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Microscopic Examination and Broth Culture of Cerebrospinal Fluid in Diagnosis of Meningitis

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We reviewed the results of microscopic Gram stain examination and routine culture for 2,635 cerebrospinal fluid (CSF) samples processed in an adult hospital microbiology laboratory during 55 months. There were 56 instances of bacterial or fungal meningitis (16 associated with central nervous system [CNS] shunt infection), four infections adjacent to the subarachnoid space, four cases of sepsis without meningitis, and an additional 220 CSF specimens with positive cultures in which the organism isolated was judged to be a contaminant. Because 121 of these contaminants were isolated in broth only, elimination of the broth culture would decrease unnecessary work. However, 25% of the meningitis associated with CNS shunts would have been missed by this practice. The most common cause of meningitis was *Cryptococcus neoformans*, followed by *Streptococcus pneumoniae* and *Neisseria meningitidis*. In 48 of 56 (88%) of cases, examination of the Gram-stained specimen revealed the causative organism. If patients who had received effective antimicrobial therapy prior to lumbar puncture are excluded, the CSF Gram stain is 92% sensitive. Microscopic examination incorrectly suggested the presence of organisms in only 3 of 2,635 (0.1%) CSF examinations. Thus, microscopic examination of Gram-stained, concentrated CSF is highly sensitive and specific in early diagnosis of bacterial or fungal meningitis.

Bacterial meningitis is a life-threatening infection. Although patients typically present with fever, headache, stiff neck, and altered mental status, these symptoms may be subtle in elderly or immunocompromised persons (1, 6, 7, 18). Early implementation of appropriate antimicrobial therapy requires prompt identification of the infecting pathogen. Although culture is considered to be the definitive diagnostic test, microscopic examination of a Gram-stained specimen of cerebrospinal fluid (CSF) may provide immediate information about the causative microorganism. Previous studies have suggested that the sensitivity of this technique ranges from 60 to 90% and the specificity approaches 100% (1, 5, 6, 8, 12, 18). Scheld concludes that the overall sensitivity is only 75% (14). It is often unclear whether earlier studies have stratified patients based upon their having received prior antimicrobial therapy. Further, the role of semiquantitative assessment of leukocytes (WBC) by microscopic examination as an indicator of infection (8, 12) is uncertain. The value of using broth culture in various populations is also questionable (9, 10, 17). In the present study, we reviewed the results of microscopic examination and routine culture of 2,635 CSF specimens to establish the predictive value of the cytocentrifuged Gram stain and the usefulness of broth culture in a veteran population.

MATERIALS AND METHODS

Results for all CSF specimens submitted to the Houston Veterans Affairs Medical Center Clinical Microbiology Laboratory from 1 January 1993 through 31 July 1997 were analyzed. CSF specimens were prepared for microscopic examination by cytocentrifugation (Cytospin 3; Shandon Scientific Limited, Cheshire, England) of 0.25 ml of CSF onto a glass microscope slide at 2,000 rpm for 10 min at room temperature. After conventional Gram staining, slides were examined by light microscopy at ×100 magnification to allow quantitation of WBC and then at ×1,000 magnification under immersion oil. The presence and morphology of organisms were noted. WBC were roughly quantified as none (0/slide), rare (<10/slide), few (<1/oil immersion field [OIF]), moderate (1 to 10/OIF), or many (>10/OIF). By laboratory policy, all CSF Gram stains showing organisms and/or at least moderate WBC were reviewed by senior staff. For culture, 0.15 ml of uncentrifuged CSF specimen was inoculated onto each of one 5% sheep blood plate and one chocolate agar plate (Becton Dickinson Microbiology Systems, Cockeysville, Md.), and 1.0 ml was inoculated into 5 ml of brain heart infusion broth with X and V factors (Carr Scarborough Microbiologicals, Inc., Decatur, Ga.). Agar plates were incubated at 35°C in 5% carbon dioxide and examined daily for 3 days. Broth cultures were incubated at 35°C and examined daily for 7 days.

