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The relationship between lung CT features and serum cryptococcal antigen titers in localized pulmonary cryptococcosis patients

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Abstract

Background To explore the associations of computed tomography (CT) image features with the serum cryptococcal antigen (CrAg) titers measured by the lateral flow assay (LFA) in localized pulmonary cryptococcosis patients.

Methods A retrospective analysis of patients with pathologically confirmed pulmonary cryptococcosis admitted to the First Affiliated Hospital of Xiamen University from January 2016 to December 2022 was performed. Clinical data, CT results, serum CrAg-LFA test results, and follow-up data were collected and analyzed.

Results A total of 107 patients with localized pulmonary cryptococcosis were included, of which 31 had a single lesion in chest CT and the other 76 had multiple lesions. The positivity rate was (94.74% vs 64.52%) and titers of serum CrAg-LFA (1.77 ± 0.87 vs 0.91 ± 0.98) in the multiple lesion group were higher than those in the single lesion group, respectively. Multivariate linear regression analysis showed that the serum CrAg titers were positively associated with the number of lesions (β, 0.08; 95% CI, 0.05 to 0.12) and the lesion size (β, 0.40; 95% CI, 0.31 to 0.50) after adjusting other covariates. The serum CrAg-LFA titers of 60 pulmonary cryptococcosis patients showed a decreasing trend with the reduction in pulmonary lesion size after effective therapy.

Conclusion In pulmonary cryptococcosis patients, the number and size of lung lesions are positively correlated with the titers of the serum CrAg-LFA test. The CrAg-LFA test could be a useful tool for the diagnosis, severity assessment, and therapeutic monitoring of localized pulmonary cryptococcosis patients.

Keywords Pulmonary cryptococcosis, Cryptococcal antigen (CrAg), Lateral flow assay (LFA), Computed tomography (CT)

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Introduction

Pulmonary cryptococcosis, caused by inhaling Cryptococcus spores into the respiratory system, was best appreciated as an opportunistic pathogen in immunocompromised populations in the past. It is noteworthy that Cryptococcus spp. also causes severe disease in immunocompetent hosts nowadays. There have been reports of pulmonary cryptococcosis occurring more frequently in immunocompetent patients than in immunocompromised patients [1, 2]. In China, Cryptococcus is the second most common invasive yeast infection [3]. Compared with the current high incidence, the diagnosis of pulmonary cryptococcosis is still challenging because the clinical manifestations and imaging of pulmonary cryptococcosis have no prominent characteristics compared with other pulmonary diseases and are prone to be missed diagnosed or misdiagnosed as lobar pneumonia, tuberculosis, tumors, or other fungal infections leading to a delay in therapy. A great many patients with pulmonary cryptococcosis were asymptomatic and presented with abnormal pulmonary imaging that was identified during physical examination or investigation for other reasons.

Cryptococcal antigen (CrAg) detection is widely recognized as having diagnostic and prognostic value for pulmonary cryptococcosis. Its positive results have been identified as one of the diagnostic criteria by the European Cryptococcal Meningoencephalitis Diagnostic Guidelines [4] and recommended by the "Consensus of Zhejiang Experts on Pulmonary Cryptococcosis" as a sign of the start of anticryptococcal treatment [5]. The cryptococcal antigen lateral flow assay (CrAg-LFA) is easily performed and has the advantage of great sensitivity and specificity, low cost, rapidity, and noninvasive compared with traditional etiological investigations (ink staining and culturing) and pathology tests. In lower-income settings, it has revolutionized the capacity of clinicians to reach accurate and timely diagnoses [6]. Recent studies have shown that the CrAg-LFA has significant clinical importance in the diagnosis of pulmonary cryptococcosis, particularly in patients with extended pulmonary invasion [7]. Based on that, we speculate that the titers of serum CrAg may be associated with the radiological manifestations in pulmonary cryptococcosis patients. A paucity of literature has comprehensively examined the relationship between radiological manifestations and serum CrAg-LFA titers. This study thus aimed to analyze the potential associations of radiological findings with the serum CrAg titers measured by the CrAg-LFA test in localized pulmonary cryptococcosis patients, hoping to provide robust support to enhance clinical diagnostic capabilities and facilitate clinical monitoring for pulmonary cryptococcosis.

Materials and methods

Study subjects

To gather a maximally extensive sample size, a retrospective study was performed on all patients with pulmonary cryptococcosis admitted to the First Affiliated Hospital of Xiamen University from January 2016 to December 2022. All patients' diagnoses had been pathologically confirmed through percutaneous transthoracic needle biopsy (PTNB) or transbronchial lung biopsy (TBLB). The exclusion criteria were as follows: (1) failure to undergo serum CrAg testing or computed tomography (CT) examination; (2) antigen testing was not synchronized with CT; (3) concomitant extrapulmonary cryptococcal infections such as cryptococcal meningitis; (4) previously received antifungal treatment; (5) underwent surgical treatment; (6) human immunodeficiency virus (HIV) test positive.

Clinical data

The following data were obtained from the medical records: (1) demographic features and past medical history; (2) environment exposure history and host immune status; (3) clinical symptoms and signs; (4) laboratory tests data; (5) serum CrAg titers; (6) CT manifestations of the lungs; (7) the follow-up data.

CrAg-LFA

The CrAg detection kit (colloidal gold method, Immy Company, USA) was used to detect serum CrAg titers according to the manufacturer's instructions regarding operation procedure and result interpretation in the clinical laboratory of the First Affiliated Hospital of Xiamen University.

