A Case of Pulmonary Cryptococcosis Caused by Capsule-deficient *Cryptococcus neoformans* in an Immunocompetent Patient

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Introduction

Cryptococcus neoformans is a common cause of opportunistic infection in patients with human immunodeficiency virus (HIV) and others with cell-mediated immunodeficiency, such as patients with solid-organ-transplant-related immunosuppression, patients receiving corticosteroids or other immunosuppressive therapy, and patients with lymphoid malignancy.[1,2] The central nervous system (CNS) is the most common clinically relevant site of cryptococcal infection, and meningoencephalitis is the most frequently encountered manifestation. Also, most of the morbidity and mortality due to cryptococcosis results from its CNS involvement. The pulmonary system is the second important site of infection, as reported by a study of 306 HIV-negative patients with cryptococcosis, which found CNS involvement in 157 patients and pulmonary involvement in 109 patients.[3] Most immunocompetent patients with pulmonary cryptococcosis are symptomatic, and the most common presenting symptoms are cough, dyspnea, and fever.[3-5] The most common radiological finding in pulmonary cryptococcosis is well defined solitary or multiple pulmonary nodules.[5,6] The capsule of *Cryptococcus neoformans* has antiphagocytic properties and is an important virulence determinant.[7] Infection by a capsule-deficient strain of *Cryptococcus neoformans* is rare in immunocompromised patients and even rarer among immunocompetent patients. We present a case of pulmonary cryptococcosis caused by capsule-deficient *Cryptococcus neoformans* in an immunocompetent patient, discovered incidentally during the diagnosis of bilateral pulmonary nodules resembling metastatic lung cancer.

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Case Summary

A 67-year-old woman was admitted to the hospital with a complaint of left leg swelling and pain for one week. She was from China and had immigrated to the United States 3 weeks prior to admission. She had a history of hypertension and cholelithiasis. Other important medical history included recurrent dry cough for 4 years, for which she was evaluated in China, but no diagnosis was established.

She did not have fever, chest/abdominal pain, bowel/bladder disturbances, change in appetite/weight, or night sweats. She denied exposure to pigeons or other animals and had no sick contacts. She had no history of receiving organ transplantation, chemotherapy, radiotherapy, or corticosteroids or other immunosuppressive agents. She denied using tobacco, recreational drugs, or drinking alcohol.

Physical examination showed an obese woman with normal vital signs and a few bibasilar crackles. Her left lower leg was edematous, erythematous, and tender, especially at the calf. Cervical, axillary, and inguinal lymph nodes were not palpable. Examinations of her other systems were normal.

Laboratory results showed normal complete blood count, including total and differential white count, serum electrolytes, renal function tests and coagulation tests. Her CD4 count was 818 cells/mL.

Doppler ultrasonography revealed thrombosis of the popliteal vein, the common femoral vein, and the superficial femoral vein, with extension into the great saphenous vein on the left side. Chest radiography showed a right perihilar radiodensity and a left-lower-lobe pulmonary nodule (Fig. 1). Computed tomogram showed multiple pulmonary nodules in both the lower lobes, up to 2.7 cm × 1.7 cm on the right and 1.8 cm × 1.6 cm on the left (Fig. 2). She underwent computed-tomography-guided percutaneous core needle biopsy of the right-lower-lobe nodule. The tissue specimens were stained with hematoxilin and eosin and Gomori's methenamine silver (GMS) stains, which revealed multiple non-necrotizing granulomas with giant cells containing budding yeasts (Fig. 3). Mucicarmine stain for capsular material was negative (see Fig. 3).

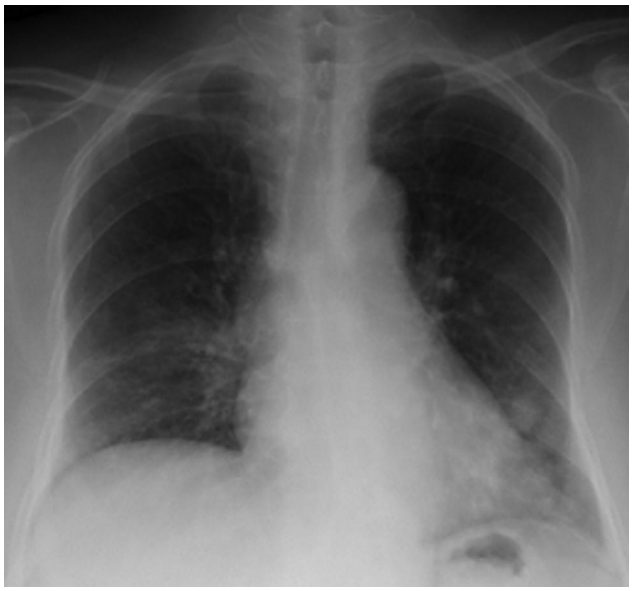

Fig. 1. Posteroanterior chest radiograph shows left-lower-lobe pulmonary nodule and right perihilar radiodensity.

