JOURNAL OF CLINICAL MICROBIOLOGY, June 1995, p. 1588–1591 Vol. 33, No. 6
0095-1137/95/$04.00+0

Direct Determination of Cryptococcal Antigen in Transthoracic Needle Aspirate for Diagnosis of Pulmonary Cryptococcosis

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Received 3 January 1995/Returned for modification 9 February 1995/Accepted 9 March 1995

Pulmonary cryptococcosis causes significant morbidity and mortality in immunocompromised patients. Definitive diagnosis of pulmonary cryptococcosis is usually difficult. The use of direct determination of cryptococcal antigen in transthoracic needle aspirate to diagnose pulmonary cryptococcosis was investigated. Over a 2-year period, we studied a total of 41 patients with respiratory symptoms and pulmonary infiltrates of unknown etiology who were suspected of having pulmonary cryptococcosis. Twenty-two patients were immunocompetent patients and 19 patients were immunocompromised. A diagnosis of pulmonary cryptococcosis was based on cytological examination, culture for *Cryptococcus neoformans*, histopathologic examination, and clinical response to antifungal therapy. All patients underwent chest ultrasound and ultrasound-guided percutaneous transthoracic needle aspiration to obtain specimens for cryptococcal antigen determination. The presence of cryptococcal antigen was determined by the latex agglutination system (CALAS; Meridian Diagnostics, Cincinnati, Ohio). An antigen titer equal to or greater than 1:8 was considered positive. The specimens were also sent for cytological examination, fungal culture, and/or histopathologic examination. A final diagnosis of pulmonary cryptococcosis was made in eight patients. Direct determinations of cryptococcal antigen in lung aspirate were positive in all eight patients with pulmonary cryptococcosis (100% sensitivity, 97% specificity, a positive predictive value of 89%, and negative value of 100%), and there was only one false-positive in noncryptococcosis patients. The diagnostic accuracy was 97.5%. Serum cryptococcal antigen was positive in only three patients with pulmonary cryptococcosis (sensitivity, 37.5%). This study showed that direct measurement of cryptococcal antigen in lung aspirate can be a rapid and useful test for diagnosis of pulmonary cryptococcosis.

Cryptococcosis is caused by the encapsulated yeast *Cryptococcus neoformans*. The clinical spectrum of the disease ranges from asymptomatic pulmonary infection in immunocompetent hosts to rapidly fatal central nervous system infection in immunosuppressed patients (5, 18). Symptomatic pulmonary cryptococcosis is seldom recognized, although the lung is the portal of entry (3, 13, 22). Pulmonary cryptococcosis causes significant morbidity and mortality in immunocompromised hosts. Even in immunocompetent individuals, it possesses a potential for dissemination (8, 20). A timely and reliable diagnosis with subsequent appropriate therapy is mandatory. Definitive diagnosis of pulmonary cryptococcosis requires a specific histopathologic examination and a positive culture. However, there are difficulties in obtaining adequate specimens, and a positive culture takes at least 1 week.

Serological diagnosis using cryptococcal antigen obtained by latex particle agglutination provides an excellent test for cerebrospinal fluid (CSF) examination (7). Detection of serum cryptococcal antigen is not as sensitive as detection of CSF antigen. It is often negative in pulmonary cryptococcosis and cryptococcal meningitis, and it may even be negative in disseminated cryptococcal disease. Some investigators have reported that direct detection of cryptococcal antigen of pleural effusion (28) and bronchoalveolar lavage (BAL) fluid (1) is helpful in diagnosing pulmonary cryptococcosis in immunocompromised hosts. Ultrasound (US) and US-guided percutaneous transthoracic needle aspiration constitute a useful and safe diagnostic method with high yield in pulmonary infection (21, 26). To our knowledge, direct detection of cryptococcal antigen in lung aspirate has not yet been reported in the literature. Thus, this prospective study was undertaken to investigate the diagnostic value of direct detection of cryptococcal antigen in lung aspirate via US-guided transthoracic aspiration.

