

# Value of Assessing Cryptococcal Antigen in Bronchoalveolar Lavage and Sputum Specimens from Patients with AIDS

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## Abstract

*Cryptococcus neoformans* has become a significant opportunistic pathogen, accounting for 8-10% of infectious complications in patients with AIDS. When encapsulated yeast cells are observed in Giemsa-stained smears of bronchoalveolar washings (BAL), or induced sputum specimens, confirmation as *C. neoformans* is germane to definitive therapy. We therefore studied 30 BAL and 9 induced sputum specimens for cryptococcal antigen. Of the 30 BAL, 3 specimens were positive for cryptococcal antigen, ranging in titer from 1:4 to 1:256, and 2 of 9 sputum samples were also smear, culture and antigen positive (titer 1:2) for *C. neoformans*. Of the 34 negative specimens, none of the seven containing *Candida* species or the one containing *H. capsulatum* or the one containing *P. carinii* cross-reacted with cryptococcal anticapsular antibody. Our results indicate that when yeast forms suggestive of *C. neoformans* are visualized on direct smears of BAL or sputum samples, rapid confirmation as *C. neoformans* may be achieved by assessment for cryptococcal antigen. A correlation may also exist between antigen titer in respiratory specimens and extent of cryptococcal infection.

**Key Words:** Cryptococcal antigen, bronchoalveolar lavage, AIDS.

### Introduction

*Cryptococcus neoformans*, an encapsulated yeast, has emerged as a significant opportunistic pathogen in patients with AIDS, accounting for 8-10% of their infectious complications (1, 2). While cryptococcal meningitis is most often the presenting complication, primary pulmonary involvement may be the initial stage in the pathogenesis of central nervous system disease (1, 2).

In the setting of AIDS, several opportunistic pathogens, including *C. neoformans*, may produce diffuse pulmonary involvement with clinical and radiologic findings indistinguishable from those produced by *Pneumocystis carinii* (3). When encapsulated yeast forms are seen on direct smears of bronchoalveolar lavage (BAL) and induced sputum specimens, rapid confirmation as *C. neoformans* is germane to definitive treatment. We therefore assessed the value of detecting and titrating cryptococcal capsular polysaccharide antigen in such respiratory specimens as a means of confirming the diagnosis of cryptococcal pulmonary infection and gauging the extent of cryptococcal infection.

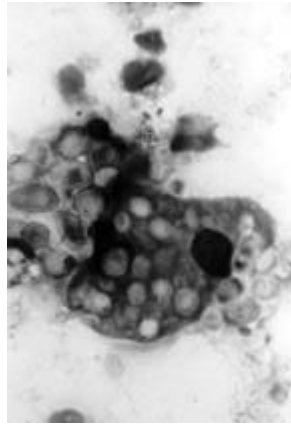
### Materials and Methods

Thirty BAL and 9 induced sputum samples were submitted for microbiologic analysis. In the laboratory, an aliquot of the BAL specimen was removed and cytocentrifuged at 2000xG onto a slide. These preparations were then Giemsa-stained (HemaColor<sup>7</sup>, EM Diagnostics Systems, Gibbstown, NJ) and examined microscopically. Induced sputa were treated with a mucolytic agent (Mucolyse<sup>7</sup>, ProLab Diagnostics, Ontario, Canada) prior to cytocentrifugation and processing as for BAL specimens. In both instances, cryptococcal antigen detection was performed by reacting filtered supernates of these specimens with latex particles coated with anticapsular cryptococcal antibody (Calas<sup>7</sup>, Meridian Diagnostics, Norwich, OH) to an endpoint titration. Titers were determined according to the manufacturer's protocol, and the highest dilution rendering clear-cut agglutination was considered positive. Positive and negative cryptococcal controls were included with each test. Controls for specificity of cryptococcal antibody consisted of growing *C. neoformans* and several *Candida* species individually in brain heart infusion broth for 72 hours and reacting culture filtrates as above. Specimens obtained directly from patients were cultured for bacterial and fungal microorganisms.

