Clinical analysis of 76 patients pathologically diagnosed with pulmonary cryptococcosis

Yuan Zhang\textsuperscript{1*}, Nan Li\textsuperscript{1*}, Yuxuan Zhang\textsuperscript{1*}, Huiping Li\textsuperscript{1}, Xueyuan Chen\textsuperscript{1}, Shanmei Wang\textsuperscript{1}, Xia Zhang\textsuperscript{1}, Rongxuan Zhang\textsuperscript{2}, Jinfu Xu\textsuperscript{1}, Jingyun Shi\textsuperscript{3}, Rex C. Yung\textsuperscript{4}

\textsuperscript{1}Department of Respiratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, \textsuperscript{2}Department of Pathology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, \textsuperscript{3}Department of Radiology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, \textsuperscript{4}Department of pulmonary and critical care medicine, Johns Hopkins University School of Medicine, Baltimore 21205 MD, USA.

*Yuan Zhang, Nan Li and Yuxuan Zhang contributed equally to this work.

Correspondence: Huiping Li, Ph.D., Department of Respiratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, 507 Zhengmin Road, Shanghai 200433, China. Phone: 086-21-65115006-2103. Fax: 086-21-55663289. Email: lihuiping1958@yahoo.com.cn.
Abstract

Objectives: Investigate the clinical characteristics of pulmonary cryptococcosis (PC) patients in China, with analysis of immunocompetent and immunocompromised subjects.

Methods: Retrospective review of 76 patients diagnosed with tissue confirmed PC at the Shanghai Pulmonary Hospital during a 9 years period (2001-2009).

Results: Of 76 patients (males 54, females 22), 41 were immunocompetent (53.95%), and 35/41 were asymptomatic. Approximately 80% patients have histories suspicious of environmental fungal exposure. Radiological (Computer Tomographic) findings showed predominantly peripheral findings (85.53%, 65/76) including nodular masses (55.26%, 42/76), pneumonic infiltrates (23.68%, 18/76) and mixed (21.05%, 16/76). 43.42% (33/76) were initially misdiagnosed, often as cancer by false-positive 18F-FDG-PET (28/46 cases). 51 patients received antifungal therapy, 25 patients were clinically observed without treatment. As of December 31, 2010, 71 cases showed total recovery, 4 cases improvement (efficacy rate of 98.68%, 75/76). One HIV-positive case died of cryptoccal meningitis.

Conclusion: Incidence of PC in China may be related to environmental fungal exposures. Most presented as asymptomatic peripheral lung lesions. False positive 18FDG-PET examinations often lead to initial clinical misdiagnosis of cancer. Unlike immunocompromised or clinically symptomatic patients, all immunocompetent patients had a good response, either to fluconazole monotherapy, or observation with a tendency of spontaneous remissions in the asymptomatic immunocompetent subjects.
Key words: pulmonary cryptococcosis, pathological diagnosis, environmental exposure, immunocompetent host, serum cryptococcal antigen test
Introduction

Pulmonary cryptococcosis (PC) is an opportunistic infection. Most are caused by Cryptococcus neoformans or C.gattii infection [1, 2]. Previously, PC was thought to occur in patients with Acquired Immune Deficiency Syndrome (AIDS) and other immunocompromised states, such as patients undergoing organ transplantation or those on immunosuppressive regimens [3, 4]. Although immunocompromised hosts are considered to be at high risk for the life-threatening complications of cryptococcal pulmonary infection, PC can also occur in immunocompetent subjects [5, 6]. Compared to Cryptococcus neoformans, C.gattii is more inclined to cause disease in healthy people [7]. C. gattii infections has drawn increased attention since 2002 [8], with cases reported in Papua New Guinea and Northern Australia, India, Brazil, Vancouver Island in Canada, Washington State, and Oregon in the United States. However, C.gattii has been rare in China, Feng et al [9] reported only 9 of 110 clinical Cryptococcus isolates (8.2%) from China as C.gattii., with the vast majority were C.neoformans var. grubii, which is in accord with the data of most studies of clinical molecular epidemiology in other geographic areas [10, 11].

