Nonvalue of Culturing Cerebrospinal Fluid for Fungi

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No studies have evaluated the efficacy of culturing cerebrospinal fluid (CSF) for fungi. Because of the facts that the most common fungi responsible for meningitis grow well in media utilized for routine bacterial cultures and that cryptococcal antigen tests are commonly ordered, the efficacy of routinely performing fungal cultures specifically to recover fungi has been questioned. We examined data from 1,225 samples of CSF which were cultured for both bacteria and fungi. Twelve specimens yielded fungi, 10 from fungal cultures and 8 from bacterial cultures. *Cryptococcus neoformans* was found in 10 specimens, *Candida albicans* was found in 1, and a *Cladosporium* sp. was found in 1. Eight of 12 positive specimens had concordant culture results. The discordant cases were one specimen that was bacterial culture positive but fungal culture negative and three specimens that were fungal culture positive but bacterial culture negative. Of the latter discrepant cultures, one had fungal contamination only and the other two were positive for cryptococcal antigen. Therefore, omitting the fungal cultures on these specimens would not adversely impact patients. When both cryptococcal antigen tests and bacterial cultures are ordered routinely, eliminating fungal cultures on CSF would have had no impact on the patients in this study. All the clinically significant fungi were detected by the cryptococcal antigen test and/or bacterial culture. With a few exceptions, the combined use of cryptococcal antigen test and bacterial cultures of CSF could replace routine fungal cultures of CSF. Exceptions include settings where fungal pathogens other than *Cryptococcus* and *Candida* remain important causes of meningitis.

Although studies have addressed the efficacy of performing mycobacterial cultures on cerebrospinal fluid (CSF), no studies have evaluated the efficacy of culturing CSF for fungi (1). Some laboratories may actually use criteria similar to those established for the rejection of mycobacterial cultures to reject cultures on CSF for fungi, but actual data to provide evidence for this process are lacking (5; K. Bromberg, Letter, Lancet **i**:1023, 1980). Because of the facts that (i) *Cryptococcus* and *Candida* spp. (by far the most common fungi responsible for meningitis) grow well in media utilized for routine bacterial cultures and that (ii) cryptococcal antigen tests are commonly ordered, the efficacy of routinely performing fungal cultures specifically to recover fungi on CSF has been questioned. In addition, the onus is now on clinical microbiology laboratories to practice evidence-based medicine, which includes concepts of evaluating tests to ensure that they yield clinically useful results. To determine the clinical utility of routinely performing fungal cultures specifically to recover fungi on CSF, we performed the present study.

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MATERIALS AND METHODS

A retrospective study of data from 1 January 1994 to 1 July 2002 was conducted at Memorial Medical Center, a 450-bed community teaching hospital in Springfield, Ill. Although this hospital has a burn unit, a transplant service, and a regional cancer center, the vast majority of its patients are from the immediate

community in central Illinois. Its pediatric population (<18 years of age) is less than 5% of all patients.

Samples of CSF which were cultured for both bacteria and fungi were examined. In addition, if a fungal pathogen were detected by the antigen test for *Cryptococcus*, then these data were included. Medical charts for all patients who had cultures positive for fungi were reviewed.

Cultures were performed by current standard accepted procedures. For bacterial cultures, CSF was spun for 15 min at $3,000 \times g$ and the sediment was inoculated onto sheep blood agar plates and chocolate agar plates and was inoculated into thioglycolate broth. These cultures were incubated at 37°C and examined daily for 3 days. For fungal cultures, the CSF sediment was inoculated onto Sabouraud 4% dextrose agar plates and sheep blood agar plates, incubated at 30°C, and examined weekly for 4 weeks. Any microorganism grown on either the fungal or bacterial culture was worked up as a potential pathogen. Standard techniques were used for yeasts and fungi, including those for determination of growth rate and colony morphology, lacto-aniline blue staining of colonies, and determination of the biochemical profile by Vitek 2 (bioMérieux, Durham, N.C.).

If requested, a latex agglutination antigen test for *Cryptococcus* (Meridian Bioscience Inc., Cincinnati, Ohio) was performed on the CSF, generally within 24 h of its receipt in the laboratory.

RESULTS

Of 1,225 samples of CSF reviewed, 12 tested positive for fungi, either by bacterial culture, fungal culture, or the cryptococcal antigen test. Of cultures from these 12 samples, 10 fungal cultures were positive and 8 bacterial cultures were positive. *Cryptococcus neoformans* was found in 10 of the specimens, *Candida albicans* was found in 1, and a *Cladosporium* sp. was found in 1. Eight of the 12 positive specimens had concordant results for each culture method, including 1 specimen which was negative by both fungal and bacterial culture but cryptococcal antigen positive (Table 1). The discordant cases were as follows: (i) on one specimen, the bacterial culture was positive for *Cryptococcus* (as was the antigen test) but the fungal culture was negative and (ii) on three specimens, the fungal cultures were positive (two for *Cryptococcus* and one for

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TABLE 1. Specimens of CSF analyzed for fungi by both bacterial and fungal cultures

Organism isolated	No. of specimens with:		
	Concordant fungal and bacterial cultures	Discordant cultures	
		Fungal culture positive, bacterial culture negative	Fungal culture negative, bacterial culture positive
<i>Cryptococcus</i>	6 ^a	2 ^a	1 ^a
<i>Candida</i>	1	0	0
<i>Cladosporium</i>	0	1	0
None	1,214 ^b	— ^c	—
Total	1,221	3	1

^a Positive antigen test for *Cryptococcus*.

^b Included in this category is one specimen which was fungal and bacterial culture negative but which had a positive antigen test for *Cryptococcus*.

^c —, not applicable.

