

Pulmonary Cryptococcosis in the Immunocompetent Patient—Many Questions, Some Answers

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Background. There are no prospective data regarding the management of pulmonary cryptococcosis in the immunocompetent patient. Clinical guidelines recommend oral fluconazole for patients with mild to moderate symptoms and amphotericin B plus flucytosine followed by fluconazole for severe disease. It is unclear whether patients who have histological evidence of *Cryptococcus neoformans* but negative cultures will even respond to drug treatment. We evaluated and managed a patient whose presentation and course raised important questions regarding the significance of negative cultures, antifungal choices, duration of therapy, and resolution of clinical, serologic, and radiographic findings.

Methods. In addition to our experience, to answer these questions we reviewed available case reports and case series regarding immunocompetent patients with pulmonary cryptococcosis for the last 55 years using the following definitions: Definite - Clinical and/or radiographic findings of pulmonary infection and respiratory tract isolation of *C. neoformans* without other suspected etiologies; Probable - Clinical and radiographic findings of pulmonary infection, histopathologic evidence of *C. neoformans*, and negative fungal cultures with or without a positive cryptococcal polysaccharide antigen.

Results. Pulmonary cryptococcosis resolves in most patients with or without specific antifungal therapy. Clinical, radiographic, and serologic resolution is slow and may take years.

Conclusions. Persistently positive antigen titers are most common in untreated patients and may remain strongly positive despite complete or partial resolution of disease. Respiratory fungal cultures are often negative and may indicate nonviable organisms.

Keywords. cryptococcosis; *Cryptococcus neoformans*; pulmonary cryptococcosis; pulmonary fungal infections.

Although the lung is almost invariably the portal of entry for cryptococcal infections, cryptococcal pneumonia is an uncommonly diagnosed disorder. In an epidemiological study of 1491 patients diagnosed with cryptococcosis between 1992 and 2000, a total of 58 (4%) presented with pulmonary disease and only 12 (0.8%) were not human immunodeficiency virus (HIV)-infected [1]. Furthermore, among non-immunocompromised subjects, Nadrous et al [2] reported that 36 (86%) of 42 with cryptococcal infection, in contrast, had isolated pulmonary involvement. Approximately one-third of the patients with pulmonary cryptococcosis are asymptomatic [3]. Of the remainder, one-half have cough or chest pain, 32% have sputum production that is bloody 18% of the time. Weight loss and fever occur in approximately 26% [4]. The most common radiographic findings in the immunocompetent host are single or multiple peripheral nodules [4]. There are no prospectively

obtained data regarding the management of pulmonary cryptococcosis in either the immunocompromised or immunocompetent patient. On the basis of available retrospective series and anecdotal reports, clinical guidelines have been published that recommend oral fluconazole for 6 to 12 months for patients with mild to moderate symptoms and amphotericin B plus flucytosine for at least 4 weeks followed by an 8-week consolidation course of fluconazole for severe disease [5]. There were no recommendations for follow up or for the management of asymptomatic, isolated pulmonary infection. It is further unclear whether immunocompetent patients who have histological evidence of *Cryptococcus neoformans* from a lung biopsy, but negative cultures of the lung tissue, will even respond to drug treatment [4]. We had the occasion to evaluate and manage a patient whose presentation and course raised some of these important issues.

CASE REPORT

A 35-year-old man without previous serious illness presented to the Medical College of Georgia Hospital of Augusta University in May 2005 with a cough of 4 months' duration preceded initially by subjective fever and sore throat. Associated symptoms included headache, night sweats, retrosternal pain, dyspnea on exertion, and weight loss. The illness had been unresponsive to several months' treatment with a macrolide and a fluoroquinolone. He had returned a year earlier from a mission trip to