MATERIALS AND METHODS

From June 1992 to May 1994, a prospective study was conducted to assess the value of direct determination of cryptococcal antigen in lung aspirate for diagnosis of pulmonary cryptococcosis. For inclusion in the study, patients had to meet the following criteria: (i) have pulmonary infiltrates of unknown etiology, (ii) be clinically suspected of having pulmonary cryptococcosis, (iii) have no bleeding tendency or coagulopathy, (iv) have nodules or infiltrates with adequate "US window" available, and (v) be cooperative. Patients with single or multiple nodules, cavitary mass, or alveolar infiltrates in chest radiographs were suspected of having pulmonary cryptococcosis; they had not been diagnosed definitively by conventional diagnostic approaches such as sputum examination and bronchoscopy with biopsy.

A total of 41 patients were available for study: 24 men and 17 women ranging in age from 15 to 79 years. Clinical features (fever, cough, sputum, dyspnea, and pleuritic pain), duration of symptoms from onset to admission, total leukocyte count with classification, and underlying disease were recorded. Most patients underwent chest US examination and needle aspiration after admission. The lesions were subjected to US-guided percutaneous transthoracic aspiration biopsy after assessment of pulmonary infiltration (21, 26, 27). The aspirate was obtained from US window-available nodules or infiltrates and was submitted immediately to microbiological examination, including cultures of aerobes and anaerobes, mycobacteria, and fungi, and cytological examination, including Gram stain, acid-fast stain, and Liu's stain (15, 23) (also known as Riu's stain; modified from the Romanowsky system). In addition, part of the lung aspirate was examined for cryptococcal antigen. Most patients had two aspirations at the same time. If the aspirate was less than 0.1 ml, it was diluted with 1 ml of normal saline (dilution factor, ≥10-fold); examinations were then carried out as described above. A routine chest radiograph was taken on the day after needle aspiration to assess any potential complication.

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TABLE 1. Clinical features and cryptococcal antigen titers in the pulmonary cryptococcosis study group

Patient no.	Age (yr)	Sex[a]	Chest X-ray findings	Lung aspirate[b]				Serum antigen (titer)[c]	CSF antigen (titer)
				Smear	Culture	Pathology	Antigen (titer)		
1	32	M	Nodules	+	+	ND[d]	1:20[e]		ND
2	48	F	Infiltrates	+	−	ND	1:2,048	1:8,192	1:2
3	50	M	Infiltrates	−	+	ND	1:128	1:4	ND
4	44	M	Nodules	+	−	ND	1:320[e]	1:4	ND
5	50	M	Infiltrates	−	−	+	1:40[e]	1:2	ND
6	27	F	Nodules	+	−	+	1:80[e]	1:64	ND
7	18	M[f]	Infiltrates	−	−	ND	1:80[e]	1:8	1:2
8	63	M[g]	Nodules	−	−	ND	1:80[e]		ND

[a] M, male; F, female.
[b] +, positive; −, negative.
[c] A titer equal to or greater than 1:8 is considered positive.
[d] ND, not done.
[e] With saline dilution (dilution factor of ≥10).
[f] With hyperimmunoglobulin E syndrome and central nervous system cryptococcosis.
[g] Clinical response to antifungal therapy.

Final diagnoses were based on the results of the histopathologic examination, cytological examination or culture of specimens obtained from the transthoracic needle biopsy, and histopathologic examination of transbronchial lung biopsy specimens obtained through fiberoptic bronchoscopy. The criteria for diagnosis of pulmonary cryptococcosis were as follows: (i) identification of *C. neoformans* by culture or cytological or histopathologic examination of lung aspirate; (ii) isolation of *C. neoformans* from other sites with pulmonary lesions not attributable to other pathogens; and (iii) clinical response to antifungal therapy. Neither mycobacteria nor other pathogenic fungi were present simultaneously. Patients fulfilling criterion i or ii were categorized as having definitive pulmonary cryptococcosis. Patients meeting criterion iii only were considered probable pulmonary cryptococcosis patients. No patients had antifungal therapy before lung aspiration.

