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# Clinical characteristics and prognostic factors of pulmonary and extrapulmonary cryptococcosis

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## Abstract

**Background** Cryptococcosis is progressively acknowledged among people, irrespective of the human with or without immunodeficiency virus (HIV). This change in epidemiology has been recorded in recent years, prompting closer examination and a broader understanding of the disease manifestations and risk factors.

**Methods** The data of cryptococcal infections in China during 11 years were retrospectively analyzed. According to the position of infection, the patients were categorized into the pulmonary infection group and extrapulmonary infection group. The composition of the two groups was compared, and the potential risk factors of disseminated infection were analyzed. Logistic regression was used to analyze the prognostic risk factors of the disease.

**Results** A total of 165 patients were enrolled. 113 (68.5%) were male, and the age was 47.49 (18–82) years. 101 cases (61.2%) had a normal immune function and 64 cases (38.8%) had impaired immune function. 45 patients had extrapulmonary infection, involving the central nervous system, bone and joint, skin and bloodstream, and 120 patients had simple pulmonary infection. The mortality of the extrapulmonary infection group (48.9%) was significantly higher than that of the pulmonary infection group (0.8%). According to univariate logistic regression analysis, immune status (hazard ratio [HR], 4.476; 95% confidence interval [CI], 1.725–11.618;  $P=0.002$ ), infection position ([HR], 113.826; [CI], 14.607–886.967;  $P<0.001$ ), white blood cell count, ([HR], 1.209; [CI], 1.054–1.386;  $P=0.007$ ), hemoglobin ([HR], 0.970; [CI], 0.955–0.986;  $P<0.001$ ), platelet count ([HR], 0.993; [CI], 0.987–0.999;  $P=0.026$ ), neutrophil percentage ([HR], 1.115; [CI], 1.065–1.168;  $P<0.001$ ), lymphocyte percentage ([HR], 0.875; [CI], 0.827–0.927;  $P<0.001$ ), neutrophil-to-lymphocyte Ratio (NLR) ([HR], 1.144; [CI], 1.072–1.221;  $P<0.001$ ), monocyte percentage ([HR], 0.752; [CI], 0.618–0.915;  $P=0.004$ ) were related to the prognosis. Multivariate logistic regression analysis showed that the infection position was remained related to the prognosis with statistical significance ([HR], 0.018; [CI], 0.001–0.384;  $P=0.001$ ).

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**Conclusion** Extrapulmonary infection of Cryptococcosis is an important risk factor for prognosis. High levels of neutrophils and NLR, and low levels of lymphocytes and monocytes may lead to disseminated infection of Cryptococcosis. Further studies are needed to reduce the occurrence rate of extrapulmonary infection and mortality.

**Keywords** *Cryptococcus*, Extrapulmonary infection, Pulmonary infection, Prognosis

## Background

*Cryptococcus* is an opportunistic pathogenic yeast that is prevalent in the environment, thriving in pigeon guano, some plants (e.g. eucalyptus trees) and soil [1]. Normally, *Cryptococcus* in the environment invade the lungs through the respiratory tract, which is often confined to the lungs or resolved by the human innate immune system. However, *Cryptococcus* may cause life-threatening illnesses in immunocompromised individuals, such as cryptococcal meningitis (CM), either from reactivation of a previously acquired latent infection or novel exposure [2–4].

The mortality rate among patients with extrapulmonary cryptococcosis is extremely high, particularly in the case of CM, where the mortality rate can reach as high as 60–80% within 6 months after diagnosis [5]. Although, in recent years, it has not only been in those with HIV and natural or iatrogenic immunosuppression but also in apparently immunocompetent individuals [6–9]. In a previous study, it was reported that 67% of instances of pulmonary cryptococcosis among immunocompetent patients progressed to disseminate into the central nervous system, leading to CM, not to mention immunocompromised patients [10].

