Original Article

Associations of serum cryptococcal antigen with different of clinical characteristics: a comprehensive analysis of 378 pulmonary cryptococcosis patients

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Background: Pulmonary cryptococcosis (PC) is an infection typically diagnosed in immunocompromised or immunocompetent patients, which can lead to severe disease if not treated appropriately. We aimed to determine the association between clinical manifestations, computed tomography (CT) findings, and host immune status with the serum cryptococcal antigen (CRAG) test results of PC patients.

Methods: The clinical data of 378 PC patients over a 12-year period were retrospectively reviewed at Shanghai Pulmonary Hospital (Shanghai, China). Serum CRAG was detected by a latex agglutination (LA) test using CryptoTrol (Immuno-Mycologics Inc., Norman, OK, USA). Patients were categorized according to their serum LA results, and their clinical characteristics were analyzed: 244 of 378 patients showed positive serum LA results and 134 had negative results.

Results: Immunocompromised hosts (ICH) were more likely to present positive LA results. The ICH group had higher titers of LA test than the non-immunocompromised host (NICH) group. Patients with negative LA results often had no symptoms and their CT findings presented a solitary nodule or mass, while LA-positive patients had variable symptoms such as cough, expectoration, fever, etc. A large diversity of CT manifestations were observed in the LA-positive patients, such as multiple nodules, patchy shadows, interstitial infiltrates, and diffuse granular shadows. Patients with a solitary nodule or mass had lower titers than did the patients with other manifestations. The clinical characteristics of LA-positive patients were different from those of LA-negative patients.

Conclusions: Serum CRAG test results were found to be associated with the clinical manifestations, CT findings, and host immune status of PC patients.

Keywords: Pulmonary cryptococcosis (PC); cryptococcal antigen (CRAG); latex agglutination test; clinical manifestations; immune status

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Introduction

Cryptococcosis is a global invasive mycosis associated with significant morbidity and mortality. Cryptococcosis is a potentially serious fungal disease, typically caused by inhalation of *Cryptococcus neoformans* or *Cryptococcus gatti*, which tend to form aerosol (1,2). Pulmonary cryptococcosis (PC) refers to acute or subacute infections of the lungs caused by *Cryptococcus* (3). PC occurs not only in immunocompromised hosts (ICH), such as people with acquired immune deficiency syndrome (AIDS), but also in some non-immunocompromised hosts (NICH). Treatment for PC needs to be tailored according to the immune status of the host site of infection, access to health care facilities and availability of antifungal drugs. Antifungal agents with activity against Cryptococcus include polyenes (amphotericin B), flucytosine, and azoles. The clinical manifestations of PC are variable and lack specificity, and can include cough, expectoration, fever, chest pain, or an absence of symptoms. The computer tomography (CT) findings of PC may easily be confused with tumor or tuberculosis, leading to misdiagnosis. Cryptococcal antigens (CRAGs) can be detected by latex agglutination (LA) tests, and the *Cryptococcus* LA test is commonly used in clinical practice as a serological detection method for PC, demonstrating high sensitivity and specificity (4). However, some PC patients show negative LA test results. This study thus aimed to analyze and identify the potential associations of clinical manifestations, CT findings, and host immune status with the LA test results in PC patients. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/apm-21-127).

Methods

Study subjects

From January 2002 to December 2013, patients with proven diagnosis or clinical diagnosis of PC at Shanghai Pulmonary Hospital (Shanghai, China) were retrospectively reviewed. The following data were obtained from the medical records: sex, age, hospitalization time, occupation, exposure history, preliminary diagnosis, basic diseases, symptoms and signs, host immune status, laboratory examination, imaging data, etc. The information from patients' relevant follow-up was obtained on regular clinic visits and by telephone follow-up. The study was approved by the institutional research ethics committee of Shanghai Pulmonary Hospital (No. K14-168). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (as revised in 2013). Informed consent was obtained from all healthy donors for inclusion in the study. Patients without complete, detailed medical records were excluded. Finally, the records of 378 patients were considered for this analysis.

Diagnostic criteria of PC

According to the international consensus obtained by the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) and the Chinese consensus obtained by the Chinese Thoracic Society, the diagnostic strategy of PC includes proven diagnosis and clinical diagnosis (5-10). The proven diagnosis must meet one of the following statements: (I) *Cryptococcus* were detected in pathological lung tissue specimens with histochemical staining or cell chemical dyeing methods; (II) *Cryptococcus* were found in sterile pathological lung tissue and pleural effusion specimens by culture or microscopy; (III) *Cryptococcus* were found in blood by culture or microscopy consistent with the cultivation of the lower respiratory tract specimens or microscopy results. Clinical diagnosis is considered in conjunction with medical history, respiratory symptoms, and chest radiographic evidence, and should meet one of the following criteria: (I) *Cryptococcus* were found in qualified sputum or bronchoalveolar lavage fluid (BALF) by culture or microscopy; (II) positive CRAG tests in lung tissue fluid, serum, BALF, cerebrospinal fluid (CSF), and pleural effusion specimens (8,10).