The presence of cryptococcal antigen was tested with a latex agglutination system (CALAS; Meridian Diagnostics, Cincinnati, Ohio). This procedure was described previously for the detection of capsular polysaccharide antigens of *C. neoformans* in serum and CSF (17). The lung aspirates were treated like serum specimens. Prior to testing, the specimen was inactivated in a boiling water bath for 5 min after centrifugation and incubation with pronase at 56°C for 15 min. After inactivation in the boiling water bath, 25 μl of patient specimen was added to each of two designated rings, followed by the addition of 1 drop of either anticryptococcal globulin reagent or normal globulin reagent to each of the designated rings. The contents were mixed, and the slides were rocked for 5 min. Agglutination of at least 2+ to 4+ was considered positive. Twofold dilution of specimens positive on the screen was repeated as described above. The highest dilutions at ≥2+ agglutination were reported. The tests for antigen in serum and, if possible, CSF were carried out within the same week. Control of the rheumatoid factor was also included during the test. A titer of cryptococcal antigen equal to or greater than 1:8 was considered positive. The sensitivity, specificity, and positive and negative predictive values of the titers of lung aspirate cryptococcal antigen were calculated.

RESULTS

There were 8 pulmonary cryptococcosis cases and 33 noncryptococcosis cases studied. In the cryptococcosis group, diagnosis was definitive in seven patients (patients 1 to 7) and probable in one (patient 8); all patients except one with hyperimmunoglobulin E syndrome were immunocompetent hosts (Table 1). Only two patients (patients 5 and 6) had adequate specimens when US-guided cutting biopsy was performed, with specific histopathologic findings. Six patients had minimal amounts of lung aspirate, less than 0.1 ml, which were subsequently diluted to 1 ml with normal saline. All eight patients had a positive cryptococcal antigen in lung aspirates, whereas only three patients had significant titers of serum cryptococcal antigen. Only two patients underwent lumbar puncture and had lower titers of cryptococcal antigens (titers of 1:2) in CSF. All patients received antifungal therapy (four were treated with fluconazole for 2 to 3 months; two, with 5-fluorocytosine for 6 months; and two, with 1.5 g of amphotericin B) after a definitive diagnosis was obtained; their clinical follow-up remained uneventful. The lesions also disappeared later from their chest radiographs.

In the noncryptococcosis group ($n = 33$), 18 patients had underlying diseases (11 with malignancy, 3 with diabetes mellitus, 2 with heart diseases, 1 with rheumatoid arthritis, and 1 with human immunodeficiency virus infection). Pulmonary pathogens were identified in 24 patients: 9 culture positive for *Mycobacterium tuberculosis*, 5 with bacterial pneumonia, 8 with lung cancer, 1 with *Aspergillus niger* infection, and 1 with *Pneumocystis carinii* infection. No definite pathogens could be found in the remaining nine patients with delayed resolved pneumonia. Tests for cryptococcal antigen in lung aspirates were negative in all patients except one. One patient who had renal cell carcinoma with lung metastasis had a false-positive cryptococcal antigen in his lung aspirate (titer of 1:16). Serum cryptococcal antigen tests, checked in 16 patients, were all negative in this group.

Overall, tests for cryptococcal antigen in lung aspirate were positive in nine patients: eight had pulmonary cryptococcosis and one had renal cell carcinoma with lung metastasis. Results for 41 patients were calculated, showing 100% sensitivity, 97% specificity, 97.5% accuracy, a positive predictive value of 89%, and a negative predictive value of 100%. Serum cryptococcal antigen was checked in 24 patients (8 with and 16 without pulmonary cryptococcosis). There was a sensitivity of 37.5%, a specificity of 100%, 79% accuracy, a positive predictive value of 100%, and a negative predictive value of 76% (Table 2). Minimal pneumothorax developed in 2 of 41 patients after needle aspiration, but no chest tube thoracostomy was required. One patient suffered from mild and self-limited hemoptysis after the procedures.

