Utility of Routine Testing of Bronchoalveolar Lavage Fluid for Cryptococcal Antigen

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All cryptococcal antigen (CrAg) testing performed at our institution between 1989 and 1994 was reviewed for utility of routinely testing of bronchoalveolar lavage fluid (BAL) for this antigen. Forty-two of 1,506 BAL specimens were positive. Seventeen of these were felt to represent false positives (sensitivity, 71%; positive predictive value, 0.59). The data on CrAg in cerebrospinal fluid and serum and the fungal culture and histological results of BAL specimens did not support continued, routine testing of BALs for CrAg to diagnose cryptococcosis.

Tests to detect the presence of cryptococcal capsular polysaccharide antigen (CrAg) have greatly enhanced prompt diagnosis of cryptococcosis (3). Since Cryptococcus neoformans enters the host via the pulmonary route, rapid testing of respiratory specimens is a desired approach to early diagnosis of cryptococcosis (6), and direct testing of respiratory specimens for CrAg has shown promise in this regard (2, 8, 10, 12).

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Beginning in mid-1989, all bronchoalveolar lavage fluid (BAL) specimens obtained from immunocompromised patients at our institution were routinely tested for the presence of CrAg (1). Since the direct cost per test performed was $10.50 (in 1996) and the number of tests on BALs had increased to >300 per year, we sought to determine whether this diagnostic effort was an efficient use of medical resources. We have undertaken a retrospective review of all CrAg test results obtained from January 1989 through December 1994.

In addition to identifying all CrAg tests performed on BAL specimens during the study period, all positive results for C. neoformans culture from BAL or bronchial washing specimens were noted. Cytology reports on all culture-positive and/or CrAg-positive BAL and bronchial washing specimens were reviewed. A review of the medical records on all culture- or CrAg-positive patients for clinical evidence of disease consistent with cryptococcosis was undertaken. Finally, all inpatient and outpatient cases of cryptococcosis identified through medical records coding and research were reviewed.

CrAg testing was performed on the BAL supernatant, following the procedure developed by Stockman and Roberts for serum (11). The CALAS (Meridian Diagnostics, Cincinnati, Ohio) assay was performed as described by the manufacturer. Repeat testing was performed on 22 available specimens after 1 to 7 years of storage in a self-defrosting freezer at −20°C. A total of 8,156 CrAg tests were performed; 1,506 of these were tests performed on BAL specimens from 995 patients. In the years 1993 and 1994, routine CrAg testing of BALs accounted for about one-quarter of all CrAg tests done in the laboratory. Overall, there were 723 positive CrAg results. Of these, 42 (from 35 patients) were from BALs. After chart review, evaluation of medical records, and cytology and microbiology laboratory reviews, 17 of these specimens (from 17 patients) were felt to represent false-positive results. The remaining 25 true-positive results were obtained from 18 patients. There were 10 specimens (nine patients) for which a negative CrAg test was obtained but which grew C. neoformans from culture. Overall, 35 (from 27 patients) of the original 1,509 BAL specimens (from 995 patients) were culture positive for C. neoformans.

Thirty-two of the positive CrAg specimens were from 25 human immunodeficiency virus (HIV)-infected individuals, whereas 10 non-HIV-infected persons each had one specimen positive for CrAg. A total of 24 specimens from 17 HIV-infected persons were considered true positives, based on the presence of pulmonary disease and previously diagnosed cryptococcosis; 8 specimens (8 patients) yielded false-positive tests. Of the 10 non-HIV patients, only one had disease consistent with cryptococcosis.

A sensitivity of 71% (25 of 35), a specificity of 99% (1,454 of 1,471), a positive predictive value of 0.59 (25 of 42), and a negative predictive value of 0.99 (1,454 of 1,464) were obtained for BAL specimens tested for CrAg. When the analysis considered patients, not specimens, being tested, a sensitivity of 66% (18 of 27), a specificity of 98% (948 of 965), a positive predictive value of 0.51 (18 of 35), and a negative predictive value of 0.99 (948 of 957) were obtained.