To further improve our comprehension of the manifestations and consequences of cryptococcosis diagnosed in Chinese people with or without HIV infection, we performed a retrospective study. The aim of this study is to compare the clinical characteristics and treatment strategies between pulmonary and extrapulmonary infection of *Cryptococcus*, explore the potential risk factors for the development of pulmonary cryptococcosis to CM, identify the prognostic factors of cryptococcosis, and provide new ideas for preventing extrapulmonary infection and improving the prognosis of patients.

## Materials and methods

### Research objects

One hundred and sixty-five patients diagnosed with cryptococcosis in the First Affiliated Hospital with Nanjing Medical University from January 2010 to December 2021 were included in the study, including 113 males and 52 females. Inclusion criteria: cryptococcal disease had to be “proven” by radiographic anomalies consistent with the disease, coupled with confirmed culture positivity from sterile fluid or biopsy confirmation. Alternatively, a diagnosis of “probable” cryptococcosis was assigned in cases exhibiting antigen positivity in blood or cerebrospinal

fluid (CSF). Instances where microbial retrieval from sputum or bronchoalveolar lavage lacked concordance with clinical and/or radiographic evidence were categorized as “possible” pulmonary disease. Diseases involving the central nervous system (CNS) with evidence of *Cryptococcus* in histological examination, antigen detection, or culture of brain tissue or CSF were also included [11, 12]. The exclusion criteria: patients under the age of 18.

The study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by The Ethics Committee of The First Affiliated Hospital with Nanjing Medical University. Because it was a retrospective nature of the study with anonymized clinical data, the informed consent was waived.

### Research methods

A retrospective analysis of the clinical data of 165 patients, including gender, age, immune status, comorbidities, clinical symptoms; laboratory indicators: blood routine examination, serum procalcitonin (PCT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fasting plasma glucose; antifungal regimens; treatment efficacy, etc. Outcomes: all-cause case fatality at 30 days after the diagnosis.

