The latex agglutination test versus counterimmunoelectrophoresis for rapid diagnosis of bacterial meningitis

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A modified latex agglutination (LA) test was compared with Gram-staining and counterimmunoelectrophoresis (CIE) for the rapid detection in the cerebrospinal fluid (CSF) of antigen to *Haemophilus influenzae* type b, *Neisseria meningitidis* groups A, B and C, *Escherichia coli* K1, *Streptococcus pneumoniae* and group B streptococci, seven frequent causes of bacterial meningitis in children. Of 50 CSF samples from patients with culture-proven bacterial meningitis 90% were correctly shown by the LA test to contain antigen of the responsible organism. Gram-staining revealed organisms in 80% of 45 of these samples. In 75% of the 40 samples that were of sufficient volume for CIE, positive results for the appropriate antigen were obtained. The concentration of antigen detected in the CSF by the LA test varied from undetectable to 800 000 ng/ml. Patients with a high concentration (more than 2000 ng/ml or a positive result at dilutions of CSF over 1/8) were significantly more likely to have a poor response to therapy (two died and two had persistent pleocytosis or bacteria in the CSF) than patients with a lower concentration (4/16 v. 0/18, $P < 0.05$). After appropriate therapy was begun the concentration of antigen fell dramatically, but measurable amounts of antigen persisted in the CSF for up to 6 days. The LA test detected bacterial antigen at concentrations 2 to 70 times below the lower limit detected by CIE. In seven additional patients who had received antibiotics before lumbar puncture was performed the LA test detected antigen from meningitis-causing bacteria even though cultures of the CSF were sterile. In another 145 patients who did not have meningitis the results of the LA test were negative. The LA test, done as described in this article, is easier to perform than CIE and should be a useful addition to the diagnostic tests carried out on the CSF of any patient suspected of having meningitis.

Un test modifié d'agglutination sur latex (AL) a été comparé à la coloration de Gram et à la contre-immunoélectrophorèse (CIE) pour détecter rapidement dans le liquide céphalo-rachidien (LCR) les antigènes de l'*Haemophilus influenzae* type b, le *Neisseria meningitidis* groupes A, B et C, l'*Escherichia coli* K1, le *Streptococcus pneumoniae* et les streptocoques groupe B, sept causes fréquentes de méningite bactérienne chez l'enfant. Sur 50 échantillons de LCR provenant de patients ayant une méningite bactérienne démontrée par culture le test AL a décelé correctement l'antigène du microorganisme responsable dans 90% des cas. La coloration de Gram a révélé des microorganismes dans 80% de 45 de ces échantillons. Des résultats positifs pour l'antigène approprié ont été obtenus dans 75% de 40 échantillons dont la quantité était suffisante pour la CIE. Les concentrations d'antigène décelé dans le LCR par le test AL variaient de quantités non décelables à 800 000 ng/ml. Les patients montrant une concentration élevée (plus que 2000 ng/ml ou un résultat positif à une dilution du LCR supérieure à 1/8) étaient, de façon significative, plus susceptibles de répondre défavorablement au traitement (deux sont décédés et deux ont présenté une pléocytose persistante ou la présence persistante de bactéries dans le LCR) que ceux qui avaient de faibles concentrations (4/16 contre 0/18, $P < 0.05$). Après la mise en route d'un traitement approprié les concentrations d'antigène se sont abaissées de façon spectaculaire, mais des quantités décelables d'antigène persistaient jusqu'à 6 jours dans le LCR. Le test AL a détecté les antigènes bactériens à des concentrations de 2 à 70 fois plus faibles que la limite inférieure de détection de la CIE. Chez sept autres patients qui ont reçu des antibiotiques avant la ponction lombaire, le test AL a décelé les antigènes de bactéries responsables de la méningite alors même que les cultures du LCR étaient négatives. Chez 145 autres patients qui ne souffraient pas de méningite les résultats du test AL sont demeurés négatifs. Le test AL, fait de la façon décrite dans cet article, est plus facile à exécuter que la CIE et devrait s'avérer une addition utile aux épreuves diagnostiques pratiquées sur le LCR des patients soupçonnés d'être atteints d'une méningite.

