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Quantitation of Bacteria in Cerebrospinal Fluid and Blood of Children with Meningitis and Its Diagnostic Significance

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Cerebrospinal fluid (CSF) specimens from pediatric patients with meningitis were examined for their concentration of microbes and the relationship of this count to the bacteremia levels, microscopy results, and polymorphonuclear leukocyte concentration. A total of 2,031 consecutive CSF specimens were analyzed, of which 63 (3.1%) were positive by culture from the same number of patients. We observed that 85% of the total CSF specimens positive for *Haemophilus influenzae* type b, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* had counts in excess of 10^3 CFU/ml, with 56% of the specimens exceeding 10^5 CFU/ml. A correlation existed between the number of organisms present in the CSF and blood. For example, from a total of 22 patients who had counts of *H. influenzae* $>10^3$ CFU/ml in the CSF, 16 or 73% had levels of bacteremia $>10^3$ CFU/ml. It was also noted that the bacterial concentration had a profound effect on the sensitivity of microscopy. The percentage of positive results increased from 25% with $\leq 10^3$ CFU/ml to 60% in the range of $>10^3$ to 10^5 CFU/ml and to 97% at concentrations of $>10^5$ CFU/ml. Furthermore, a significant correlation ($P < 0.01$) was noted between the concentration of bacteria in the CSF and the number of polymorphonuclear leukocytes observed on microscopy.

Acute bacterial meningitis in children remains an important cause of death and major neurological damage among survivors (6, 12, 13, 16). Microscopic examination of the cerebrospinal fluid (CSF) cultures and antibiotic susceptibility tests are cornerstones for diagnosis and therapy of these infections. Of the various parameters which affect microscopy results, one of the most significant is the concentration of organisms in the body fluid (11). Only limited information is available on the concentration of microbes in CSF and their relationship to microscopy (2, 3).

The three major aims of this study were (i) to determine the magnitude of organisms in the CSF of pediatric patients with meningitis; (ii) to evaluate the relationship between bacterial concentration in CSF and microscopy results; and (iii) to elucidate whether an association exists between the bacterial counts in the CSF and the magnitude of concurrent bacteremia.

MATERIALS AND METHODS

Patient population and specimen processing. A total of 2,031 inpatient and outpatient CSF samples were analyzed at the Children's Hospital of Buffalo, Buffalo, N.Y., for a 16-month period. CSF specimens were procured once per patient before antibiotic therapy. The samples were placed in sterile tubes and had an average volume of 0.5 to 1.0 ml. Blood cultures were processed as previously described (9, 10). In brief, the blood cultures consisted of BACTEC aerobic bottles that had attached quantitative direct plating heparin tubes. A total of 1 to 3 ml of blood was inoculated into the BACTEC bottle, and 0.2 to 1 ml was injected into the quantitative direct plating heparin tube. The time elapsed between the collection of the specimens and their arrival in the laboratory was usually 1 h or less.

Quantitation of bacteria in CSF and blood. The CSF volume was noted, and the specimen was inoculated on a chocolate agar plate by using a 0.01 calibrated loop and spread with a routine bacteriological loop. The plates were incubated at 37°C in 5 to 10% CO_2 and monitored for growth. The number of bacteria per milliliter of CSF was calculated. Calibration of the 0.01 loops was periodically checked by a pour plate procedure. The bacterial count of blood was determined by the quantitative direct plating procedure with sheep blood and chocolate agar plates as previously described (9, 10). The plates were incubated at 37°C in 5 to 10% CO_2 and monitored for growth. The number of bacteria per milliliter of blood was calculated.

Sedimentation and microscopy of CSF. The CSF was centrifuged at 1,500 × *g* for 15 min. The sediment was inoculated onto conventional bacteriological culture media, including chocolate agar, and incubated at 37°C in 5 to 10% CO_2. The average volume for all the CSF specimens was determined to be 0.75 ml, which indicated that the average concentration factor upon sedimentation was 0.75. The observations by microscopic examination of the sediment were related to the number of bacteria determined in the uncentrifuged specimen. Methylene blue and Gram stains were performed on the sediment, and each smear was read by two technologists, both being unaware of the other's results. Several fields were examined per smear. Positive and negative controls were included in every staining procedure. A specimen was considered positive if bacteria were seen in one or more smears. Identification and antibiograms of all isolates were performed by conventional procedures. The data obtained in Table 3 were statistically examined by the chi-square method (15).