There was only one instance in which the CrAg result from a BAL specimen was the first positive test suggesting the diagnosis of cryptococcosis. The culture of the BAL from this HIV-negative, nonimmunocompromised patient was positive for C. neoformans on day 7, and there were yeast cells consistent with Cryptococcus organisms on the cytological smear of the BAL specimen.

Three clustered episodes accounted for 12 of the 17 false-positive results. Repeat testing with a second lot of CALAS at the time of the original positive confirmed the results. For the third cluster, pretreatment with 2-mercaptoethanol failed to
eliminate the false-positive results (14). Microbiology records failed to reveal positive cultures for *Trichosporon* sp. or *Candida* (formerly DF-2), organisms known to cross-react in the CrAg latex agglutination test (9, 13). The results of repeat testing of five available specimens after storage in a self-defrosting freezer at -20°C for 2 to 4 years were negative. True-positive specimens stored in the same manner maintained CrAg positivity within one tube of the original titer. During the cluster time periods, false-positive results were not obtained from the cerebrospinal fluid or serum specimens processed in the same laboratory runs. A review of procedures used in the endoscopy suite where bronchoalveolar lavages were performed did not reveal any changes that might explain the false positives, such as the use of different cleaning preparations or detergents, which have been associated with false positives (4).

Cost-efficient medical care is predicated on the effective use of diagnostic tests and reasonable rationing of laboratory personnel time. The CrAg test can be a valuable tool in the delivery of prompt care to a patient affected with cryptococcosis. The goal is to increase the efficiency of diagnosis of disease without significantly affecting the delivery of care or increasing the cost of care. In our series, either the positive predictive value for any positive test was low, other diagnostic tests for cryptococcosis were obtained within a clinically reasonable time period, or the patient was known to have cryptococcosis. In only one case, that of a nonimmunocompromised patient, was the BAL CrAg the first positive test; however, cytological examination performed within 24 h of specimen collection also revealed the presence of *Cryptococcus* organisms. Further, the savings to the laboratory achieved by discontinuing routine CrAg testing of BAL specimens is significant due to the cost of materials and the reduction in work load; importantly, in this case, there does not seem to be an adverse impact on prompt diagnosis.

Although the retrospective nature of this study may have limited our ability to determine a cause for clustering of false-positive episodes at the end of the study period, each of the three episodes was detected at the time of occurrence and efforts to identify known causes of false positivity for CrAg, such as syneresis fluid or detergents, were unsuccessful (4, 5, 7). Further, the retrospective nature of our study also made an analysis of true economic cost-effectiveness difficult, in that there could be no accounting of the additional tests and/or therapeutic modalities resulting directly from the false-positive CrAg results on the BAL specimens from the 17 patients. The cost of a potentially missed case of cryptococcosis is difficult to factor into an economic analysis. Therefore, only laboratory cost savings were determined.

The false-negative specimens from patients with culture-positive disease may represent a loss of sensitivity due to dilution. The manufacturer of CALAS notes the test to be sensitive to 25 ng/ml. Procurement of BAL involves the introduction of a fluid vehicle to lavage the portion of the bronchial tree being studied. This might dilute the specimen below the level of detection.

BAL sampling of immunocompromised patients with respiratory syndromes may be a useful diagnostic procedure in appropriate clinical situations (1). However, the routine testing of the BAL for the presence of CrAg is not warranted. Many of the patients in our series had disseminated cryptococcal disease, and a number of them had evidence of extrapulmonary cryptococcal disease before the bronchoscopy. Our institution ceased doing routine testing of BALs for the presence of CrAg beginning in January 1996 but continued to offer the test. In the year 1996 only 8 BAL specimens were tested for CrAg at the request of the bronchoscopist, versus 315 in 1994. At a direct cost of $10.50 per test, this translates into a direct cost savings of $>3,200.00 per year for the laboratory. Most importantly, our review demonstrates that the removal of this test from a routine testing panel should not affect the delivery of medical care to patients.

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REFERENCES