Microbiologic data, results for all CSF analyses, and patients' medical records were reviewed in every case for which there was bacterial or fungal growth on routine media or in which at least moderate WBC were recorded on microscopic examination of centrifuged CSF. The only exception to this was that medical records were not reviewed if the CSF Gram stain showed no more than few WBC and the culture yielded ≤4 colonies of coagulase-negative staphylococci or growth of these organisms in broth only.

Based on this evaluation, cases were separated into those with and without infection of the central nervous system (CNS). Cases of infection were further stratified as follows: (i) bacterial or fungal meningitis, (ii) infection adjacent to the subarachnoid space (such as brain abscess or subdural empyema), or (iii) sepsis without meningitis (positive CSF culture and positive blood culture with no more than few WBC in CSF by microscopic examination and all other results of CSF analysis normal).

RESULTS

During the 55 months studied, 2,635 CSF specimens were sent for Gram stain and routine culture (Table 1). Based on the criteria presented above, there were 64 cases of infection, including 56 cases of bacterial or fungal meningitis, 4 cases of infections adjacent to the subarachnoid space, and 4 cases of sepsis without meningitis. Bacterial or fungal meningitis was further stratified into that associated or not associated with a CNS shunt. As shown in Table 2, *Cryptococcus neoformans* was the most common cause of meningitis in the absence of a

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TABLE 1. Overall results of 2,635 CSF specimens submitted for routine culture

Gram stain	Routine culture	Infection present	Total no.
+	+	+	50
+	+	−	2
+	−	−	1
−	+	+	14
−	+	−	218
−	−	+	4[a]
−	−	−	2,346

[a] Acid-fast bacillus culture positive for *Mycobacterium avium* in three specimens and *Mycobacterium tuberculosis* in one specimen.

shunt, followed by *Streptococcus pneumoniae* and *Neisseria meningitidis*. Coagulase-negative staphylococci and *Enterobacter cloacae* were the most common causes of meningitis associated with a CNS shunt.

Organisms were detected by microscopic examination of the Gram-stained CSF in 35 of 40 (88%) instances of bacterial or fungal meningitis and in 13 of 16 (81%) instances of shunt-associated meningitis (Table 3). The Gram stain morphology was correctly interpreted in all cases. If patients who were receiving effective antimicrobial therapy (as determined by retrospective review of susceptibility data) at the time the CSF specimen was obtained are excluded, microscopic examination was 92% sensitive (35 of 38 without and 13 of 14 with CNS shunts). Of the three patients without CNS shunts whose CSF Gram stain failed to reveal organisms, two had cryptococcal meningitis which was diagnosed by simultaneous cryptococcal antigen testing and thus would not have gone undetected. The remaining patient had presumptive meningitis due to *Streptococcus sanguis* II; however, additional studies (such as computed tomagraphy or magnetic resonance imaging) were not done to rule out an abscess, and all other cultures were negative. Microscopic examination showed at least moderate WBC

TABLE 2. Results of 284 CSF specimens with positive routine cultures

Category and organism(s) isolated	No. of specimens				
	Culture positive				With organisms seen on Gram stain
	Total	On broth and solid medium	On solid medium only	On broth only	
Meningitis					
C. neoformans	27	8	17	2	23
S. pneumoniae	6	6	0	0	6
N. meningitidis	2	2	0	0	2
Others[a]	5	5	0	0	4
Total	40	21	17	2	35
Shunt-associated meningitis					
Coagulase-negative staphylococci	5	5	0	0	5
E. cloacae	4	4	0	0	4
Others[b]	7	1	2	4	5
Total	16	10	2	4	14
Adjacent pyogenic infections					
Streptococcus bovis	1	0	1	0	0
S. aureus	1	1	0	0	0
Escherichia coli	2	2	0	0	1
Total	4	3	1	0	1
Sepsis without meningitis: total[c]	4	4	0	0	0
Contaminants					
Coagulase-negative staphylococci	141	31	33	77	1
Coryneforms	23	1	5	17	0
Alpha-hemolytic streptococci	12	2	2	8	0
S. aureus	9	3	1	5	0
Acinetobacter sp.	5	0	2	3	0
Enterococcus sp.	4	2	0	2	0
Bacillus sp.	4	0	4	0	0
Stenotrophomonas maltophilia	4	0	0	4	0
Pseudomonas sp.	3	3	0	0	0
Others[d]	15	1	9	5	1
Total	220	43	56	121	2
Grand total	284	81	76	127	52