RESULTS

During a 16-month period, CSF specimens from pediatric patients with meningitis were examined for their concentration of microbes and the relationship of these results to bacteremia levels and microscopy results. A total of 2,031

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TABLE 1. Quantitation of bacteria in CSF[a]

Bacteria	Total no. of specimens	No. of specimens with the following no. of CFU/ml: ≤10^3	>10^3–10^5	>10^5	% >10^3 CFU/ml
H influenzae type b	36	3	9	24	92
S. agalactiae	9	2	1	6	78
S. pneumoniae	9	3	2	4	67
N. meningitidis	7	2	4	1	72
E. coli	2	0	2	0	100
% of total specimens		16	29	56	

[a] CSF is not centrifuged.

CSF samples was examined, and 63 (3.1%) were found to be positive by culture. All bacteria considered to be contaminants by analysis of the clinical data were excluded from this study.

The distribution of bacteria, the frequency of recovery, and the quantitation of microbes in the CSF are shown in Table 1. The predominant bacteria, *Haemophilus influenzae* type b, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*, represented 97% of the total positive CSF specimens. It is evident from the data in Table 1 that 85% of the total specimens had bacterial counts in excess of 10^3 CFU/ml. In fact, 56% of the specimens had >10^5 CFU/ml. Ninety-two percent of the specimens yielding *H. influenzae* type b had counts in excess of 10^3 CFU/ml (Table 1). This characteristically high magnitude of organisms in the CSF was also observed with certain other bacteria causing meningitis; however, for *Escherichia coli*, more CSF samples are required to obtain a meaningful analysis.

The correlation of the quantity of bacteria in the sediment of the CSF and microscopy results is shown in Table 2. Viable counts below or equal to 10^3 CFU/ml for all organisms were associated with poor microscopic results; only two out of eight specimens were positive. However, as the number of bacteria increased, we observed a dramatic increase in positive microscopy (Table 2). In the range of >10^3 to 10^5 CFU/ml 60% of the CSF specimens were positive. At the level of >10^5 CFU/ml, 97% of the specimens were positive. In contrast, only 25% with counts ≤10^3 CFU/ml were positive. This pattern was observed regardless of the type of organism isolated from the CSF. Thus, the results obtained by microscopy depend closely on the number of organisms present in the sediment of CSF.

TABLE 2. Correlation of CSF[a] colony counts and microscopic[b] results

Bacteria	No. of specimens positive/total no. of specimens with the following no. of CFU/ml: ≤10^3	>10^3–10^5	>10^5
H. influenzae type b	0/3	7/9	23/24
S. agalactiae	0/1	2/2	6/6
S. pneumoniae	2/2	1/3	4/4
N. meningitidis	0/2	2/4	1/1
E. coli		0/2	
% Positive by microscopy	25	60	97

[a] CSF is centrifuged with an average concentration factor of 0.75.
[b] Both Gram and methylene blue stains.

The presence of high numbers of polymorphonuclear leukocytes in the CSF as seen by microscopy is a significant diagnostic laboratory indication of bacterial meningitis. It also permits the observance of intracellular organisms. The relationship between the concentration of bacteria in the CSF and the number of polymorphonuclear leukocytes visualized by microscopy is shown in Table 3. Out of a total of 61 patients, 41 or 67% had a high leukocyte concentration when bacterial counts were >10^3 CFU/ml ($P < 0.01$). In contrast, when the bacterial count was only ≤10^3 CFU/ml, leukocytes were rarely seen. In 10 patients, the bacterial counts of >10^3 CFU/ml were not associated with the presence of numerous leukocytes (Table 3). This result could be a manifestation of the ages of the patients or due to the stage or nature of the infection.

Patients with meningitis invariably have bacteremia, which usually precedes seeding of the CSF by the infecting organism (17). The relationship between bacterial counts in the CSF and the blood is shown in Table 4. Sufficient quantitative data in blood and CSF were available only for *H. influenzae*. As indicated in Table 4, from a total of 22 patients who had counts >10^3 CFU/ml in the CSF, 16 or 73% had levels of bacteremia in excess of 10^3 CFU/ml. Furthermore, three patients whose CSF counts were <10^3 CFU/ml also had correspondingly low numbers of bacteria in the blood. Thus, there was a close association between the magnitude of organisms present in the blood and CSF.