Interpretation of chest CT scans

Two investigators (C Dai and C Lin) independently conducted the interpretation of chest CT scans. The findings of chest CT scans were assessed for the following items: (1) the location and distribution of parenchymal lesions; (2) the number and size of the parenchymal lesions (the lesion size was defined as the average value of the longest axis and the axis perpendicular to it on the cross-section of CT); (3) the inflammatory infiltrating type of the lesions; (4) related thoracic abnormalities such as halo sign, cavitation, and pleural effusion. All disagreements were resolved through discussion or, if required, adjudicated by a third reviewer (Y Lin).

Treatment

In accordance with the recommendations of the "Consensus of Zhejiang Experts on Pulmonary Cryptococcosis" [5], antifungal agents are administered for 6 to 12 months. For immunocompetent hosts, even in asymptomatic cases, aggressive therapy is warranted.

Fluconazole is administered at a dosage of 200 to 400 mg/day for a six-month course [5]. For patients with mild to moderate symptoms, a higher dosage of fluconazole, 400 mg/day, is administered for a duration ranging from 6 to 12 months [5]. In cases of severe illness, the initial induction phase favors the combination of amphotericin B and flucytosine, administered for a minimum of four weeks [5]. Following this, consolidation therapy with fluconazole is administered for eight weeks, followed by maintenance therapy with fluconazole for a period of 6 to 12 months [5].

Statistical methods

Statistical analyses were performed using SPSS 26.0 software (IBM, Armonk, NY, USA), the statistical software packages R (http://www.R-project.org, The R Foundation), and Empower Stats (http://www.empowerstats.com, X&Y Solutions, Inc, Boston, MA). In order to conform to the normal distribution, the CrAg titer was subjected to log transformation (Log [Antigen titer^{-1} + 1]).

Normally distributed measurement data were expressed as means ± standard deviation, non-normally distributed continuous variables were presented as medians (Q1, Q3) and categorical data were reported as numbers (percentage). A paired Student t-test was used for comparison between normally distributed continuous variables, a Mann–Whitney U test was used for comparison between non-normally distributed continuous variables, and a χ^2 test or Fisher's exact probability test was used for categorical data. Correlation significances between serum CrAg titers and the size and number of the parenchymal lesions were calculated using Pearson's correlation analysis. Generalized linear models were conducted to assess the potential relationship between the radiological findings and the serum CrAg titer levels. Different models were built by adjusting various risk factors. Non-adjusted, mini-adjusted, and multivariable-adjusted models were listed. A P-value < 0.05 (two-sided) was considered statistically significant.

Results

Demographic information

From January 2016 to December 2022, 179 patients were diagnosed with PC at the First Hospital of Xiamen University. 72 patients were excluded due to the following reasons: 11 patients did not undergo CrAg test or CT examination, in 40 patients serum CrAg titer detection did not coincide with pathological confirmation, two patients had concomitant extrapulmonary cryptococcal infections, five patients had previously received antifungal treatment, 13 patients underwent surgical resection treatment, and one patient tested positive for HIV. Finally, 107 patients were included in this study, containing 59 males and 48 females in total. Based on the number of lung CT lesions, these patients were divided into two groups, with 31 patients in the single lesion group and 76 patients in the multiple lesion group (as shown in Fig. 1). The demographic data including age, sex, BMI,

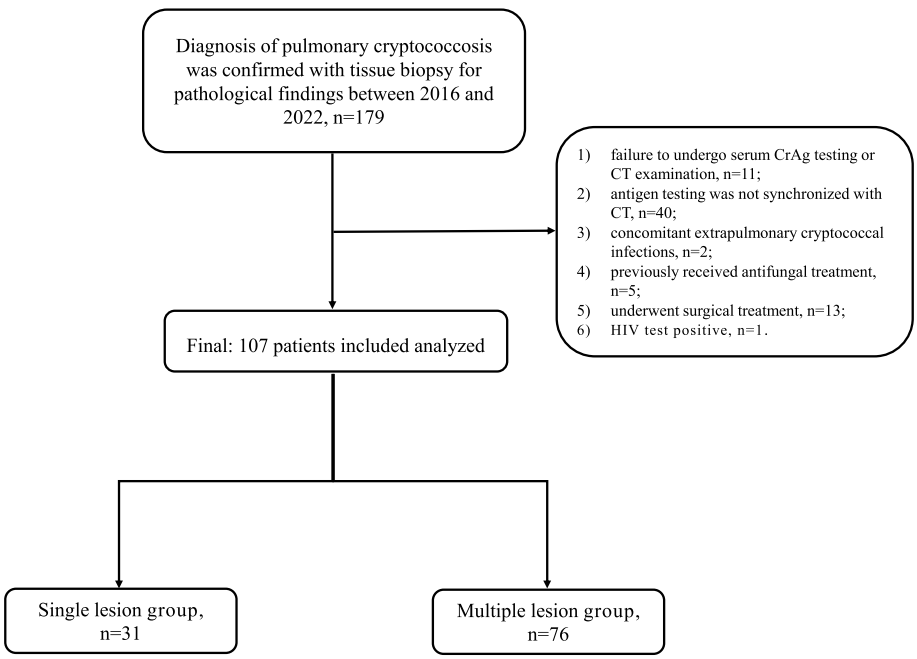

Fig. 1 Flow diagram of the study patient selection. CrAg: cryptococcal antigen; CT: computed tomography; HIV: human immunodeficiency virus

the coexisting disease, immune status, and environmental exposure history were listed in Table 1. There was no significant difference between the two groups of patients on these basic data ($P > 0.05$). As well, less than 7% of patients had a definite history of environmental exposure (an evident history of close contact with poultry, or exposure to a humid environment).

Clinical manifestations and laboratory investigations

The clinical manifestations of pulmonary cryptococcosis were non-specific and variable. As shown in Table 2, approximately half of the pulmonary cryptococcosis patients (42.06%) did not present with any symptoms and signs, and their pulmonary diseases were detected by an incidental radiological examination (chest radiography or CT scan). Cough (49.53%) and expectoration (34.58%) were the most common presenting symptoms followed by dyspnea (15.89%), chest pain (14.02%), and fever (7.48%). Subsequently, no significant difference in symptoms or signs was observed between the two groups ($P > 0.05$).

