CLINICAL REPORT

Pulmonary cryptococcosis in non-AIDS patients

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Abstract

Purpose: To clarify the clinical features and imaging characteristics of non-AIDS patients with pulmonary cryptococcosis.

Methods: We retrospectively collected 15 HIV-negative patients with pathology-proved pulmonary cryptococcosis from Sep1992 to Jan 2008. Their medical records and radiological data were reviewed and analyzed.

Results: Only one patient was asymptomatic. Thirteen patients were immunocompetent and two were immunosuppressed. Three patients had associated cryptococcosis meningitis. The most common radiographic abnormalities were multiple pulmonary nodules or masses, seen in 8 and 5 cases of patients respectively. 14 patients received specific therapy for *Cryptococcus neoformans*. Two patients died. In the 11 patients with isolated pulmonary cryptococcosis, treatment consisted of fluconazole alone (n=7), in combination with amphotericin B (n=2), and both 5-flucytosine and amphotericin B (n=2). For the other 2 patients with cryptococcosis meningitis, one was treated with amphotericin B alone and the other with fluconazole combined with amphotericin B and 5-flucytosine.

Conclusions: Non-AIDS patients might also be susceptible to cryptococcosis infection. Histological examination is the principal method of diagnosis. The most common CT findings are solitary or multiple nodules with or without cavitation in the subpleural areas of the lung.

Cryptococcosis is caused by infection with the encapsulated fungus, *Cryptococcus neoformans*, an organism with a worldwide distribution. Inhalation of or-

ganisms is the usual route of the infection that may remain isolated to the lungs or undergo hematogenous spread to involve the central nervous system (CNS), bones, and skin, depending on the host immune status.¹ Cryptococcal infection can occur in individuals with normal immunity but is more common in the immunocompromised host. Predisposing factors include acquired immunodeficiency syndrome (AIDS), hematological malignancy, organ transplantation, corticosteroid therapy, diabetes mellitus, and other conditions that impair T-cell mediated immunity.^{1,2} In particular, cryptococcosis is most commonly associated with AIDS.³ However, only a few case reports or small-scale studies of pulmonary cryptococcosis in non-AIDS individuals have been published. The characteristics of pulmonary cryptococcosis in this group are still unclear. Therefore, respectively, we accumulated the clinical features, computed tomography (CT) findings, treatment and prognosis of 15 patients with pathology-proved pulmonary cryptococcosis between Sep 1992 and Jan 2008 in our hospital. The purpose was to search for the characteristics of pulmonary cryptococcosis in non-AIDS patients, especially aspects that may distinguish it from other pulmonary diseases. In addition, antifungal therapy and prognosis in immunocompetent host were evaluated.

Materials and methods

This retrospective study was carried out between Sep. 1992 and Jan. 2008 in our institution, an affiliated hospital of University. The study protocol was approved by Ethics Committee at our institution. During the study period, all patients with histological proved pulmonary cryptococcosis were eligible for inclusion in this investigation. The diagnosis of pulmonary cryptococcosis was confirmed by histological demonstration of the organisms in a lung biopsy specimen (obtained by surgery, percutaneous needle biopsy or thoracoscopic biopsy). All resected specimens were stained with Gomori methenamine silver stain (GMS), haematoxylin-eosin stain (HE), and periodic acid-Schiff (PAS), and reviewed by one pulmonary pathologist. Patients with sputum culture positive for *Cryptococcus neoformans* without clinical and radiologic manifestations of pneumonia were excluded from the study.

For all studied patients, data were collected by two physicians using specially designed case report forms (CRF). This included review of their laboratory data, chest radiograph and other reports, orders, treatment records, progress notes, nursing notes and follow-up flow sheets, which verified the timing of specific events. All forms were checked by another researcher for errors. Then, data were double entered manually into a Microsoft Excel master sheet to build our database. The CRF contained the following information: baseline characteristics, clinical presentation, radiographic images and histological results, in-hospital department, concomitant medication, source of specimen (sputum, cerebrospinal fluid and histology) and diagnostic methods (culture or pathology). Patients with following comorbidities were considered immunocompromised: malignancy, cirrhosis, end-stage renal failure, autoimmune disorders, diabetes mellitus, chronic use of corticosteroid or other immunosuppressive therapies, idiopathic CD4 T-cell lymphopenia, organ transplant recipient, HIV infection.

Chest radiographs and computed tomography (CT) images were reviewed by two thoracic radiologists for the presence and type of pulmonary infiltrates and the presence of nodules, cavitations, mass densities, and pleural effusions. Microbiology data included the date, specimen site, and results of cultures. Pathology data included date, specimen type, and result of specific stains (HE, Grocott, and PAS). Treatment, prognosis and follow-up information were also extracted from the medical charts. The primary cause of death was obtained from the discharge summary or autopsy report.