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Guadalajara, Mexico, where he had heavy exposure to pigeon guano while sweeping the church floor, which was a converted garage where the birds roosted. His physical exam revealed only cervical and axillary lymphadenopathy. A chest radiograph showed bilateral, patchy, alveolar infiltrates. However, computed tomography (CT) of the chest demonstrated several bilateral pulmonary nodules, some of which were cavitating (Figure 1). Laboratory studies including examination of cerebrospinal fluid (CSF) were unremarkable except for a serum cryptococcal antigen titer of >1:64. He was seronegative on HIV testing. A CD4 cell count was 761/ μ L and immunoglobulin levels were normal. Histopathology of a transbronchial biopsy disclosed innumerable, encapsulated, budding yeast of varying sizes indicative of *Cryptococcus* spp, which failed to grow in culture. Given the significant exposure to pigeon guano, infection by *Cryptococcus gattii* was deemed unlikely, although it is entirely possible that his infection occurred at some other time. He was given a 9-month course of 400 mg oral fluconazole therapy per day without improvement. Because of the remote possibility of a fluconazole-resistant yeast, he then received 8 additional weeks of 200 mg of oral voriconazole twice daily. Dry cough, fatigue, and dyspnea continued. Physical examination and chest radiographs were unchanged. Serum cryptococcal antigen titers were repeatedly >1:64 at 3-month intervals. Accordingly, he was given 310 mg (4 mg/kg) of intravenous (IV) liposomal amphotericin B (L-AmB) per day after AmB deoxycholate at 0.6 mg/kg per day resulted in an abrupt rise in serum creatinine on the third hospital day. He received a total of 5 weeks of L-AmB along with 1250 mg of oral flucytosine every 6 hours, again without improvement. Because he was clinically stable, further antifungal therapy was withheld. On a follow-up exam in November 2007, his only remaining symptom was occasional, nonproductive cough, and he was considered clinically resolved even though his chest radiograph remained unchanged. His serum cryptococcal antigen titer was 1:32. Although in the ensuing months he became completely asymptomatic, a repeat radiograph in October 2008 disclosed “. . . two

more prominent nodules in the right middle or right lower lobe area and another in the left lower lobe area.” Accordingly, a repeat CT scan of the chest was performed 2 weeks later, which showed “. . . Overall improvement. . . Solidification of (the) previously cavitary lesions suggestive of partially-treated fungal infection.” In summary, 3 years after his initial symptoms, he became asymptomatic although the pulmonary nodules persist to the present. The serum cryptococcal antigen titer remained at 1:32 by May 2013.

Because of the failure of aggressive therapy to result in complete resolution of the clinical and radiographic manifestations of cryptococcal pneumonia over a period of 6 years in our patient, we sought to review what is known about the natural history of treated and untreated disease and answer the following relevant questions:

In immunocompetent patients:

1. Does symptomatic pulmonary cryptococcosis resolve in most individuals without antifungal therapy?
2. Does asymptomatic pulmonary cryptococcosis resolve in most individuals without antifungal therapy?
3. Does antifungal therapy hasten clinical and radiographic resolution of pulmonary disease in both symptomatic and asymptomatic individuals?
4. How often can negative fungal cultures be expected when pulmonary tissues or secretions reveal encapsulated yeast and what is their significance?
5. Can antifungal therapy be discontinued despite the presence of significant radiographic abnormalities?
6. How long do pulmonary lesions persist with and without therapy?
7. What is the significance of persistently positive serum cryptococcal antigen testing with pulmonary cryptococcosis?

METHODS

We reviewed available case reports and case series regarding immunocompetent patients with pulmonary cryptococcosis for

