Factors Affecting the False Negative of Cryptococcal antigen in Patients with Cryptococcosis: Immunology and Radiology

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Abstract

Study objectives The purpose of this article is to investigate the factors associated with false negatives in cryptococcal antigen detection in patients with pulmonary cryptococcosis by studying the effects of immune status and radiology on cryptococcal antigen results. **Methods** The clinical records, serum cryptococcal antigen result, laboratory findings and computed tomography findings of 61 immunocompetent and 17 immunocompromised patients with a diagnosis based on biopsy confirmation of the presence of pulmonary cryptococcosis were reviewed during the course of the study. Chi-square test was used to analyze categorical variable. The independent t-test was obtained to analyze continuous variable. Logistic regression models was used to analyze the effects of immunity and radiology imaging on cryptococcal antigen results. **Results** We studied the differences between these two groups. No difference were found in baseline characteristics, clinical symptoms, and laboratory findings. Logistic regression analysis showed that the significant independent variables related to serum cryptococcal antigen result were the immune status and radiology findings. **Conclusions** Our study indicates that immune status and lung imaging findings are important factors influencing cryptococcal antigen detection.

Introduction

Pulmonary cryptococcosis caused by infection with *Cryptococcus neoformans* C. *Cryptococcus* complexes is common in patients with immune dysfunction and it has become a emerging disease in patients with good immune function.[1-3]Some reports[4, 5] revealed that pulmonary cryptococcosis occurs more frequently in immunocompetent patients than in immunocompromised patients. Compared to its current high morbidity, the diagnosis of pulmonary cryptococcosis is still not fully diagnosed due to the limitations of diagnostic tools. Pulmonary cryptococcosis usually presents with nonspecific symptoms and computed tomography signs.[6-8] The methods used to confirm the infection are culture, direct microscopic, histopathology, serology, and molecular detection.[9, 10]Some research shows that serum CrAg is a useful diagnostic tool[11-13],and CrAg can be an effective non-invasive means of detecting pulmonary cryptococcosis. Research on serum CrAg was not a rare in the study of cryptococcal diseases. However, false negative serum CrAg is common among patients with Pulmonary cryptococcosis. The purpose of our study was to explore the factors associated with serum CrAg test.

Methods

Study subjects

We retrospectively reviewed the hospitalized patients with pulmonary cryptococcosis between January 2012 and March 2019 in Zhujiang Hospital of Southern Medical University, Nanfang Hospital of Southern Medical University and Guangzhou People's Hospital. All patients' diagnoses were confirmed by percutaneous transthoracic needle biopsy (PTNB) or postoperative biopsy .The data and information were collected including the following: (1) demographic features and past medical history; (2) clinical

features and symptoms; (3) laboratory tests, including white blood cell (WBC) counts, neutrophils counts, concentrations of hemoglobin (Hb), platelet counts (PLT), plasma procalcitonin (PCT); (4) serum CrAg detected by lateral flow assay (IMMY, Norman, OK, USA); (5) chest radiological findings. According to Tao Zhu's research, we divided the imaging findings into diffuse lesions and limited lesions.[11] In the end, 78 patients with non-HIV were enrolled; 61 patients had normal immunity and 17 had immunodeficiency. There were 54 patients in the CrAg positive group (CrAg+ group) and 24 patients in the negative group (CrAg-group). [Figure 1]

Statistical analysis

Statistical analyses were performed with SPSS software, version 20.0. Continuous data are presented as the mean $\pm$ standard deviation or range. Chi-square test was used to analyze categorical variable. The independent t-test was obtained to analyze continuous variable. Logistic regression models were used to analyze the effects of immunity and imaging on CrAg results. $P < 0.01$ was considered statistically significant.