### Results

Three of the 30 BAL specimens showed the presence of oval yeast cells with varying degrees of encapsulation which were morphologically suggestive of *C. neoformans* (Fig.). Oval yeast forms were also observed within pulmonary macrophages; these morphologically resembled *Histoplasma capsulatum*. Interaction of the 3 filtered supernates with anticapsular antibody resulted in endpoint titrations ranging from 1:4 to 1:256 (Table). *C. neoformans* only was subsequently recovered from culture of these specimens. Among the 27 negative BAL specimens, 1 showed *P. carinii*, 3 grew *Candida albicans*, 1 *Candida glabrata*, 1 *Candida* species, and 1 *H. capsulatum* (Table).

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**Fig.** Giemsa stain of bronchoalveolar lavage showing a cluster of encapsulated yeast cells of *Cryptococcus neoformans*.

**TABLE**

*Results of cryptococcal antigen detection in respiratory specimens showing encapsulated yeast cells on smear and C. neoformans on culture*

Tested	Positive (titer)	Negative Species present		
	Tested (n)	Positive (titer) (n)	n	in negative specimens (n)
Bronchoalveolar Lavage	30	3 (1:4, 1:4, 1:256)	27	<i>P. carinii</i> (1), <i>C. albicans</i> (3), <i>C. glabrata</i> (1), <i>Candida</i> spp:(1) <i>H. capsulatum</i> (1)
Sputum	9	2 (1:2, 1:2)	7	<i>C. albicans</i> (2)
Total	39	5	34	<i>Candida</i> spp. (7) <i>H. capsulatum</i> (1) <i>P. carinii</i> (1)

n = number

Two of the 9 sputum samples tested, reacted with anticryptococcal antibody to a titer of 1:2. Of the 7 negative specimens, 2 grew *C. albicans*. Taken together, of 39 respiratory specimens tested, 5 were smear, culture, and antigen positive for *C. neoformans*. None of the 9 specimens containing other mycotic agents or *P. carinii* cross-reacted with cryptococcal anticapsular antibody. Furthermore, 72-hour-old filtrates of 1 strain each of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* grown in brain heart infusion broth were non-reactive in the latex agglutination test while *C. neoformans* grown similarly was strongly reactive.

### Discussion

The pathogenesis of cryptococcosis begins by inhalation of sparsely or non-encapsulated particles of *C. neoformans* from contaminated environments, usually soil (4). Despite this route of

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acquisition, clinically apparent pulmonary disease is a rare occurrence among immunologically competent individuals (5). In the setting of immunosuppression, however, cryptococcal infection may target the lung, or concomitantly disseminate hematogenously to other organs (1, 3, 5, 6). In patients with AIDS, cryptococcal pneumonia may radiologically mimic *Pneumocystis* pneumonia or even coexist with *P. carinii* infection (1, 6, 8). Because of the difficulty in making a specific etiologic diagnosis of pulmonary infiltrates in patients with AIDS, specimens obtained by bronchoscopy or sputum induction are often submitted for microbiologic evaluation. When yeast cells morphologically compatible with *C. neoformans* are visualized in Giemsa-stained smears of respiratory specimens, a tentative diagnosis of pulmonary cryptococcosis can be made. Strong support for this diagnosis can be readily achieved through demonstrating cryptococcal antigen in such respiratory specimens.

The pulmonary phase of cryptococcosis may resolve spontaneously prior to dissemination or coexist with disseminated disease (1, 3, 5). In this regard, a correlation may exist between the titer of cryptococcal antigen present in respiratory specimens and the degree of pulmonary involvement and/or dissemination. For instance, in the case previously reported (3), the patient whose bronchoalveolar specimen showed a cryptococcal antigen titer of 1:256 showed widespread cryptococcal disease involving numerous organs and the meninges. Cerebrospinal fluid cryptococcal antigen titer was 1:8192. This patient expired within 48 hours of hospital admission. Baughman et al. (9) and Meyohas et al. (6) also found a correlation between the height of the BAL cryptococcal antigen titer and extent of extrapulmonary involvement.