DISCUSSION

Quantitation of microbes in clinical specimens has been proven clinically important in cystitis (1, 8), pyelonephritis (1, 7, 18), bacteremia (3, 17), pneumonia (3), endocarditis (19), and the analysis of burn and wound infections (3). The quantitation of bacteria in CSF of patients with meningitis has received limited attention. In one study by Feldman (4), it was noted that 27 patients with meningitis caused by *H.*

TABLE 3. Relationship between the number of polymorphonuclear leukocytes detected by microscopy and the quantitation of bacteria in the CSF of 61 patients

Bacteria	No. of patients with the following levels of bacteria and polymorphonuclear leukocytes: ≤10^3 CFU/ml, Rare	≤10^3 CFU/ml, Numerous	>10^3 CFU/ml, Rare	>10^3 CFU/ml, Numerous
H. influenzae	1	2	6	27
S. agalactiae	2	0	2	5
S. pneumoniae	2	1	1	5
N. meningitidis	1	1	1	4

TABLE 4. Correlation of the level of *H. influenzae* bacteremia with patients having bacterial counts <1,000 and >1,000/ml of CSF

CSF bacterial counts	No. of patients	No. of patients with the following levels of bacteremia (CFU/ml):			% in excess of 10^3 CFU/ml
		$\leq 10^3$	$>10^3$–10^5	$>10^5$	
<1,000	3	3	0	0	0
>1,000	22	6	11	5	73

influenzae type b, *S. agalactiae*, *S. pneumoniae*, or *N. meningitidis* had CSF bacterial counts ranging from 4.5×10^3 to 3.0×10^8 CFU/ml before antibiotic therapy. It was also observed that patients with pretreatment concentrations of 10^7 CFU/ml or greater often continued to have positive cultures indicative of therapy failure (4). Feldman (5) subsequently reported a relationship between bacterial cell, antigen concentration, and prognosis in meningitis; increases in these parameters led to more neurological damage, subdural effusions, prolonged fever, and greater mortality.

In this study, the patients with meningitis had high concentrations of bacteria in the CSF, with 85% of all specimens having counts in excess of 10^3 CFU/ml and with 56% having $>10^5$ CFU/ml. The high magnitude of bacteria present in the CSF is consistent with clinical data obtained by Feldman (4) and a report by Smith et al. (14), who used an infant rat model with *H. influenzae* type b. Furthermore, in relation to one of the objectives of the study to elucidate whether an association existed between bacterial counts in the CSF and the magnitude of bacteremia, 73% of the patients with *H. influenzae* bacteremia and meningitis had counts in excess of 10^3 CFU/ml in blood as well as CSF. Similarly high concentrations of organisms in blood were previously noted by La Scolea et al. (10) in children with *H. influenzae* and *N. meningitidis* disease. The reason why such large concentrations of bacteria occur in CSF and blood is unclear. Factors such as the rate of bacterial multiplication in vivo and host defenses remain largely undefined. Previous information indicates that the rate of multiplication may be relatively rapid, since CSF levels of *H. influenzae* in infant rats have obtained 10^7 CFU/ml within 24 h after intraperitoneal inoculation (14). High concentrations of organisms are not limited to blood or CSF in that similar results have been reported for urine and wound and burn infections of humans (3).

The diagnosis of meningitis by microscopic examination of CSF is important for the detection and the preliminary characterization of the infecting agent. This facilitates the choice of antibiotic therapy. However, as previously reported (11), the sensitivity of microscopy, in particular the use of the Gram stain, requires a threshold concentration of 10^5 CFU of bacteria per ml for detection, with the possible exception of the acridine orange stain, which is capable of detecting bacteria in concentrations of about 10^4 CFU/ml (11). The data in this study support the concept that the concentration of bacteria has a profound effect on the sensitivity of microscopy. Regardless of the type of organism in the CSF, the percentage of positive microscopic results increased from 25% with $\leq 10^3$ CFU of organisms per ml to 60% in the range of $>10^3$ to 10^5 CFU/ml and to 97% at concentrations of $>10^5$ CFU/ml. A correlation was noted between the concentration of bacteria in the CSF and the number of polymorphonuclear leukocytes observed on microscopy. Large numbers of leukocytes were seen on microscopy with higher bacterial concentrations, and low bacterial numbers were associated with rare leukocytes. High counts of bacteria, however, were associated with low numbers of leukocytes in 10 instances. It should be noted that the interpretation of these data is subject to limitations based on the different age groups of the patients, the nature of the infection, and the procurement of the lumbar puncture(s) in relation to the stage of the disease. The relationship of bacterial concentration in the CSF to other diagnostic tests and to clinical outcome is currently being investigated in our laboratory.

