Original Article

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Imaging and serum antigen levels that influence the treatment and prognosis of cryptococcosis in immunocompetent and immunocompromised patients: A 10-year retrospective study

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SUMMARY: This article was to summarize the treatment course and prognosis of immunocompetent and immunocompromised patients with pulmonary cryptococcal infections and to analyse the relevant factors. The chi-squared test was used to test for differences in categorical variables, and the independent samples t test was used to compare continuous variables. Multivariable analyses using the Cox proportional hazards model were used to estimate the effect of prognostic factors on treatment time and improvement time. A total of 243 patients were included in the analysis. Immunocompetent patients with diffuse imaging infiltrates had an extension of the treatment course within six months ($P = 0.048$) and an extension of the improvement days within four weeks ($P = 0.008$). In immunocompromised patients, an antigen assay ≥ 40 ($P = 0.013$) is an unfavourable factor leading to an extension of treatment by nine months. The serum antigen assay in 26/98 (26.53%) immunocompetent patients who did not turn negative when the treatment had finished was significantly lower than that in 14/29 (48.28%) immunocompromised patients ($P = 0.027$). All patients who underwent surgical resection had a good prognosis. Diffuse imaging infiltrates suggest longer treatment days and a longer improvement time in immunocompetent patients. Higher serum antigen levels in immunocompromised patients indicate longer treatment. Serum antigen assays in immunocompromised patients are difficult to negative.

Keywords: pulmonary cryptococcosis, immunocompetent, immunocompromised, treatment, prognosis, serum antigen assay

1. Introduction

Cryptococcosis is an invasive fungal infection caused by *Cryptococcus neoformans* or *Cryptococcus gattii* and has become increasingly common in both immunocompetent and immunocompromised patients. In addition to causing localized respiratory disease, combined lung and central nervous system (CNS) infection is common (*1*). Other sites of infection include the musculoskeletal system, skin and soft tissues, prostate, abdominal organs and the eye, either in combination with lung and/or CNS infection or in isolation (*2,3*).

During prolonged treatment adhering to clinical guidelines (*4*), physicians may encounter several inquiries, such as whether to continue treatment when the capsule antigen assay is still positive, risk of disease recurrence, and should it be replaced immediately or continued if inefficacy of fluconazole occurs in a short period. While some literature has reported on the clinical manifestations of cryptococcosis in both immunocompetent and immunocompromised patients (*5,6*), there are scarce articles available on the treatment course and prognosis due to the extended treatment time and follow-up period needed. This article presents a summary of the treatment course and prognosis of patients who had pulmonary cryptococcal infections in our hospital over the previous decade. It also investigates the factors that influence their treatment course and prognosis, with the aim of lending insights for the management of cryptococcal infections among both immunocompetent and immunocompromised patients.

2. Methods

2.1. Case series

Patients were eligible if the patients were admitted to the Infectious Diseases Department at Zhongshan Hospital, Fudan University, between January 1, 2012, and December 31, 2021. Data regarding patient demographics,

clinical features, laboratory results, pathogenic findings, treatments, and outcomes were obtained from the Zhongshan Hospital Information System. This project received approval from the Ethics Committee of Zhongshan Hospital and informed consent was obtained from all subjects or their legal guardians. All research was performed in accordance with relevant guidelines and the Declaration of Helsinki. All data were reviewed by two physicians (QQW and YS), while any discrepancies in interpretation between the primary reviewers were resolved by a third researcher (JP). The data that support the findings of this study are available from the corresponding author.

2.2. Case definition

Cryptococcosis patients are comprised of confirmed and clinical patients. Confirmed cryptococcosis was identified as a positive result of *Cryptococcus* culture from any site. Clinical cryptococcosis was identified by positive histopathology or cryptococcal antigen results, together with clinical or radiographic evidence of disease (*7*). Immunocompromised individuals were identified as having at least one underlying condition, which included AIDS, liver cirrhosis, haematologic malignancy, malignant solid tumour, chronic steroid use, and rheumatologic diseases such as rheumatoid arthritis, lupus, psoriatic arthritis, ankylosing spondylitis and inflammatory myopathy (*8*). The treatment duration refers to the period between starting the medication and discontinuing it. Improvement days denote the period between the initiation of medication and the improvements seen on chest imaging. The CT scan's morphological features have been categorized as either solitary nodule/mass, multiple nodules/masses, consolidation, or diffuse infiltrates (nodules/mass with consolidation) (*9*).