PC is not rare on the China mainland [12-14]. Our present understanding of PC is mostly limited to typical clinical manifestations and radiologic presentations. In fact, the great variations and protean manifestations of these clinical features also often lead to misdiagnosis. In addition, the treatment protocol for the PC especially for immunocompetent patients is not clearly stated. Here, we performed a retrospective evaluation of 76 patients with a tissue-confirmed diagnosis of pulmonary
cryptococcosis over a period of 9 years (2001-2010) at our hospital. Our specific aims are to provide an updated review and to characterize the epidemiological, clinical, immunological, radiological characteristics as well as treatment on PC in China.

Materials and Methods

1.1 Patients

A retrospective review based on discharge summary from January 2001 to December 2009, 76 consecutive patients with tissue proven PC verified by biopsy at Shanghai Pulmonary Hospital were identified and included in this review. Patients who were diagnosed with PC on the basis of cryptococcal antigen plus radiographic (CT/PET) findings that are consistent with PC, but without histological confirmations were excluded. The medical records of all patients, including demographics, underlying diseases, respiratory symptoms, laboratory tests including immune status studies, imaging data, treatment and outcome were collected. The relevant follow-up patient information was obtained on regular clinic visits and by telephone follow-up. The last follow-up was on December 31, 2010. The study plan has been approved by Shanghai Pulmonary Hospital's Ethics Committee (2011-FK-112), and the patients’ informed consents for participating of the study were obtained prior to data collection or analysis.

1.2 Pathological diagnosis

Histology: Diagnosis of PC was confirmed in 76 patients with tissue biopsy for pathology findings. Of the 76 cases, 68 cases received either a thoracoscopic surgery or
open-lung biopsy; 7 cases underwent transthoracic aspiration; and one case was
diagnosed bronchoscopically with Transbronchial Lung Biopsy (TBLB). All samples
were fixed by conventional 4% neutral formalin, embedded in paraffin, stained by
hematoxylin-eosin (HE), and histochemically stained by periodic acid Schiff (Pas),
mucus card Red (Mc) and Methenamine Silver (GMS). The samples were then
examined under light microscopy [14]. Thus, the presence of the cryptococcal pathogen
in the tissues sections is the direct histologic evidence of PC.

Culture: The specimen (serum, blood, BALF, tissue, sputum from patients and air
samples from patients’ living environments) were cultured in glucose AGAR culture
medium at room temperature (25 °C) or 37 °C for 2 ~ 5 days for fungi.

1.3 Radiologic Assessment

All PC patients during this period had a CT scan. The CT scans were carried out
with one of the two multi-slice detection scanners (Philips, Billiance 40, or Philips,
Billiance 64) in the Shanghai Pulmonary Hospital. Consecutive 8-mm-thick sections
were obtained from the lung apices to the lung bases with additional 1-2 mm thin
sections through areas with abnormal lesions. The main manifestations of CT finding
were mainly classified into three patterns, namely nodular, pneumonia and mixed
morphological characteristics. (1) The nodular imaging finding with clear boundary
was further subdivided divided into single nodular (Figure 1 a, b) and multi- nodular
(Figure 1 c), (2) the pneumatic infiltrates with ill-defined margin were subcategorized
as single and multifocal pneumonia (Figure 1 d, e), (3) in mixed morphological
patterns, both nodular and patchy infiltrates were detected (Figure 1 f). Cavity, pleural
effusion, mediastinal lymphadenopathy, air bronchograms and were also recorded accordingly. Two thoracic radiologists who were unaware of the patients’ immune status and clinical symptoms assessed all findings independently.

The brain CT and Magnetic Resonance Imaging (MRI) when performed, were recorded to evaluate who may have Central Nervous System (CNS) involvement.

1.4 Immune function tests and evaluation

The following tests for humoral or cellular immunity impairments were measured to determine status of immune function and impairment.

a) Humoral immune parameters, including serum IgG, IgA and IgM were quantitatively measured using immunoturbidimetry. Patients were classified as immune impaired when any two of the following values are below the lower limits of normal range (IgG: 7-16 g/l, IgA: 0.4-2.3 g/l and IgM 0.7-4.0 g/l).

b) Cellular immune parameters, included peripheral blood count and differential, (the total count and classification of white blood cells); serum CD3, CD4 and CD8 measured using flow cytometry. The cellular immune functions were regarded as normal when the ratio in percentage of CD3 cells ranges from 62 to 76%, the percentage of CD4 cells ranges from 30 to 40%, and the CD4/CD8 ratio ranged from 1.5 to 2.0.

c) Clinical history of immune suppression: The patients with a past history of at least one of the predisposing conditions, including use of immunosuppressive drugs (treatment with corticosteroids or disease modifying drugs with immunosuppressive effects), severe diabetes mellitus associated with organ damage, malignancies, HIV
infection, history of organ transplantation, severe respiratory system limitation or other systemic disorders eg. collagen vascular disease such as Lupus, on steroids, were considered immunocompromised. Patients were also considered immunodeficient if their peripheral Absolute Neutrophil count (ANC) was below $2.0 \times 10^4/\mu l$ or Absolute lymphocyte count (ALC) was below 1000/\mu l.