DISCUSSION

Definitive diagnosis of pulmonary cryptococcosis remains difficult, requiring identification of the organism by specific histopathologic findings in lung tissue and positive culture. Diagnosis from culture of pulmonary secretions was only 19%, and it was 70% from 101 specimens obtained during surgery or at autopsy (3). Even open lung biopsy may fail to detect the responsible organisms. A rapid presumptive diagnosis can be made by using cytological smears or serology. Cryptococcal

TABLE 2. Diagnostic value of determination of cryptococcal antigen in lung aspirate or serum

Cryptococcal antigen in:	No. of patients positive for antigen/no. tested (%)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Lung aspirate (n = 41)	8/8 (100)	32/33 (97)	8/9 (89)	32/32 (100)
Serum (n = 24)	3/8 (37.5)	16/16 (100)	3/3 (100)	16/21 (76)

antigens detected in body fluids are derived from the capsular polysaccharides of *C. neoformans*. Antibodies cross-reacting with four serotypes are used to detect *C. neoformans* (2). Cryptococcal antigen may be detected by a simple and reliable latex agglutination test for detection of cryptococcal antigen in the CSF (12). Diagnosis of pulmonary cryptococcosis has been suggested by detection of cryptococcal antigen in serum (10, 16), pleural effusion (28), and BAL fluid (1).

In patients with subpleural cryptococcal lesions, percutaneous computerized tomography- or US-guided aspiration was reported to have a diagnostic yield greater than that of bronchoscopy (19). In this study, the specificity of lung aspirate cryptococcal antigen was comparable to that of serum antigen (97 versus 100%), and the sensitivity of lung aspirate antigen was superior to that of serum antigen (100 versus 37.5%). The serum cryptococcal antigen test appears to be much less useful for screening patients with pulmonary cryptococcosis. Although cytological examination is also a rapid test for pulmonary cryptococcosis, small fungi may be overlooked during routine microscopic screening unless a large number of the organisms are present or there is already clinical suspicion of the infection (15). Four of eight patients with pulmonary cryptococcosis had negative results by cytological examination, and they were diagnosed by culture, histopathologic examination, and in association with central nervous system cryptococcosis and clinical response to antifungal therapy, respectively. Although two patients underwent US-guided cutting biopsy, yielding a definitive diagnosis, they had taken time to wait for the results of histopathologic examination. In addition, cutting biopsy can entail risks of pulmonary hemorrhage and pneumothorax (25). Considering the limitation of detection of serum cryptococcal antigen, culture, and cytological and histopathologic examinations, direct determination of cryptococcal antigen proved to be a rapid and reliable diagnostic method. A correct and timely diagnosis of pulmonary cryptococcosis can promote an appropriate and effective treatment.

Culture for *C. neoformans* should be done with all specimens including sputum, pleural effusion, BAL fluid, lung aspirate, CSF, urine, blood, skin lesions, and material from any other suspected sites of infection. Negative cultures do not necessarily rule out cryptococcal infection. In this study, only two of eight patients had positive cultures of *C. neoformans* from lung aspirates. Several factors may have contributed to a false-negative culture from the percutaneous transthoracic needle aspirates in the remaining six patients: (i) inadequate volume of lung aspirate, (ii) saline dilution, or (iii) small numbers of fungal organisms in lung lesions (6). Some investigators reported that the rate of positive cultures of BAL fluid ranged from 63 to 100% in immunocompromised hosts (4, 9, 11). However, those studies were limited to a small number of patients and focused on immunodeficient patients. Contamination of colonized *C. neoformans* in the airway should be cautiously considered in BAL fluid because this fungus may be a saprophyte. It is emphasized that direct lung aspiration is a more reliable method and avoids possible contamination.