2.3. Statistical analysis of the data

Depending on the data distribution, sex, clinical symptoms at onset, and treatment prescription, categorical variables were presented as percentages, while continuous variables comprising age, leukocyte count, neutrophil count, lymphocyte count, CD4 count, erythrocyte sedimentation rate, high-sensitivity C-reactive protein, interleukin-2, interleukin-6 and interferon-gamma were presented in terms of the mean and standard deviation. Median and quartile values were used to present the serum antigen assay and treatment duration. Categorical variables were screened for differences using the chi-square test, while the independent samples *t* test was employed to compare continuous variables between immunocompetent and immunocompromised patients. Prognostic factors' effects on treatment time and improvement time were estimated using multivariable analyses with the Cox proportional hazards model. Statistical analyses were executed using IBM's SPSS 23.0 software (Armonk, NY, USA), and the graphs were produced utilizing GraphPad Prism 8.0.

3. Result

3.1. Case selection and classification

A total of 243 patients were analysed. Among them, 238 patients with pulmonary *Cryptococcus* infection or other site infection in combination with pulmonary infection were further analysed (Figure 1).

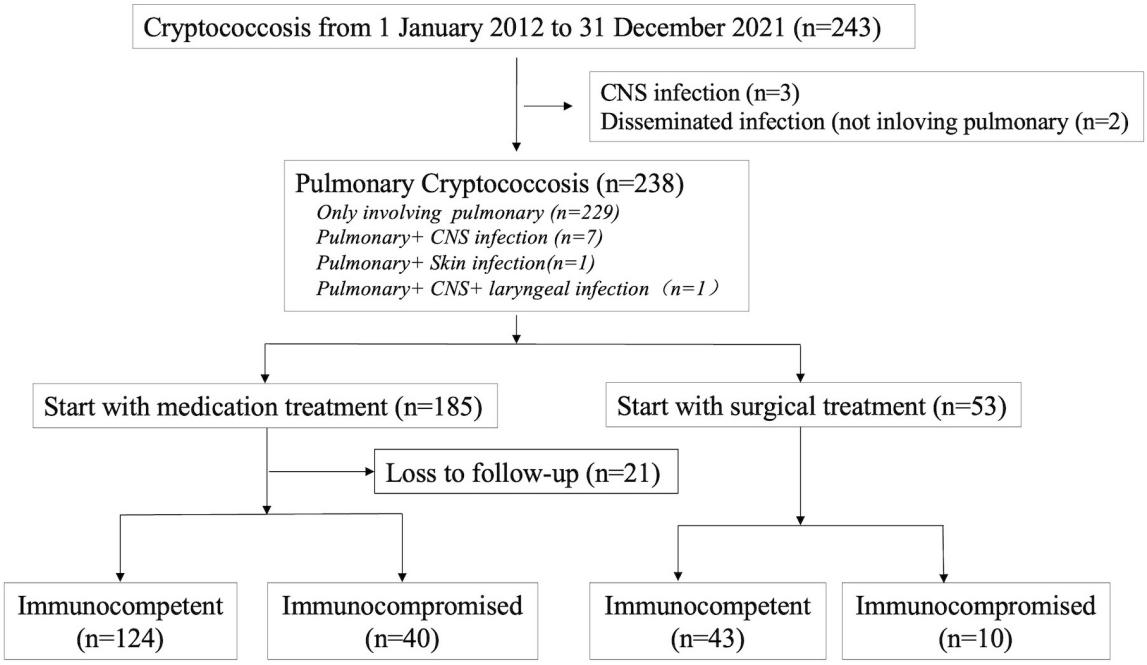

Figure 1. Flow chart of cases selection and classification. CNS: central nervous system.