Serum cryptococcal antigen test (sCRAG): The result of Serological latex agglutination test was considered positive when titer equal to or greater than 1:8.

1.5 Clinical outcome evaluating criteria

**Cured/complete response (CR):** clinical symptoms resolved, and imaging findings indicating that all lesions have completely disappeared; **Partial Response (PR):** clinical symptoms are improved and imaging findings indicated that the lesions had partially resolved; **No change / progressive disease (NC/PD):** clinical symptoms and imaging findings did not change or the clinical symptoms have deteriorated, and or imaging indicated the lesions have progressed.

1.6 Statistical analysis

All the information was entered in an approved research database by 3 individuals independently, after verification, with the SPSS16.0 statistical analysis software for data processing. All data were expressed as mean ± SD. The Chi-square test was used for ordinal data, the t-test was used for numerical data and nonparametric test for Ranked data. Significance was set to $p<0.05$ for all statistical analysis.

**Results**
2.1 Demographic information

Our retrospective analysis identified 264 patients with a hospital discharge of pulmonary cryptococcosis, these included 76 patients with tissue proven PC. These 76 patients (males 54 and females 22) ranged from 19 to 72 years old (median, 50 years; mean age, 49.76±10.87 years). 21 patients (27.63%) had a history of smoking.

2.2 Immune function

Forty-one patients (53.95%) had no comorbidity or immune dysfunction; 35 cases (46.05%) that had abnormal testing results of the humoral immune or cell immune dysfunction and were considered immunocompromised (Table 1). These 35 patients had past history of at least one of the predisposing conditions, including use of immunosuppressive drugs, severe diabetes mellitus with organ damage, malignancy, HIV infection, respiratory system or other system serious disorders.

Table 1. The Past Medical History (PMH) of n=35 Immunocompromised Patients with Pulmonary Cryptococcosis

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Only humoral immune dysfunction (n=12, 34.29%)</th>
<th>Only cellular immune dysfunction (n=14, 40%)</th>
<th>Combined humoral and cellular immune dysfunction (n=9, 25.71%)</th>
<th>Total (n=35, 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunosuppressive drugs therapy a</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Severe Diabetes mellitus b</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HIV infection c</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory system disorders d</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>
Other system disorders
disorders 
\[a\] glucocorticoids, \(>10\text{mg}, >\text{three-month course, within the past half year;}
\[b\] Diabetes mellitus with organ damage;
\[c\] CD4+T cell account <200/\text{mm3 at time of PC diagnosis;}
\[d\] Respiratory system disorders including: old tuberculosis, 2 cases; chronic bronchitis, 2 cases; bronchial asthma, 1 case.
\[e\] Other system disorders including: autoimmune diseases and collagen diseases, 4 cases (systemic lupus erythematosus 1 case, dermatomyositis 1 case, scleroderma 2 cases); rheumatoid arthritis, 3 cases; hepatitis B, 2 cases; nephrotic syndrome, 1 case; cardiovascular diseases, 9 cases (coronary heart disease, 4 cases; severe arrhythmia, 2 cases; hypertension, 3 cases).

2.2 Environmental exposures

Approximately 80% of patients had a history of environmental exposure (a clear history of exposure to pigeon droppings, dust or fungal spores or keeping cats, dogs or poultry) (Table 2).