False-positive detection of cryptococcal antigens in body fluids is well documented and can be due to cross-reactions with another fungus and immunoglobulins, especially the rheumatoid factor. In our study, one patient with metastatic lung cancer (renal cell carcinoma) had cryptococcal antigens (titer, 1:16) in lung aspirate. The factors responsible for false-positives remained uncertain, whereas the possibility of nonspecific interference with immunoglobulin was reduced in lung aspirates and pronase-treated samples. False-positive detection of cryptococcal antigen in the CSF of patients with malignant diseases had been reported previously (14, 24). However, cryptococcal antigens in lung aspirate may mean subclinical or potential subsequent infection. As Baughman et al. reported (1), a significant titer of 1:8 or more for cryptococcal antigen in lung specimens, including lung aspirate and BAL fluid, may signal subsequent full-blown dissemination of infection. However, the clinical significance needs further study.

Pulmonary cryptococcosis, in both immunocompetent individuals and immunocompromised hosts, is not a rare disease and may become a fatally disseminated disease. A timely and correct diagnosis is mandatory. The latex agglutination test for *C. neoformans* antigen is more rapid and the results are more reliable than isolation of this organism, and antigen is occasionally detected in patients from whom no organisms can be isolated. Although this test has not yet been approved by the Food and Drug Administration for determination of cryptococcal antigen in lung aspirates, we suggest that it should be included as an adjunct test for evaluation of patients with pulmonary cryptococcosis. This prospective study proves that direct determination of cryptococcal antigen in lung aspirate, through US-guided percutaneous needle lung aspiration, is useful for diagnosis of pulmonary cryptococcosis.

REFERENCES

1. **Baughman, R. P., J. C. Rhodes, M. N. Dohn, H. Henderson, and P. T. Frame.** 1992. Detection of cryptococcal antigen in bronchoalveolar lavage fluid: a prospective study of diagnostic utility. Am. Rev. Respir. Dis. **145:**1226–1229.
2. **Bhattacharjee, A. K., J. E. Bennett, and C. P. J. Glaudemans.** 1984. Capsular polysaccharides of *Cryptococcus neoformans*. Rev. Infect. Dis. **6:**619–624.
3. **Campbell, G. D.** 1966. Primary pulmonary cryptococcosis. Am. Rev. Respir. Dis. **94:**263–273.
4. **Chechani, V., and S. L. Kamholz.** 1990. Pulmonary manifestations of disseminated cryptococcosis in patients with AIDS. Chest **98:**1060–1066.
5. **Chuck, S. L., and M. A. Sande.** 1989. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N. Engl. J. Med. **321:** 794–799.
6. **Conces, D. J., S. A. Clark, R. D. Tarver, and G. R. Schwenk.** 1989. Transthoracic needle biopsy: value in the diagnosis of pulmonary infections. AJR (Am. J. Roentgenol.) **152:**31–34.
7. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. Ann. Intern. Med. **80:**176–181.
8. **Diamond, R. D., and S. M. Dlevitz.** 1994. *Cryptococcus neoformans* pneumonia, p. 551–568. *In* J. E. Pennington (ed.), Respiratory infection: diagnosis and management. Raven Press, New York.
9. **Duperval, R., P. E. Hermans, N. S. Brewer, and G. D. Roberts.** 1977. Cryptococcosis, with emphasis on the significance of isolation of *Cryptococcus neoformans* from the respiratory tract. Chest **72:**13–19.
10. **Fisher, B. D., and D. Armstrong.** 1977. Cryptococcal interstitial pneumonia: value of immunologic detection of cryptococcal antigen determination. N. Engl. J. Med. **297:**1440–1441.
11. **Gal, A. A., S. Evans, and P. R. Meyer.** 1987. The clinical evaluation of cryptococcal infections in the acquired immunodeficiency syndrome. Diagn. Microbiol. Infect. Dis. **7:**249–254.