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LITERATURE CITED

1. **Barry, A. L., P. B. Smith, and M. Turck.** 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coordinating ed., T. L. Gavan. American Society for Microbiology, Washington, D.C.
2. **Dietzman, D., G. Fischer, and F. Schoenknecht.** 1974. Neonatal *Escherichia coli* septicemia—bacterial counts in blood. J. Pediatr. **85**:128–130.
3. **Edberg, S. C.** 1981. Methods of quantitative microbiological analyses that support the diagnosis, treatment, and prognosis of human infection. Crit. Rev. Microbiol. **8**:339–397.
4. **Feldman, W. E.** 1976. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. J. Pediatr. **88**:549–552.
5. **Feldman, W. E.** 1977. Relation of concentrations of bacteria and bacterial antigen in cerebrospinal fluid to prognosis in patients with bacterial meningitis. N. Engl. J. Med. **296**:433–435.
6. **Haggerty, R. J., and M. Ziai.** 1964. Acute bacterial meningitis. Adv. Pediatr. **13**:129–181.
7. **Jones, S. R., J. W. Smith, and J. P. Sanford.** 1974. Localization of urinary-tract infection by detection of antibody coated bacteria in urine sediment. N. Engl. J. Med. **290**:591–593.
8. **Kass, E. H.** 1956. Asymptomatic infections of the urinary tract. Trans. Assoc. Am. Physicians **69**:56–64.
9. **La Scolea, L. J., Jr., D. Dryja, and E. Neter.** 1981. Comparison of the quantitative direct plating method and the BACTEC procedure for rapid diagnosis of *Haemophilus influenzae* bacteremia in children. J. Clin. Microbiol. **14**:661–664.
10. **La Scolea, L. J., Jr., D. Dryja, T. D. Sullivan, L. Mosovich, N. Ellerstein, and E. Neter.** 1981. Diagnosis of bacteremia in children by quantitative direct plating and a radiometric procedure. J. Clin. Microbiol. **13**:478–482.
11. **Lauer, B. A., L. B. Reller, and S. Mirrett.** 1981. Comparison of acridine orange and Gram stains for detection of microorganisms in cerebrospinal fluid and other clinical specimens. J. Clin. Microbiol. **14**:201–205.
12. **Sell, S. H. W., R. E. Merrill, E. O. Doyne, and E. P. Zimsky, Jr.** 1972. Long-term sequelae of *Haemophilus influenzae* meningitis. Pediatrics **49**:206–211.
13. **Sell, S. H. W., W. W. Webb, J. E. Pate, and E. O. Doyne.** 1972. Psychological sequelae to bacterial meningitis; two controlled studies. Pediatrics **49**:212–217.
14. **Smith, A. L., D. H. Smith, and D. R. Averill.** 1973. Production of *Haemophilus influenzae* b meningitis in infant rats by intraperitoneal inoculation. Infect. Immun. **8**:278–290.

15. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods, 6th ed. Iowa State University Press, Ames.
16. **Sproles, E. T., III, J. Azerrad, C. Williamson, and R. E. Merrill.** 1969. Meningitis due to *Haemophilus influenzae*: long-term sequelae. J. Pediatr. **75:**782–788.
17. **Sullivan, T. D., L. J. La Scolea, Jr., and E. Neter.** 1982. Relationship between the magnitude of bacteremia in children and the clinical disease. Pediatrics **69:**699–702.
18. **Thomas, V., A. Shelokov, and M. Forland.** 1974. Antibody coated bacteria in the urine and the site of urinary-tract infection. N. Engl. J. Med. **290:**588–590.
19. **Werner, A. S., C. G. Cobbs, D. Kaye, and E. W. Hook.** 1967. Studies on the bacteremia of bacterial endocarditis. J. Am. Med. Assoc. **202:**199–203.