Determining the causative organism is an important initial step in the management of bacterial meningitis. Although the identity of the organism is often suggested in a Gram-stained specimen of cerebrospinal fluid (CSF), confirmation requires culture, and it is usually 18 hours or more before the culture results can be interpreted. Bacterial growth may, however, be delayed or inhibited if the patient received an antibiotic before the sample of CSF was obtained. Therefore, a number of investigators have employed immunologic or chemical methods to detect various bacterial antigens in the CSF.[1-15]

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Many of these techniques are difficult to perform in the usual diagnostic microbiology laboratory. The latex agglutination (LA) test, as described by Severin,[13] has been used to diagnose meningitis due to *Haempphilus influenzae* type b, *Neisseria meningitidis* groups A and C, and group B streptococci.[7,10,14,15] The test is easily performed and can be interpreted within 10 minutes. In pediatric centres where *H. influenzae* type b is responsible for most cases of meningitis a test for only one organism in the CSF is a useful diagnostic tool. In our centre, however, at least seven types of bacteria (*H. influenzae* type b, *N. meningitidis* groups A, B and C, *Escherichia coli* K1, *S. pneumoniae* and group B streptococci) frequently cause meningitis.[16] Considerable inconvenience and risk of contamination would result if seven LA tests were performed in the conventional manner with microscope slides. For this reason we have modified the procedure described by Severin: we place ready-to-use sensitized latex particles in sealed microtitre wells. We have compared the sensitivity of the modified LA test, Gram-staining and another immunologic method, counterimmunoelectrophoresis (CIE), and report here our experience with these methods over 2½ years.

Patients, materials and methods

Patients

A CSF sample was obtained for culture from 207 patients suspected of having bacterial meningitis on the basis of clinical and CSF findings; 190 of the samples were obtained at the Izaak Walton Killam Hospital for Children, Halifax, and the remainder were forwarded from other centres for confirmation of the diagnosis or because the patient's illness constituted a diagnostic challenge. Approximately 30% of the patients had received antibiotics orally before the CSF sample was obtained.[16] Smears of CSF were prepared and stained and cultures performed by routine microbiologic techniques for isolation and identification.[17] Patients over 4 weeks of age who were considered to have meningitis were initially given ampicillin (400 mg/kg daily) and chloramphenicol (100 mg/kg daily) intravenously until the results of culture and bacterial sensitivity testing were available. They were then treated with one antibiotic given by intravenous infusion for at least 10 days. Neonates were treated with ampicillin plus an aminoglycoside in appropriate dosages for age.

LA testing and CIE

The samples of CSF were tested immediately or stored at 4°C until tested by LA and CIE, usually within 12 hours after they had been obtained. The volume was not always adequate for both tests.

A technologist who was unaware of the clinical or other laboratory findings tested the samples, using all of the available antiserum preparations for each one. Samples with marked pleocytosis but negative reactions for bacterial antigen in one or both of the immunologic tests were concentrated fivefold with an Amicon CS-15 concentrator (Amicon Corporation, Lexington, Massachusetts).

For the LA test, latex particles (Difco Laboratories, Detroit) were sensitized with antiserum by the method described by Severin.[13] Latex reagents prepared in this manner were stable when stored in glass containers at 4°C for at least 6 months. After 10 μl of each type of sensitized latex particles had been placed by Gilson pipette (Mandel Scientific Company, Montreal) in each well of a flat-bottomed polystyrene microtitre plate (Fisher Scientific Company, Mississauga, Ont.) 25 μl of the CSF sample or purified antigen was added to each well. The microtitre plate was then gently agitated for 3 minutes. Each well was examined closely for agglutination, indicating a positive reaction, with a Leitz inverted microscope (Wild Leitz Canada Ltd., Willowdale, Ont.) at 100× magnification.

CIE was carried out with 1% agarose in a sodium barbital buffer (pH 8.6).[8] Fresh agarose slides were prepared for each study, and the reservoir barbital buffer was changed weekly. Slides were examined with a 4× lens and oblique light after refrigeration at 4°C for 45 minutes and again the next day after storage in a moist chamber at 4°C.

Antigens

For the LA test and CIE, positive and negative controls with purified or partially purified bacterial antigen were used. *H. influenzae* type b polysaccharide was provided by Dr. P. Anderson, Rochester, New York. *N. meningitidis* groups A and C polysaccharides were donated by Merck Frosst Laboratories, Dorval, PQ; the group B polysaccharide, which is identical to the *E. coli* K1 polysaccharide,[18] was provided by Dr. J.R. Robbins, Bethesda, Maryland. For the *S. pneumoniae* antigen a vaccine composed of 14 pneumococcal capsular types (Merck Sharpe & Dohme, Montreal) was employed. Crude group B streptococcal antigen was prepared by an acid extraction method described by Lancefield.[19] *H. influenzae* type b antiserum was provided by Dr. D. Scheifele, Vancouver. Antisera to *N. meningitidis* groups A and C were provided by the Canadian Communicable Diseases Centre, Ottawa; the group B antiserum was provided by Dr. J.R. Robbins and was also used to detect *E. coli* K1. *S. pneumoniae* omniserum was obtained from Statens Seruminstitut, Copenhagen. Group B streptococcal antiserum was obtained from Hyland Laboratories, Montreal. For the LA test the antiserum was diluted, whereas for CIE it was used undiluted.