Results

Demographics and Clinical Data

Patient demographic and clinical information is summarized in Table 1. Our patients ranged in age from 15 to 81 years (mean age, 44.44 years). Forty-nine patients were male and 29 were female. Sixty-one patients had no comorbidity, and 17 patients were considered to be immunocompromised with at least one predisposing condition, including kidney transplantation use with immunosuppressive drugs using in 15 patients, Langerhans cell histiocytosis and Acute myeloid leukemia in 1 patient. None of the patients included in this study had acquired immune deficiency syndrome (AIDS) at the time of diagnosis of pulmonary cryptococcosis. There was no significant difference in WBC, neutrophils, Hb, PLT, and plasma PCT level between two groups. Cough was the most common presenting symptom, occurring in 44 immunocompetent patients (72.13%) and 15 in immunocompromised patients (88.24%), followed by expectoration (55.74% vs 58.82%), chest pain (22.95% vs 23.53%), and fever (13.11% vs 17.65%). Nineteen people were asymptomatic, 17 of whom had no immunodeficiency disease.

Serum cryptococcal antigen and human status

The relationship of serum cryptococcal antigen expression and human status were analyzed. For patients with normal immune function and immunodeficiency, the expression of cryptococcal antigen was compared and a significant correlation was found between the two groups. ($P < 0.01$). And higher positive expression of cryptococcal antigen was observed in immunocompetent patients when compared with immunocompromised ones (Table 2).

Radiological findings

Table 3 showed that in the normal immune group, there were 47 diffuse extent lesion in the CrAg+ group and 7 in the CrAg- group ($P < 0.01$). Which demonstrated diffuse extent lesion was more common in CrAg+ group and limited extent lesion was more frequently observed in CrAg- group among normal immune group. On the other hand in Table 4, there were 4 diffuse extent lesion in the CrAg+ group and 10 in the CrAg- group among immunodeficiency group ($P > 0.01$). Chest radiological findings and biopsy results in patients with pulmonary cryptococcosis were shown in Figure 2.

Binary logistic regression analysis

The results of binary logistic regression analysis are summarized in Table 5. The significant independent variables related to serum CrAg results were the immunity [odds ratio = 0.89 (95% CI: 0.024– 0.325), $P = 0.0001$] and CT [odds ratio = 8.697 (95% CI: 1.755– 43.084), $P = 0.008$].

Discussion

Many studies [14, 15] have shown that serum CrAg diagnosis of pulmonary cryptococcosis has a higher sensitivity and specificity for HIV-positive patients, but lack of studies on HIV-negative patients. In this study, a retrospective research of 78 patients with HIV-negative cryptococcosis was conducted to investigate the effects of immune status and lung CT findings on serum CrAg detection, which directly compared the CrAg results of patients with pulmonary cryptococcosis under different immune conditions, and evaluated the effects of CT on CrAg results by comparing the CT findings of each group. The results of our study demonstrated that serum CrAg positive results were significantly more common in immunocompetent patients than in immunocompromised ones, and diffuse lung lesions were more common in immunologically normal CrAg+ patients. Additional multiple logistic regression analysis demonstrated that immune status and lung CT findings were the independent variables significantly related to serum CrAg test.

Similar to previous reports, our study found that a relatively low proportion of cryptococcosis infected patients were immunocompromised patients. The most frequent symptoms in our patients with pulmonary cryptococcosis included cough and expectoration were also found in our series and in those in previously published reports. [4, 16-19]

Our data on laboratory findings, including WBC, neutrophils, Hb, PLT, and plasma PCT level suggested that there were no significant difference between two groups (Table 1). It may confirm that cryptococcosis could dampen pulmonary neutrophil recruitment and inflammatory cytokine production in immunocompetent hosts, which affects the immunity of a healthy subject. [18, 20]

Our findings in normal immune patients have expanded the value of serum CrAg in the diagnosis of extensive disease in patients with normal immune function, supporting the results of previous studies that the most common CT findings of pulmonary cryptococcosis was multiple pulmonary nodules/masses of different diameters. [21-24]

We consider that the results of our study suggest two important factors with clinical usefulness. Firstly, serum CrAg was valuable for the diagnosis of extensive extent lesion in immunocompetent patients with pulmonary cryptococcosis. Secondly, cryptococcus infection is also common in normal immune populations; thus, Patients with pulmonary CT lesions should undergo CrAg examination.

On the other hand, our study had several limitations. First, it is a retrospective study with a small number of patients collected over a long period and our results should not be generalized to the worldwide population. Secondly, our study lacks the classification of cryptococcal varieties. Moreover, criteria for immunocompetency were based on the patient's medical records and thus may be prone to selection bias. Therefore, further study is wanted to confirm the findings reported here.