The specimens were inoculated on Sabouraud Dextrose Agar and cultured at 25 or 37 ℃ for one week to identify whether there was a growth of cryptococcus. CRAG was detected by LA test using CryptoTrol (Immuno-Mycologics Inc., Norman, OK, USA). The result of LA test was considered positive if the titer ≥1:8. The patient was defined as ICH when he or she suffered from one of the following conditions: malignancy, organ transplantation, diabetes, liver cirrhosis, connective tissue disease, low peripheral blood cluster of differentiation 4 $(CD4)^+$ cell count or $CD4^+/CD8^+$ <1.5. If the patient did not meet any of the above criteria, the patient was defined as NICH (4,11).

Statistical analysis

All data were independently entered by three individuals

Table 1 Demographic data of pulmonary cryptococcosis patients from 2002 to 2013 (n=378)

Variable	All patients	Serum LA positive	Serum LA negative	P
Subjects (n)	378	244	134	
Age, y	46.45±13.28	44.32±13.64	50.93±10.99	<0.001
Sex, F/M (n)	131/247	86/158	45/89	0.973
Smoking, n (%)	111 (29.36)	74 (30.33)	37 (27.61)	0.579
Drinking, n (%)	75 (19.84)	47 (19.26)	28 (20.90)	0.703
Occupation, n (%)				0.506
Farmer	108 (28.57)	64 (26.23)	44 (32.84)	
Housekeeper	59 (15.61)	40 (16.39)	19 (14.18)	
Office worker	31 (8.20)	19 (7.79)	12 (8.96)	
Construction/industrial worker	28 (7.41)	17 (6.97)	11 (8.21)	
Retiree	26 (6.88)	14 (5.74)	12 (8.96)	
Porter	22 (5.82)	15 (6.15)	7 (5.22)	
Cleaner	21 (5.56)	15 (6.15)	6 (4.48)	
Motor-vehicle driver	15 (3.97)	10 (4.10)	5 (3.73)	
Civil servant	13 (3.44)	9 (3.69)	4 (2.99)	
Medical worker	11 (2.91)	8 (3.28)	3 (2.24)	
Student	12 (3.17)	12 (4.92)	0 (0)	
Teacher	8 (2.12)	6 (2.46)	2 (1.49)	
Unknown	24 (6.35)	15 (6.15)	9 (6.72)	
No underlying disease or other risk factors, n (%)	230 (60.85)	125 (51.23)	105 (78.36)	<0.001
ICH, n (%)	126 (33.33)	102 (41.80)	24 (17.91)	<0.001
Pet owners, n (%)	28 (7.41)	19 (7.79)	9 (6.72)	0.704

Data are presented as numbers (%) or mean ± SD. ICH, immunocompromised host; y, years; F, female; M, male.

into an approved research database, after verification with SPSS 25.0 statistical analysis software (IBM Corp., Armonk, NY, USA) for data processing. All measurement data are presented as mean ± SD. All numeration data are presented as counts and percentages. The chi-squared test was used for ordinal data, the unpaired *t*-test was used for numerical data, and the nonparametric test was used for ranked data. Significance was set at a P value <0.05 for all statistical analyses.

Results

Demographic information

In this study, 171 patients with LA-positive CRAG results and clinical manifestations had an integrated diagnosis of pulmonary cryptococcosis, 207 cases had a proven diagnosis of PC, 11 of which were confirmed by cryptococcus microscopy or culture of sterile specimens. Pathological diagnosis occurred in 196 cases; among these cases, 7 were confirmed with transbronchial lung biopsy (TBLB), 47 were confirmed by percutaneous lung biopsy, 83 were confirmed by video-assisted thoracic surgery (VATS) lung biopsy, and the other 59 cases were confirmed by open thoracic operation. The demographic data of the 378 PC patients, including age, sex, smoking and drinking history, occupation, basis of disease, immune status, and pet ownership, collected from 2002 to 2013, are shown in *Table 1*. Among the 378 PC patients, 244 patients had

Table 2 Host factors of pulmonary cryptococcosis patients (n=378)

Host factors	No. (%)
With comorbidities	148 (39.15)
Immunocompromised host	126 (33.33)
CD4/CD8$^+$ <1.5	50 (13.23)
DM	17 (4.50)
Glucocorticoid treatment	13 (3.44)
MT	7 (1.85)
DM + CD4/CD8$^+$ <1.5	10 (2.65)
CTD + glucocorticoid treatment	9 (2.38)
Glucocorticoid treatment + CD4/CD8$^+$ <1.5	5 (1.32)
AIDS + CD4$^+$/CD8$^+$ <1.5	1 (0.26)
MT + CD4$^+$/CD8$^+$ <1.5	3 (0.79)
MT + chemotherapy	4 (1.06)
CTD + DM + glucocorticoid treatment	3 (0.79)
DM + MT + chemotherapy + CD4/CD8$^+$ <1.5	1 (0.26)
CTD + glucocorticoid treatment + immunosuppressive therapy + CD4$^+$/CD8$^+$ <1.5	2 (0.53)
MT + transplantation + CD/CD8$^+$ <1.5 + immunosuppressive therapy	1 (0.26)

Data are presented as number (%). DM, diabetes mellitus; CTD, connective tissue diseases; MT, malignant tumor; AIDS, acquired immunodeficiency syndrome.

positive serum LA findings and 134 patients had negative findings. The average age of the LA-positive group was 44.32±13.64, with 51.23% showing no basis of disease, and 41.8% being ICH. Meanwhile, the average age in the LA-negative group was 50.93±10.99, with 78.36% showing no basis of disease, and only 17.91% being ICH.

