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Case Report

Pulmonary Infection With Capsule-Deficient *Cryptococcus neoformans*

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Summary. A case of bilateral lobar pneumonia due to *Cryptococcus neoformans* is presented. The capsule-deficient fungal organisms within tissue were suggestive of *Histoplasma capsulatum*. Cultures grew *Cryptococcus neoformans* and there was cryptococcal antigen in the serum. Pulmonary cryptococcosis and the occurrence of unencapsulated cryptococci are reviewed. The important role both culture and the latex agglutination test for cryptococcal antigen play in the differential diagnosis of systemic fungal infections with over-lapping histopathology are discussed.

Key words: Capsule-deficient *Cryptococcus neoformans* – Pulmonary cryptococcosis – Pulmonary fungal infection – Morphologic overlap in fungi.

Introduction

Cryptococcosis limited to the lungs is an uncommon clinical and pathologic diagnosis. The radiologic spectrum of disease ranges from a solitary pulmonary nodule to massive bilateral infiltrates. A review of the English literature (Campbell, 1966) listed a total of 101 cases reported since the original case of primary pulmonary cryptococcosis was described (Sheppe, 1924). Three-fourths of these cases were diagnosed only after histological examination at surgery or autopsy. More recent reports and reviews have confirmed the usual method of diagnosis of invasive disease to be microscopic examination of tissue (Hammerman et al., 1973; Smith et al., 1976; Tynes et al., 1968) or aspiration cytology (Prolla et al., 1970; Whitaker et al., 1976). The morphology of typical *Cryptococcus neoformans* (oval to round budding yeasts ranging in size from 5 μ to 10 μ with a large mucicarmine positive capsule) is distinctive and diagnostic. However, cases of cryptococcosis morphologically indistinguishable from blastomycosis (Farmer et al., 1973) and histoplasmosis (Gutierrez et al., 1975) have been reported. This report describes the case of a man with bilateral lower lobe pneumonia caused by a capsule-deficient *C. neoformans* that on biopsy histologically mimicked *Histoplasma capsulatum* and illustrates the role of culture and serologic procedures in obtaining an accurate diagnosis.

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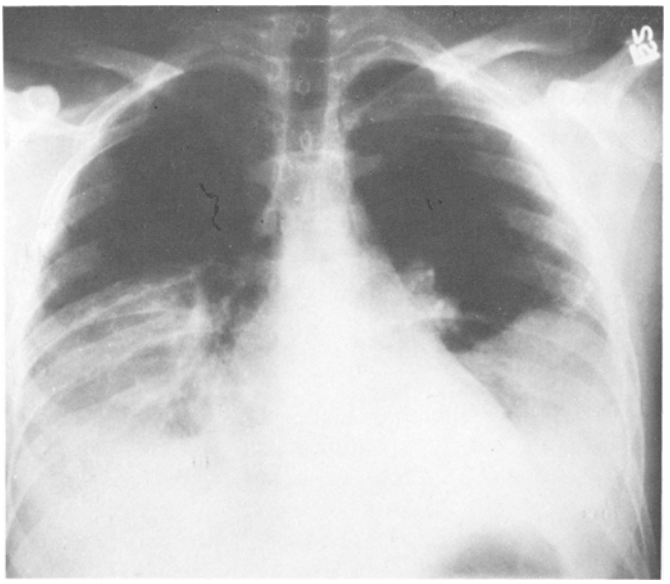

Fig. 1. Posteroanterior chest roentgenogram obtained on admission. Bilateral dense lower lobe infiltrates are present

Case Report

A 21-year-old white male with fever to 105° F, productive cough and shortness of breath was admitted to the University of Virginia Hospital following 2 weeks of progressive symptoms and a work-up in another hospital for a bilateral pneumonia of undetermined etiology. One month before admission he participated in rescue squad maneuvers and was exposed to dust contaminated with bird droppings in an abandoned building.