Table 2. History of Environmental Exposure of 76 patients with Diagnosis of Pulmonary Cryptococcosis

<table>
<thead>
<tr>
<th>Occupational environment exposure history</th>
<th>Number</th>
<th>Immuno-competent patients</th>
<th>Immuno-compromised patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of exposure to pigeon droppings</td>
<td>26(34.21%)</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>History of dust or fungal spores</td>
<td>19 (25.00%)</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>History of keeping cats, dogs or poultry</td>
<td>16(21.05%)</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>No exposure history</td>
<td>15(19.74%)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>76(100%)</td>
<td>41</td>
<td>35</td>
</tr>
</tbody>
</table>

2.4 Clinical manifestations
In this group, 45 patients (59.21%) had a variety of symptoms, including cough (41 cases), productive of sputum (35 cases), chest pain (16 cases), fever (14 cases), shortness of breath (12 cases), chest tightness (12 cases), fatigue and discomfort (9 cases), sweating (7 cases), minimum blood streaking in the sputum (7 cases), and gross hemoptysis (4 cases). All of the 35 immunocompromised patients had at least one or more of the above symptoms. Conversely most of the immunocompetent PC patients (31/41, 82.93%) did not present with any symptom, and their pulmonary diseases were detected by incidental radiological examination (chest X-ray or CT scan). 1 HIV-positive patient showed abnormal CNS imaging by head CT. Overall symptoms are much more likely in immunocompromised patients (35/35, 100%) than in immunocompetent patients (10/41, 24.39%) p<0.05.

2.5 Serological latex agglutination test: Of the 76 patients reviewed, 7 had sCRAG test, 5 showed positive results (titer ranged 1:320-1:1280).

2.6 Sputum examination: A sputum examination was performed on all patients in this group. *Cryptococcus neoformans* grew from only two cultures, for a culture positive rate of 2.63%.

2.7 Findings on chest imaging CT scan

1) Distribution of lesions: Of the 76 patients, lung lesions of 85.53% (65/76) patients located mostly in the peripheral lung field (outer 1/3 of the lung), close to the pleura. Lesions in 39 patients (51.32%) involved only the right lung, 22 patients (28.9%) only the left lung, and bilateral in 15 (19.74%). (Table 3).
Table 4. The relationship between imaging lesion patterns and immune functions

<table>
<thead>
<tr>
<th>Lesion patterns</th>
<th>Total (%)</th>
<th>Immuno-competent patients (n=41)</th>
<th>Immuno-compromised patients (n=35)</th>
<th>Size of nodules (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1.5</td>
</tr>
<tr>
<td><strong>Nodular</strong></td>
<td>42(55.2%)</td>
<td>28</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Single nodular</td>
<td>34(44.73%)</td>
<td>21</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Multi-nodular</td>
<td>8(10.53%)</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Pneumonic</strong></td>
<td>18(23.6%)</td>
<td>6</td>
<td>12</td>
<td>--</td>
</tr>
<tr>
<td><strong>Mixed</strong></td>
<td>16(21.05%)</td>
<td>7</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

2) Lesions were varied in shape (Figure 1), including single or multiple nodules, pneumonic infiltrates or both (mixed) (Table 4). 42 cases had nodular masses; 18 cases had pneumonic infiltrates; and 16 cases were of a mixed type. 68.30% of immunocompetent patients (28/41) showed either single or multi nodular lesions. 40% of immunocompromised patients displayed nodular lesions, the other 60% cases showed pneumonia or mixed-type.
Other accompanying signs

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>▲</th>
<th>▲</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavity</td>
<td>10(13.16%)</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>3(3.95%)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mediastinal</td>
<td>7(9.21%)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air bronchogram</td>
<td>4(5.26%)</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

▲ The difference between two groups is significant when p<0.05

3) FDG-PET: 46 patients in this group of 76 patients underwent $^{18}$Fluoro-deoxyglucose positron emission tomography ($^{18}$F-FDG-PET). In total, 28 cases (60.87%) displayed abnormal uptake standardized uptake value (SUV) > 2.5 and the lesion-to-normal tissue ratio (T/N) ≥1.3) in the lung lesions, which heightened the suspicion for lung cancer; 4 cases showed abnormal uptake with mild increase in SUV suspicious for non-malignant inflammation; 14 cases showed normal 18 FDG uptake indicating no active inflammation.

2.8 Tissue Acquisition: Overall, 68 cases of PC were confirmed by surgical biopsy, 7 by percutaneous needle lung biopsy, and 1 by Transbronchial Lung Biopsy (TBLB) via fiber-optic bronchoscope. Postoperative pathology by periodic acid Schiff (Pas), mucus card Red (Mc), six-ammonium silver staining or Grocottmethenamine silver nitrate (GMS) revealed cryptococcal-infected granuloma (Figure 2) or a more mucus-like substance in association with cryptococcal pathogen.