Table 1 Baseline characteristics of pulmonary cryptococcosis patients ($n = 107$)

Variable	All patients	Single lesion, n (%)	Multiple lesions, n (%)	t/χ^2	*P* value
Subjects	107	31(28.97)	76(71.03)		
Age, y	48.12 ± 13.73	45.94 ± 14.02	49.01 ± 13.61	-1.052	0.295[t]
Sex, F/M	48/59	16/15	32/44	0.805	0.370[a]
BMI, kg/m^2	23.62 ± 3.28	23.42 ± 2.96	23.70 ± 3.42	-0.396	0.693[t]
Comorbidities				0.005	0.941[a]
Without comorbidities	42(39.25)	12(28.57)	30(71.43)		
With comorbidities	65(60.75)	19(29.23)	46(70.77)		
Hypertension	19(17.76)	6(31.58)	13(68.42)	0.076	0.782[a]
DM	9(8.41)	2(22.22)	7(77.78)	0.007	0.934[a]
CAD	5(4.67)	1(20.00)	4(80.00)	0.000	1.000[a]
Hyperlipidemia	41(38.32)	13(31.71)	28(68.29)	0.242	0.623[a]
Lung cancer	4(3.74)	3(75.00)	1(25.00)	2.270	0.132[a]
Other tumors	14(13.08)	3(21.43)	11(78.57)	0.123	0.725[a]
Liver insufficiency	4(3.74)	1(25.00)	3(75.00)	0.000	1.000[a]
Renal insufficiency	5(4.67)	3(60.00)	2(40.00)	1.127	0.288[a]
CTD	11(10.28)	1(9.10)	10(90.90)	1.401	0.237[a]
Radiotherapy or chemotherapy	5(4.67)	2(40.00)	3(60.00)	0.003	0.959[a]
Hemopathy	5(4.67)	2(40.00)	3(60.00)	0.003	0.959[a]
Tuberculosis	5(4.67)	1(20.00)	4(80.000	0.000	1.000[a]
ILD	1(0.93)	0(0.00)	1(100.00)		1.000[b]
Immune state				0.018	0.895[a]
Immunocompromised host	68(63.55)	20(29.41)	48(70.59)		
Immunocompetent host	39(36.45)	11(28.21)	28(71.79)		
Environmental exposure history					
No exposure history	29(27.10)	7(24.14)	22(75.86)		
History of close contact with poultry	6(5.61)	0(0.00)	6(100.00)		
History of exposure to a humid environment	1(0.93)	1(100.00)	0(0.00)		
No data	71(66.36)	23(32.39)	48(67.61)		

Data are presented as numbers (%) or mean ± SD

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), CTD, severe respiratory system limitation such as tuberculosis or ILD, severe DM associated with organ damage, CAD, hypertension, hyperlipidemia, malignancies and being on radiotherapy or chemotherapy, hemopathy, liver or renal insufficiency, were considered immunocompromised. Otherwise, immunocompetent was defined

F female, *M* male, *DM* diabetes mellitus, *CAD* coronary atherosclerotic heart disease, *CTD* connective tissue diseases, *ILD* interstitial lung disease

[a] Chi-squared test

[b] Fishers exact test

[t] t-test

Table 2 Clinical manifestations of pulmonary cryptococcosis patients ($n=107$)

Clinical manifestation	Total ($n=107$), n (%)	Single lesion($n=31$), n (%)	Multiple lesions($n=76$), n (%)	χ^2	P
Symptoms					
No symptoms	46(42.99)	17(54.84)	29(38.16)	2.500	0.114[a]
Fever	8(7.48)	1(3.23)	7(9.21)	0.439	0.508[a]
Shivering	2(1.87)	1(3.23)	1(1.32)		0.497[b]
Cough	53(49.53)	12(38.71)	41(53.95)	2.045	0.153[a]
Expectoration	37(34.58)	10(32.26)	27(35.53)	0.104	0.747[a]
Dyspnea	17(15.89)	2(6.45)	15(19.74)	1.999	0.157[a]
Hemoptysis	4(3.74)	1(3.23)	3(3.95)	0.000	1.000[a]
Chest pain	15(14.02)	4(12.90)	11(14.47)	0.000	1.000[a]
Headache	5(4.67)	0(0.00)	5(6.58)	0.917	0.338[a]
Signs					
No signs	95(88.79)	26(83.87)	69(90.79)	0.478	0.489[a]
Shortness of breath	3(2.80)	0(0.00)	3(3.95)		0.555[b]
Dry pulmonary rale	2(1.87)	2(6.45)	0(0.00)		0.082[b]
Wet pulmonary rale	7(6.54)	3(9.68)	4(5.26)	0.165	0.684[a]
Signs of pulmonary consolidation	2(1.87)	1(3.23)	1(1.32)		0.497[b]
No symptoms and no signs	45(42.06)	17(54.84)	28(36.84)	2.926	0.087[a]

Data are presented as numbers (%)

[a] Chi-squared test

[b] Fishers exact test

Among all the patients, 92 cases (85.98%) were positive for the serum CrAg-LFA test, including 20 out of 31 cases in the single lesion group (64.52%), and 72 out of 76 cases in the multiple-lesion group (94.74%). The median (range) serum CrAg titer was 1:40 (1:5–1:160). Of note, the median serum CrAg titers in patients with multiple lesions (1:80) were significantly higher than those in the single lesion group (1:5) ($P<0.0001$). There was no significant difference in the levels of white blood cell, neutrophils, lymphocytes or procalcitonin between the two groups (Table 3).