Results

Incidence

Between 1992 and 2008, 15 non-AIDS patients with pulmonary cryptococcosis were identified histologically. All were hospitalized. The incidence of pulmonary cryptococcosis by year during the study period is shown in Figure 1. There was no patient enrolled in 1993-2000, 2005 and 2007. All patients were negative for HIV by serological tests and none had received organ transplantation. Two immunocompromised patients were included in the current cohort, one with diabetes mellitus and severe hepatitis, and another with SLE receiving corticosteroid therapy. Thus, 86.7% were immunocompetent patients (n=13).

Patient characteristics

Seven patients were male and eight were female. The mean age at the time of diagnosis was 43 ± 15.5 yr (range, 15-62 yr). Thirteen patients (86.7%) were non-smokers. Four patients had a close contact with birds, one with partridges and pigeons. Four patients were from the department of infectious diseases, five from the department of thoracic surgery, five from the department of respiratory disease and one from the department of rheumatology.

Clinical manifestation are summarized in Table 1. Presenting symptoms at the time of diagnosis were

FIGURE 1. Annual incidence of pulmonary cryptococcosis (1992-2008) per 10,000 hospital admissions.

reported in 14 patients. The most common symptoms were cough and chest pain, followed by sputum production and fever. Chest pain was usually bearable with dull, blunt or tingling sensation. In most patients, cough was not serious and dry cough was more common. No one suffered from hemoptysis.

Fever was presented in four cases. One patient with liver failure and infectious shock presented with high-grade fever. The three patients with cryptococcal meningitis suffered low to moderate fever (37.5-38.5°C) and headache. One patient, with SLE, was asymptomatic and was detected by an abnormal radiography on routine examination. No patient without extrapulmonary involvement was pyrexial.

TABLE 1. Symptoms in patients with pulmonary cryptococcosis.

Symptoms	n	%
Cough	8	25
Sputum production	5	15.6
Chest pain	8	25
Fever	4	12.5
Headache	3	9.38
Chest distress	1	3.13
Asymptomatic	1	3.13
Total	32	100

Radiological features

The pulmonary abnormalities seen on the initial CT scans are summarized in Table 2. Nodules were defined as round or oval opacities < 3 cm in diameter. Opacities $\geq$ 3 cm in diameter were considered as masses. Overall, the most common radiographic abnormalities were multiple pulmonary nodules or masses, seen in 38.1 and 23.8% of patients respectively. Five patients presented as masses with peripheral small nodules of 10 to 20 mm diameter. Diffuse miliary lesions were seen in two patients and pleural involvement in three patients. The lower lobes were involved in 14 patients and upper lobes in four. Most lesions were located in the region surrounding the pleura. The areas of consolidation were predominantly subpleural. Typical radiological images of a 34-yr-old woman and a 23-yr-old man with solitary nodule and multiple nodules respectively are shown in Figures 2 and 3.

As described above, radiographs of pulmonary cryptococcosis commonly presented as pulmonary nodules, consolidation or even cavitation, which may be easily misdiagnosed as lung tumours. In addition, mass densities and infiltration may be considered to be pneumonia. In the current cohort, a primary diagnoses of lung cancer was made in 6 cases, pneumonia in 5, pulmonary tuberculosis in 3 and inflammatory pseudotumour in 1 case. Pulmonary cancer and pneumonia were the most common misdiagnoses at first visit.

TABLE 2. Chest radiographic findings in patients with pulmonary cryptococcosis.

Abnormality	n	%	Location	n	%
Nodules	8	38.1	Left upper lobe	4	16.7
Masses	5	23.8	Left lower lobe	8	33.3
Consolidation	4	19	Right upper lobe	1	4.2
Cavitation	3	14.3	Right lower lobe	6	25
Lobulation and speculation	1	4.8	Bilateral lung involvement	2	8.3
			Pleural invasion	3	12.5
Total	21	100	Total	24	100

FIGURE 2. A chest radiograph (A) and (B) CT of 34-yr-old woman, shows a solitary nodule in the middle third of his left pulmonary field.