PC is an opportunistic infection and many host factors may contribute to its emergence. The host factors of the 378 patients are summarized in *Table 2*. The data showed that 39.15% of PC patients had comorbidities and 33.33% of PC patients were ICH, which included patients with CD4$^+$/CD8$^+$ <1.5, diabetes mellitus, glucocorticoid treatment, malignant tumor, AIDS, etc.

Clinical manifestations

The clinical manifestations of PC were non-specific and variable. More than 257 cases had cough, sputum, fever, chest pain, and shortness of breath as the main symptoms. Other rare symptoms included weight loss, hemoptysis, and headache. There were no abnormal signs in 235 cases. As shown in *Table 3*, all patients were categorized into two groups according to the serum LA test result. In the LA-positive group, 15.98% of patients had no symptoms; however, in the LA-negative group, 61.19% of patients had no symptoms, which was a significant difference between the two groups (P<0.001). Furthermore, physical examination on admission revealed 52.46% of the LA-positive patients had no signs, while 79.85% of LA-negative patients had no signs, which was also a significant difference (P<0.001). These differences combined with other results (*Figure 1*) indicate that LA-positive PC patients might be more likely to have symptoms and signs than LA-negative patients.

Laboratory investigations

All patients underwent a serum LA test, with 64.55% showing positive results (titer from 1:10 to 1:2,560). In relation to immune status, 56.35% of the NICH patients and 80.95% of the ICH patients had positive LA test results, with this difference being significant (P<0.001). In addition, the ICH group had higher titers of serum LA test than did the NICH group (*Figure 2*).

Chest imaging findings

Chest CT was performed in all patients, and the characteristics of the images are summarized in *Table 4*, including solitary nodule or mass, multiple nodules and masses, patchy consolidation opacity, patchy mixed nodular shadows, interstitial infiltrates and diffuse granular shadows. Round or oval opacities <3 cm in diameter were considered to be nodules. Masses were defined as opacities ≥3 cm in diameter. Lung lesions of 63.23% patients were located mostly in the peripheral lung field (outer third of the lung), close to the pleura. In the LA-positive group, 35.65% of patients presented a nodule/mass in CT findings and 15.57% of patients had a solitary nodule or mass. In the LA-negative group, 79.10% of patients presented with a nodule/mass and 65.67% of patients had a solitary nodule or mass. Furthermore, patients with negative serum LA results were more likely to form solitary nodule/mass lesion than those with positive LA results (P<0.001). Meanwhile, patients with positive serum LA results were more likely to form patchy consolidation opacity lesions or combined patchy and nodular shadows than those with negative LA results (P<0.001). Also, patients with positive serum LA

Table 3 Clinical manifestations and serum LA findings in pulmonary cryptococcosis patients (n=378)

Clinical manifestation	Total (n=378), n (%)	Serum LA positive (n=244), n (%)	Serum LA negative (n=134), n (%)	χ^2	P
Symptoms					
No symptoms	121 (32.01)	39 (15.98)	82 (61.19)	81.24	<0.001
Cough	223 (58.99)	181 (74.18)	42 (31.34)	65.61	<0.001
Expectoration	197 (52.12)	162 (66.39)	35 (26.12)	56.22	<0.001
Fever	80 (21.16)	74 (30.33)	6 (4.48)	34.64	<0.001
Chest pain	76 (20.11)	60 (24.59)	16 (11.94)	8.62	0.003
Dyspnea	65 (17.20)	55 (22.54)	10 (7.46)	13.81	<0.001
Emaciation	43 (11.38)	34 (13.93)	9 (6.72)	4.47	0.034
Hemoptysis	34 (8.99)	30 (12.30)	4 (2.99)	2.73	0.099
Headache	21 (5.56)	21 (8.61)	0 (0)		<0.001$^{\Delta}$
Signs					
No signs	235 (62.17)	128 (52.46)	107 (79.85)	27.59	<0.001
Wet pulmonary rale	67 (17.72)	51 (20.90)	16 (11.94)	4.76	0.029
Diminution of breath sounds	58 (15.34)	45 (18.44)	13 (9.70)	5.09	0.024
Dullness to percussion	36 (9.52)	29 (11.89)	7 (5.22)	4.45	0.035
Nervous system signs	4 (1.06)	4 (1.64)	0 (0)		0.302$^{\Delta}$

Data are presented as numbers (%). $^{\Delta}$, Fisher's exact test.

results were more likely to have lesions in the combined lung fields (P<0.001).

After analysis of the LA titers and the CT findings of all the PC patients (*Figure 3*), it was revealed that those with a solitary nodule or mass had lower titers than patients with other CT manifestations (P<0.001). The same analysis was performed on ICH and NICH PC patients (*Figure 3*), respectively, which clearly showed that in both the ICH (P<0.001) and NICH (P<0.001) group, patients with a solitary nodule or mass had lower titers than patient with the other five manifestation types (*Table 4*).