[a] One instance each of *Listeria monocytogenes*, *S. aureus*, *Morganella morganii*, *S. sanguis* II, and *S. bovis*.
[b] One or two instances each of *S. aureus*, *Corynebacterium amycolatum*, *C. albicans*, and *Klebsiella pneumoniae*.
[c] One instance each of *Cryptococcus*, *S. pneumoniae*, *N. meningitidis*, and *E. coli*.
[d] One or two instances each of *Propionibacterium acnes*, *Micrococcus* sp., *C. albicans*, *Candida parapsilosis*, *F. oryzihabitans*, *Proteus mirabilis*, *Sphingomonas paucimobilis*, and *Curvularia* sp.

TABLE 3. Summary of Gram stain results for 284 CSF specimens with positive routine cultures

Category	No. of Gram-stained specimens with:			
	Organisms seen and:		No organisms seen and:	
	≥ moderate WBC	≤ few WBC	≥ moderate WBC	≤ few WBC
Meningitis	23	12	4	1
Meningitis with CNS shunt	7	6	1	2
Adjacent pyogenic infection	1	0	3	0
Sepsis without meningitis	0	0	0	4
Infection absent	1	1	28	190

in 12 of 13 (92%) cases of bacterial meningitis without a CNS shunt, 15 of 27 (56%) cases with cryptococcal disease, and 8 of 16 (50%) cases with shunt infection.

Microscopic examination revealed no organisms in 218 of 220 CSF specimens in which the organisms isolated were judged to be contaminants (Table 3). In 28 cases, WBC were quantitated as at least moderate. Review of the medical records for these patients indicated a corresponding elevated WBC count in the CSF of 10 who were subsequently diagnosed with viral meningitis (3 patients), tuberculous meningitis (2 patients), and neurosyphilis, toxoplasmosis, lymphocytic meningitis, systemic lupus erythematosus, and CNS lymphoma (1 patient each). For the remaining 18, CSF cell counts by hemocytometer were normal ($\leq$4 WBC/mm^3) and no associated disease was present. Generally, estimation of WBC as no more than few on Gram stain correlated with normal cell counts by hemocytometer, but less agreement was seen when WBC were quantified as at least moderate. Errors tended to be caused by uneven distribution of cells by cytocentrifugation and misidentification of other cell types and/or debris as WBC.

Microscopic examination was equivocal for two CSF specimens in which Gram stain and culture results were eventually found to be discrepant. In the first case, the Gram stain was initially interpreted as many WBC and no organisms seen. The senior reviewer identified possible rare gram-positive forms (suggestive of *Listeria* organisms, which are often found in low numbers). With uncertainty conveyed to the clinician, the results were reported as rare gram-positive rods and/or cocci and many WBC. All cultures were negative, and the patient was diagnosed with a cerebrovascular accident. In the second case, microscopic examination revealed two clumps of gram-negative rods with moderate WBC in CSF from a patient thought to have recurrence of a brain abscess. Doubt that these organisms represented infection was expressed because they were not evenly dispersed or associated with the WBC. Broth culture grew *Flavimonas oryzihabitans*, which infrequently causes infection but more often occurs as a contaminant of laboratory materials and Gram-staining equipment (3, 11, 16). The positive culture in this case was interpreted as contamination. Aside from these two cases, microscopic examination was thought to yield a misleading false-positive result in only one additional CSF specimen. In this case, the Gram stain showed rare gram-positive cocci with no more than few WBC, but all other CSF values were normal, and culture grew only one coagulase-negative *Staphylococcus* colony.