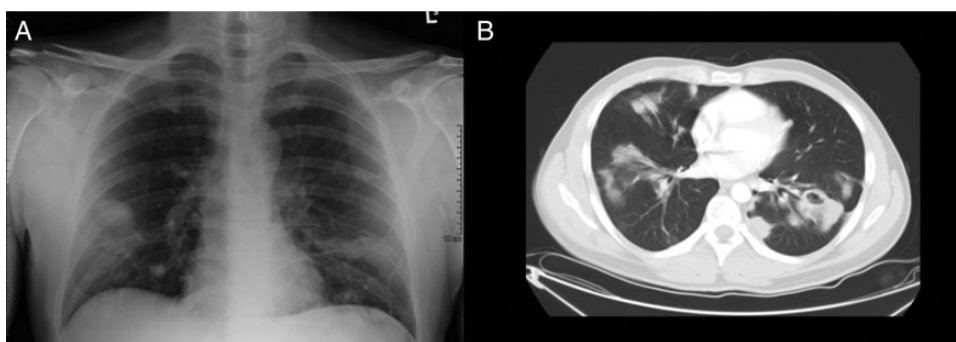


Figure 1. (A) Chest radiograph at presentation showing numerous nodules and masses. (B) Computed tomography showing numerous nodules and masses with areas of cavitation.

the last 55 years. In the evaluation of the literature, definitions of proven or presumed cryptococcal pneumonia were variable. In as much as India ink mounts of CSF and serum and CSF cryptococcal antigen may be detectable long after apparent successful therapy [6], it remains unclear whether fungal organisms seen histopathologically in pulmonary tissue can be proven to be alive or dead. Indeed, it could be argued that a continuing inflammatory reaction against antigenic components of nonviable organisms explains clinical and radiographic findings in some patients. Therefore, in our examination of the literature, we categorized pulmonary cryptococcosis as follows: (1) Definite - Clinical and/or radiographic findings consistent with pulmonary infection and isolation of *C. neoformans* from respiratory secretions or tissue without other suspected etiologies; and (2) Probable - Clinical and radiographic findings consistent with pulmonary infection, histopathologic findings of encapsulated yeast having the morphology of *C. neoformans*, and negative fungal cultures with or without serologic evidence of cryptococcal polysaccharide antigen.

According to these definitions, our patient had probable pulmonary cryptococcosis.

RESULTS

According to the above definitions of disease, we found 28 patients with definite pulmonary cryptococcosis and 75 patients with probable pulmonary cryptococcosis who had no identifiable immunocompromising condition at the time of evaluation. Responses to the posed questions were as follows:

1. **Does symptomatic pulmonary cryptococcosis resolve in most individuals without antifungal therapy?** The literature search disclosed 9 patients with respiratory symptoms attributed to pulmonary cryptococcal infection (5 proven, 4 probable) [2,7–9] who received no antifungal therapy. All clinical findings resolved in the patients, but the time to resolution was variable in the 8 patients in whom it was reported. Residual evidence of cryptococcal lung infection in these individuals ranged from approximately 5 months to as long as 5.08 years. One patient who became asymptomatic continued to have radiographic evidence of disease after 9 years of follow up. There were no deaths in this group of patients. We conclude that symptomatic pulmonary cryptococcosis resolves in the majority of patients without specific antifungal therapy, but that complete resolution of all radiographic findings may take years.

2. **Does symptomatic pulmonary cryptococcosis resolve in most individuals with antifungal therapy?** Clinical details were available in our review of the literature for 50 patients symptomatic from pulmonary cryptococcosis (17 proven; 36 probable) [2, 7–26] who received various forms of antifungal therapy. Treatment led to complete resolution of clinical and radiographic evidence of pulmonary infection in 6 of 16 patients with culture-proven disease in from 6 to 52 weeks. Follow-up

data were not provided in 3. Resolution of all but residual radiographic evidence of disease occurred in the remainder. Nineteen of the patients with probable pulmonary cryptococcosis had a complete response after treatment in 6 weeks to 2 years, whereas the radiographs remained abnormal in 12 after 4 to 6 weeks. There were 2 deaths and an individual who failed to respond to 8 weeks of fluconazole and underwent resection of the pulmonary lesion. We conclude that resolution of symptomatic pulmonary cryptococcosis is accelerated with appropriate treatment, but it is slow.