In our study, fluorodeoxyglucose positron emission tomography (FDG-PET) was performed in 169 cases, including 97 with serum-positive LA tests and 72 with serum-negative LA tests: anomalous radioactive concentrations or standardized uptake values (SUV) >2.5 were found in 66 cases (39.05%), in whom lung cancer was suspected; mild radioactive concentrations was found in 74 cases (43.79%), indicating the presence of inflammation and other benign diseases; and no obvious radiological signs were present in 29 cases (17.16%). The rate of mild and normal FDG uptake in the LA-positive group was significantly higher than in the LA-negative group (P<0.05). The FDG-PET results of the PC patients are shown in *Table 5*.

Discussion

Cryptococcus neoformans is an encapsulated budding yeast with a worldwide distribution. It is found in the soil, particularly soil contaminated with poultry droppings, and also within decaying plants or vegetables (12,13). In the environment, *C. neoformans* has a thin capsule and can easily form aerosol. However, within an organism, it can form a thick and adherent capsule. *Cryptococcus* infection occurs through the inhalation of desiccated yeast cells or spores, and is considered to be a primary pulmonary infection. Direct transmission between people or animals appears to be rare, but a previous study showed that symptoms of *Cryptococcus* infection can occur after birth from an asymptomatic mother, possibly indicating that placental infection can lead to a potentially serious condition (14).

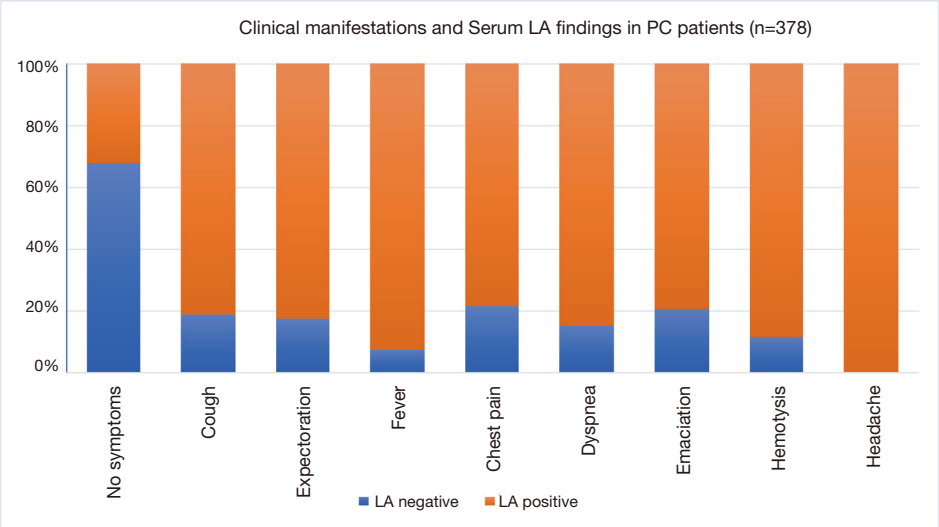


Figure 1 Clinical manifestations and serum latex agglutination test findings in pulmonary cryptococcosis patients (n=378) (corresponding to *Table 3*). We analyzed the clinical manifestations and serum latex agglutination test results of 378 pulmonary cryptococcosis patients. About 67.8% of asymptomatic pulmonary cryptococcosis patients were found to be latex agglutination test-negative. On the contrary, latex agglutination test–positive patients accounted for a large proportion the patients with symptoms, which included cough, expectoration, fever, chest pain, dyspnea, emaciation, hemoptysis, and headache.