Analytic methods

We estimated the concentration of bacterial antigen in the CSF by titrating the appropriate latex preparation against serial twofold dilutions of CSF and comparing the results with those of serial twofold dilutions of bacterial antigen. We then multiplied the reciprocal of the final dilution of CSF giving a positive reaction by the lowest concentration of purified bacterial antigen having the same degree of reactivity.

Sensitivity was defined with the formula TP/(TP + FN), where TP is the number of true-positive results and FN the number of false-negative results. The "gold

standard" used to indicate meningitis was the culture of bacteria from the CSF. Fisher's exact test was used to determine the significance of differences between groups of patients.

Results

In 145 of the 207 patients suspected of having bacterial meningitis no organisms were seen by Gram-staining, the cultures were sterile, and the LA tests and CIE had negative results. In all but 4 of the 145 the clinical condition was judged not to be meningitis, and antibiotic therapy was either not begun or was stopped within 3 days after the CSF sample was obtained. The four patients who had received antibiotics before the CSF sample was obtained were treated for meningitis even though the laboratory findings did not support this diagnosis.

In five cases bacteria were isolated from the CSF for which appropriate antisera were not available; these included a nontypable strain of *H. influenzae*, *N. meningitidis* group W135, *Citrobacter freundii*, *Staphylococcus epidermidis* and *Mycobacterium tuberculosis*. In all five cases the CSF sample gave negative results with the LA test and CIE.

In 50 of the 57 remaining cases microorganisms for which antisera were available were identified in the CSF (Table I). The LA test correctly identified the organism in 44 of the 50 cases when the CSF was used unconcentrated and in 1 case only when the CSF had been concentrated. Gram-staining revealed organisms in 80% of 45 cases. CIE had positive results in 30 of 40 cases; however, the result was initially negative in 3 cases, becoming positive only after the slides had been stored at 4°C for 18 hours. Three CSF samples gave a positive reaction in the LA test for more than one organism — *N. meningitidis* group B plus another species; the correct species was easily identified by the strength of the reactions. The antiserum used to prepare the latex particles was not affinity purified and therefore could cross-react to some extent with human tissue or bacterial antigens common to several species.

In the other seven cases the CSF cultures were sterile, but bacterial antigen was detected in the CSF (Table II). Each of the patients had received antibiotics before the CSF sample was obtained. In three patients bacterial meningitis was considered the likely final diagnosis on the basis of strong supportive clinical and laboratory evidence. In the remaining four patients bacterial meningitis due to the organism identified by the LA test or CIE could not be excluded, and the patients were given adequate treatment for this disease.

The concentration of bacterial antigen detected in the CSF by the LA test varied widely at the time of diagnosis, from 3 to approximately 800 000 ng/ml. It was extremely high (800 000 and 3200 ng/ml respectively) in the two patients who died (one within a day of the diagnosis of meningococcal meningitis and the other several months after the diagnosis of severe hydrocephalus) and was similarly high in only four of the patients who survived.

Repeat lumbar punctures were not routinely done during the initial management of bacterial meningitis. However, when available, CSF from follow-up lumbar punctures was tested for bacterial antigen. The concentration of antigen decreased dramatically in most instances within 48 hours after therapy was started (Fig. 1); although it persisted in the CSF for up to 6 days, at the end of therapy bacterial antigen was not detected in the CSF of any patient. One of the patients in whom a high concentration of bacterial antigen persisted was subsequently discovered to have been treated with an antibiotic to which the organism was resistant.