Three of the 5 patients whose smears and cultures were positive for *C. neoformans* showed cryptococcal antigen titers of 1:2 to 1:4 in their respiratory specimens. Two of these patients with titers of 1:2 had no evidence of disseminated disease and their serum antigen titers were negative. The third patient (titer 1:4) had been treated for cryptococcal pneumonia and meningitis that had been diagnosed earlier. The presence of both cryptococci on smear with concomitant antigen may reflect a persistent pulmonary focus of cryptococcosis or reactivation of the earlier disease, necessitating bronchoscopy as a diagnostic endeavor. This patient's concurrent serum antigen titer was 1:2.

Baughman et al. (9), in their study of the diagnostic utility of detecting cryptococcal antigen in BAL fluid, encountered 3 patients with titers of 1:2 to 1:4 whose smears were negative, but whose cultures of the BAL fluid were positive for *C. neoformans*. None of these patients had serum antigenemia, suggesting an early pulmonary focus without dissemination. Similarly, in their study of 27 patients with pulmonary cryptococcosis, Meyohas et al. (6) diagnosed 2 patients only by detection of cryptococcal antigen (titers not given) in BAL fluid. These patients were subsequently shown to have systemic cryptococcosis. In this report, we have shown that a diagnosis of pulmonary cryptococcosis may be readily confirmed by demonstrating cryptococcal antigen in respiratory specimens showing yeast cells morphologically resembling *C. neoformans*. Furthermore, although limited data exist from this and previous reports (6, 9), there does appear to be a correlation between antigen titer in respiratory specimens and extent of cryptococcal infection. Because cross-reactions did not occur in the presence of other mycotic agents which may be confused with cryptococci in direct smears, there was a high degree of both sensitivity and specificity accorded to the detection of

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antigen in respiratory specimens, especially if antigen titers of  $\geq 1:8$  are obtained (9). Although we did not correlate the presence of cryptococcal antigen with CD4<sup>+</sup> lymphocyte count, Meyohas et al. (6) recommend that cryptococcal antigen be assayed in all swab samples of BAL and pleural samples of HIV-infected patients with CD4<sup>+</sup> lymphocyte counts of  $< 100/\text{mm}^3$ . A positive reaction may signal the need for a more extensive evaluation of the patient(s) for cryptococcal infection.

### References

1. Masci JR, Poon M, Wormser GP, Bottone EJ. *Cryptococcus neoformans* infections in the era of AIDS. In: Wormser GP, editor. AIDS and other manifestations of HIV infection. 2nd ed. New York: Raven Press; 1992. pp. 393-409.
2. Powderly WG. Cryptococcal meningitis and AIDS. *Clin Infect Dis* 1993; 17:837-842.
3. Loerinc AM, Bottone EJ, Finkel LJ, Teirstein AS. Primary cryptococcal pneumonia mimicking *Pneumocystis carinii* pneumonia in a patient with AIDS. *Mt Sinai J Med* 1988; 55:181-186.
4. Farhi F, Bulmer GS, Tacker JR. *Cryptococcus neoformans* IV. The not-so-encapsulated yeast. *Infect Immun* 1970; 1:526-531.
5. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS: 100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995; 8:515-548.
6. Meyohas M, Roux P, Bollens D, et al. Pulmonary cryptococcosis: Localized and disseminated infections in 27 patients with AIDS. *Clin Infect Dis* 1995; 21:628-633.
7. Special Report: Pulmonary complications of the acquired immunodeficiency syndrome. Report of a National Heart, Lung and Blood Institute Workshop. *N Engl J Med* 1984; 310:1682-1688.
8. Clark RA, Greer DL, Valainis GT, Hyslop NE. *Cryptococcus neoformans* pulmonary infection in HIV-infected patients. *J Acquir Immune Defic Syndr* 1990; 3:480-484.
9. Baughman RP, Rhodes JC, Dohn MN, et al. Detection of cryptococcal antigen in bronchoalveolar lavage fluid: A prospective study of diagnostic utility. *Am Rev Respir Dis* 1992; 145:1226-1229.