Table 1. Clinical manifestations and laboratory results

Items	Immunocompetent (n = 163)	Immunocompromised (n = 46)	P
Medical treatment	n = 124 (%)	n = 40 (%)	
Male/Female	74/50	21/19	0.424
Age, years	47.69 ± 13.06	59.00 ± 13.66	< 0.001*
Involving other parts	4 (3.23)	4 (10.00)	0.191
Clinical symptoms at onset	56 (45.16)	21 (52.50)	0.419
Fever	21(16.94)	7 (17.50)	0.934
Cough	48 (38.71)	10 (25.00)	0.115
Expectoration	38 (30.65)	8 (20.00)	0.193
Chest pain	10 (8.06)	2 (5.00)	0.766
Shortness of breath	1 (0.81)	4 (10.00)	0.016*
Serum antigen assay	20 (10,160)	80 (25,640)	0.028*
Leukocyte × 10^9/L	6.77 ± 2.55	7.52 ± 3.22	0.209
Neutrocyte × 10^9/L	4.38 ± 2.27	5.79 ± 3.18	0.018*
Lymphocytes × 10^9/L	1.71 ± 0.56	1.06 ± 0.65	< 0.001*
CD4 (cells/μL)	535.98 ± 348.67	412.49 ± 338.47	0.158
ESR (mm/H)	17.78 ± 19.85	30.50 ± 23.17	0.004*
CRP (mg/L)	6.54 ± 17.51	23.27 ± 39.02	0.022*
IL-2 (U/mL)	388.75 ± 220.20	739.05 ± 725.38	0.052
IL-6 (pg/mL)	2.77 ± 2.20	7.32 ± 6.48	0.009*
INF-γ (pg/mL)	8.57 ± 8.16	28.58 ± 79.28	0.287
Surgical treatment	n = 43	n = 10	
Male/Female	31/12	3/7	0.033*
Age, years	53.60 ± 9.26	53.00 ± 17.89	0.919
Leukocyte *10^9/L	6.71 ± 2.39	6.19 ± 1.92	0.523
Neutrocyte *10^9/L	4.44 ± 2.45	4.27 ± 1.77	0.840
Lymphocytes *10^9/L	1.27 ± 0.47	0.87 ± 0.38	0.193
CRP (mg/L)	6.26 ± 9.86	4.38 ± 6.67	0.664

The values in brackets are the percentage except for the values in brackets of the serum antigen assay, which are the quartiles. Bold and * indicate significant differences. ESR: erythrocyte sedimentation rate, CRP: high-sensitivity C-reactive protein, IL-2: interleukin-2, IL-6: interleukin-6, INF-γ: interferon-gamma. Involving other parts: Four patients of pulmonary and CNS infections were included in immunocompetent patients, while immunocompromised patients had two cases of pulmonary and CNS infections, one case of pulmonary and skin infections, and one case of pulmonary, CNS, and laryngeal infections. One immunocompromised patient was lost to follow-up and therefore was not included in the study.

3.2. Clinical manifestations and laboratory results

Table 1 shows clinical manifestations and laboratory results of immunocompetent and immunocompromised patients. This study comprised 53 patients who had undergone surgical resection. Before surgery, 19 patients underwent serum antigen assay testing, of which only 2 had positive results. Following surgery, 21 patients were treated with antifungal therapy for a median duration of 81 days. The remaining patients were not administered any medication.

3.3. Antifungal treatment and treatment course

Anticryptococcal treatment and treatment course in patients with different immune states were shown in Table 2. The median duration of treatment for immunocompetent patients was 207 (137.5, 319) days, whereas for immunocompromised patients, it was 253.5 (156.25, 344.25) days. After ineffective treatment with fluconazole at 400 mg qd initially, five immunocompetent patients were prescribed fluconazole and flucytosine. Four out of seven immunocompromised patients received both fluconazole and flucytosine as initial treatment, while the remaining three received fluconazole alone, which proved ineffective and was subsequently switched to the combination therapy. Three immunocompromised patients were administered amphotericin B/amphotericin B liposomes after one month of ineffective fluconazole treatment. The duration of their treatment course was significantly shorter than others.

3.4. Related factors of treatment course and prognosis

The study analysed the relevant factors affecting the treatment duration of immunocompetent patients within 180 days and immunocompromised patients within 270 days based on the data provided in Table 2. The results showed that immunocompetent patients with diffuse imaging infiltrates had a significantly longer treatment course (P = 0.048) (Figure 2). Meanwhile, in immunocompromised patients, an antigen assay level of ≥ 40 (P = 0.013) was associated with an unfavourable outcome leading to prolonged treatment (Figure 2).

Among 124 immunocompetent patients, 2 out of 120 (1.67%) with pulmonary infection only and 1 out of 4 (25.00%) with pulmonary and CNS infection progressed. Among 40 immunocompromised patients, 1 out of 36 patients (2.78%) with only pulmonary infection progressed because of the underlying disease, and 1 out of 4 patients (25.00%) with pulmonary and skin infection progressed.