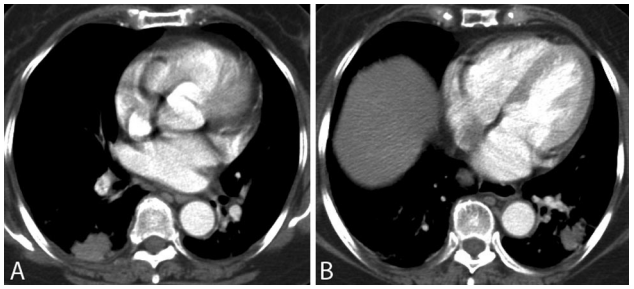

Fig. 2. Computed tomogram shows (A) right and (B) left lower lobe pulmonary nodules.

Serum cryptococcal antigen was positive, with a titer of 1:32. Serology was negative for *Coccidiodes immitis*, *Blastomyces dermatitidis*, and HIV. Urine histoplasma antigen was also negative. Since the fungal elements stained negative with mucicarmine, we considered the possibility of a capsule-deficient strain of *Cryptococcus*. The lung biopsy was repeated to obtain tissue for Fontana-Masson silver (FMS) stain, as there was not enough tissue left from the first biopsy specimen. FMS stain came back strongly positive (see Fig. 3).

Although tissue fungal culture did not grow *Cryptococcus*, the diagnosis of pulmonary cryptococcosis from capsule-deficient *Cryptococcus neoformans* was made on the basis of the presence of recurrent cough, radiologic evidence of pulmonary nodules, positive histopathology, and repeatedly positive serology for *Cryptococcus*. She was treated with intravenous amphotericin B for 2 days, which was then changed to fluconazole. She had no neurological symptoms, low serum cryptococcal antigen titer, and did not undergo lumbar puncture. She also received anticoagulation therapy to treat venous thrombosis.

Discussion

Lungs are the initial site of infection by *Cryptococcus neoformans* in humans. It is believed that humans become infected by inhalation of the besidiospores (smaller sexual spore form) of this organism, which deposit in the terminal bronchioles and alveoli and cause focal pneumonitis. The subsequent course of the infection (resolution of pneumonitis, latent infection, or symptomatic dissemination) largely depends on the host's immune status, along with the inoculum and virulence of the infecting strain.[1] Immunocompromised patients with pulmonary cryptococcosis present with more severe symptoms and more rapid disease progression than immunocompetent patients,[4] and are more likely to have extrapulmonary disease. Pulmonary *Cryptococcus* infection is more prevalent in patients with compromised cell-mediated immunity, such as those with HIV, steroid or other immunosuppressive therapy, chemotherapy, lymphoid malignancies, and solid-organ-transplant-related immunosuppression.[1,2] However, hematopoietic-stem-cell transplant recipients may be much less affected by cryptococcosis, compared to solid-organ-transplant recipients.[8]

Most immunocompetent patients with pulmonary cryptococcosis are symptomatic; the common symptoms are cough, dyspnea, chest pain, increased sputum production, fever, weight loss, and hemoptysis.[1,3-5] Although rare, Pancoast's syndrome[9] and chest-wall involvement[10] have also been reported. It is unclear which patients will develop symptomatic infection, but it may depend on the size of the *Cryptococcus* inoculum, the virulence of the strain, and the host response. The pathological response to *Cryptococcus neoformans* infection is characteristically minimal or no tissue inflammation, and the capsule stains deep red with mucicarmine.[11] The capsule has antiphagocytic properties and it also prevents the exposure of the underlying protein antigen.[12] Loss of capsular material elicits an intense inflammatory reaction characterized by early suppuration and phagocytosis, and tends to form granulomas,[13-15] as seen in our patient. Although, the capsule is the most important virulence determinant, and acapsular strains are less virulent in animal models,[16] capsule-deficient *Cryptococcus neoformans* can produce clinically important pulmonary lesions, which suggests that other factors might be involved in *Cryptococcus* pathogenicity in lung tissue. Capsule-deficient *Cryptococcus neoformans* pulmonary cryptococcosis in immunocompetent patients has been reported in very few prior studies.[17]

The most common radiographic finding in pulmonary cryptococcosis in the immunocompetent host is well defined solitary or multiple non-calcified pulmonary nod-