3. **Does asymptomatic pulmonary cryptococcosis resolve in most individuals without antifungal therapy?** Our literature search yielded 10 patients (2 proven cryptococcal pneumonia, 8 probable) [2, 14, 27–29], in which chest radiography disclosed an incidental abnormality in an asymptomatic, previously healthy individual that on evaluation was shown to be caused by *C. neoformans*. Six of the 10 x-rays cleared completely, 4 within 13–15.3 months. Time to resolution was not given for 2 of these 6 patients. Partial resolution occurred in 2 of the remaining patients after 14 months to 7 years, respectively, of follow up. Residual nodules remained in the latter patient. Details regarding the partial resolution in the last 2 patients were not available. We conclude that complete resolution can be expected in the majority of untreated, asymptomatic patients with pulmonary cryptococcosis, but that the healing process may take more than 1 year. Residual nodules have been noted in as long as 7 years of follow up in these individuals.

4. **Does asymptomatic pulmonary cryptococcosis resolve in most individuals with antifungal therapy?** We found 30 patients without respiratory symptoms whose radiographs disclosed abnormalities, which were demonstrated by attending physicians to be caused by *C. neoformans* (1 proven; 29 probable) [2, 18, 23, 25, 30]. Twenty-two received various courses of fluconazole ranging from 2 weeks to more than 6 months.

5. Two were given AmB plus flucytosine for an unknown length of time, and 1 patient received AmB alone in a total course of 2.8 grams. The remaining 5 patients received combinations of fluconazole and voriconazole or a 2-week induction course of AmB followed by 1–2 weeks of fluconazole.

6. Chest x-rays returned to normal in 6 and partially cleared in the remainder. We conclude that antifungals are associated with complete or partial resolution of clinical and radiographic findings in asymptomatic patients with pulmonary cryptococcosis.

7. **How often can negative fungal cultures be expected when pulmonary tissues or secretions reveal encapsulated yeast and what is their significance?** In an analysis of all groups of pulmonary cryptococcosis, criteria for proven or probable disease diagnosis were variable and differed from ours. We were able to identify 48 patients [2, 12, 17, 26, 31, 32] whose pulmonary cryptococcosis included histopathology or smears of infected secretions demonstrating the characteristic morphology of cryptococci along with culture results. Of those

patients, 29 specimens (60%) yielded the organism in culture. We conclude that cultures are often negative in patients who have histopathologic evidence of cryptococcal lung disease. These findings are consistent with, but do not prove, the notion that some organisms may be nonviable and yet incite an ongoing inflammatory response in the lung that may be either symptomatic or asymptomatic.

8. Can antifungal therapy be discontinued despite the presence of significant radiographic abnormalities? A total of 81 patients with proven or probable cryptococcal pneumonia received antifungal therapy. Forty-three had complete remission, 36 had partial remission, 1 died, and 1 failed therapy and required lobectomy. Therapy was discontinued in the 36 patients with partial remission despite persistent radiographic abnormalities. We conclude that the vast majority of patients with proven or probable cryptococcal pneumonia do not develop progressive disease after a course of therapy and that antifungal treatment can be safely discontinued. Until randomized, controlled studies are available to define the appropriate single or combination of antifungal drugs and the appropriate duration, the management strategy must be individualized to each patient and left to the judgment of the physician caring for the patient.

9. How long do pulmonary lesions persist with and without therapy? We found 8 untreated patients with pulmonary cryptococcosis for whom follow-up information was sufficient to provide time to complete radiographic resolution of pulmonary lesions [14, 26, 28, 29, 33–35]. Complete resolution occurred among these patients within 4.5–60 months (mean, 18.2 months; median, 13.3 months). For 13 patients receiving antifungal therapy, the time to complete resolution ranged from 2 to 18 months (mean, 11.5; median, 12.5). We conclude that complete resolution of pulmonary lesions can be expected within 1–2 years in most patients irrespective of whether therapy has been given, but that there are many patients who are left with residual pulmonary abnormalities for much longer periods of time.