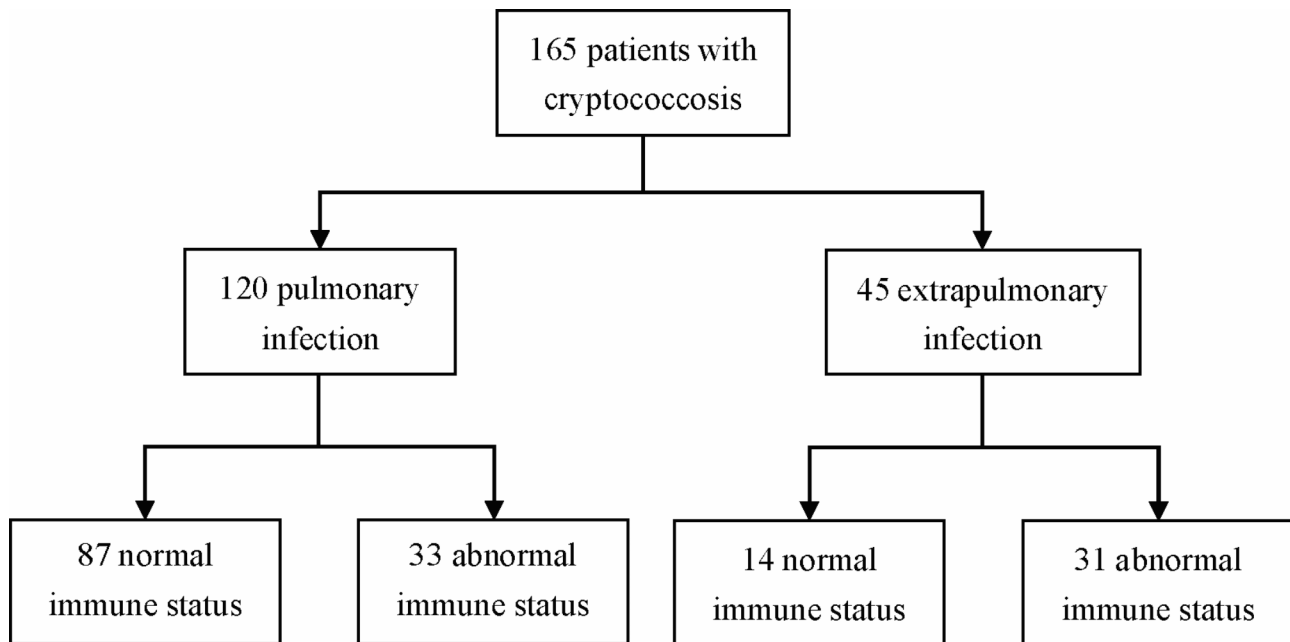
### Statistical methods

All data statistics used are carried out by SPSS 26.0 software. Count data were expressed as  $n$  (%) and  $\chi^2$  test or Fisher’s exact probability test was used. Normally, distributed measurement data were expressed as  $\bar{x} \pm s$  and analyzed with independent samples t-test. Non-normally distributed measurement data were expressed as median (M) and interquartile range (Q1–Q3) indicates that the non-parametric test was used. Logistic regression analysis was used for multivariate analysis.  $P < 0.05$  was considered statistically significant.

## Results

### General information

According to the inclusion and exclusion criteria, a total of 165 adult cases of cryptococcosis were screened, as shown in Fig. 1. Subject demographic characteristics, exposures, and immunosuppressive conditions are summarized in Table 1. Among 165 patients, 113 (68.5%) were male, the age was 47.49 (18–82) years, and 58 (35.2%) patients had a history of smoking. Normal immune function was observed in 101 cases (61.2%), whereas 64 cases (38.8%) exhibited impaired immune function. The



**Fig. 1** Flow chart of study sample selection

**Table 1** Demographic characteristics of 165 patients with cryptococcosis

Variables	Values
Mean age, (year)	47.49 (18–82)
Male sex, no (%)	113 (68.5)
Smoker, no (%)	58 (35.2)
Alcohol, no (%)	32 (19.4)
Underlying diseases, no (%)	
Diabetes mellitus	27 (16.4)
Autoimmune disease	13 (7.9)
solid organ transplantation	7 (4.2)
Hematologic malignancy	5 (3.0)
Decompensated liver disease	3 (1.8)
Solid tumor	4 (2.4)
Invasive Fungal Infection history	3 (1.8)
Acquired immunodeficiency syndrome	8 (4.8)
Immunosuppressive medications	
Glucocorticoid therapy	16 (9.7)
Cytotoxic chemotherapy	6 (3.6)
Calcineurin/mTOR inhibitors	7 (4.2)

majority of patients with abnormal immune status had conditions such as diabetes mellitus, autoimmune diseases, and glucocorticoid therapy.

#### Characteristics at admission

Of the 165 patients, 45 individuals were afflicted with extrapulmonary infection, which affected their central nervous system, bones and joints, skin, or bloodstream. Meanwhile, the remaining 120 patients suffered from a more straightforward pulmonary infection.

Upon analyzing the composition of the pulmonary infection group and the extrapulmonary infection group, it was observed that the proportion of patients with abnormal immune function in the extrapulmonary infection group was higher, and the clinical manifestations of the two groups were significantly different. The main manifestations of the pulmonary infection group were cough (57.5%), expectoration (38.4%), and chest pain (16.8%). In contrast, the extrapulmonary infection group primarily exhibited symptoms such as headache (80.0%), fever (68.9%), nervous system symptoms (57.8%), and vomiting (42.2%). Furthermore, in the laboratory examination, the percentage of neutrophils (82.5%) and NLR (6.7131) in the extrapulmonary infection group were higher, hemoglobin (115 g/L), platelet count ( $179.04 \times 10^9/L$ ) and lymphocyte percentage (14.17%) were lower. Additionally, an increase in PCT, CRP and fasting blood glucose was more common. (Table 2).