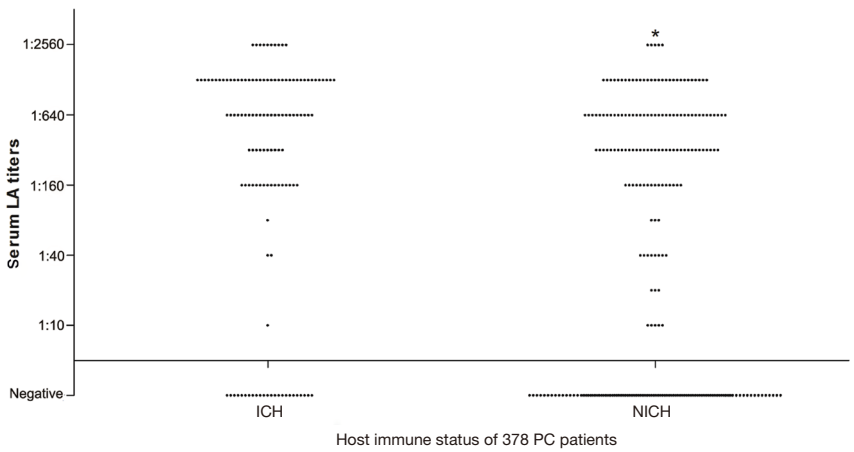
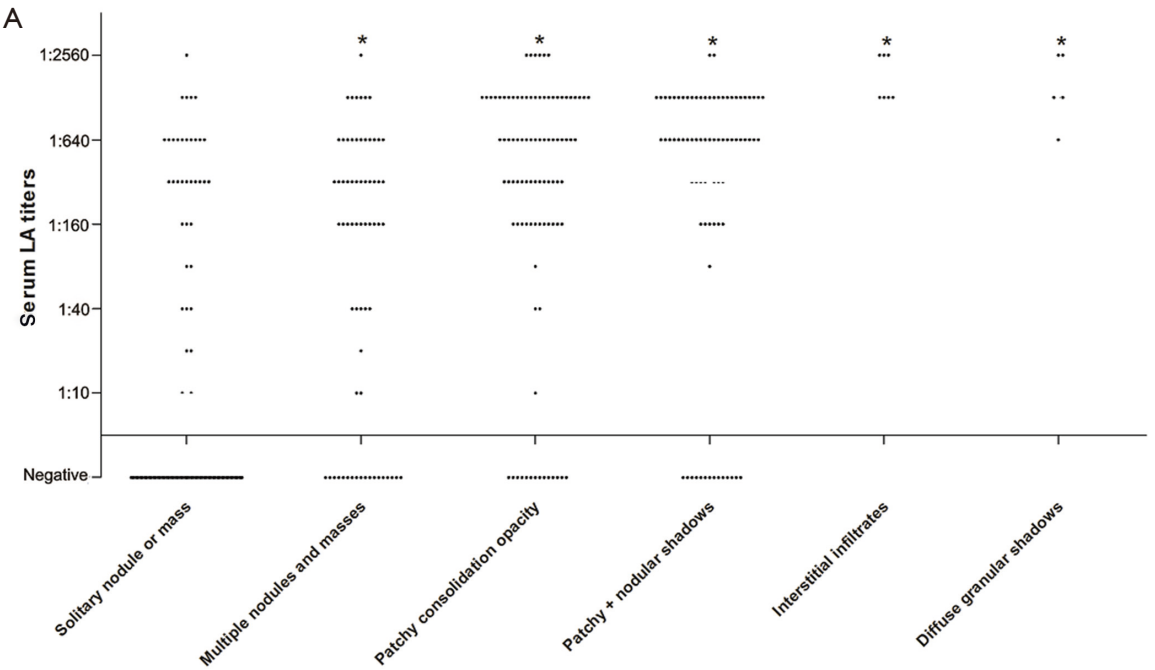


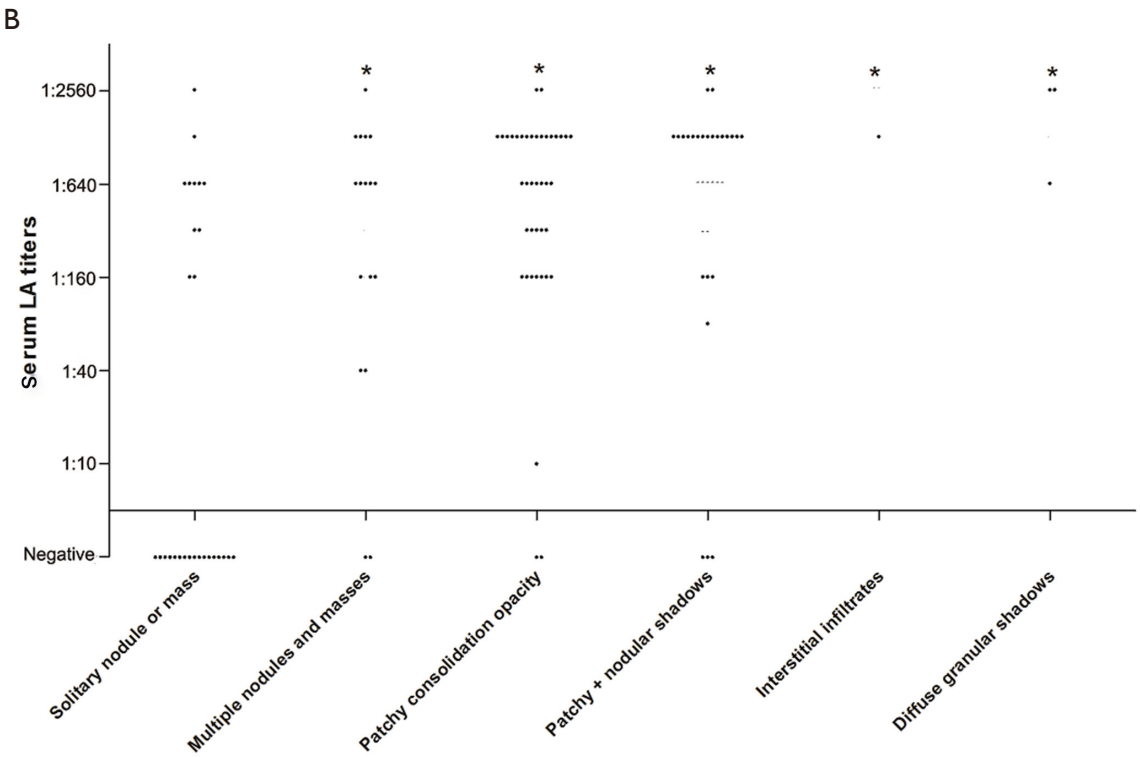
Figure 2 Serum latex agglutination test titers of immunocompromised hosts and non-immunocompromised hosts in 378 pulmonary cryptococcosis patients. We divided the 378 pulmonary cryptococcosis patients into two groups according to host immune status and analyzed the serum latex agglutination test titers (ranging from negative to 1:2,560). The immunocompromised hosts group had significantly higher titers in serum latex agglutination testing than the non-immunocompromised hosts group (*, P<0.001).