10. What is the significance of persistently positive serum cryptococcal antigen testing with pulmonary cryptococcosis? Data were available to attempt to answer this question on 28 patients. Six had received no antifungal therapy [10, 29, 31, 35], 1 of whom underwent resection of the residual pulmonary nodular consolidation. Cryptococcal antigen titers declined in 5 of the 6 untreated patients from >1:32 by at least 3-fold. Only 1 patient's serum cryptococcal antigen became negative.

Unexpectedly, the cryptococcal antigen titer in the patient treated surgically, after an initial titer of 1:256, declined to 1:32 by the follow-up visit at 10 months, remained stable at that titer at the time of resection at 24 months, and persisted at 1:32 at 48 months with the patient completely well.

Twenty-five patients received antifungal therapy [8, 10, 18, 19, 22, 26]. Cryptococcal antigen titers declined from >1:32 in 22

patients, became negative in 19, but remained persistently positive at 1:4–1:8 in 4 patients. Curiously, 1 patient had a rise in cryptococcal antigen titer—1 after a month of therapy to 1:256 from an initial result of 1:64 [26].

We conclude that cryptococcal antigen titers decline gradually in a majority of patients with or without antifungal chemotherapy. Persistently positive antigen titers are most commonly seen in untreated patients, and in some of these cases they may remain strongly positive despite complete or partial resolution of disease. Specific antifungal therapy ordinarily results in negative serum cryptococcal antigen in most patients within 1 year. An occasional treated individual will remain persistently positive for cryptococcal antigen at low titer for at least 1–2 years. Testing of treated individuals within 1 month may demonstrate a rise in titer from baseline and then ultimate decline. Whether this rise reflects polysaccharide antigen shedding from dying organisms remains speculative.

DISCUSSION

Our patient presented with a chronic cough, fever, night sweats, and weight loss presumably after exposure to large amounts of aerosolized pigeon droppings. Multiple, bilateral nodular densities on chest radiography led to a transbronchial biopsy, which showed innumerable, mucicarmine-positive, encapsulated, yeast consistent with pulmonary cryptococcosis. If he acquired this infection in Mexico, the incubation period was 4 months. Such a presentation has been reported in multiple case series, but a 1965 series of 4 patients with culture-proven cryptococcal pneumonia by Campbell [3] is noteworthy. At the time of his review of the literature, there had been a total of 101 reported cases, only 19 (19%) of which were proven culturally. Our review of 103 cases of pulmonary cryptococcosis identified 49 patients with histopathologic evidence of cryptococcal infection in whom culture results were reported. Only 29 (60%) specimens were culture-positive. Thus, failure to grow the yeast seen histopathologically is commonplace. Inasmuch as cryptococci grow readily from CSF in patients with cryptococcal meningitis [36–39] and from other infected human tissues and fluids [40–44], it is difficult to explain why cultures of respiratory secretions for the yeast seen in pathologically proven pulmonary cryptococcosis are so frequently negative. A possible explanation is that the yeast seen in pulmonary tissue in many instances are not viable, but continue to evoke an inflammatory response. In one study of experimental pulmonary cryptococcosis, when 10^6 heat-killed cryptococci were inoculated intratracheally, the yeast cells were phagocytosed by 24 hours, cytopathic changes were present inside macrophages, and neutrophils surrounded them [45]. In another model of pulmonary cryptococcosis [46], a suspension of heat-killed *C. neoformans* was inoculated intratracheally into mice and inflammatory markers were subsequently monitored. Macrophages, T and B lymphocytes increased, peaked at day 7,

and gradually decreased to baseline levels. There was evidence of macrophage activation as indicated by enhanced expression of major histocompatibility complex class II, intercellular adhesion molecule-1, and Fc receptor. T cells were also activated as shown by the presence of interleukin IL-2 receptor. In addition, CD4⁺ T cells preferentially accumulated in the lungs of the animals. In further work from the same laboratory [47], once again heat-killed cryptococci were inoculated intratracheally into mice, and an increase of the same inflammatory markers was observed. Histopathologically, approximately two thirds of macrophages were found to have ingested an average of 3.77 ± 0.12 yeast cells per macrophage. The phagocytosis could be significantly reduced by injecting mice with monoclonal antibodies to interferon- γ or to depletion of CD4⁺ and CD8⁺ cells. Such studies support the concept that nonviable cryptococci in human lungs are capable of producing a substantial inflammatory response in the surrounding tissue, which could conceivably include clinical symptoms.