2.9 Diagnosis
The duration from the presentation in clinic to final tissue diagnosis ranged from 17 days to 1 year (average 65 ± 52 days; 67 ± 45 for immunocompetent vs. 64 ± 60 immunocompromised groups, \( p > 0.05 \)). Of all 76 cases, cryptococcal infection was considered in only 4 cases (5.26%) on the initial visits. The diagnosis of the preliminary testing in 39 cases (51.32%) were uncertain, although they were later confirmed as PC by biopsy. The misdiagnosis rate at presentation was 43.42% (33/76); 23 cases were misdiagnosed as lung cancer (FDG-PET also suggested lung cancer in these cases); 8 cases as lung bacterial infection; and 2 cases as tuberculosis.

2.10 Treatment and outcome

As shown in Table 5, 51 patients received antifungal treatments. The duration of the treatment ranged from 2 weeks to 2 years, with a mean of 4.8 months and a median of 3 months. Follow-ups ended on December 31, 2010 (12-114 months, average 58.82 ± 36.98 months). In the 76 patients, 71 cases were cured, and 4 cases showed improvement (overall efficiency, 98.68%). There was only one mortality, in the HIV+ patient (CD4 < 200 / mm³) complicated by cryptococcal meningitis. This patient died of cryptococcal encephalitis on the 4th day after a VATS lung biopsy.

| Table 5. Treatment and Outcome of 76 patients with Pulmonary Cryptococcosis |
|--------------------------------|-------------------|---------|---------|---------|
| Treatment methods             | Cases (%)         | CR      | PR      | NC/PD   |
| Anti-fungal treatment         |                   |         |         |         |
| Fluconazole                   | 39(51.32%)        | 39      | 0       | 0       |
| Itraconazole                  | 7(9.21%)          | 7       | 0       | 0       |
| Amphotericin B + 5-fluorocytosine | 5(6.58%)   | 1       | 3       | 1*      |
| Clinical observation without therapy | 25(32.89%) | 24      | 1       | 0       |
Discussion

In the present study of a single institution retrospective review of 76 patients pathologically diagnosed with Pulmonary Cryptococcosis, we found that pulmonary cryptococcosis is not rare in immunocompetent persons, whose disease is often an asymptomatic radiologic presentation, and whose clinical outcome is most often favorable. Recent data showed that the incidence of PC is increasing. An epidemiological demographic study in British Columbia, Canada [2] showed the annual incidence of PC has increased from 6 per million in 1999 to 38 per million in 2006. This greater than six fold increase cannot be explained by the common association with poorly controlled HIV patients, as it was reported that the incidence of people with HIV infection is stable. The increased incidence is mainly due to non-HIV infection [2]. At present, there are no data in China on the incidence of PC based on comprehensive epidemiological survey. Zhang et al’s [12] review summarized overall 113 reports and total 728 cases with PC in China from 1981-2009. The first PC case was reported by Zhu [15] in 1981; additional 65 cases were reported from 1981 to 2001; and another 668 cases reported from 2001 to 2009. While the number of PC cases being reported in China had increased significantly during recent decades, it is uncertain if this is a true increase in incidence or secondary to presumed infections based on wider serologic testing and more detailed chest imaging with greater access to...
CT imaging. In Zhang et al [12] review of Chinese PC patients, 102 reports included
detailed medical history and they found that a total of 404/580 (69.7%) cases had no
comorbidities that could explain an underlying immunosuppression. Our present cohort
of 76 tissue proven PC patients is the largest single group of patients with PC diagnosed
by pathological examination in China. Notably, similar to the Zhang series,
immunocompetent patients accounted for more than half (53.95%) of the patients in the
series. Even so, the single HIV patient in our cohort may seem inordinately low,
however medical care is organized in China in such a way that know HIV+ patients are
preferentially referred to Infectious Diseases hospitals and centers whereas our
institutions is specialized in pulmonary-focused diseases.

With regard to sex distribution of PC, our cohort of 76 PC patients included 54
males and 22 females. The male: female ratio (2.5:1) in this group is similar to a
previous PC report's ratio in China (M: F=2.3:1, males 487 and females 216) by Zhang
et al, [12].

