there was 3·5 and 5 ng/ml polysaccharide, respectively, and thus below the detection limit of the LX assay. Samples from meningitis caused by *N. meningitidis* group A, on the one hand, and *N. meningitidis* group C or *H. influenzae* type b on the other, seem to differ. Among the latter, the capsular polysaccharide could be detected after antibiotic treatment in seven out of 11 samples, whereas only one out of nine such samples was positive for *N. meningitidis* group A polysaccharide (p < 0·05). In fact a slow elimination of *H. influenzae* type b polysaccharide has been described before and was connected with a poor antibody response to this antigen in infancy (O'Reilly *et al.*, 1975; Granoff *et al.*, 1977). Also the *N. meningitidis* group C polysaccharide is a poor immunogen in infants (Gold *et al.*, 1975) whereas that of group A is definitely better (Mäkelä *et al.*, 1977).

In all the above samples (patient groups I and II) the serological findings were specific, so that the antigen detected corresponded with the organism found on culture and/or suggested by antibody determination.

The third group consists of 20 CSF samples, which would not be expected to be positive in the specific serological tests applied because they came from patients with serous meningitis (8) or purulent meningitis caused by *N. meningitidis* groups B or Y (12). RIA was indeed negative in all of these samples, but two positive LX reactions were seen (both in samples from which *N. meningitidis* group B had been cultivated). One of these nonspecific LX reactions was indicative of *N. meningitidis* group A, the other of group C.

Discussion

How useful is antigen determination in the diagnosis of bacterial meningitis? Firstly, the severity of the condition favours the use of any additional methods that could appreciably increase the number of positive findings. In this series the correct antigen was detected by RIA in 40% (9 out of 23) and by LX in 30% (7 of 23) of culture-negative CSF samples. In nearly all these cases the samples were negative on culture because intensive antimicrobial treatment had been started before sampling, and the amount of bacterial antigen present was relatively low. It has been shown elsewhere that the longer the treatment lasts, the less antigen is left (Granoff *et al.*, 1977). Under other conditions, for example, in areas less experienced in the prompt taking and correct handling of specimens for CSF culture, or where distances to the laboratory are greater, there would be more negative samples on culture but they would contain large amounts of bacterial antigen. Among such samples, the serological demonstration of antigen by either RIA or LX

would be expected to succeed in approximately 90% of cases. However, bacterial culture should not be neglected for antigen determination—in three cases (7%) the latter was negative by both RIA and LX although culture was positive. We suspect that these samples were taken very early in the course of the disease when the number of bacteria in the CSF was low, but we cannot verify this interpretation because of a lack of accurate records of the timing of the samples. An equally compelling reason for culture is the importance of obtaining the bacteria for antibiotic sensitivity determination, especially with the now increasing resistance of *H. influenzae* (Thomas *et al.*, 1974).

The second and, in most instances, main argument for antigen determination is the time factor. All three serological methods produce results more quickly than culture. In this respect LX is the best test for it can be performed literally as a bedside assay, while CIEP requires 15 to 60 minutes in the laboratory, and RIA two to three hours. In the present series a standard overnight incubation was used in the RIA, but we have shown previously that although shortening the incubation time decreases the sensitivity slightly it does not influence the result (Käyhty *et al.*, 1977).

The value of antigen determination as a routine method would be increased if there were assays for as many potential CSF pathogens as possible. In children 3 months to 5 years old, the assays tested here would cover the majority of cases of purulent meningitis (60% in Finland in 1977—(Peltola and Mäkelä, to be published)) whereas in the neonatal period streptococci and enteric bacteria prevail, and in older patients *H. influenzae* is rare. The capsular polysaccharide of *N. meningitidis* group B is a poor immunogen, and it is difficult to develop serological assays for it. With a good antiserum (for example, serum produced in the horse (Sivonen *et al.*, 1977)) CIEP can be positive (Feldman, 1977) and also a latex reagent can be produced (Leinonen, in preparation). These assays have not yet been tested for sensitivity sufficiently to allow a comparison with the assays used here. Both CIEP and LX based on a multivalent capsular polysaccharide antiserum have revealed pneumococcal antigen in CSF and also in patient serum (Coonrod and Rylko-Bauer, 1976).

SPECIFICITY OF SEROLOGICAL ASSAYS

All the assays use essentially the same kind of antiserum, produced in animals by immunisation with whole bacteria and probably containing antibodies to many other bacterial components besides the capsular polysaccharide. A purer immunogen could not be used because these polysaccharides are poorly immunogenic in animals. The specificity of RIA is increased by the radioactive polysaccharide whose specific binding to the antiserum is prevented by the bacterial polysaccharide to be assayed. All the 47 RIA-positive reactions in this series were specific. CIEP measures precipitation of soluble electronegative material only and is therefore also expected to be rather specific for capsular polysaccharide but false-positive and cross-reactions have been described with commercial antimeningococcal sera (Shackelford *et al.*, 1974).

The LX agglutination might be expected to be least specific. However, among the 86 CSF samples tested we found only two that gave an apparently nonspecific positive reaction in the LX assay, whereas a specific LX reaction (corresponding with the causative agents otherwise identified) was seen in 45 samples. The two nonspecific LX reactions indicated *N. meningitidis* group A (1 case) or group C (1 case) and occurred in samples positive for *N. meningitidis* group B. We think that they could have been caused by antibodies directed to bacterial components other than the capsular polysaccharide. This could also explain the two CSF samples that were negative in RIA but positive in LX (one for *H. influenzae* type b, one for *N. meningitidis* group A). These latter two reactions were, however, specific for the organism identified on culture. If this interpretation of the origin of nonspecific reactions is correct, they can be expected mainly between closely related bacteria likely to share many antigens. Important distinctions in terms of treatment are those between haemophilus and *N. meningitidis* or *D. pneumoniae*, for haemophilus must not be treated with penicillin. In this series we had 19 cases of *H. influenzae* meningitis, of which 14 were positive by LX for the specific antigen, none giving misleading nonspecific reactions.

It seems that the LX assay is an important assay in every hospital to which patients with purulent meningitis might be sent. It is easy to perform and read and no complex equipment is required. RIA is demanding in respect of reagents since the radioactive antigen has to be prepared and standardised every two months. The LX reagents are stable for at least six months in the refrigerator and do not need standardisation or controls at the time of testing. Unfortunately, however, the LX reagents are not commercially available. In Finland they are prepared and provided through the Public Health Laboratory system, and this could be followed in other countries.

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