Lung CT manifestations

The lung CT manifestations of the pulmonary cryptococcosis patients were shown in Table 4. The mean lesion size was 2.41 ± 1.48 cm. Lung lesions of pulmonary cryptococcosis were more inclined to cluster on one side of the lung. There was no significant difference in the lesion size between the two groups ($P>0.05$). The inflammatory infiltrating types and accompanying signs were similar between the two groups ($P>0.05$).

Relationship between radiological features and serum CrAg titers

After analyzing the correlation between the serum LFA titers and the CT features, it was revealed that the titers of patients with multiple lesions were higher than those with a single lesion ($P<0.0001$) (Fig. 2a). Meanwhile, analyses were performed to identify the relationship between serum CrAg titers and lesion size. It showed that titers of patients with larger lesions were higher than those with a smaller lesion ($P<0.0001$) (Fig. 2b). In addition, titers of patients with lesions involving multiple lung lobes were higher than those with lesions involving only one lobe ($P<0.05$) (Fig. 2c). Meanwhile, whether the lesion is distributed in bilateral lungs did not seem to affect the titer level (Fig. 2d). Further correlation analyses showed that serum CrAg titer was positively correlated with the number ($r=0.08$, $P<0.0001$) (Fig. 3a) and the size ($r=0.39$, $P<0.0001$) (Fig. 3b) of parenchymal lesions. More data were shown in Table 5.

In order to further investigate whether the serum CrAg titers were independently associated with the number of lesions, the lesion size, and the number of involved lobes, generalized linear models were conducted. And in the single-factor linear regression analysis, three models were established to separately analyze the effects of the number of lesions, the lesion size, and the number of involved lobes on serum CrAg titers (Table 6). In model II, a positive association between serum CrAg titers and the number of lesions (β, 0.08; 95% CI, 0.05 to 0.12) and the lesion size (β, 0.40; 95% CI, 0.31 to 0.50) was detected, respectively. While there was no obvious correlation between the serum CrAg titers and the number of involved lobes ($P>0.05$). Finally, the number and size of lesions were included in the multivariate linear regression analysis.

Table 3 Laboratory investigations of patients with pulmonary cryptococcosis

Characteristics	Total	Single lesion	Multiple lesions	U/t/χ^2	P
WBC (10^9/L)	6.65(5.69,7.84)	6.65(5.22,8.23)	6.64(5.71,7.84)	1146.000	0.826[c]
Neutrophils	4.11(3.25,5.37)	4.06(2.96,5.65)	4.11(3.32,5.34)	1116.000	0.866[c]
Lymphocytes	1.85(1.35,2.18)	1.55(1.32,2.07)	1.89(1.38,2.21)	958.000	0.202[c]
Hb(g/L)	136.72 ± 18.98	136.52 ± 18.64	136.80 ± 19.24	-0.070	0.944[t]
PLT (10^9/L)	260.31 ± 65.91	244.52 ± 63.72	266.75 ± 66.11	-1.594	0.114[t]
MPV	10.20 ± 0.97	10.52 ± 0.91	10.07 ± 0.97	2.207	0.029[t]*
PCT	0.04(0.03,0.07)	0.04(0.03,0.06)	0.04(0.03,0.07)	410.000	0.219[c]
Serum CrAg(positive/negative) % Serum CrAg titers	92/15(85.98%) 1:40 (1:5–1:160)	20/11(64.52%) 1:5(0, 1:40)	72/4(94.74%) 1:80(1:10, 1:160)	14.271 -4.422	< 0.0001[a]**** < 0.0001[t]****

WBC white blood cells, *Hb* hemoglobin, *PLT* platelet, *MPV* mean platelet volume, *PCT* procalcitonin, *CrAg* cryptococcal antigen

[a] Chi-squared test

[c] Mann-Whitney U test

[t] t-test

* $P < 0,05$

**** $P < 0.0001$

Table 4 Chest CT manifestation in pulmonary cryptococcosis patients ($n = 107$)

Radiological characteristics	Total ($n = 107$), n (%)	Single lesion ($n = 31$), n (%)	Multiple lesions($n = 76$), n (%)	χ^2/t	P
Lesion distribution					
Lesions in the middle zone of the lung	71(66.36)	16(51.61)	55(72.37)	4.249	0.039[a]*
Subpleural lesions	94(87.85)	23(74.19)	71(93.42)	5.932	0.015[a]*
Lesion location					
Both lungs	21(19.63)	0(0.00)	21(27.63)	10.657	0.001[a]**
Single lung	86(80.37)	31(100.00)	55(72.37)	10.657	0.001[a]**
Lesion size(cm)	2.41 ± 1.48	2.24 ± 1.59	2.47 ± 1.44	-0.732	0.466[t]
> 3 cm	23(21.50)	6(19.35)	17(22.37)	2.905	0.234[a]
1-3 cm	72(67.29)	19(61.29)	53(69.74)	2.905	0.234[a]
< 1 cm	12(11.21)	6(19.35)	6(7.89)	2.905	0.234[a]
Inflammatory infiltrating type					
Patchy shadow or ground glass shadow	67(62.62)	15(48.39)	52(68.42)	3.775	0.052[a]
Consolidation shadow along the lung lobes or segment	19(17.76)	5(16.13)	14(18.42)	0.079	0.778[a]
Shadow of exudation or consolidation along the bronchovascular bundle	42(39.25)	9(29.03)	33(43.42)	1.912	0.167[a]
Accompanying signs					
Halo sign	8(7.48)	0(0.00)	8(10.53)	2.169	0.141[a]
Cavitations	23(21.50)	4(12.90)	19(25.00)	1.909	0.167[a]
Pleural effusions	5(1.87)	1(3.23)	4(5.26)	0.000	1.000[a]