The antigen concentration in the CSF predicted the clinical course, in that it was high (either the level was more than 2000 ng/ml or the LA test had a positive result at dilutions over 1/8) in all of the patients who had a poor response to therapy (two died and two had

Table I—Detection of bacteria or bacterial antigen by Gram-staining, latex agglutination (LA) test and counterimmunoelectrophoresis (CIE) in samples of cerebrospinal fluid (CSF) from patients with meningitis

Organism isolated	No. of positive results/total no. of tests		
	Gram-staining*	LA test	CIE†
Haemophilus influenzae type b	19/25	26/29	19/23
Neisseria meningitidis			
Group A	1/2	2/2	2/2
Group B	3/3	3/3	1/2
Group C	6/6	6/6	4/6
Escherichia coli K1	2/2	3/3	3/3
Streptococcus pneumoniae	3/4	2/4	0/1
Group B streptococci	2/3	3/3	1/3
Total	36/45	45/50	30/40
Sensitivity of test (%)	80	90	75

*The results were not recorded for five patients.
†In 10 patients this test was not performed because of an insufficient sample of CSF.

Table II—Data for patients with sterile CSF in which bacterial antigen was detected, the CSF samples having been obtained after antibiotics were given*

Patient no.	Likely organism	CSF pleocytosis	Result of test			Final diagnosis
			Gram's staining	LA test	CIE	
1	*H. influenzae* type b	++	NBS	++	+	Meningitis
2	*H. influenzae* type b	+	NBS	+	–	?
3	*N. meningitidis* group A	+	NBS	+	–	?
4	*E. coli* K1	++	Gram-negative rods	+	–	Meningitis
5	*E. coli* K1 or *N. meningitidis* group B	+	NBS	+	+	Meningitis
6	*N. meningitidis* group C	+	NBS	+	–	?
7	*N. meningitidis* group C	–	NBS	–	+	?

*++ = marked or strongly positive; + = less marked or positive; – = not present or negative; NBS = no bacteria seen.

persistent pleocytosis or bacteria in the CSF), whereas it was lower in all the patients who responded rapidly to therapy (4/16 v. 0/18, $P < 0.05$).

When the LA test and CIE were performed in parallel with freshly prepared reagents and purified bacterial antigen a marked difference was noted in the results: the LA test gave positive reactions at concentrations of bacterial antigen 2- to 70-fold lower than those needed for a positive reaction with CIE (Table III). The variance of serial estimates of the *H. influenzae* type b antigen concentration was 2.4 ng/ml with the LA test.

Discussion

Our results suggest that the modified LA test is useful for rapid identification of the bacteria that commonly cause meningitis in children. It proved convenient for testing several types of bacterial antigen at the same time and was more sensitive than CIE for CSF samples from patients with meningitis. The limit for detection of purified bacterial antigen was lower with the modified LA test. In addition, the test can be performed and its result interpreted within 10 minutes of receipt of the CSF sample, whereas CIE requires several hours.

The LA test may be particularly useful in evaluating CSF samples obtained from patients who have received antibiotic therapy, for even though the CSF may be sterile the bacterial antigen may still be present. It persisted for up to 6 days in the CSF of patients in our series who were receiving intravenous antibiotic therapy. In the patients who were adequately treated the concentration of bacterial antigen fell dramatically within 48 hours of the start of treatment. Of the 16 patients with high concentrations of antigen 4 had a poor response to therapy, whereas all 18 of the patients with a lower concentration did well. In addition, one patient with a persistently high antigen concentration was shown to have been inappropriately treated. Because the concentration appears to correlate well with the number of viable bacteria and the severity of the disease,[20] the detection and quantitation of bacterial antigen in the CSF may also be useful in predicting sequelae. Long-term assessment of these patients is under way to evaluate this possibility.

Our findings are in accord with those of others who have found an LA test useful, when combined with Gram's staining and culture, in establishing the cause of meningitis.[4,7,9,10,13-15] The test's sensitivity depends on the quality of the antiserum employed. Since only IgG is adsorbed at the surface of the polystyrene latex particles, high-titre antiserum predominantly of this class is desirable.[21] The sera that we used were selected because of their sensitivity when tested with purified bacterial antigen.

Before any diagnostic test is introduced for routine use in a clinical laboratory a full assessment of the cost/benefit relation should be considered. The LA test, as described, has been introduced and monitored in a carefully controlled laboratory setting. Under these conditions its sensitivity was found to be 90%. Since the test did influence medical management in cases of partially treated meningitis and hastened the institution of specific antibiotic therapy, its clinical usefulness seems apparent. The reagents required for each test cost approximately 10¢.

The staphylococcal coagglutination test has also recently been described as a rapid method for diagnosis of meningitis.[14] It appears to be just as sensitive as the LA test for detecting antigen in the CSF. Both tests have advantages over CIE and can be performed in diagnostic microbiology laboratories with limited facilities. In the modified LA test, as described here, the use of flat-bottomed microtitre plates and an inverted microscope, although adding to the expense of the test, offers considerable advantages in convenience and safety when multiple tests are to be done. In centres where several different organisms cause meningitis this system may be the only practical means of performing such tests.