12. **Goodman, J. S., L. Kaufman, and M. G. Koenig.** 1971. Diagnosis of cryptococcal meningitis: value of immunologic detection of cryptococcal antigen. N. Engl. J. Med. **185:**434–436.
13. **Gordonson, J., W. Birnbaum, G. Jacobson, and E. N. Sargent.** 1974. Pulmonary cryptococcosis. Radiology **112:**557–561.
14. **Hopfer, R. L., E. V. Perry, and V. Fainstein.** 1982. Diagnosis value of cryptococcal antigen in the cerebrospinal fluid of patients with malignant disease. J. Infect. Dis. **145:**915.
15. **Hsu, C. Y.** 1993. Cytological diagnosis of pulmonary cryptococcosis in immunocompetent hosts. Acta Cytol. **37:**667–672.
16. **Jensen, W. A., R. M. Rose, S. M. Hammer, and A. W. Karchmer.** 1985. Serological diagnosis caused by *Cryptococcus neoformans*. Am. Rev. Respir. Dis. **132:**189–191.
17. **Kaufman, L., and E. Errol.** 1992. Serodiagnosis of fungal diseases, p. 517–519. *In* N. R. Rose, E. Conway de Macario, J. L. Fahey, H. Friedman, and G. M. Penn. (ed.), Manual of clinical laboratory immunology, 4th ed. American Society for Microbiology, Washington, D.C.
18. **Kerkering, T. M., R. J. Duma, and S. Shadomy.** 1981. The evolution of pulmonary cryptococcosis: clinical implications from a study of 41 patients with and without host factors. Ann. Intern. Med. **94:**611–616.
19. **Lee, L. N., P. C. Yang, S. H. Kuo, K. T. Luh, D. B. Chang, and C. J. Yu.** 1993. Diagnosis of pulmonary cryptococcosis by ultrasound guided percutaneous aspiration. Thorax **48:**75–78.
20. **Lewis, J. L., and S. Rabinovich.** 1972. The wide spectrum of cryptococcal infections. Am. J. Med. **53:**315–322.
21. **Liaw, Y. S., P. C. Yang, Z. G. Wu, C. J. Yu, D. B. Chang, L. N. Lee, S. H. Kuo, and K. T. Luh.** 1994. The bacteriology of obstructive pneumonitis: a prospective study using ultrasound-guided transthoracic needle aspiration. Am. J. Respir. Crit. Care Med. **149:**1648–1653.
22. **McDonnell, J. M., and G. M. Hutchins.** 1985. Pulmonary cryptococcosis. Hum. Pathol. **16:**121–128.
23. **Riu, C. H.** 1956. A study of staining blood film (Romanowsky system). J. Niigata Med. Assoc. **70:**635–643.
24. **Sachs, M. K., C. M. Huang, D. Ost, and D. L. Jungkind.** 1991. Failure of dithiothreitol and pronase to reveal a false-positive cryptococcal antigen determination in cerebrospinal fluid. Am. J. Clin. Pathol. **96:**381–384.
25. **Yang, P. C., D. B. Chang, C. J. Yu, Y. C. Lee, H. D. Wu, S. H. Kuo, and K. T. Luh.** 1992. Ultrasound-guided core biopsy of thoracic tumors. Am. Rev. Respir. Dis. **146:**763–767.
26. **Yang, P. C., K. T. Luh, D. B. Chang, C. J. Yu, S. H. Kuo, and H. D. Wu.** 1992. Ultrasonographic evaluation of consolidation. Am. Rev. Respir. Dis. **146:** 757–762.
27. **Yang, P. C., K. T. Luh, J. C. Sheu, S. H. Kuo, and S. P. Yang.** 1985. Peripheral pulmonary lesions: ultrasonography and ultrasonically guided aspiration biopsy. Radiology **155:**451–456.
28. **Young, E. J., D. D. Hirsh, V. Fainstein, and T. W. Williams.** 1980. Pleural effusion due to *Cryptococcus neoformans*: a review of literature and report of two cases with cryptococcal antigen determinations. Am. Rev. Respir. Dis. **121:**743–747.