#### Treatment

The lung infection mainly accepts azole monotherapy, while extrapulmonary infection only 19 cases (42.2%) use azole monotherapy. Out of the remaining 26 patients with extrapulmonary infections, 19 patients were subjected to treatment using amphotericin B alone or in conjunction with azoles or in combination with 5-FC. 2 patients were subjected to triple therapy. 4 patients were left untreated as a consequence of either discontinuing their treatment prematurely or the rapid progression of their disease. In addition to antifungal treatment, adjunctive treatment was also provided (Table 3). 11 patients

**Table 2** Comparison of clinical characteristics between patients with cryptococcosis

	Total cohort (n = 165)	Pulmonary infection (n = 120)	Extrapulmonary infection (n = 45)	PValue
Mean age, year (range)	47.49 (18–82)	47.6 (18–80)	47.2 (19–82)	0.866
Male, no (%)	113 (68.5)	84 (70.0)	29 (64.4)	0.494
Smoking, no (%)	58 (35.2)	45 (37.5)	13 (28.9)	0.302
Alcohol drinking, no (%)	32 (19.4)	23 (19.2)	9 (20)	0.904
Epidemiological, no (%)	8 (4.8)	5 (4.2)	3 (6.7)	0.796 <sup>b</sup>
Immune status, no (%)	64 (38.8)	33 (27.5)	31 (68.9)	<0.001
Underlying diseases, no (%)				
Diabetes mellitus	27 (16.4)	22 (18.3)	5 (11.1)	0.264
Autoimmune disease	13 (7.9)	5 (4.2)	8 (17.8)	0.010 <sup>b</sup>
solid organ transplantation	7 (4.2)	1 (0.8)	6 (13.3)	0.002 <sup>b</sup>
Hematologic malignancy	5 (3.0)	1 (0.8)	4 (8.9)	0.029 <sup>b</sup>
Decompensated liver disease	3 (1.8)	1 (0.8)	2 (4.4)	0.372 <sup>b</sup>
Solid tumor	4 (2.4)	4 (3.3)	0	0.502 <sup>b</sup>
Invasive Fungal Infection history	3 (1.8)	1 (0.8)	2 (4.4)	0.372 <sup>b</sup>
Acquired immunodeficiency syndrome	8 (4.8)	0	8 (17.8)	<0.001 <sup>b</sup>
Immunosuppressive medications, no (%)				
Glucocorticoid therapy	16 (9.7)	4 (3.3)	12 (26.7)	<0.001 <sup>b</sup>
Cytotoxic chemotherapy	6 (3.6)	2 (1.7)	4 (8.9)	0.082 <sup>b</sup>
Calcineurin/mTOR inhibitors	7 (4.2)	3 (2.5)	4 (8.9)	0.168 <sup>b</sup>
Diagnosis.no. (%)				
Proven	133 (80.6)	89 (74.2)	44 (97.8)	<0.001
Probable	27 (16.4)	26 (21.7)	1 (2.2)	<0.001
Possible	5 (3.0)	5 (4.2)	0	0.378 <sup>b</sup>
Concurrent (non-lung) site of infection, no (%)				
CNS	39 (23.6)		39 (86.7)	
Bloods tream	9 (5.5)		9 (20.0)	
Bone/joint	2 (1.2)		2 (4.4)	
Skin	3 (1.8)		3 (6.7)	
Clinical characteristic, no (%)				
Fever	50 (20.3)	19 (15.2)	31 (68.9)	<0.001
Cough	80 (48.5)	69 (57.5)	11 (24.4)	<0.001
Expectoration	56 (33.9)	48 (38.4)	8 (17.8)	0.012
Neurological symptoms <sup>d</sup> , no (%)	26 (15.8)	0	26 (57.8)	<0.001
Vomit	19 (11.5)	0	19 (42.2)	<0.001
Headache	37 (22.4)	1 (0.8)	36 (80.0)	<0.001
Dyspnea	22 (13.3)	17 (14.2)	5 (11.1)	0.607
Chest pain	21 (12.7)	21 (16.8)	0	0.003
Skin lesion	5 (3.0)	0	5 (11.1)	<0.001 <sup>b</sup>
Time to diagnosis				
Mean number of days (IQR)	4 (0.5–10)	5 (0.25–10)	3 (0.5–8)	0.515
Laboratory results				
WBC (×10 <sup>9</sup> /L)	6.77 (5.34–8.92)	6.67 (5.51–8.18)	8.38±4.26	0.074
Hb (g/L)	135 (121–148)	142 (131–153)	115 (85.5–125)	<0.001
PLT (×10 <sup>9</sup> /L)	213(162.75–268)	222(179–278)	181(108–283.4)	0.002
Neutrophils <sup>c</sup> (%)	65.95 (58.9–76.9)	63.05(56.23–70.85)	82.5 (71.8–88.65)	<0.001
Lymphocytes <sup>c</sup> (%)	24.7 (14.40–31.00)	27.75(20.26–33.77)	12.2(5.75–19.6)	<0.001
NLR	2.72 (1.90–5.25)	2.26 (1.61–3.32)	6.7131 (3.61–15.34)	<0.001
Monocytes <sup>c</sup> (%)	6.43(4.75–8.10)	6.60 (5.18–8.41)	5.50(3.20–7.95)	0.008
CRP <sup>a</sup>	57/136	33/98	24/38	0.002
PCT <sup>a</sup>	38/63	17/35	21/28	0.033
ESR <sup>a</sup>	28/51	15/30	13/21	0.400
GLU <sup>a</sup>	38/155	21/113	17/42	0.005