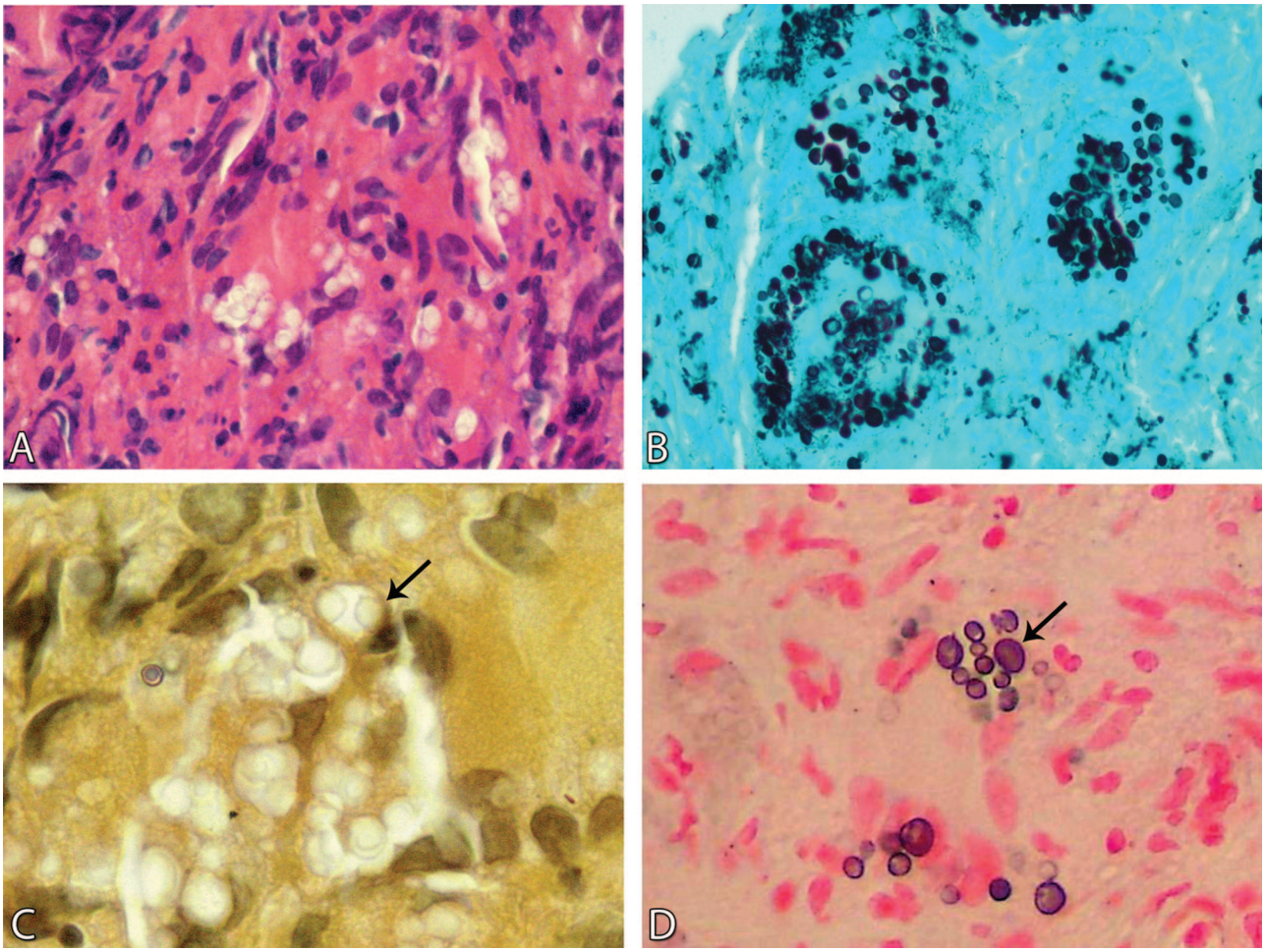

Fig. 3. A: Hematoxylin and eosin stained lung biopsy specimen shows giant cells containing multiple fungal spores (× 200). B: Multiple fungal colonies with some budding yeasts, strongly stained by Gomori's methenamine silver (GMS) stain (× 200). C: Mucicarmine stain shows multiple yeast forms of *Cryptococcus neoformans* with unstained capsule (× 400, arrow). D: Fontana-Masson silver (FMS) stain shows strongly positive staining of the cryptococcal organisms (× 400, arrow).

ules,[1,5] which may resemble metastasis. Other chest radiographic abnormalities may be diffuse reticulonodular opacities, pleural effusion, hilar and mediastinal lymphadenopathy, and, rarely, cavitations within cryptococcal nodules.[1]

The diagnosis of cryptococcal infection is made by culturing the organism in the sputum, tissue, serum, cerebrospinal fluid, or other specimen. Culture does not always grow the organism, so histopathology and staining of the tissue sample provide additional support in making the diagnosis and should always be sought along with serology.