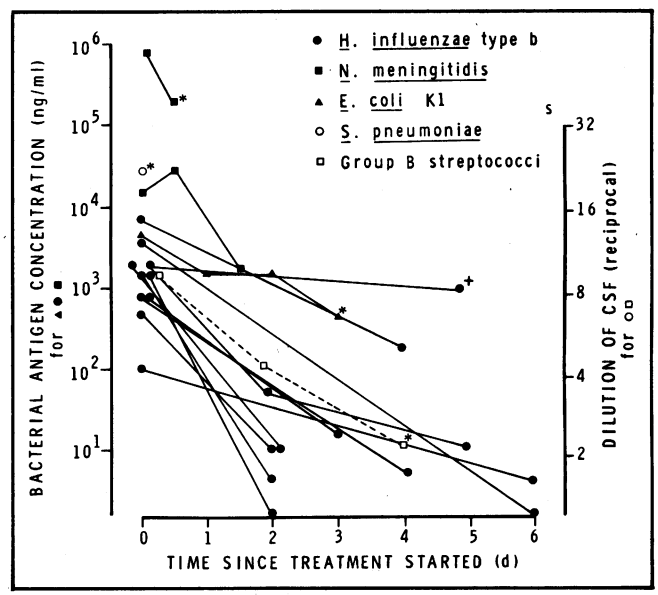

FIG. 1—Concentration of bacterial antigen in cerebrospinal fluid (CSF) before and during antibiotic treatment, determined by modified LA test and calculated as concentration of purified polysaccharide or as final dilution of CSF giving a positive reaction. H. = Haemophilus; N. = Neisseria; E. = Escherichia; S. = Streptococcus. Asterisks indicate abnormal outcome: death (■* and ▲*) or persistence of pleocytosis and bacteria in CSF (○* and □*). Plus sign indicates inappropriate antibiotic therapy.

Table III—Lowest concentration or final dilution of bacterial antigen giving a positive reaction in the LA test and in CIE performed in parallel

Bacterial antigen	LA test	CIE
	Lowest concentration (ng/ml)	
H. influenzae type b	4.7	12.5
N. meningitidis		
Group A	75	150
Group B	36	2500
Group C	23	780
E. coli K1	36	2500
	Final dilution	
S. pneumoniae	1/4000	1/2000
Group B streptococci	1/900	1/50

Although rapid identification of bacterial antigen in the CSF may suggest the correct diagnosis it should be attempted only after the CSF has been inoculated on appropriate media. Isolation of the causative organism will remain the single most valuable diagnostic test.

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Why is the number of pregnancies among teenagers decreasing?

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The issue of pregnancy among adolescent women has received considerable attention from the media. Contrary to common belief, both the numbers and the rates of such pregnancies, even when data on abortion are included, have been declining. Patterns of contraception may account for some of the decrease; however, more study is required. In the past, unmarried teenagers who became pregnant either got married or put the baby up for adoption. Now they can either have an abortion or keep the baby. Solutions to the problems of pregnancy among teenagers must therefore be addressed to these altered social consequences rather than to misleading comments about "epidemics", with their suggestion of increased rates of pregnancy.

La question de la grossesse chez les adolescentes a passablement retenu l'attention des média d'information. Contrairement à la croyance répandue et même si l'on tient compte des statistiques d'avortement, le nombre aussi bien que le taux de ces grossesses ont décliné. Les modes de contraception peuvent expliquer une partie de cette baisse; toutefois, de nouvelles études sont nécessaires. Autrefois, les adolescentes célibataires qui devenaient enceintes se mariaient ou laissaient leur bébé en adoption. Maintenant, elles peuvent soit obtenir un avortement ou garder leur bébé. Les solutions aux problèmes de la grossesse chez les adolescentes doivent donc être envisagés en fonction de ces conséquences sociales modifiées plutôt que par rapport à des commentaires trompeurs sur une "épidémie", qui laissent supposer une augmentation du taux des grossesses.

Social and health agencies and the media have recently been paying a great deal of attention to the issue of pregnancy among adolescent women. Phrases such as "epidemic of teenage pregnancies", "babies having babies" and "schoolgirl mothers" have been coined and popularized, and calls for action have been issued by government ministries, voluntary agencies and concerned physicians.[1-4] Because of this perception of "crisis", we decided to review the comprehensive and highly reliable data on pregnancy, abortion and fertility provided by Statistics Canada.

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