Data are presented as numbers (%)

[a] Chi-squared test

[t] t-test

* $P < 0,05$

** $P < 0.01$

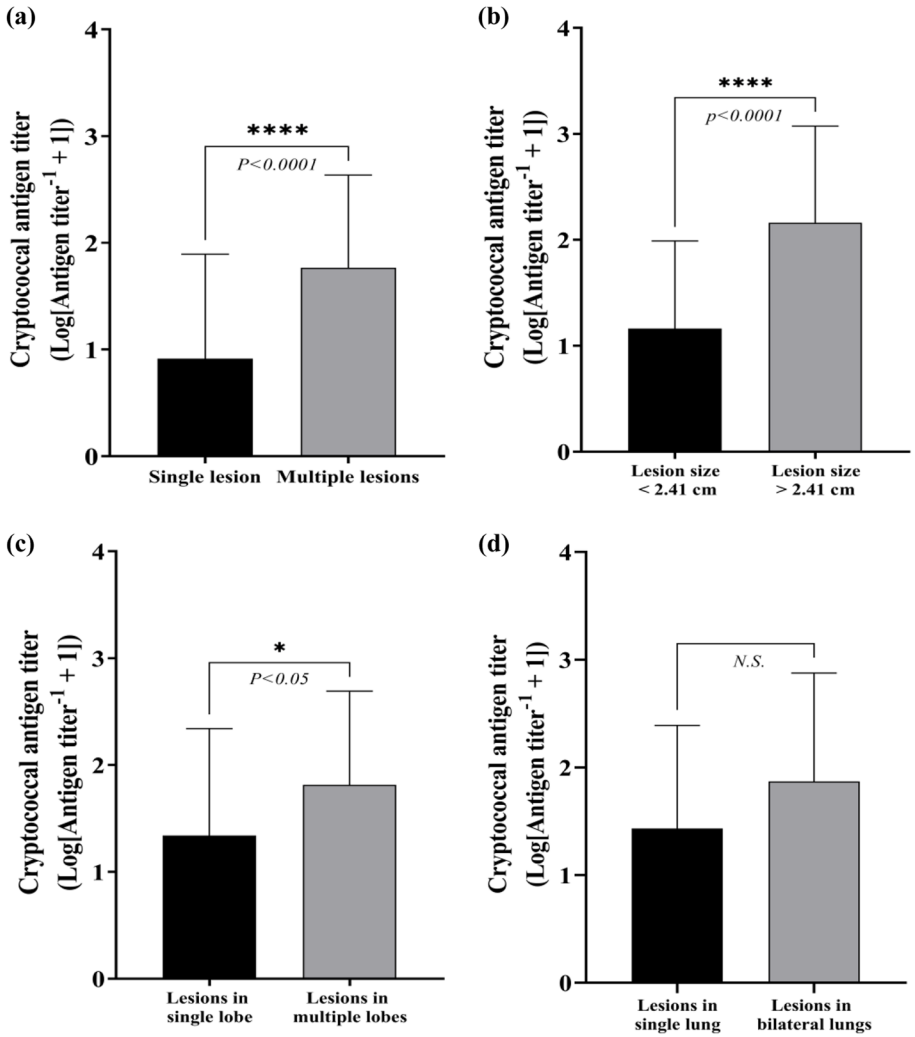

Fig. 2 Relationships between radiological findings and serum CrAg titers. CrAg: cryptococcal antigen. **a** The serum CrAg titers of the multiple-lesion group were higher than those of the single lesion group ($P < 0.0001$). **b** Serum CrAg titers were significantly elevated in the larger-lesion group ($P < 0.0001$). **c** Serum CrAg titers of patients with lesions involving multiple lung lobes were higher than those with lesions involving only one lobe ($P < 0.05$). **d** There was no significant difference in CrAg titers between the group of bilateral lung lesions and the group of unilateral lung lesions

The results showed that serum CrAg titers were still positively associated with the number of lesions (β, 0.08; 95% CI, 0.05 to 0.12) and the lesion size (β, 0.40; 95% CI, 0.31 to 0.50) after adjusting other covariates.

Variation of serum CrAg titers before and after antifungal therapy

Sixty pulmonary cryptococcosis patients who were radiologically effective after treatment were followed for up to 12 months. The remaining 47 patient data were not presented due to incomplete record. Dynamic changes of CrAg titers before and after treatment were depicted in Fig. 4. Nine of the 60 patients were consistently negative for the serum CrAg test. The remaining 51 patients showed a decrease in CrAg titer after antifungal treatment, with 26 patients turning negative for CrAg testing and the other 25 patients maintaining a low level of titer for long periods after successful therapy.

Discussion

Previous studies have focused on evaluating the diagnostic performance of CrAg test [8–10]. In our study, we tried to explore the association of lung involvement with the serum CrAg titer. To the best of our knowledge, this is the first article that systematically elucidates the relationship between the chest CT image feature and the serum CrAg titer measured by the LFA test in patients with localized pulmonary cryptococcosis.