The majority (28 of 34 [82%]) of the bacterial isolates causing true infection were recovered both in broth and on solid media and were too numerous for accurate quantitation (Table 2). Of the four cases of shunt-associated meningitis in which organisms were isolated in broth only, only one (*Candida albicans*) was detected in additional specimens. The observation that only one-third (10 of 30) of the fungal isolates causing infection were recovered on both media may be artifact, as it is laboratory policy to refer fungal isolates to the mycology section as soon as the first culture is positive and to discontinue incubation and examination of the remaining media.

In the remaining 220 CSF specimens with positive cultures, bacterial or fungal growth was judged to reflect contamination (Table 2). In 80% (177 of 220), the isolates grew in broth only or on solid medium only with a colony count of $\leq$4. Coagulase-negative staphylococci were isolated in 154 cultures (as the only organism in 141, with other common contaminants in 11, and with potential pathogens in 2). Other common contaminants were coryneforms, alpha-hemolytic streptococci, and *Staphylococcus aureus*. Although bacterial growth was obtained from both broth and solid media in 43 cases, review of medical records indicated that these were the result of contamination.

DISCUSSION

The present study shows that microscopic examination of Gram-stained CSF is likely to suggest that correct etiologic agent in the great majority (88%) of cases of bacterial or fungal meningitis. When cases in which effective antimicrobial treatment had already been given at the time of lumbar puncture were excluded, the sensitivity increased to 92%. During the time of this study, only 3 of 2,635 Gram-stained specimens were thought to be falsely positive—a specificity for this technique which exceeds 99%. The sensitivity may be related to use of the cytocentrifuge for slide preparation, as shown previously (2, 15); however, the present study was not designed to compare cytocentrifugation to other techniques. Semiquantitative assessment of WBC, which in itself is nonspecific for infection, may contribute to this sensitivity by automatically leading to review by a senior microbiologist. This laboratory policy may also contribute to the high specificity, as review was sometimes necessary to determine that bacterium-shaped forms actually represented staining artifacts.

Although current guidelines for processing and interpreting CSF recommend inoculation of either brain heart infusion or thioglycolate broth in addition to blood and chocolate agar medium (4, 13), this practice has been questioned (9, 10, 17). In this study, 284 of 2,635 (1.1%) routine CSF cultures yielded growth of bacteria or fungi, but in 220 the growth was felt to be due to contamination. This classification was based on type of isolate (common skin flora), quantity of growth (on one medium only), microscopic examination (negative Gram stain with no more than few WBC), and clinical review. Within this group, 121 grew in broth only. Eliminating the broth culture would eliminate the identification and antimicrobial susceptibility testing associated with these isolates. However, whereas all 13 cases of acute bacterial meningitis unassociated with CNS shunts would have been detected in the absence of a broth culture, 4 of 16 (25%) cases of shunt-associated meningitis would have been missed. Only one of these could have been diagnosed by additional specimens. The two cases of cryptococcal meningitis in which broth cultures were positive when cultures on blood and chocolate agar were still negative were, in fact, detected earlier by the cryptococcal antigen test. Thus, there are two cost-saving measures which might be enacted: (i) clinicians might indicate which submitted specimens could be associated with a shunt or adjacent CNS infection and broth could be inoculated for these, and/or (ii) growth found in broth only without elevated WBC would not be worked up without further clinician involvement.

In conclusion, microscopic examination of gram-stained,

concentrated CSF, a rapid and inexpensive test, is 92% sensitive and >99% specific in diagnosing untreated bacterial or fungal meningitis. The presence and quantity of WBC on the CSF Gram stain can be useful to identify specimens that deserve closer scrutiny in the laboratory. CSF specimens should be cultured in broth in special cases only, such as patients with CNS shunts and those for whom infection adjacent to the subarachnoid space is suspected. Extensive identification and antimicrobial susceptibility testing of isolates grown on only one medium from specimens with negative Gram stains and no more than few WBC could probably be eliminated as a cost-saving measure. Because the laboratory is unable to distinguish cases in which the growth of small numbers of coagulase-negative staphylococci or other common contaminants indicates true infection, it would be wise to hold such isolates pending a request for identification and susceptibility testing by the clinician.

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