**Table 2** (continued)

	Total cohort (n = 165)	Pulmonary infection (n = 120)	Extrapulmonary infection (n = 45)	PValue
Serum CrAg	76/93	65/79	11/14	1.000 <sup>b</sup>
CSF CrAg	15/25		15/25	
CSF smear	29/34		29/34	
CSF culture	8/12		8/12	
Antifungal regimen, no (%)				
Azole monotherapy	131 (79.4)	112 (93.3)	19 (42.2)	< 0.001
Non-azole monotherapy	27 (16.4)	1 (0.8)	26 (57.8)	
None	11 (6.7)	7 (5.8)	4 (8.9)	
Outcome, no (%)				
Survivors	142 (86.1)	119 (99.2)	23 (51.1)	< 0.001
Non-survivors	23 (13.9)	1 (0.8)	22 (48.9)	

a. The detection value was higher than the reference range (CRP 0-8 mg/L, PCT 0-0.05ng/ml, ESR 0-20 mm/h, GLU 3.9-6.1mmol/L)

b. Fisher exact probability method

c. Percentage of each type of cell in blood cells

d. Neurological symptoms: hearing loss, vision loss, cognitive impairment, personality change, coma, delirium, epilepsy, etc

**Table 3** The therapeutic methods of patients with cryptococcosis

Antifungal regimen, no (%)	Total cohort (n = 165)	Pulmonary infection (n = 120)	Extrapulmonary infection (n = 45)
Amphotericin B + 5FC	6 (3.5)	0	6 (13.3)
Azole monotherapy	136 (80.0)	112 (93.3)	19 (42.2)
Amphotericin B monotherapy	2 (1.2)	0	2 (4.4)
Amphotericin B + azole	12 (7.1)	1 (0.8)	11 (24.4)
Azole + 5FC	1 (0.6)	0	1 (2.2)
Triple therapy	2 (1.2)	0	2 (4.4)
None	11 (6.5)	7 (5.6)	4 (8.9)
Adjunctive management			
High-dose corticosteroids	15 (8.8)	0	15 (33.3)
Serial LP	13 (7.6)	0	13 (28.9)
CSF shunt/ventriculostomy	1 (0.6)	0	1 (2.2)
Surgical lung resection	12 (7.1)	11 (8.8)	1 (2.2)

with pulmonary infection underwent lung resection and resection of lesions. In cases of extrapulmonary infection, especially for patients with cryptococcal meningitis, 15 patients were administered high-dose corticosteroids to control inflammation, and 13 patients received serial LP to manage high-pressure and relieve symptoms such as headaches. The mortality of extrapulmonary infection group (48.9%) was significantly higher than that of pulmonary infection group (0.8%).