In conclusion, we have demonstrated that the immune status and lung CT findings of patients can affect serum CrAg results. When immune normal patients has extensive lung lesions and serum CrAg positive, it is highly suggestive of cryptococcal infection. Moreover, immunocompromised patients usually have Our findings in normal immune patients have expanded the value of serum CrAg in the diagnosis of extensive disease in patients with normal immune function, supporting the results of previous studies that the most common CT findings of pulmonary cryptococcosis was multiple pulmonary nodules/masses of different diametersmore extensive pulmonary involvement and serum CrAg negative result than do immunocompetent patients in the setting of cryptococcal infection.

Declarations

Abbreviations

CrAg: cryptococcal antigen; CT: computed tomography; WBC: white blood cell; Hb: hemoglobin; PLT: platelet counts; PCT: plasma procalcitonin; HIV: human immunodeficiency virus; AIDS: Acquired immune deficiency syndrome.

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Not applicable.

Authors' contributions

All of the authors had access to the full dataset (including the statistical Reports and tables) and take responsibility for the integrity of the data and the accuracy of the data analysis. JQM and HJD conceived the study. KLH, SMC, LFL and TZ collected the data and designed the analysis. JQM interpreted the data. JQM wrote the first draft of the paper. HJD review and approved the final report. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This cross-sectional study was approved by research Southern Pearl River Hospital Ethics Committee Medical University (No. 2016HXNK007). Anonymous analysis of clinical data, records, and data for this study was performed without intervention. So informed consent was waived.

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Tables

Table 1: Baseline characteristics of patients with pulmonary cryptococcosis

characteristics	Normal immunization	Immunodeficiency	P
n	61	17	
Male(n%)	38(62)	11(64)	0.856*
Age	44.82±14.06	43.06±14.10	0.650#
Asymptomatic(n%)	17(27.87)	2(11.76)	0.294*
Cough(n%)	44(72.13)	15(88.24)	0.294*
expectoration (n%)	34(55.74)	10(58.82)	0.820*
Chest pain(n%)	14(22.95)	4(23.53)	1*
fever(n%)	8(13.11)	3(17.65)	0.936*
WBC(10 ⁹ /L)	7.27±1.96	7.93±2.57	0.255#
Neutrophils	4.87±1.92	5.83±1.94	0.074#
Hb(g/L)	131.00±17.87	121.70±23.47	0.081#
PLT(10 ⁹ /L)	291.98±107.67	258.41±116.67	0.260#
Plasma procalcitonin level (ng/ml)	0.05±0.04	0.09±0.12	0.290#

*:Chi-square test; # :t-test.WBC: White blood cell; Hb: Hemoglobin.

Table 2:Relationship between CrAg expression and immune status of patients

CrAg expression	Normal immunization	immunodeficiency	P
CrAg+(n)	49	5	0.0001*<0.01
CrAg-(n)	12	12	

*:Chi-square test; CrAg: Cryptococcal antigen

Table 3: Chest radiological findings of Normal immunization group

Characteristics	CrAg+	CrAg-	P
Diffuse extent lesion(n)	47	7	0.002*<0.01
Limited extent lesion(n)	2	5	

*:Chi-square test; CrAg: Cryptococcal antigen

Table 4☐Chest radiological findings of immunodeficiency group

Characteristics	CrAg+	CrAg-	P
Diffuse extent lesion(n)	4	10	1*
Limited extent lesion(n)	1	2	

*:Chi-square test; CrAg: Cryptococcal antigen

Table 5☐Results of binary logistic regression analysis

Independent variable	Partial regression coefficient	Significance probability	Odds ratio	95% CI	
				Lower limit	Upper limit
Immunity	-2.146	0.0001	0.89	0.024	0.325
CT	2.163	0.008	8.697	1.755	43.084

CI: cofidence interval.

Figures

Figure 1

Flow diagram of the study patient selection.

Figure 2

The chest radiological findings and biopsy results in patients with pulmonary cryptococcosis. (a) lesion (the diameter <3 cm) in a single lobe (limited extent lesion). (b) lesions in multiple lobes (diffuse extent); Lesion (black arrows). Biopsy results (c-d), *Cryptococcus* sp. yeasts (black arrows).