Cladosporium spp.) but the bacterial cultures were negative (Table 1). The two specimens which grew *Cryptococcus* on the fungal culture only were also positive for *Cryptococcus* by the antigen test.

The patient who had the fungal culture positive for the *Cladosporium* sp. was thought not to have fungal meningitis and was not treated for this. This isolate was considered clinically to be a contaminant.

DISCUSSION

Cryptococcus is by far the most common fungus responsible for meningitis, followed by *Candida* spp. as a distant second, except in pediatric patients, where *Candida* spp. account for the vast majority of infections (3, 7). Our study has shown that, in the population studied, the combined use of the antigen test for *Cryptococcus* and bacterial cultures of CSF could replace routine fungal cultures of CSF. Exceptions to this policy would be in settings or clinical practices where fungal pathogens other than *Cryptococcus* and *Candida* remain important causes of meningitis, such as areas where *Coccidioides* spp. are endemic. However, even in these regions, fungal cultures rarely yield *Coccidioides* from CSF (D. Pappagianis [University of California, Davis], personal communication; M. Saubolle [Department of Clinical Pathology, Good Samaritan Medical Center, Phoenix, Ariz.], personal communication). One source indicates that the culture is positive in only one-third of the cases (8). In fact, even culturing a large volume (~10 ml) of CSF recovers the fungus in only approximately one-half the cases of confirmed meningitis due to *Coccidioides* (D. Pappagianis, personal communication). Another source indicates that the best way to make a diagnosis of meningitis due to *Coccidioides* is by special communication between the laboratory and the physician and the use of repeated cultures of large volumes of CSF (2). Generally, it is acknowledged that the test of choice to diagnose meningitis due to *Coccidioides* is serology done on the serum and/or CSF (2, 8; D. Pappagianis, personal communication; M. Saubolle, personal communication).

Although this study was performed in an area of endemicity for histoplasmosis, none of the samples of CSF cultured for fungi grew *Histoplasma capsulatum*. In fact, in our collective memory (spanning >25 years here), we cannot remember any

CSF which grew *H. capsulatum*. Meningitis due to *H. capsulatum* is a rare complication of histoplasmosis, but, when it occurs, it is as a chronic meningitis, not an acute meningitis (4, 9). Patients may present to their physicians weeks to many months after the onset of symptoms. CSF is positive for the fungus in only 27 to 65% of the cases. The detection of meningitis due to *H. capsulatum* may be better addressed by consultation with the ordering physician rather than routinely culturing all CSF specimens for fungi. Detection of serum and CSF antibodies against the fungus is the most sensitive test for histoplasmosis (4).

If patients reside in an area of endemicity for other fungal pathogens, such as *Blastomyces dermatitidis*, then this issue should be examined by using the local data (evaluating the yield of clinically useful information, as was done in this study). A feature in *B. dermatitidis* infections useful for differentiation is that the lesions are usually focal in the CNS, as opposed to the more-diffuse meningitis caused by other fungi (4).

Other exceptions or potential problems with routinely eliminating fungal cultures on CSF might include situations where the patients have immunodeficiencies or are immunosuppressed (such as treatment centers for human immunodeficiency virus patients or cancer centers). Another pertinent issue may be the distinction between acute meningitis and chronic meningitis, which this study did not address. The diagnosis of chronic meningitis should be seriously considered if the neurologic findings persist or progress and the CSF remains abnormal for at least 4 weeks (4). Fungal cultures are considered mandatory in chronic meningitis (4). Unfortunately, the test requisitions which most clinical microbiology laboratories receive currently do not indicate whether acute or chronic meningitis is suspected. It is remotely possible that, with the newer requirements for test requisitions, this information will be available in the future.

Practice guidelines for the management of cryptococcal disease indicate that fungal cultures should be done, but they did not specify which type of media would suffice to detect *Cryptococcus* (6). In addition, the use of the antigen test for *C. neoformans* is indicated by the guidelines.

Of the 12 patients in this study positive for fungi, 1 was positive only by the cryptococcal antigen test, 10 were positive by fungal culture, and 8 were positive by bacterial culture. All specimens which grew *Cryptococcus* in cultures for either fungi or bacteria were positive for *Cryptococcus* by the antigen test. If no fungal cultures had been done on these specimens, three potentially positive specimens may have been missed by performing only bacterial cultures. However, one of these three specimens was considered a contaminant (*Cladosporium* sp.), and the other two had positive cryptococcal antigen tests. Therefore, omitting the fungal cultures on these three specimens would have had no adverse clinical impact on these patients. If the cost of performing fungal cultures is \$70, then, in the last 8.5 years, the cost avoidance of eliminating fungal cultures of CSF in this setting would have been over \$85,000 (\$70 × 1,225 cultures) or over \$10,000 annually.

If antigen tests for *Cryptococcus* are not done routinely, then the use of fungal cultures is still justified since this study indicates that there is a 30% likelihood that using bacterial cultures alone will result in a missed diagnosis of meningitis due to *Cryptococcus*. Before routinely eliminating fungal cultures on CSF, an educational program should be conducted by ap-

appropriate people in the institution, including the microbiology personnel, to require the antigen test for *Cryptococcus* when bacterial cultures are ordered on CSF. In summary, when both cryptococcal antigen tests and bacterial cultures are ordered routinely, the elimination of fungal cultures on CSF would have had no impact on patients in this study. All the clinically significant fungi were detected by the cryptococcal antigen test and/or bacterial culture. This study suggests that, in areas where *Candida* and *Cryptococcus* cause the vast majority of fungal meningitis, the combined use of cryptococcal antigen test and bacterial cultures of CSF could replace routine fungal cultures of CSF. However, before any widespread conclusions or recommendations can be made from this study, other studies should be done to validate these findings.

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