Table 2. Anticryptococcal treatment and treatment course in patients with different immune states

Medicine	Treatment			Treatment days		
	Immunocompetent (n = 124)	Immunocompromised (n = 40)	P	Immunocompetent	Immunocompromised	P
Triazoles ± Fluorocytosine						
Fluconazole	87 (70.16)	24 (60.00)	0.232	200 (137.5,300)	274 (183.25,356.25)	0.128
Voriconazole	7 (5.65)	5 (12.50)	0.272	173 (154.75,347.25)	179 (158,222)	0.373
Itraconazole	1 (0.81)	/	/	264	/	/
Fluconazole + Fluorocytosine	5 (4.03)	7 (17.50)	0.013*	335 (315,596)	311 (253.5,374.5)	0.512
Voriconazole after Fluconazole	1 (0.81)	/	/	573	/	/
Amphotericin B/Amphotericin B Liposome ± Fluorocytosine						
Amphotericin B/Amphotericin B Liposome after Fluconazole	17 (13.71)	3 (7.50)	0.444	170 (131,285)	111 (105,132.5)	0.022*
Amphotericin B/Amphotericin B Liposome	5 (4.03)	/	/	199 (91,366)	/	/
Amphotericin B/Amphotericin B Liposome + Fluorocytosine	1 (0.81)	1 (2.50)	0.429	654	1642	/

The values in brackets of treatment are thepercentages. The values in brackets of treatment days are the quartiles. Bold and * indicate significant differences. Voriconazole after fluconazole: Fluconazole was ineffective in treating the condition, and thus, the treatment was switched to voriconazole. Amphotericin B/Amphotericin B Liposome after fluconazole: Fluconazole was ineffective in treating the condition, and thus, the treatment was switched to Amphotericin B/Amphotericin B Liposome.

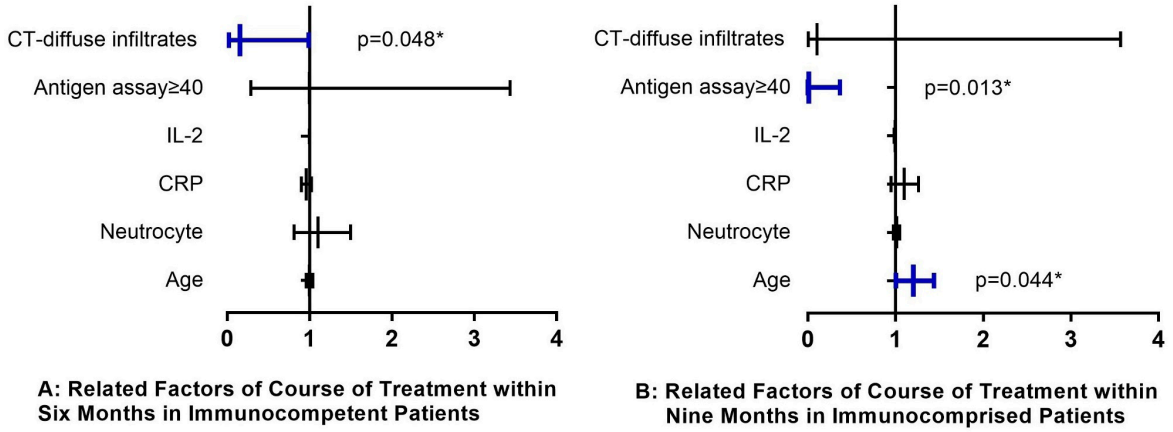

Figure 2. Related factors of treatment course.

Of the eight patients with *Cryptococcus* infection affecting both the lungs and other sites, the disease of one patient advanced due to aplastic anaemia, and the others were treated for more than one year. Due to the limited sample size, the results cannot be subjected to statistical analysis.

Ninety-eight immunocompetent patients were monitored by serum antigen assay, and 26 (26.53%) of them remained positive. Twenty-nine immunocompromised patients were monitored by serum antigen assay, and 14 (48.28%) remained positive (P = 0.027).

3.5. Related factors of improvement days

The median time for pulmonary infection improvement was 32.5 days in immunocompetent patients and 34.5 days in immunocompromised patients. Diffuse pulmonary infiltrates (P = 0.008) and CRP (P = 0.024) were significant factors for improvement days exceeding 4 weeks in immunocompetent patients, whereas age (P = 0.033) and neutrocyte count (P = 0.038) were significant factors for improvement days exceeding 4 weeks in immunocompromised patients (Figure 3).

Five immunocompetent patients (5/124) demonstrated spontaneous improvement in their pulmonary imaging prior to receiving medication. Chest imaging of these patients showed infections affecting one or two lobes. In the immunocompromised patients, no spontaneous improvement was observed.