Besides host immune factors, PC could also be related to environmental exposure
of contaminated airspace including close contact with animals [16-19], green plants
[20] or other natural sources contaminated by fungi [21]. Cryptococcus is an
encapsulated yeast that can be found in the feces of birds, especially pigeons through
dropping [16, 17]. Although there have been no reports of direct animal-to-human
transmission, animal can carry cryptococcal spores and may contaminate the
surrounding environments including trees, water, and soil through bird droppings.
Their ubiquitous presence in the environments could also become an important source
of cryptococcal infections as the flying birds can increase fungal spore counts in the air [22]. In addition, household pets such as cats [18] can carry pathogenic Cryptococcus. As Figure 3 shows, the cryptococcal spores can spread freely in the air and eventually infect humans by inhalation.

Fungal contamination can occur not only in the living area (Figure 4) or office environment but also in automobiles, trains and other modern means of transportation. In particular, heating, ventilation and air-conditioning systems tend to foster fungal and bacterial contamination [23, 24]. Brazilian scholars reported that dust in the home environment also carried of the potential for cryptococcus contamination and subsequent exposure risk as high as 13% [21].

Of those surveyed in this study, 34.21% of patients (26/76) had a history of direct or indirect exposure to pigeon droppings (e.g., from breeders who feed pigeons and sell pigeons at the markets, veterinarians, and bird fanciers feeding pigeons at the square), which is much higher than the 12.8% from previous report [12]. For approximately 25% of our patients in the present series, their working or living environments also were potentially contaminated by fungal spores, two case examples are illustrated in figure 3 bottom right, and figure 4. This number is again higher than the 13.5%, previously reported in China [12]. Although in this study the environmental exposure was not significantly different ($p=0.11$) for immunocompromised and the immunocompentent patients, a large number (81.26%) of PC patients had environmental exposure history, suggested that clinicians should ask detailed questions about occupational and environmental exposure history.
In this group, most immunocompetent PC patients (31/41, 82.93%) were without any pulmonary symptoms to suggest a lung infection and their PC were only detected by incidental imaging finding ordered as a part of routine annual examination or for other non-pulmonary indications.

The radiologic presentations varied, however, the lesion patterns had some relevance to host immune function ($p<0.05$). 68.30% (28/41) of immunocompetent patients showed nodular mass. Pneumonic and mixed types were more common in immunocompromised patients (12+9/35, 60%) versus immunocompetent patients (6 + 7/41, 31.71%), $p<0.05$. Cavitation combined with pulmonary infiltrates is again more common in immunocompromised cases (8/35 vs. 2/41, $p<0.05$).

Although tissue biopsy and or a positive fungal culture for cryptococcal organisms are the most accurate means of confirming a definitive PC diagnosis, there are other non-invasive diagnostic tests, such as sCRAG test. sCRAG testing, is considered an effective non-invasive diagnostic tool, and is less complex and costly than the invasive procedures [25]. However, sCRAG testing has not been widely available in China, for example, during the period under study, only three hospitals in metropolitan Shanghai offered this test, and the cost of 400 RMB per test is often prohibitively expensive for the average self-paying patient. This explains one of the shortcomings of our study in that, only 7 patients in our group underwent sCRAG test, and 5 cases yielded positive results (titer> 1:320) at a sensitivity of 71.4%. Another limitation to our study is that few samples from either BALF or tissue obtained by biopsies were sent for culture. Most of the surgical excisions were directed towards
resection of a suspected lung cancer, and this is the main reason that PC or other granulomatous infection had not been considered prior to the final pathologic diagnosis.

Here, a major question is how one should choose among the different biopsy approaches appropriately. Different methods have respective advantages and disadvantages. Surgical morbidities are serious risks, and the costs are high, especially when applied to non-malignant conditions. Both TBLB and percutaneous lung biopsy are less invasive, economical and practical, although smaller tissue samples are obtained. As our data suggested, 85.53% (65/76) lesions of PC are located in the peripheral regions of the lungs. Percutaneous lung biopsy operation in experienced hands is relatively simple, less invasive, and effective. With definitive diagnosis rate of 74% to 95% [4-6], percutaneous lung biopsy may be the preferred initial biopsy method.