Microbiology and pathology

Diagnosis of pulmonary cryptococcosis was made by direct tissue examination in all 15 patients (surgery - 2, percutaneous needle biopsy - 7, and thoroscopic resection - 6). Microscopic examination of lung tissue showed pulmonary cryptococcosis by Gomori methenamine silver stain (GMS), haematoxylin-eosin stain (HE), and PAS stain, and was performed in all patients (Figure 4). Testing for cryptococcal capsular antigen

in serum or cerebrospinal fluid (CSF) was not performed. Three patients with cryptococcosis meningitis had positive findings of Indian ink and culture on CSF examination. Among the 15 patients, positive culture for *Cryptococcus neoformans* was identified from sputum in three patients. Sputum cultures obtained at the time of pulmonary cryptococcosis diagnosis yielded an additional pathogen of *P. aeruginosa* in one patient.

Treatment and prognosis

Of the 15 patients, 14 received specific therapy for *Cryptococcus neoformans* and 2 died (Table 3). Improvement was confirmed in 13 patients presenting with normal temperature, amelioration of CNS symptoms and absorption of lung lesion in the chest radiology. Of the two deaths, one died of multiple organ dysfunction without antifungal therapy, and one with cryptococcosis meningitis died after receiving 10 days of fluconazole. Of other 2 patients with cryptococcosis meningitis, one was treated with amphotericin B alone and another with fluconazole combined with amphotericin B and 5-flucytosine. The length of hospitalization in these two patients was 117 and 260

FIGURE 3. Transverse CT scan in 23-yr-old man shows multiple irregularly marginated nodules in the left low pulmonary field.

FIGURE 4. (A) Methenamine silver (GMS) staining ($\times 400$). (B) Red staining in cell wall of *C. neoformans* by Periodic acid-Schiff (PAS) staining ($\times 400$). (C) Yeast cells of *C. neoformans* without staining of capsule in HE stained tissue section of lung ($\times 400$).

days, respectively. In the 11 patients with isolated pulmonary cryptococcosis, treatment consisted of fluconazole alone ($n=7$), combined with amphotericin B ($n=2$), or both 5-flucytosine and amphotericin B ($n=2$). These 11 patients recovered as confirmed by clinical symptomatology and radiograph, with the length of hospitalization ranging from 11-48 days.

Seven patients completed follow-up. The length of treatment ranged from 25 days to 6 months for six patients with isolated pulmonary cryptococcosis, and 12 months for one patient with cryptococcosis meningitis. No patient showed progressive dissemination or relapse during follow-up. Asymptomatic duration ranged from 6 to 48 months for six patients with isolated pulmonary cryptococcosis, and 72 months for the one patient with cryptococcosis meningitis.

Discussion

Our data confirmed that cryptococcosis infection may be present in immunocompetent patients without AIDS. The diagnosis relies upon histological examination because of the low positive culture rate. The most common radiographic abnormalities were multiple pulmonary nodules or masses, that may be misdiagnosed as tumours or tuberculosis. Antifungal therapy should be given to these non-immunosuppressed and symptomatic patients.

Clinical descriptions regarding pulmonary cryptococcosis with non-AIDS individuals are limited because of the rarity of the disease. The incidence of pulmonary cryptococcosis in our cohort was 0.7-3 per 10,000 hospital admissions between 1992 and 2008. Vilchez RA et al. reported that the annual incidence in non-AIDS patients over a 9-year period ranged from 0.5-7.5 per 10,000, with < 3 per 10,000 in 7 years.⁴ The prevalence of cryptococcosis is markedly increased among patients with defects in the cell-mediated arm of the immune system such as occurs in patients with AIDS, lymphoma, chronic leukemias, collagen vascular disease, sarcoidosis, and those receiving immunosuppressive drugs.^{1,2} Vilchez RA et al.

TABLE 3. Treatment and prognosis of 15 patients with pulmonary cryptococcosis.

#	Treatment	Duration wks	Prognosis	Resection
1	None	6	Died	
2	Fluconazole 400mg	4	Lost to follow up	Resect
3	AmBisome 150mg Fluconazole 300mg	4 28	Alive and well	Resect
4	Fluconazole 400mg	28	Alive and well	Resect
5	Fluconazole 400mg	24	Alive and well	
6	AmBisome 150mg Fluconazole 400mg 5-FC 4.5	2 6 2	Alive and well	
7	Fluconazole 200mg	4	Alive and well	Resect
8	AmBisome 100mg Fluconazole 400mg 5-FC 4.5	2 6 5	Alive and well	Resect
9	Fluconazole 400mg	2	Died	
10	Fluconazole 400mg	3	Lost to follow up	Resect
11	Fluconazole 200-400mg AmB 50mg 5-FC 4.5	26 24 24	Alive and well	
12	Fluconazole 200mg AmB 45mg	2 2	Lost to follow up	Resect
13	Fluconazole 400mg	2	Lost to follow up	
14	AmB 60mg	8	Lost to follow up	Resect
15	Fluconazole 400mg	2	Lost to follow up	

included 25 (66%) solid-organ transplant (SOT) recipients in their cohort.⁴ Most patients in our cohort were healthy with no known risk factors for cryptococcosis.