The diagnosis of capsule-deficient *Cryptococcus neoformans* via tissue section analysis alone is difficult because many other nonencapsulated fungi, such as *Coccidiodes immitis*, *Blastomyces dermatitidis*, and *Histoplasma capsulatum* can mimic capsule-deficient *Cryptococcus neoformans* morphologically and in the type of the tissue response they elicit; and they all stain negative with mucicarmine, like capsule-deficient *Cryptococcus neoformans*.[13-15,18] In such cases, the FMS stain is very helpful to differentiate capsule-deficient *Cryptococcus neoformans*[18] and can be used as a specific stain for the histological diagnosis of cryptococcosis.[19] The FMS stain, originally developed by Fontana in 1912 and later modified by Masson in 1928,[18] detects melanin and other silver reducing substances. *Cryptococcus neoformans* possesses the enzyme phenol oxidase that catalyzes conversion of phenolic compounds to melanin. Melanin accumulates in the cell wall of the organism and can be detected with the FMS stain. Among the other pathogenic fungi, only the cell wall of *Sporothrix schenckii* stains positive with the FMS stain, but it can be differentiated by its light brown color, versus the dark brown cell wall of *Cryptococcus neoformans*.[19] In the present case, the lung biopsy tissue culture did not

grow any organism, and the pathology examination revealed multiple granulomatas with fungal forms that stained with GMS stain but not with mucicarmine. These organisms stained strongly positive with the FMS stain.

Serum cryptococcal antigen testing is sensitive and specific (90%), and titer ≥ 1:4 in biological fluid strongly suggests infection.[1] This patient had a positive serology and a serum cryptococcal antigen titer of 1:32, although the histopathology showed absence of the capsule. The presence of capsular cryptococcal antigen in capsule-deficient *Cryptococcus neoformans* infection has been reported.[15,20] A patient with a high serological titer is more likely to have a high burden of infection and thus a higher likelihood of extra-pulmonary spread, especially to the CNS. A patient with a high serum cryptococcal antigen titer, neurological symptoms, and underlying predisposing conditions such as immunodeficiency should undergo lumbar puncture to rule out CNS infection by *Cryptococcus neoformans*. A serum cryptococcal antigen titer higher than 1:64 is associated with extra-pulmonary dissemination in non-HIV patients with pulmonary cryptococcosis.[21] We did not pursue lumbar puncture for our patient because she had low serum cryptococcal antigen titer, no neurological symptoms, and no underlying immunodeficiency.

According to the practice guidelines of the Infectious Disease Society of America[22] for the management of cryptococcal disease, asymptomatic immunocompetent patients with pulmonary cryptococcosis may require careful observation and no systemic therapy. Symptomatic patients, serum cryptococcal antigen titer of > 1:8, and positive lung cultures for *Cryptococcus neoformans* are indications to start therapy with oral azoles.[22] For patients with mild to moderate symptoms, oral therapy with fluconazole (200–400 mg/d) is indicated for 6–12 months.[22] If the patient cannot tolerate fluconazole, an acceptable alternative is itraconazole (200–400 mg/d). For patients with more severe symptoms, progressive disease and intolerance to azoles, amphotericin B (0.5–1 mg/kg/d, total dose 1,000–2000 mg) is recommended.[22] This patient was initially treated with amphotericin B for 2 days, which might not have been necessary. Then we changed it to fluconazole for the duration of therapy, in accord with the Infectious Disease Society of America guidelines.[22]

We attributed the deep venous thrombosis in this case to prolonged immobilization during her recent flight from China to the United States. We do not draw any causal relationship between the cryptococcosis and the deep venous thrombosis.

Teaching Points

Cryptococcal infection should be a part of the differential diagnosis of pulmonary nodules. Definitive diagnosis of pulmonary cryptococcosis is made via fungal cultures. The FMS stain can be used as a specific stain for the histological diagnosis of cryptococcosis. This case emphasizes the role of FMS stain in diagnosing cryptococcal infection when fungal cultures are negative. In addition, this case illustrates a rare presentation of pulmonary cryptococcosis caused by capsule-deficient *Cryptococcus neoformans* in an immunocompetent host.

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