PC occurs commonly in ICH individuals, such as in AIDS patients, but can occur in NICH individuals as well (15-17). In the present study, there was only 1 AIDS case, while the others were non-HIV patients. There are two main human pathogenetic species of *Cryptococcus*: *C. neoformans* and *C. gattii* and both are associated with PC. It is well known that *C. neoformans* is found primarily in pigeon guano, is globally distributed, and is a common cause of infection in ICH; meanwhile *C. gattii* is linked mainly with eucalyptus trees and afflicts mostly immunocompetent individuals in tropical and subtropical regions (18-21).

In this study, PC was found to mainly affect middle-



Chest CT manifestation of 378 PC patients



Chest CT manifestation of ICH PC patients

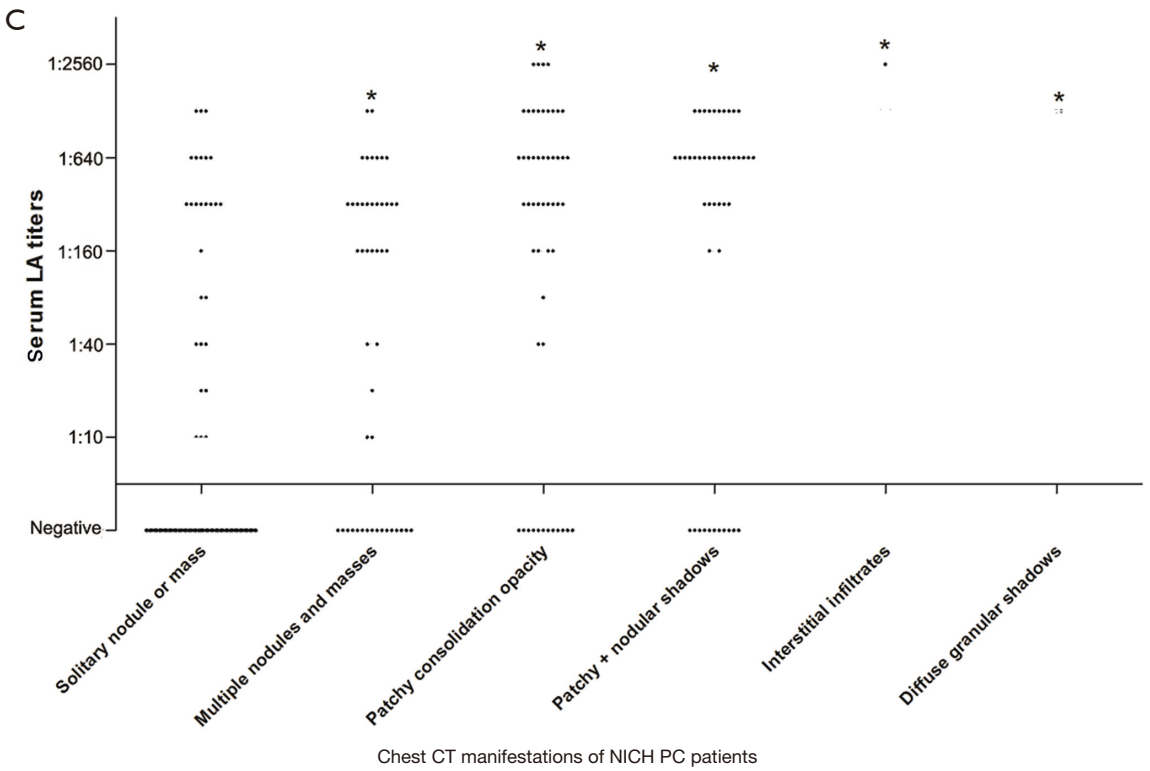


Figure 3 Serum latex agglutination test titers and chest CT manifestations of pulmonary cryptococcosis patients. (A) Serum latex agglutination titers and chest CT manifestations of 378 pulmonary cryptococcosis patients; (B) serum latex agglutination test titers and chest CT manifestations of immunocompromised pulmonary cryptococcosis patients; (C) serum latex agglutination test titers and chest CT manifestations of non-immunocompromised pulmonary cryptococcosis patients. After analyzing the latex agglutination test titers and the CT findings of all the pulmonary cryptococcosis patients (A), it was found that those with a solitary nodule or mass had lower titers than the other patients (*, P<0.001). The same analysis was performed on the immunocompromised (B) and non-immunocompromised pulmonary cryptococcosis patients (C), respectively. In both immunocompromised (*, P<0.001) and non-immunocompromised hosts (*, P<0.001), patients with a solitary nodule or mass had lower titers than the other five chest CT manifestation types.

aged individuals, and occurred more often in males than in females. The male-female ratio (1.89:1) was similar to that in previous reports in China or other Western countries (13,22). The possible explanation for the skewed distribution is associated with the differences of the immune system and physiology between males and females. Although the *C. neoformans* extracted from pigeon guano is regarded as the most important source of infection (23,24), our study only had 14 patients with a history of direct exposure to pigeon droppings. However, 194 patients were farmers, construction/industrial workers, porters, or cleaners, who might have had a history of indirect exposure to soil or dust contaminated with pigeon or other poultry droppings.