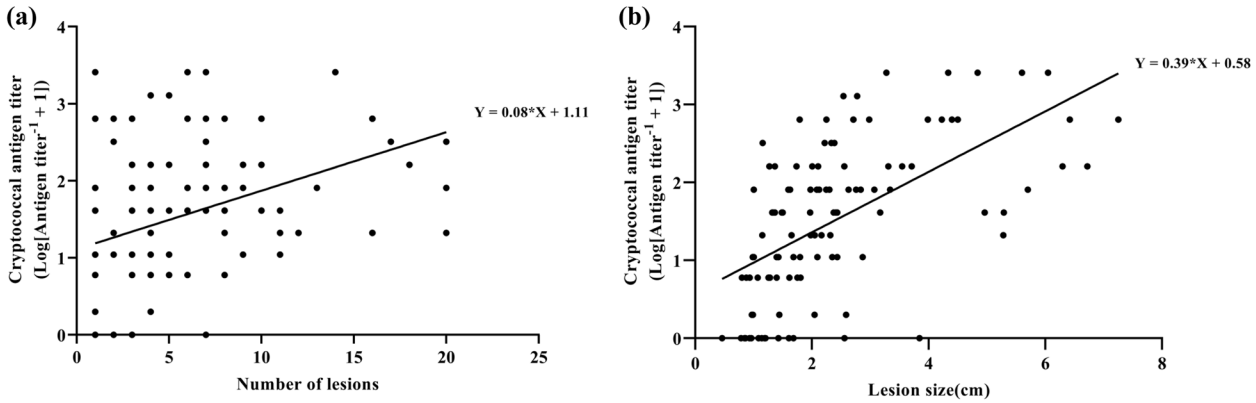

Fig. 3 Correlation analysis between radiological findings and serum CrAg titers. CrAg: cryptococcal antigen. **a** The correlation between serum CrAg titers and the size of the parenchymal lesions ($r = 0.39$, $P < 0.0001$). **b** The correlation between serum CrAg titers and the number of the parenchymal lesions ($r = 0.08$, $P < 0.0001$). The correlation significance was calculated using Pearson's correlation analysis

Table 5 Relationship between radiological findings and serum cryptococcal antigen titers ($n = 107$)

Radiological characteristics		Total ($n = 107$), n (%)	Cryptococcal antigen titer (mean ± SD)	t	P
Number of lesions	**Single lesion**	31(28.97)	0.91 ± 0.98	-4.422	< 0.0001^{t****}
	Multiple lesions	76(71.03)	1.77 ± 0.87		
Lesion size (mean diameter/cm)	**< 2.41**	69(64.49)	1.16 ± 0.83	-5.765	< 0.0001^{t****}
	> 2.41	38(35.51)	2.16 ± 0.91		
Lesion location	**Single lobe**	67(62.62)	1.34 ± 0.99	-2.474	0.015^{t*}
	Multiple lobes	40(37.38)	1.81 ± 0.88		
	Single lung	86(80.37)	1.43 ± 0.96	-1.851	0.067^{t}
	Both lungs	21(19.63)	1.87 ± 1.01		

Data are presented as numbers (%)

^{t} t-test

* $P < 0,05$

**** $P < 0.0001$

The titer value of CrAg detection is positively correlated with the antigen released into the blood from cryptococcal lesions. Previous studies have demonstrated that high CrAg titers were associated with disseminated cryptococcosis involving the central nervous system and the skin [11–14]. These findings suggested that the CrAg titers are strongly related to the severity of disseminated cryptococcosis. However, the assessment of the severity of localized pulmonary cryptococcosis remains challenging due to the continued lack of specificity in clinical presentation and chest CT image features [15–17], and there is still a paucity of corresponding studies. Therefore, this study was designed to investigate the associations of chest CT image features with the serum CrAg titers measured by the LFA test in patients with pulmonary cryptococcosis and to explore whether the serum CrAg-LFA titer could be a microbiological criterion for the diagnosis and severity assessment of pulmonary cryptococcosis.

Our research results showed that serum CrAg titers were positively associated with the number of pulmonary lesions and the lesion size after adjusting other covariates. These findings indicated that CrAg can not only serve as a diagnostic standard for pulmonary cryptococcosis, but its titer detection can also supplement clinical judgment for evaluating lung lesions in localized pulmonary cryptococcosis. Moreover, the follow-up results in our study also confirmed the consistency between CrAg-LFA titers and lung lesions. Therefore, during clinical practice, if access to chest radiography or CT is limited, the test of CrAg-LFA titer can be considered as a valid tool to evaluate the intrapulmonary lesions in patients with localized pulmonary cryptococcosis.

Consistent with the results of previous studies [18, 19], our study demonstrated a correlation between serum CrAg titers and radiological severity in patients with pulmonary cryptococcosis. Patients whose CT images

Table 6 Association of radiological findings with serum cryptococcal antigen titers in different models

	Single-factor linear regression analysis[A]					
	Crude Model	***P* for trend**	**Model I**	***P* for trend**	**Model II**	***P* for trend**
Number of lesions	0.08(0.04,0.11)	< 0.0001****	0.08(0.04,0.11)	0.0001***	0.08(0.05,0.12)	< 0.0001****
Lesion size	0.39(0.29,0.49)	< 0.0001****	0.39(0.28,0.49)	< 0.0001****	0.40(0.31,0.50)	< 0.0001****
The lesion-involved lung lobe	0.20(0.03,0.38)	0.0250*	0.21(0.03,0.39)	0.0230*	-0.12(-0.35,0.12)	0.3317
	Multifactor linear regression analysis[B]					
	Crude Model	***P* for trend**	**Model I**	***P* for trend**	**Model II**	***P* for trend**
Number of lesions	0.07(0.05,0.10)	< 0.0001****	0.08(0.05,0.10)	< 0.0001****	0.08(0.05,0.12)	< 0.0001****
Lesion size	0.39(0.29,0.48)	< 0.0001****	0.39(0.29,0.48)	< 0.0001****	0.40(0.31,0.50)	< 0.0001****

[A] **P*<0,05; ***P*<0.001; ****P*<0.0001. Crude Model, no covariates were adjusted. Model I: model adjusted for age and gender. Model II: model in the number of lesions adjusted for gender, age, BMI, comorbidities, immune state, the lesion involved both lungs, lesion size, the lesion-involved lung lobe; the lesion size adjusted for gender, age, BMI, comorbidities, immune state, the lesion involved both lungs, the number of lesions, the lesion-involved lung lobe; and the lesion involved in the lung lobe adjusted for gender, age, BMI, comorbidities, immune state, the lesion involved both lungs, lesion size, the number of lesions