Admission chest X-ray revealed bilateral lower lobe infiltrates (Fig. 1). Gram stain of sputum obtained by transtracheal aspirate revealed numerous neutrophils but no organisms. Examination of cerebrospinal fluid was normal. Fever persisted to 40° C and the patient remained in respiratory distress despite broad spectrum antibacterial therapy. Bacterial and viral cultures and serological studies for Histoplasma and Blastomyces were negative. Bronchoscopy revealed diffusely hyperemic bronchial mucosa and material exuding from both lower lobe bronchi. A lower lobe transbronchial lung biopsy was reported as consistent with histoplasmosis. A bone marrow revealed no fungi or granulomas. Amphotericin B therapy was initiated at 20 mg IV daily.

Sixteen days after admission a positive sputum fungal culture isolate was identified as *Cryptococcus neoformans*. The admission serum cryptococcal antigen titer, performed after the culture became positive, was 1:32. CSF was again normal and CSF cryptococcal antigen was negative.

The patient progressively improved over the next 2 weeks; fever abated over one week, the cough and dyspnea decreased. The patient was discharged on the twenty-seventh hospital day. A total dose of Amphotericin B to exceed 1 g is planned on an outpatient basis. When seen 2 months after discharge he was further improved but the chest x-ray revealed only minimal clearing of the infiltrates. The serum cryptococcal antigen titer has fallen to 1:2.

Pathological Findings

Sections of the trans-bronchial lung biopsy stained with hematoxylin and eosin showed intense granulomatous inflammation. Numerous multinucleated giant

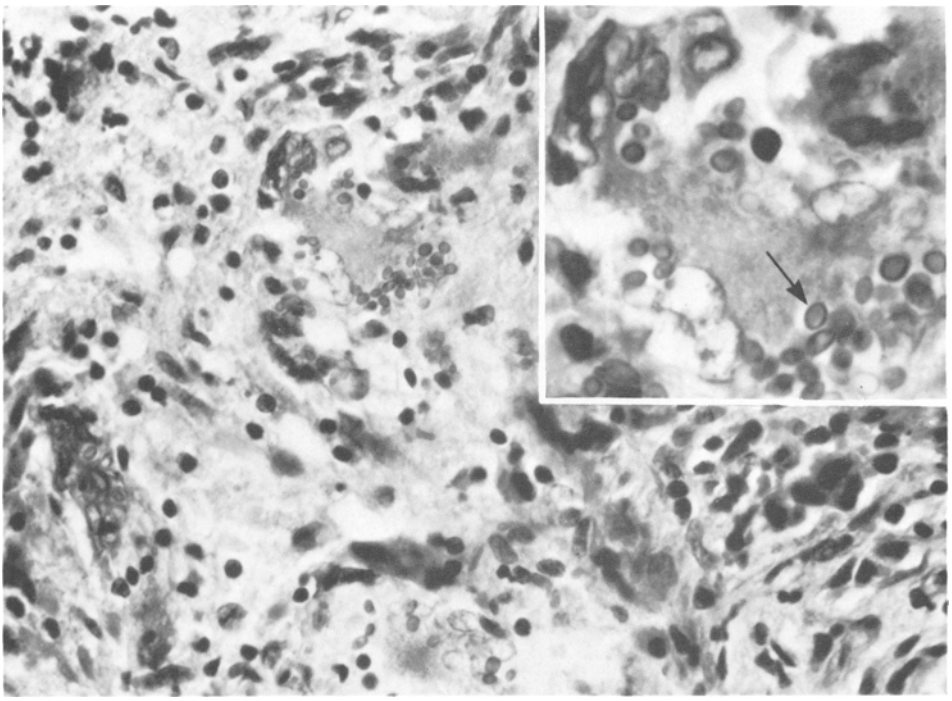

Fig. 2. Microscope section of lung, showing dense granulomatous inflammation. Multinucleated giant cell containing many unencapsulated yeast. Periodic acid-Schiff × 520. Inset × 1,300

cells surrounded by lymphocytes, epithelioid cells and occasional scattered polymorphonuclear leukocytes and plasma cells were seen (Fig. 2). Caseation and necrosis were absent. Small vacuoles containing very faintly staining oval structures were present within giant cells.