Clinical manifestations of PC are variable and mostly associated with the host's immune status. It was reported that approximately one-third of immunocompetent patients with PC were asymptomatic (25). Our study revealed that 38.4% of NICH were asymptomatic, while symptomatic patients presented with cough, expectoration, fever, chest pain, and dyspnea. These symptoms may also manifest in other common diseases, including lung cancer, pneumonia, and pulmonary tuberculosis. Therefore, PC is likely to be misdiagnosed. Furthermore, 21 (5.56%) patients presented with headache, 8 of whom had cryptococcal meningitis as a complication. Thus, headache often suggests an increased risk for cryptococcal meningitis, but occurs too rarely with concurrent meningeal irritation to be noticeable.

Routine laboratory investigations, such as peripheral white blood cell counts and C-reactive protein, were

Table 4 Chest CT manifestation and serum LA findings in pulmonary cryptococcosis patients (n=378)

Radiological characteristics	Total (n=378), n (%)	LA positive (n=244), n (%)	LA negative (n=134), n (%)	χ^2	P
Abnormality					
Nodule/mass	193 (51.06)	87 (35.66)	106 (79.10)	65.35	<0.001
Solitary nodule or mass	126 (33.33)	38 (15.57)	88 (65.67)	97.69	<0.001
Multiple nodules and masses	67 (17.72)	49 (20.08)	18 (13.43)	2.62	0.105
Patchy consolidation opacity	93 (24.60)	79 (32.38)	14 (10.45)	22.42	<0.001
Patchy shadows + nodular shadows	79 (20.90)	65 (26.64)	14 (10.45)	13.72	<0.001
Interstitial infiltrates	7 (1.85)	7 (2.87)	0 (0)		0.054$^{\Delta}$
Diffuse granular shadows	6 (1.59)	6 (2.46)	0 (0)		0.094$^{\Delta}$
Other accompanying signs					
Cavitations	56 (14.81)	39 (15.98)	17 (12.69)	0.75	0.388
Air bronchogram	41 (10.85)	31 (12.70)	10 (7.46)	2.46	0.117
Lobulation	37 (9.79)	18 (7.38)	19 (14.18)	4.53	0.033
Spiculation sign	33 (8.73)	16 (6.56)	17 (12.69)	4.08	0.043
Pleural indentation sign	30 (7.94)	15 (6.15)	15 (11.19)	3.02	0.083
Enlarged mediastinal lymph node	23 (6.08)	17 (6.97)	6 (4.48)	0.94	0.33
Halo sign	22 (5.82)	16 (6.56)	6 (4.48)	0.68	0.409
Pleural effusions	20 (8.20)	17 (6.97)	3 (2.24)	3.86	0.049
Pericardial effusion	2 (0.53)	2 (0.82)	0 (0)		0.541$^{\Delta}$
Lesion area (right/left)				33.82	<0.001
Right lung	170 (44.97)	95 (38.93)	75 (55.97)	10.14	0.001
Left lung	103 (27.25)	57 (23.36)	46 (34.33)	5.25	0.022
Combination	105 (27.78)	92 (37.70)	13 (9.70)	33.81	<0.001
Lesion area (upper/middle/lower)				44.07	<0.001
Upper lung	73 (19.31)	27 (11.07)	46 (34.33)	30.04	<0.001
Middle lung	19 (5.03)	13 (5.33)	6 (4.48)	0.131	0.717
Lower lung	191 (50.53)	122 (50)	69 (51.49)	0.077	0.781
Combination	95 (25.13)	82 (33.61)	13 (9.70)	26.27	<0.001
Centre of lesion (lung field)					
Near the hilum	72 (19.05)	58 (23.77)	14 (10.45)	9.96	0.002
Middle third	67 (17.72)	46 (18.85)	21 (15.67)	0.6	0.439
Outer third	239 (63.23)	140 (57.38)	99 (73.88)	10.13	0.001

Data are presented as numbers (%). $^{\Delta}$, Fisher's exact test.

Table 5 FDG-PET findings in pulmonary cryptococcosis patients (n=169)

Radiological characteristics	Total (n=169), n (%)	LA-positive (n=97), n (%)	LA-negative (n=72), n (%)	χ^2	P
Higher FDG uptake	66 (39.05)	24 (24.74)	42 (58.33)	19.59	<0.001
Mild FDG uptake	74 (43.79)	65 (67.01)	9 (12.50)	49.89	<0.001
Normal FDG uptake	29 (17.16)	8 (8.25)	21 (29.17)	12.72	<0.001

Data are presented as numbers (%).

