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Comparison of Slide Coagglutination Test and Countercurrent Immunoelectrophoresis for Detection of Group B Streptococcal Antigen in Cerebrospinal Fluid from Infants with Meningitis

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The usefulness of Phadebact streptococcus reagents for the detection of group B streptococcal antigen in cerebrospinal fluid was evaluated in 54 infants with meningitis and in 22 normal infants. Antigen was detected by slide coagglutination in 19 (82.6%) and by countercurrent immunoelectrophoresis in 20 (87.0%) of 23 cerebrospinal fluid specimens from infants with group B streptococcal meningitis at admission. After initiation of antimicrobial therapy, antigen could be detected in 11 of 19 (by slide coagglutination) and 7 of 18 (by countercurrent immunoelectrophoresis) cerebrospinal fluids. False-positive reactions were noted by slide coagglutination in one infant with *S. bovis* meningitis and one with group B streptococcal bacteremia without meningitis; none occurred with countercurrent immunoelectrophoresis. The commercial availability, simplicity, sensitivity (82.6%), and specificity (96.4%) of the Phadebact slide coagglutination test for detecting group B streptococcal antigen in cerebrospinal fluid suggest that it may be useful for the early and rapid diagnosis of group B streptococcal meningitis.

The finding that the protein A antigen in the cell wall of several strains of *Staphylococcus aureus* combines with the Fc portion of human (4) and rabbit (6) immunoglobulin G, leaving the Fab fragment available for interaction with homologous antigens, has led to the development of several methods to detect bacterial antigens by agglutination of specific antibody-coated staphylococci. A commercially available slide coagglutination (CAT) method (Phadebact Streptococcus Test) has been reported to allow the serological identification of isolates of group A, B, C, and G streptococci from plate (1) and broth (9) culture media. Although the detection of bacterial antigen in cerebrospinal fluid (CSF) of patients with meningitis has provided the rapid determination of the causative agent in several reported studies, CAT methods have been evaluated infrequently (7, 10), and Phadebact reagents have never been tested. Since group B streptococci are associated frequently with meningitis in the first three months of life, the present study was designed to determine the usefulness of the Phadebact group B reagent in detecting group B streptococcal antigen in CSF specimens. In addition, since countercurrent immunoelectrophoresis (CIE) has been previously reported as a sensitive method for the rapid diagnosis of group B streptococcal meningitis (2, 3, 8), CSF specimens were tested concomitantly by slide CAT and CIE.

MATERIALS AND METHODS

Clinical specimens. CSF specimens were collected from 79 infants on admission for testing by CAT and CIE. Thirty-nine of these infants had culture-proved bacterial meningitis, whereas the remainder had diagnoses of aseptic meningitis (15), group B streptococcal bacteremia without meningitis (2), and fever with normal CSF (22). The group B streptococcal isolates were serogrouped and serotyped by the capillary precipitin method of Lancefield, employing hyperimmune rabbit antisera prepared in our laboratory (5). Portions of CSF obtained after the initiation of antimicrobial therapy in several of the 23 patients with group B streptococcal meningitis were also tested. All specimens were analyzed immediately or were stored at 4°C until testing.

CAT. Phadebact Streptococcus Test reagents (Pharmacia Diagnostics, Piscataway, N.J.) were provided through the courtesy of Gary Britton and were prepared according to the test package insert. For each test, a drop of each reconstituted streptococcal reagent (groups A, B, C and G) was mixed on a glass slide with a drop of CSF, rotated manually for 60 s, and examined for agglutination. Agglutination was graded as 1+ (fine granularity, milky background), 2+ (small clumps, milky background), 3+ (large and small clumps, clear background), or 4+ (large clumps, clear background) and recorded. Only 3 to 4+ reactions were regarded as positive. The lack of significant agglutination by the group A, C, and G reagents served as controls. All tests were performed by two different observers, and results were averaged.

CIE. CIE was performed by using a plastic electrophoresis chamber (Hyland Laboratories, Costa Mesa,

Calif.) in plates prepared with sodium barbital buffer (pH 8.6) in 1% agarose and hyperimmune group B streptococcal antisera prepared in rabbits in our laboratory. These methods have been detailed elsewhere (2). Appropriate antigen and antisera controls were included in each test plate.

RESULTS

Group B streptococcal antigen was detected by CAT in 19 (82.6%) of the CSF specimens obtained at the time of hospital admission from 23 infants with group B streptococcal meningitis. Of the CSF specimens from 16 patients with non-group B streptococcal meningitis, only one was positive by CAT; *Streptococcus bovis* was isolated from this specimen. The organisms isolated from the 15 other patients included *Streptococcus pneumoniae* (3 patients), *Haemophilus influenzae* type b (5 patients), *Neisseria meningitidis* (5 patients), *Proteus mirabilis* (1 patient), and *Citrobacter diversus* (1 patient). An additional false-positive reaction was observed in the CSF of one of three infants with group B streptococcal bacteremia without meningitis. No coagglutination was noted in CSF specimens from 15 infants with aseptic meningitis or from 22 febrile infants with normal CSF.