[B] ****P* < 0.0001. Crude Model, no covariates were adjusted. Model I model adjusted for age and gender. Model II model adjusted for gender, age, BMI, comorbidities, immune state, the lesion involved both lungs and the lesion-involved lung lobe

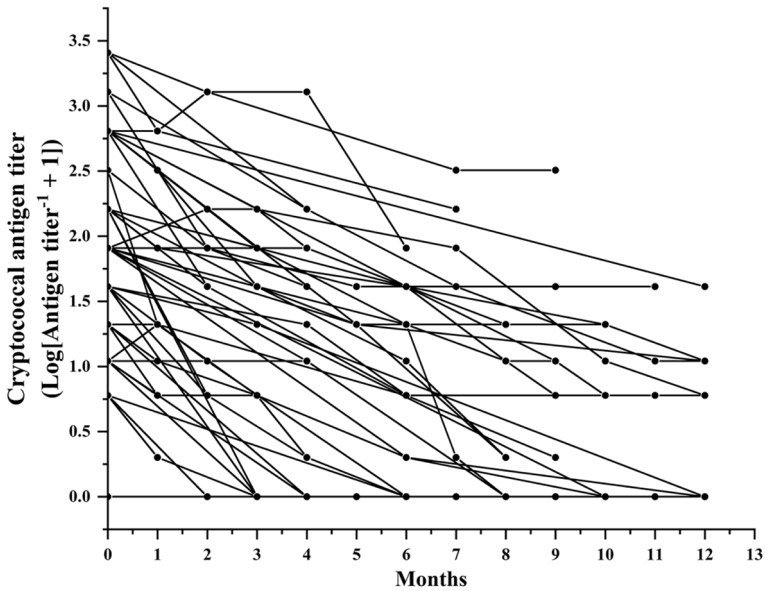

Fig. 4 Variation of serum CrAg titers before and after antifungal therapy of sixty pulmonary cryptococcosis patients who were radiologically effective after treatment. CrAg: cryptococcal antigen

showed more and larger lesions exhibited higher positive rates and antigenic titers detected by the serum CrAg-LFA test, which may be related to the characteristics of localized infiltration and growth of cryptococcus in the lung lesion and the difficulty of releasing CrAg into human blood in the focal lesion, while in patients with more lesions or further extensive involvement, more CrAg is released into the blood [7]. However, in a retrospective study from Japan, a comparison of CrAg titers revealed no significant difference between solitary and multiple nodules [13]. The negative result of this early study may be due to the small sample size and the fact that the antigen detection method used was latex agglutination test. Moreover, in our study, 107 cryptococcal patients were all pathologically confirmed, further enhancing the credibility of the results.

In accordance with the research results of Li et al. [19], serum CrAg titers showed continuously decreasing trend with the reduction in pulmonary lesion size after effective therapy in this study. It reconfirms that the CrAg-LFA titers

correlate with the organism burden. However, titers in patients with pulmonary cryptococcosis can remain at low titers for long periods after successful treatment. The situation has arisen because dead cryptococcus keep releasing capsular polysaccharide antigens, and the host clears them relatively slowly. Hence CrAg titers can be used as a marker of the effectiveness of antifungal treatment, but not as an indicator of cure or a decision to discontinue treatment.

Meanwhile, the proportion of negative CrAg-LFA test results in our patients is noteworthy. In our study, 15 patients (14.02%) were found to be CrAg-negative but confirmed by pathology, including 11 out of 31 patients in the single lesion group (35.48%), and 4 out of 76 patients in the multiple- lesions group (5.26%), which could possibly be attributed to a concentration of CrAg below the kit detectability limit, a masking effect by unknown nonspecific proteins in vivo, a prozone phenomenon arising from high concentrations of CrAg, or a poorly encapsulated strain with low production of polysaccharide [13, 20]. These findings highlight the potential limitations of relying solely on the CrAg test for the diagnosis, severity assessment and therapeutic monitoring of pulmonary cryptococcosis with solitary lesion, and highlight the importance of incorporating clinical and pathological information in the diagnostic algorithms, especially for patients with only a single lung lesion.

Approximately 42% of patients diagnosed with PC exhibited no respiratory symptoms in this study, with their conditions being serendipitously uncovered during routine health assessments or non-respiratory health investigations. In China, the enduring effects of the COVID-19 pandemic have significantly amplified the public's concern for pulmonary health, leading to an increased demand for low-dose CT scans of the lungs. Upon the identification of pulmonary nodules in individuals aged 40 and above, respiratory specialists and thoracic surgeons sometimes advocate for a lung biopsy. This invasive diagnostic procedure aims to definitively ascertain the etiology, which could range from infectious diseases such as tuberculosis, to neoplastic growths, or even rarer conditions like pulmonary cryptococcosis. Nonetheless, this heightened scrutiny and the resultant increase in diagnostic procedures may introduce a selection bias in studies related to lung nodules.

Limitations of this study include being conducted in a single, southeast academic tertiary hospital, which may limit generalizability. The retrospective nature of this study may result in incomplete records and documentation, which subsequently imposes inherent limitations on the scope and validity of the research findings. Meanwhile, this study did not include patients with extrapulmonary cryptococcal infections or HIV infection, which would limit the application of its results.

Conclusions

Localized pulmonary cryptococcosis patients whose CT images showed more and greater lesions exhibited higher antigenic titers detected by the serum CrAg-LFA test. The CrAg-LFA test could be a useful tool for the diagnosis, severity assessment, and therapeutic monitoring of localized pulmonary cryptococcosis patients.