4. Discussion

In the past, many patients with pulmonary cryptococcosis presented as a solitary nodule, leading to misdiagnosis as a tumour and subsequent surgical resection. In recent years, the use of serum antigen assays and percutaneous lung puncture technology (*10,11*) has become widespread,

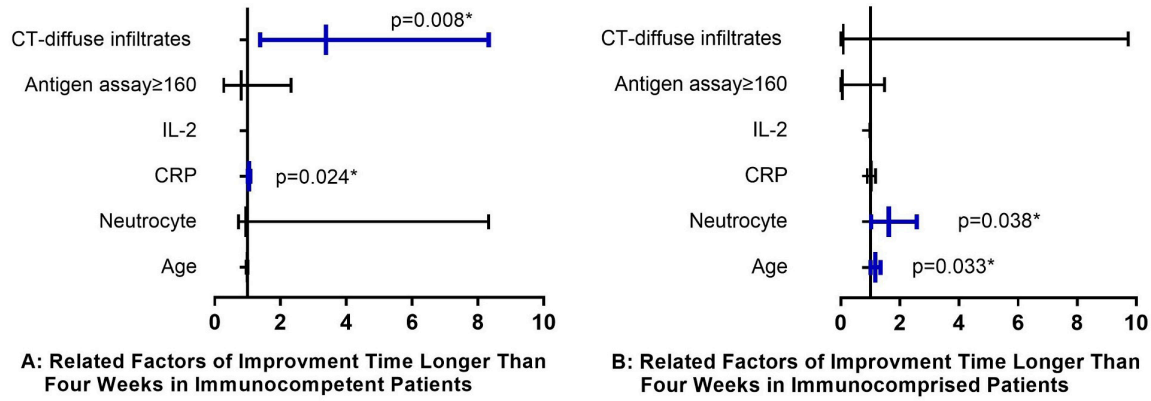

Figure 3. Related factors of improvement days.

resulting in increased presurgical diagnoses of cryptococcosis presenting as solitary nodules. However, our study demonstrated that 89.5% of patients who underwent thoracic surgery presented negative serum antigen assays. This is because serum antigen assays from patients with pulmonary cryptococcosis are seldom positive when disseminated disease is absent (*12*). As such, a diagnosis of cryptococcosis cannot be excluded with a negative serum antigen assay if a patient visits the thoracic surgery department with a single nodule. Therefore, a lung biopsy is needed for a clear diagnosis.

There is insufficient evidence in evidence-based medicine to support the use of fluconazole and flucytosine for cryptococcal infections affecting multiple sites. The World Health Organization now recommends a high dose of fluconazole (1,200 mg) and flucytosine as induction therapy for cryptococcal meningitis in HIV-infected patients to be administered over 14 days (*13*). Fluconazole and flucytosine were investigated as viable therapies for cryptococcal meningitis for non-HIV and non-transplant-associated cryptococcal meningitis (*14*). According to those studies, the combination of fluconazole and flucytosine can be used for multiple sites of cryptococcal infection. In our case study, a kidney transplant recipient with infections in the lungs, central nervous system, and larynx was treated with fluconazole and flucytosine, exhibiting a positive outcome. However, two out of three patients who had cryptococcal infections in more than one site used fluconazole only as initial treatment and had a poor prognosis in our study, and the conclusions drawn from the literature were consistent with ours (*15*).

Nodular lesions were the predominant CT (computed tomography) type in immunocompetent patients. Consolidation and multiple lesions indicated infection spread, reflecting the challenge in managing fungal infections, which were more prevalent in immunocompromised patients (*16*). Immunocompromised patients exhibited a higher prevalence of multiple lesions, air bronchial signs, and cavitation (*17*). In our study of immunocompetent patients, those with diffuse imaging infiltrates who had an increased burden of fungal infection needed a treatment course sixty to ninety days longer than patients with solitary or multiple nodules. The imaging features of patients with varying immune statuses have been examined (*18,19*), and the possibility of forecasting prognosis through different imaging presentations merits additional investigation.

The serum and CSF lateral flow cryptococcal antigen assay is now the preferred test because of its speed, accuracy and cost (*20,21*). The antigen assay has been reported to predict meningitis and death in cryptococcal meningitis, and based on our research, an increased antigen assay in immunocompromised patients with pulmonary cryptococcal infection signifies a prolonged treatment time. Therefore, it is important to use the antigen assay in the future to personalize therapy for prevention, treatment and prognosis (*22*). Additionally, our research has revealed that almost 50% of immunocompromised patients do not achieve negative results in serum antigen assays at the end of treatment. Therefore, negative conversion of serum antigen assays should not be relied upon as the sole basis for discontinuing medication. Instead, a comprehensive evaluation of clinical symptoms, improvements in imaging, and inflammatory indicators should be conducted.

In conclusion, diffuse infiltrates in imaging in immunocompetent patients indicated longer treatment days and longer improvement time. A higher serum antigen assay of immunocompromised patients indicates a longer treatment time. Solitary nodules from surgical resection have a good prognosis.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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