These above results for CAT were compared with those for CIE to contrast their sensitivity and specificity (Table 1). Antigen was detected in 20 of 23 (87%) initial CSF specimens from infants with group B streptococcal meningitis. No false-positive reactions were observed with CIE. Both methods of antigen detection had similar sensitivities (CAT, 82.6%; CIE, 87%) and specificities (CAT, 96.4%; CIE, 100%).

False-positive reactions (3 to 4+) were observed by CAT with group A, C or G reagents in 5 of the 23 CSF specimens from patients with group B streptococcal meningitis. In four of these specimens, the reaction with the group B reagent was detectably stronger; three of the four had agglutination with the group G reagent alone and one with both group C and G reagents. The remaining CSF had 4+ CAT reactions noted with both group A and B reagents, and the quantity of CSF remaining was insufficient for testing with group C and G reagents.

TABLE 1. *Comparison of CAT and CIE in detecting group B streptococcal antigen in CSF specimens at admission*

Diagnosis	No. of positives/no. tested	
	CAT	CIE
Meningitis:		
Group B *Streptococcus*	19/23	20/23
S. pneumoniae	0/3	0/3
S. bovis	1/1	0/1
H. influenzae, type b	0/5	0/5
N. meningitidis	0/5	0/5
P. mirabilis	0/1	0/1
C. diversus	0/1	0/1
Aseptic	0/15	0/15
Group B streptococcal bacteremia without meningitis	1/3	0/3
Normal infants	0/22	0/22

The persistence of antigen in the CSF of several patients with group B streptococcal meningitis was evaluated by CAT and CIE, and the results are summarized in Table 2. Group B streptococcal antigen was detected in 11 of 19 (CAT) and 7 of 18 (CIE) CSF specimens obtained after the initiation of antimicrobial therapy. Of these 19 specimens, only 3 had group B streptococci isolated from cultures. Antigen persisted in the positive specimens for a mean duration of 6.3 days by CAT and 5.1 days by CIE (range, 1 to 18 days).

DISCUSSION

The results of this study indicate that CAT is a simple and sensitive technique for the detection of group B streptococcal antigen in CSF. When compared with the widely used technique of CIE, CAT was comparable in sensitivity (82.6%) to CIE (87%) in this investigation and in those reported by others (64 to 81%) (8; P. G. Shackelford and B. W. Stechenberg, Pediatr. Res. **11**:505, 1977; D. L. Ingram, E. L. Pendergrass, J. D. Thullen, and C. D. Yoder, Pediatr. Res. **12**:494, 1978; C. J. Baker, B. J. Webb, C. V. Jackson, and M. S. Edwards, Pediatrics, in press). However, the results from the present study are quite different from those reported by Thirumoorthi and Dajani (10) in which none of 10 patients with group B streptococcal meningitis had group B antigen detected in CSF, serum, or urine specimens by CAT or CIE. Reasons for this difference are not readily apparent, but, in the instance of CIE, the best results for the detection of group B streptococcal antigen

TABLE 2. *Temporal detection of group B streptococcal antigen in CSF*

Time (days)	No. of specimens positive/no. tested (%)	
	CAT	CIE
0	18/23 (82.6)	19/23 (87)
1	2/3	1/2
2	5/6	4/6
3–10	1/1	1/1
>10	3/9	1/9
Total specimens after admission	11/19 (57.9)	7/18 (38.9)

in CSF have been reported with use of laboratory rather than commercially prepared antisera (8; C. J. Baker, et al., Pediatrics, in press). Even with high-titered laboratory group B streptococcal antisera, CAT was more sensitive than CIE (57.9 versus 38.9%) in detecting antigen after the initiation of antimicrobial therapy. This point may be of particular importance to tertiary care centers in the evaluation of neonates who have received therapy before collection of cultures.

One problem encountered during the study was the occurrence of false-positive CAT reactions with group A, C or G reagents and CSF of patients with group B streptococcal meningitis. In all specimens tested except one, however, the reaction with the group B reagent was detectably stronger. Therefore, these specimens were considered positive for group B antigen as is instructed by the Phadebact package insert. In addition, no false-positive reactions were noted with these reagents in specimens from infants with non-group B streptococcal meningitis or for those from normal infants. Therefore, the infrequent occurrence of these cross-reactions and the rare false-positive test noted with the group B reagent would not alter the accepted therapeutic approach to patients. Although it is possible that additional cross-reactions might occur with specimens from patients with meningitis due to other streptococci (enterococci, viridans streptococci, etc.), the unusual occurrence of these agents as meningeal pathogens suggest that this possibility would be unlikely to influence the usefulness of CAT for group B streptococcal antigen.

In summary, the commercial availability of CAT (Phadebact), its sensitivity and acceptable specificity, and the accessibility of results within minutes of specimen collection suggest that this technique for detecting group B streptococcal antigen in CSF specimens will facilitate the diagnosis of meningitis.

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