After the tissue sections were stained with period acid-Schiff (PAS) and Gomori methenamine-silver (GMS) numerous oval or round fungal organisms ranging in size from approximately 2 μ to 4 μ were identified in clusters within giant cells (Fig. 2 inset) and scattered throughout the tissue. Mucicarmine stain did not demonstrate any capsules. The histologic diagnosis of histoplasmosis was made on the basis of the size of the organisms and their lack of encapsulation.

Bronchial washings, and lung tissue from the trans-bronchial biopsy were cultured. Bacterial and mycobacterial cultures were negative. Fungal cultures grew non-mucoid yeast colonies after 1 week of incubation. The isolates were identified biochemically as *Cryptococcus neoformans*. Sub-cultures on Staib's *Guizotia abyssinica* seed agar showed characteristic brown pigment formation within 5 days. India ink preparations were initially negative for capsule formation. After repeated subculture on Sabouraud's dextrose agar some of the cells showed the formation of very thin, attenuated capsules by India ink preparation and electron microscopy (Fig. 3).

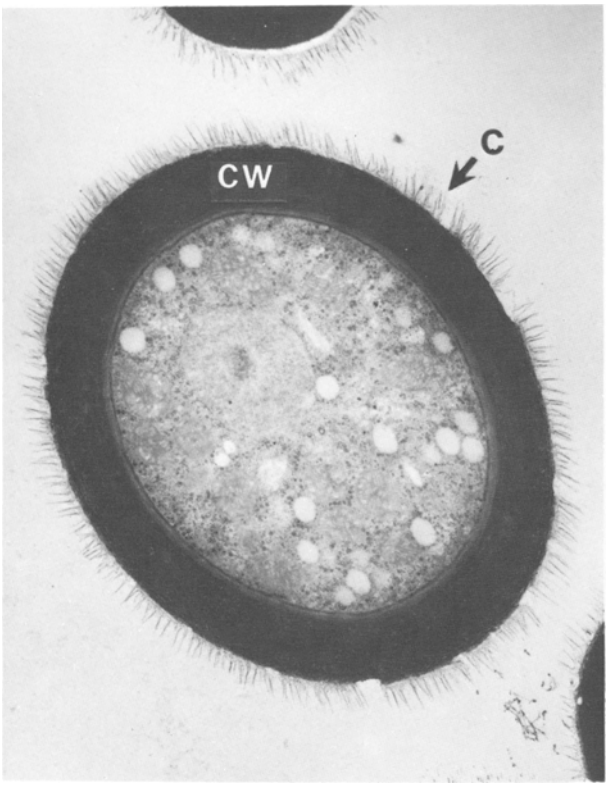

Fig. 3. Electron micrograph of cultured organism. Laminated cell wall (*cw*) and cytoplasmic organelles are well preserved. The capsule (*c*) is markedly attenuated and less than one-fifth of normal thickness × 20,000

Discussion

Cryptococcus neoformans is both an opportunistic invader in patients with severe underlying disease and a pathogen in apparently normal hosts. Cryptococcal meningitis and disseminated cryptococcosis are much more frequently associated with an immunologically compromised host than is pulmonary infection. Only 11 of the 101 patients in Campbell's review (1966) of primary pulmonary cryptococcosis had severe co-existing diseases. This case report describes the typical patient with pulmonary cryptococcosis; a white adult male, with no underlying immunologic impairment or co-existing severe disease. In addition, this case illustrates the morphologic overlap of the tissue forms of *Cryptococcus neoformans* and other systemic pathogenic fungi, in this instance *Histoplasma capsulatum*. It serves as a reminder that unencapsulated or capsule deficient cryptococci can cause disease and their accurate recognition depends on the use of cultural and serologic techniques.