Clinical presentation of pulmonary cryptococcosis is variable, ranging from asymptomatic abnormal CXR findings to evidence of acute infection, such as fever, chest pain, cough, body weight loss and sputum production.⁵⁻⁸ About one-third of immunocompetent patients with pulmonary cryptococcosis were asymptomatic^{5,6} and were often identified during physical examination. In contrast, the majority of our patients (93.3%) with *Cryptococcus* lung involvement were symptomatic at the time of diagnosis: cough and chest pain were the most common symptoms. Asymptomatic patients occurred only with localized disease, implying a more indolent and benign course. By comparison, fever was more common in patients with disseminated disease.^{6,8,9}

Although the radiographic characteristics of pulmonary cryptococcosis have been well described, only a few studies focused on CT findings of pulmonary cryptococcosis in non-AIDS individuals.^{7,10,11} Zinck et al. demonstrated that the most common CT finding was solitary or multiple pulmonary nodules without cavitation in 10 of 11 cases and the margin of nodules was smooth in five cases.¹⁰ Our study demonstrated that pulmonary nodules were the most frequent CT findings of pulmonary cryptococcosis.^{5,11,12} The nodules may vary in size, ranging from 0.5 to 4 cm in diameter.^{12,13}

Lesions were most commonly distributed in the lower lung, followed by upper the field.^{5,11,13,14} Similarly, Chu HQ et al.¹⁴ found the predominance of lesions in the lower lobes. In the present study, the nodules presented in peripheral and subpleural regions, as described previously.¹¹⁻¹⁶ Less frequent radiographic features include segmental or lobar pneumonia, cavi-

tation, lymphadenopathy or pleural effusion. Radiographs of patients with pulmonary cryptococcosis are non-specific and can easily be confused with pulmonary tuberculosis, pneumonia, Wegener's granulomatosis, sarcoidosis, and especially lung cancer.^{12,17}

Treatment of pulmonary cryptococcosis depends on the host's immune status and the anatomical site of involvement.^{6,18,19} When the patient is immunocompromised or has dissemination of infection to the CNS or other organs, active therapy is warranted. Recommended treatment is with amphotericin B plus flucytosine for 6 – 10 months in patients with the CNS involvement. In the three patients with cryptococcosis meningitis in our cohort, the one treated with fluconazole alone died while the two patients treated with amphotericin B alone or combined with fluconazole survived.

Although immunocompromised hosts are considered to be at greatest risk for life-threatening complications they may also occur in healthy hosts.^{6,8,19} The rationale for treatment is twofold: to promote rapid resolution of clinical symptoms and signs that may be disabling, and to prevent the development of potentially life-threatening complications.

Therapeutic options include follow-up observation, antifungal therapy or surgical resection of the lesion in immunocompetent patients. Preferred treatment regimens are oral -azole therapy (fluconazole or itraconazole) in immunocompetent hosts with isolated pulmonary cryptococcosis and symptomatic infection. In our cohort, 3 immunocompetent patients were treated with fluconazole alone for 25-60 days and no progressive dissemination or relapse occurred during the follow-up period. For eight patients receiving surgical resection, the periods of antifungal therapy were 28 weeks, 28 weeks, 6 weeks and 4 weeks, respectively. No relapse occurred in the 2 patients with < 8 weeks of antifungal therapy. This suggests that the therapeutic duration in this population might be reduced.²⁰ Radiographic findings revealed improvement in all patients treated with oral-azole antifungal

agents alone without side effects, even in immunocompromised hosts. Pulmonary lesions hardly cleared even after treatment over 6 months in immunocompetent hosts. Thus, the therapeutic goal is the control of symptoms, rather than complete resolution of the pulmonary shadow.

Our study had several limitations. This was a retrospective study with a small number of patients collected over a long time. Patients in whom pulmonary cryptococcosis has been diagnosed and those who have been treated more recently may have a better prognosis, because progress in imaging technology and improvements in radiographic-guided biopsy methods may permit earlier diagnosis and improved outcomes. Only two patients with < 8 weeks antifungal therapy after surgical resection had no dissemination during follow-up. We cannot support full therapy (>6 months) after surgery in immunocompetent patients. Further study is required.

In conclusion, pulmonary cryptococcosis in non-AIDS patients is rare. Presentation imaging and management strategy are uncertain.

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