### Prognostic analysis

According to univariate Logistic regression analysis, several factors were found to be related to the prognosis of cryptococcosis, including immune status (hazard ratio [HR], 4.476; 95% confidence interval [CI], 1.725–11.618;  $P=0.002$ ), infection position ([HR], 113.826; [CI], 14.607–886.967;  $P<0.001$ ), white blood cell count,

**Table 4** Univariate analysis of factors for cryptococcosis patients prognosis

Variables	PValue	95%CI
Age	0.222	1.021 (0.988–1.055)
Male	0.399	1.480 (0.595–3.680)
Smoking	0.667	1.220 (0.493–3.019)
Immune status	0.002	4.476 (1.725–11.618)
Diagnosis of time	0.513	0.977 (0.913–1.047)
Infection position	< 0.001	113.826 (14.607–886.967)
WBC	0.007	1.209 (1.054–1.386)
Hb	< 0.001	0.970 (0.955–0.986)
PLT	0.026	0.993 (0.987–0.999)
N <sup>c</sup>	< 0.001	1.115 (1.065–1.168)
L <sup>c</sup>	< 0.001	0.875 (0.827–0.927)
NLR	< 0.001	1.144 (1.072–1.221)
M <sup>c</sup>	0.004	0.752 (0.618–0.915)
CRP <sup>a</sup>	0.049	2.743 (1.005–7.484)
PCT <sup>a</sup>	0.085	0.385 (0.846–13.544)
GLU <sup>a</sup>	0.003	4.359 (1.678–11.325)
Serum CrAg	0.577	1.846 (0.214–15.902)
Azole monotherapy	< 0.001	0.134 (0.052–0.344)

([HR], 1.209; [CI], 1.054–1.386;  $P=0.007$ ), hemoglobin ([HR], 0.970; [CI], 0.955–0.986;  $P<0.001$ ), platelet count ([HR], 0.993; [CI], 0.987–0.999;  $P=0.026$ ), neutrophil percentage ([HR], 1.115; [CI], 1.065–1.168;  $P<0.001$ ), lymphocyte percentage ([HR], 0.875; [CI], 0.827–0.927;  $P<0.001$ ), Neutrophil-to-Lymphocyte Ratio (NLR) ([HR], 1.144; [CI], 1.072–1.221;  $P<0.001$ ), Monocyte percentage ([HR], 0.752; [CI], 0.618–0.915;  $P=0.004$ ) were related to the prognosis (Table 4). Multivariate logistic regression analysis showed that the infection position remained was related to the prognosis, with statistical significance ([HR], 0.018; [CI], 0.001–0.384;  $P=0.001$ ), fasting blood glucose ([HR], 4.359; [CI], 1.678–11.325;  $P=0.003$ ), and azole monotherapy ([HR], 0.134; [CI], 0.052–0.344;

$P < 0.001$ ) were related to the prognosis. Further multivariate logistic regression analysis revealed that infection position remained significantly related to the prognosis ([HR], 0.018; [CI], 0.001–0.384;  $P = 0.010$ ). (Fig. 2).