McMullan et al [48] reported on the use of microscopy and the use of vital dyes to determine the viability of *C. neoformans* in CSF in the clinical laboratory. Viable cryptococci are able to exclude trypan blue; nonviable yeast cannot. Yeast cells containing the dye were rapidly identified by light microscopy and counted in a hemocytometer. In an additional experiment, these investigators explored the use of flow cytometry to quantitate viable cells, which were able to take up a dye and enzymatically convert it to a fluorescent derivative. Using adaptations of such methods, it would be of interest if yeast suggestive of pulmonary cryptococcosis obtained from secretions or biopsy specimens from the respiratory tract could be tested for viability when cultures are negative. Results could affect patient management.

With respect to symptomatic pulmonary cryptococcosis, our review of the literature indicates that resolution is enhanced with antifungal treatment, but that residual pulmonary lesions may require years to fully resolve. Cryptococcal antigen titers gradually decline especially with therapy. Our patient is a rather dramatic example of how slowly the resolution process of treated cryptococcal pneumonia can occur. Whether the bilateral extent of his disease and ongoing inflammation against nonviable organisms was the explanation for his sluggish clinical response is purely speculative. Nevertheless, complete resolution of pulmonary lesions can be expected in most patients, treated or untreated, within 1–2 years if it will occur, but residual pulmonary abnormalities are common.

In retrospect, based upon the response of our patient and the toxic potential of antifungal agents and finding similar patients in our literature review, a strong case could have been made for a single course of treatment with close follow up. It appears that stable patients such as ours with rather mild clinical findings could have been simply treated symptomatically after the first treatment regimen. We believe that a more aggressive approach

with prolonged initial treatment or change to an alternative antifungal agent should be reserved for patients with severe symptoms and continued isolation of *C. neoformans* from respiratory secretions.

CONCLUSIONS

In conclusion, we believe that patients with proven or probable cryptococcal pneumonia, whether symptomatic or not, should receive a single course of antifungal therapy. The guidelines of the Infectious Diseases Society of America for the treatment of pulmonary cryptococcosis in immunocompetent patients along with the strength of the data supporting them [5] can be summarized as follows:

- (a) Mild to moderate symptoms: fluconazole 400 mg/day orally $\times 6$ –12 months (B-II)
- (b) Severe disease: such as cryptococcal meningitis (ie, amphotericin B deoxycholate [AmBd]), 0.7–1.0 mg/kg IV + flucytosine 100 mg/kg per day in 4 divided doses $\times 4$ weeks; followed by fluconazole 800 mg daily $\times 8$ weeks; then fluconazole 200 mg daily for 6–12 months (B-III)
- (c) Alternatives: itraconazole or voriconazole 200 mg twice daily orally, posaconazole 400 mg twice daily orally (if fluconazole unavailable or contraindicated) (B-II)
- (d) Surgery considered for diagnosis, persistent radiographic abnormalities, and symptoms not responding to antifungal therapy (B-III)
- (e) Consider lumbar puncture except in asymptomatic pulmonary nodules or infiltrate, no central nervous system symptoms, and negative or very low serum cryptococcal antigen (B-II). It is appropriate to point out that some apparently immunocompetent patients are being increasingly recognized to be at greater risk for cryptococcal central nervous system infection [49–51]. Accordingly, routine screening for occult immunosuppression may need to be expanded in the future.
- (f) For acute respiratory distress syndrome, consider corticosteroid treatment.

After any of the above treatment regimens, protracted symptoms, and signs may occur, but further treatment is ordinarily not necessary.

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