The initial clinical misdiagnosis rate of patients in this group was quite high, 43.42% (33/76) for lung cancer (23 cases, 30.26%), pneumonia (8 cases, 10.53%) or tuberculosis (2 cases, 2.63%). Of special interest is that a 18F-FDG-PET examination was obtained in 46/76 of this cohort, based on a clinical suspicion of cancer. SUV, the standardized uptake value and is used as a quantitative indicator of the 18F-FDG tracers that is absorbed by tissue is usually used to distinguish malignant and benign lesions. For example, cancer is suggested when 18F-FDG-PET SUV in lung nodules is greater than 2.5. Studies have reported that a 94% accuracy of the use of this one criteria for lung cancer [26]. However, 18F-FDG is a non-specific tumor-imaging agent, and in
addition to tumor tissues, non-malignant tissue outside the tumor and some benign but inflammatory or infectious lesions can also demonstrate increased tissue uptake of 18F-FDG [27]. Some reports suggested that 18F-FDG PET had a fairly low specificity of 58.97% while good sensitivity of 87.5% in a multicenter clinical trial on the diagnostic value of Dual-Tracer FDG-PET in Pulmonary Lesions (n=55) [28]. In 60.87% (28/46) of the patients in our cohort, a high degree of abnormal uptake in the lung lesions (SUV>2.5, T/N>1.3), which typically indicates malignancies resulted in rushing the patient directly to the operating table. Therefore, although 18F-FDG-PET have a definite role in directing the diagnosis and staging of thoracic malignancies, caution in false positive misdiagnosis of PC and other granulomatous diseases must be considered, especially in areas endemic for these infections.

In recent decades, the treatment of PC in China follows the cryptococcal treatment guidelines established by the Infectious Diseases Society of America (IDSA), initially published in 2000 [29] and that has been updated in 2010 [1]. These guidelines recommend that patients with PC should first be evaluated in accordance to the patient’s immune status and for the extent of systemic spread. Patients should receive step-wise graded treatment according to the severity of respiratory symptoms and the presence of extrapulmonary spread and manifestation of disease. Accordingly the immunocompromised patients (n=35) in this present cohort were given a sufficient course of anti-fungal treatment. Of the overall 76 cases, 39 patients with mild to moderate symptoms were prescribed fluconazole monotherapy and had good response, a typical case of which is presented (Figure 5). There is controversy regarding the need
for therapy in asymptomatic patients with normal immune function. In the immunocompetent patients in this series (n=41), 25 asymptomatic cases did not receive any antifungal therapies but were observed. Among them, 24 patients had complete resolution of radiographic findings; and 1 patient had improvement. Therefore, it appears that the majority of immunocompetent asymptomatic patients with PC may undergo spontaneous remission. 6 of 31 of the asymptomatic patients did receive empiric antifungal therapy at their treating physicians’ discretion. The cure rate of 100% using oral fluconazole 400 mg/day for 3 to 10 months in these six was similar to other reports from East Asia [30]. Hence, our data suggest that for most immunocompetent patients, fluconazole monotherapy is sufficient when clinically indicated, but observation alone can be justified for asymptomatic patients. One HIV-positive case with low CD4 count (<200 /mm$^3$) died of cryptoccal meningitis which is an expectable outcome.

In addition, positive rate of sputum culture in this study was only 2.63% (2/76) that were all of *Cryptococcus neoformans* and no *C gattii* was detected. Unfortunately, no tissues were cultured at the time of surgical resection of what is thought to be malignancies. This reinforces the recognition that there is often insufficient clinical awareness of the broader differential diagnosis of abnormal radiologic findings found in asymptomatic patients without overt risk factors for opportunistic pulmonary infections. Therefore, once tissues were obtained, culture should be done at the same time in order to exclude infectious diseases accurately.
Conclusions

In our retrospective series and review of literature, in the last decade, pulmonary cryptococcosis in immunocompetent persons is becoming more commonly identified. The risk factors might be related to fungus-contaminated environmental exposure. False positive FDG-PET examinations often lead to initial clinical misdiagnosis of cancer. A careful understanding of the diagnostic options available can lead to fewer lung resections of non-malignant diseases as the first invasive procedure. Lung lesions occur mostly in the outer lung fields, varying in shapes. Nodular lung masses were relatively common in people with normal immune functions. For patients with tissue confirmed PC with normal immune function, some have exhibited spontaneous remission and the majority have respond well to fluconazole monotherapy, further prospective studies should be directed towards understanding who may be carefully observed without pharmacologic interventions.

Acknowledgments

The study was funded by grants from the National Science Foundation of China (No: 30800405, 30971323, 81170011); Science and Technology Commission of Shanghai Municipality (No: 11430702100, 114119b2200, 09411951500); and International Science & Technology Cooperation Program of China (2011DFB30010). The authors thank Dr. Dajun Li and Dr. Xuemei Wang for their helpful discussions.
References


Figure 1
Figure 4

Figure 5