Abbreviations

CT	Computed tomography
CrAg	Cryptococcal antigen
LFA	Lateral flow assay
PTNB	Percutaneous transthoracic needle biopsy
TBLB	Transbronchial lung biopsy
HIV	Human immunodeficiency virus

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None.

Authors' contributions

Dai C.M. and Lin Y.H. wrote the main manuscript text and Dai C.M. prepared figures 1-4. All authors reviewed the manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Ethics Commission of the First Affiliated Hospital of Xiamen University, China (approved NO. 2021 -67). The need for participants' informed consent was waived because of the retrospective nature of this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Mo Z, Li C, Liang Z, Cui J, Yu L, Chen L. Clinical analysis of non-AIDS patients with pulmonary cryptococcosis and the change in their clinical features over 30 years in a tertiary hospital in Beijing, China. Jpn J Infect Dis. 2022;75(5):476–83.
2. Wang DX, Zhang Q, Wen QT, Ding GX, Wang YG, Du FX, Zhang TY, Zheng XY, Cong HY, Du YL, et al. Comparison of CT findings and histopathological characteristics of pulmonary cryptococcosis in immunocompetent and immunocompromised patients. Sci Rep. 2022;12(1):5712.
3. Chen M, Xu Y, Hong N, Yang Y, Lei W, Du L, Zhao J, Lei X, Xiong L, Cai L, et al. Epidemiology of fungal infections in China. Front Med. 2018;12(1):58–75.

4. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813–21.
5. Zhejiang Medical Association Society of Respiratory Diseases. Expert consensus on diagnosis and treatment of pulmonary cryptococcosis. Chin J Clin Infect Dis. 2017;05:321–6.
6. Jitmuang A, Panackal AA, Williamson PR, Bennett JE, Dekker JP, Zelazny AM, Diekema DJ. Performance of the cryptococcal antigen lateral flow assay in non-HIV-related cryptococcosis. J Clin Microbiol. 2016;54(2):460–3.
7. Zhu T, Luo WT, et al. Extent of lung involvement and serum cryptococcal antigen test in non-human immunodeficiency virus adult patients with pulmonary cryptococcosis. Chin Med J. 2018;131(18):2210–5.
8. Hu Q, Li X, Zhou X, Zhao C, Zheng C, Xu L, Zhou Z. Clinical utility of cryptococcal antigen detection in transthoracic needle aspirate by lateral flow assay for diagnosing non-HIV pulmonary cryptococcosis: a multicenter retrospective study. Medicine. 2022;101(37):e30572.
9. Dubbels M, Granger D, Theel ES, Gilligan P. Low Cryptococcus antigen titers as determined by lateral flow assay should be interpreted cautiously in patients without prior diagnosis of cryptococcal infection. J Clin Microbiol. 2017;55(8):2472–9.
10. Zhou Y, Lin PC, Ye JR, Su SS, Dong L, Wu Q, Xu HY, Xie YP, Li YP. The performance of serum cryptococcal capsular polysaccharide antigen test, histopathology and culture of the lung tissue for diagnosis of pulmonary cryptococcosis in patients without HIV infection. Infect Drug Resist. 2018;11:2483–90.
11. Akintilo L, Femia A. Disseminated Cryptococcosis. N Engl J Med. 2021;385:1699.
12. Wang C, Li R, Ma C, Du J, Gu L. Early diagnosis of disseminated cryptococcosis by cryptococcal antigen lateral-flow assay. J Microbiol Immunol Infect. 2022;55(1):177–9.
13. Kohno S, Kakeya H, Izumikawa K, Miyazaki T, Yamamoto Y, Yanagihara K, Mitsutake K, Miyazaki Y, Maesaki S, Yasuoka A, et al. Clinical features of pulmonary cryptococcosis in non-HIV patients in Japan. J Infect Chemother. 2015;21(1):23–30.
14. Xu M, Peng Z, Xu C, Chen Y, Cheng J, Chi Y, Wei H, Chen W, Hu Z. Underlying cryptococcal diseases and the correlation with serum cryptococcal antigen titers in hospitalized HIV-infected patients screened positive for cryptococcal antigenemia. Front Cell Infect Microbiol. 2020;10:170.
15. Marr K, Sun Y, et al. A multicenter, longitudinal cohort study of cryptococcosis in human immunodeficiency virus-negative people in the United States. Clin Infect Dis. 2020;70(02):252–61.
16. Hou X, Kou L, Han X, Zhu R, Song L, Liu T. Pulmonary cryptococcosis characteristics in immunocompetent patients-a 20-year clinical retrospective analysis in China. Mycoses. 2019;62(10):937–44.
17. Thomaz DY, Grenfell RC, Vidal MS, Giudice MC, Del Negro GM, Juliano L, Benard G, de Almeida Junior JN. Does the capsule interfere with performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of Cryptococcus neoformans and Cryptococcus gattii? J Clin Microbiol. 2016;54(2):474–7.
18. Yan Y, Wu Y, Wang Q, Zhu X, Li H, Jiang H. Lesion size as a prognostic factor in the antifungal treatment of pulmonary cryptococcosis: a retrospective study with chest CT pictorial review of 2-year follow up. BMC Infect Dis. 2023;23(1):153.
19. Li Z, Wang M, Zeng P, Chen Z, Zhan Y, Li S, Lin Y, Cheng J, Ye F. Examination of a Chinese-made cryptococcal glucuronoxylomannan antigen test in serum and bronchoalveolar lavage fluid for diagnosing pulmonary cryptococcosis in HIV-negative patients. J Microbiol Immunol Infect. 2022;55(2):307–13.
20. McMullan BJ, Halliday C, Sorrell TC, Judd D, Sleiman S, Marriott D, Olma T, Chen SC. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. PLoS ONE. 2012;7(11):e49541.

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