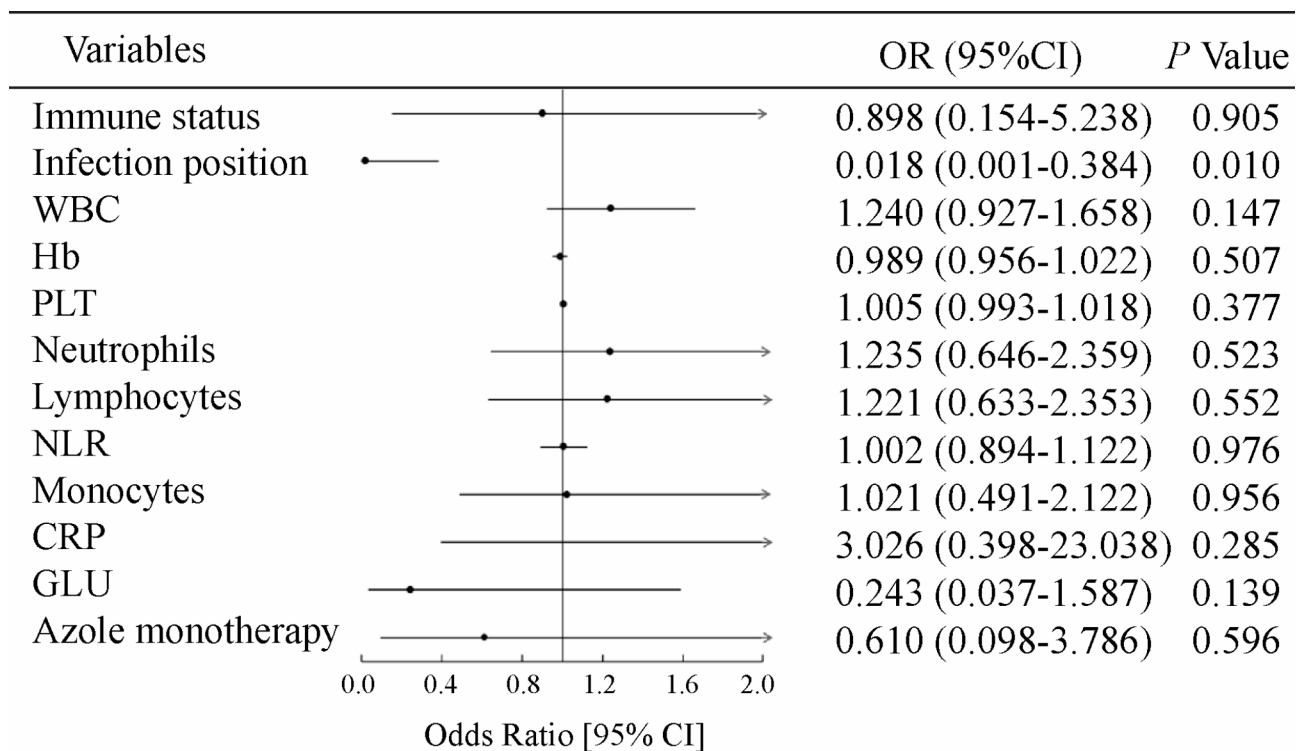
## Discussion

*Cryptococcus* is an opportunistic pathogen that invades the lungs through the respiratory tract and gives rise to pulmonary cryptococcosis, which is the most prevalent form of cryptococcosis. When the immune system is compromised, *Cryptococcus* can breach the pulmonary immune defenses and disseminate to other body sites such as the central nervous system, lymph nodes, bones and joints, and skin, leading to disseminated cryptococcosis.

*Cryptococcus* is a lethal fungus whose polysaccharide capsules are considered the primary virulence factors [13]. Polysaccharides are immunomodulators, One of their target cell populations for modulation are macrophages, Polysaccharide capsules affect the immune function of macrophages by reducing the antigen presentation function, changing co-stimulatory molecules, regulating the secretion of pro-inflammatory and anti-inflammatory cytokines, and up-regulating the death receptor Fas/FasL to stimulate cell apoptosis [14–18]. Various studies have proved that cryptococcal antigen (CrAg) titer in blood and CSF could act as risk factors for predicting the extent of pulmonary cryptococcosis and outcome in non-HIV

patients [19, 20]. We also analyzed the association of CrAg with cryptococcosis, but we found that there was no significant difference between the two groups for CrAg in serum, and no relationship was shown between CrAg and cryptococcosis outcome, which might due to the small size and individual difference.