That *C. neoformans* exists in pigeon excreta and soil in the unencapsulated state was documented by Emmons (1962) and later Ishaq et al. (1968). The possibility that this smaller, lighter, more readily airborne and therefore inhaled form of the organism is the infectious particle has been postulated (Bulmer

et al., 1968). Experimental studies (Farhi et al., 1970) have shown that this unencapsulated yeast is capable of producing cryptococcosis in mice and that capsule development occurred subsequently in vivo. However, another study in mice (Farmer et al., 1973) using a capsule-deficient isolate from a human case that morphologically resembled blastomycosis showed no tendency for in vivo capsule development. While this strain of the organism was not lethal for the mice when injected intracerebrally, an intense chronic inflammatory response resulted. Many of the organisms were phagocytized in contrast to encapsulated *C. neoformans*.

In human cryptococcosis, the inflammatory response varied from minimal to an intense chronic inflammatory reaction with granuloma formation, fibrosis and in a few cases caseous necrosis (Baker et al., 1955). Inoculum size, organism virulence and host response are probably some of the determining factors of the pathologic process. This patient may have received an overwhelming number of organisms since significant disease developed despite the fact that microscopically visible capsule formation did not occur in vivo to inhibit phagocytosis. Sufficient polysaccharide antigen was produced to give a positive serum cryptococcal antigen test, however, and perhaps the presence of this virulence associated antigen is more important than the size of the capsule seen morphologically.

The latex agglutination test for cryptococcal antigen has been instrumental in the diagnosis of cryptococcal meningitis. The assay (when performed on CSF) is currently sensitive and highly specific when appropriately controlled (Bennett et al., 1971; Goodman et al., 1971). The sensitivity in serum (for CNS infections) is much lower, however the specificity remains. The sensitivity of this assay in pulmonary or disseminated disease is unknown. Because of the increased frequency of isolation of *C. neoformans* in cultures of sputum and bronchial secretions without evidence of invasive disease (Randhawa et al., 1977), the diagnostic importance of positive cultures alone is diminishing. The presence of cryptococcal Ag in serum can, as in this case, be highly significant (Kaufman et al., 1968; Fisher et al., 1977). If circulating cryptococcal Ag is present in a patient with pulmonary disease and no evidence of meningitis the diagnosis of pulmonary cryptococcosis is strongly suggested by this simple non-invasive procedure. The number of false negatives in the presence of severe or progressive pulmonary disease has not been defined and there may be many. However, one would postulate the test to be more frequently positive in this setting than in solitary pulmonary foci or cryptococcomas. Total organism load has been correlated with positivity and titer in this assay (Kaufman et al., 1968).

The therapeutic implications of accurately diagnosing the fungal pathogen are several. When primary histoplasmosis is present, the patient will most likely resolve even moderately severe disease without the need for antimycotic therapy. However, if symptomatic blastomycosis is diagnosed, amphotericin B therapy is indicated because local progression and dissemination are more likely. If cryptococcosis is established, a careful search for meningeal involvement should be performed. In symptomatic cryptococcal pulmonary disease, antimycotic therapy is indicated, and therapeutic options include 5-Fluorocytosine in combination with lower total doses of Amphotericin B as well as Amphotericin B

alone (Utz et al., 1975). The diagnosis of cryptococcosis necessitates investigations for host immunologic impairment or severe underlying diseases. Pulmonary histoplasmosis or blastomycosis in any form are less frequently associated with the compromised host.

Other techniques such as specific fluorescent antibody stains have been applied to confirm the tissue diagnosis (Gutierrez et al., 1975; Pidcoe et al., 1968). These antisera are not generally available and the demand in most institutions would be very low. Material for these procedures should be sent to a reference laboratory such as the Center for Disease Control if serologic procedures are not definative and cultures at the time of biopsy are not performed or are negative. This is particularly important when the morphology suggests *Histoplasma capsulatum* or *Blastomyces dermatitidis* and capsule deficient or unencapsulated *Cryptococcus neoformans* cannot be excluded.

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