generally nonspecific in this study, which is consistent with a previous study (26). Moreover, 36.14% (73/202) of patients presented with serum $CD4^+/CD8^+$ <1.5 suggesting that PC may be associated with cellular immune function, especially T lymphocyte cell immunity. Several studies have shown that *Cryptococcus* LA tests can be used not only for auxiliary diagnosis of deep cryptococcosis, but also for semi-quantitative detection of CRAG in serum, CSF, BALF, and urine to evaluate the treatment efficacy and monitor the outcome of disease (27-29). In patients with cryptococcal meningitis, the positive rate of CRAG is as high as 94.1–100% in CSF and 86–93.6% in serum (30-32), but the serum-positive rate in HIV-negative isolated PC patients without meningitis is only about 25–56% (33), while the positive rate in BALF or lung tissue fluid is as high as 100% (34-36). In our study, the positive LA serum rates in BALF, pleural fluid, lung tissue fluid, and CSF were 64.55%, 80%, 85%, 95%, and 100%, respectively. The positive rate was much higher in ICH patients than in NICH patients, and the ICH group generally had a high titer of serum LA test, which is similar to the findings of Hung *et al.* (37). In this study, symptoms and signs were much more likely to appear in LA-positive patients than in LA-negative patients, suggesting that patients with serum LA-positive results had a more severe and higher load of *Cryptococcus* infection. The results also act as a reminder to clinicians that even in the absence of symptoms or serum LA indications, the diagnosis of PC still cannot be ruled out, and further LA testing in other specimens should be performed. The proportion of negative LA tests in our patients is noteworthy and could possibly be attributed to a concentration of CRAG below the kit detectability limit, a masking effect by unknown non-specific proteins in vivo, a prozone phenomenon arising from high concentrations of CRAG, or a poorly encapsulated strain with low production of polysaccharide (38,39).

The radiologic manifestations of PC are variable, and include nodular shadow, mass-like opacity, patchy consolidation opacity, diffuse infiltration, hilar adenopathy, and pleural effusions (4). Nodular lesions are the most common of these findings in immunocompetent patients, whereas immunosuppressed patients are more likely to have alveolar or interstitial opacities and evidence of cavitation. In the present study, 13 patients had interstitial pneumonia or diffuse granular shadows, 11 of them (included the AIDS patient) were ICH. Of the non-AIDS patients, 51.06% had nodules or masses, and the high frequency of these lesions was consistent with other studies (21,37,40). Of the nodular shadows found, 65.28% were a solitary pulmonary nodule (SPN), and were usually accompanied by lobulation, spiculation sign, or pleural indentation sign, with most being mistaken for evidence of lung cancer. Most patients had lesions mainly in subpleural areas, involving the unilateral lung, and lesion were more frequently located in the right lung or the lower lung than in the left lung or the upper lung. These results are consistent with Fox's findings (41). However, the lesion patterns had some relevance to the result of the serum LA tests. Interstitial pneumonia or diffuse granular shadows were seen in the LA-positive patients. Patchy consolidation opacity or mixed lesion were the most common findings in the LA-positive patients, whereas LA-negative patients mainly showed nodules and/or masses. This is perhaps because the lesions of the nodules/masses were so limited that the associated CRAG released was also minute. The incidence of pleural effusion in the LA-positive group was greater than that in the LA-negative group, and the incidence of lobulations or spiculation sign in the LA-positive group was lower. Thus, serum LA testing is helpful for the diagnosis of PC, especially in the patients with radiological findings of multiple lesions or non-nodules/mass shadows. However, if the radiologic manifestations include a solitary pulmonary nodule and the serum LA test is negative, PC still cannot be excluded until more conclusive evidence can be found.

It is common for 18FDG-PET to be performed for detection of lung malignant lesions with high sensitivity and

low specificity. PC patients can show higher FDG uptake, misleading physicians to presumptively diagnose lung cancer (42-44). In our study, 66 (39.05%) of the 169 patients that underwent 18FDG-PET scans had high FDG uptake or SUV >2.5. Moreover, the positive rate of 18FDG-PET in the LA-negative group was higher than that in the LA-positive group. The high positive rate might be associated with lesions in LA negative group being mainly nodules or masses.

The present study had some limitations. First, there was only 1 AIDS patient in our cohort; however, medical care is organized in China in such a way that known HIV-positive patients are preferentially referred to infectious diseases hospitals and centers, whereas our institution is specialized in pulmonary-focused diseases. Second, the rate of sputum culture positivity in this study was only 2.65% (10 out of 378), and our clinical laboratory does routinely identify *Cryptococcus* to the species level. Furthermore, this study was a single-center retrospective analysis, and some selection bias was inevitable. Thus, further multicenter, large sample, prospective research will be performed to improve the diagnosis and treatment strategies of PC in the future.

Conclusions

PC is more frequently found in middle-aged male non-immunocompromised hosts, whose clinical features and radiographic features are non-specific; thus, misdiagnosis or negligence of this condition is possible. Serum CRAG tests by LA may be helpful for accurate diagnosis, and our study found significantly different clinical characteristics between serum LA-positive and serum LA-negative patients. The serum LA titers were associated with clinical manifestations, CT findings, and host immune status of PC patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the institutional research ethics committee of Shanghai Pulmonary Hospital (No. K14-168). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (as revised in 2013). Informed consent was obtained from all healthy donors for inclusion in the study.

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