Macrophages are vital cellular components of innate immunity, serving as the primary barrier against pathogens and regulating homeostasis and inflammatory responses. Macrophages hold an integral role in the defense against *Cryptococcus*, especially alveolar macrophages (AMs) as first responders, whose surface complement binds to mannose receptors, mediates phagocytosis, clears *Cryptococcus* or forms inflammatory granulomas to confine *Cryptococcus* to the lungs [21, 22]. Host cytokines kill *Cryptococcus* by inducing macrophage polarization. It has been shown that activation of M1 macrophages and downstream Th1 response effectively control *Cryptococcus* infection [23–26]. In addition, macrophages release high levels of reactive nitrogen intermediates (RNI), nitric oxide, reactive oxygen intermediates (ROI) and superoxide, which damage DNA and a number of chemical moieties of *Cryptococcus* [27]. Malfunction in macrophage development, activation, proliferation, and signaling collectively contribute to a heightened risk of disseminated cryptococcal infection [28–31]. Monocytes in the blood give rise to macrophages, which escape from blood vessels and infiltrate



**Fig. 2** Multivariable analysis of factors for cryptococcosis patient prognosis

the tissues following further differentiation. We have observed a statistically significant decrease in the number of monocytes in the extrapulmonary infection group compared to that in the pulmonary infection group, which consequently leads to a secondary decrease in macrophages that could potentially facilitate the development of disseminated cryptococcosis.

NLR is regarded as a diagnostic and predictive marker of disease severity in patients with inflammatory ailments, which can mirror the balance between innate and adaptive immune reactions [32, 33], and the increase in NLR may indicate the inflammatory response to fungal infection, revealing, to some extent, the imbalance between inflammation and immune response in our investigation. Some studies found that an increase in the NLR during treatment may be used as an indicator of treatment failure for patients with cryptococcal meningitis [34]. In HIV-negative immunocompetent cryptococcal meningitis, they got the same conclusion, in addition, they proved the baseline high IgM indicated a low risk of post-infectious inflammatory response syndrome (PIIRS) development during the treatment [35]. Lymphopenia and monocytopenia may be risk factors for cryptococcal dissemination [36], which agrees with our study. In our study, the percentage of neutrophils in the extrapulmonary infection group was higher than pulmonary infection group, hemoglobin, platelet count and lymphocyte percentage were lower. And univariate Analysis found that elevated white blood cells and neutrophils, decreased lymphocytes may serve as risk factors for the prognosis of cryptococcosis, which is consistent with the findings of prior investigations [37, 38]. Evidence suggests that neutrophils promote non-specific immunity and initiate the body's response to inflammation, while lymphocytes are protective elements against inflammation. The increase of white blood cells and neutrophils in patients with cryptococcosis may be closely related to lymphopenia. It is well known that the patients with lymphopenia easily leads to microbial infection which can directly induce the recruitment of neutrophils to tissue sites and further promote the activation of neutrophils, resulting in the increase of the total number of white blood cells and the percentage of neutrophils [39–41]. To a certain extent, we can predict the prognosis by assessing the patient's condition based on high neutrophils and low lymphocytes. Previous study also has proved that low CRP levels and high haemoglobin levels were risk factors for developing CM in cryptococemia patients [42]. But in our study, we found that there was no significant difference between the two groups for CRP, and no relationship was shown between CRP and cryptococcosis outcome, which might due to the small size and individual difference.

Tian et al. found that underlying disease, dyskinesia, anemia, high peripheral blood neutrophils, low platelet

count, high CSF fungal burden (measured by CrAg titer), and high CSF OP, were independently associated with prognosis in patients infected with ST5 lineage without HIV [20]. The results of another study in HIV-negative CM patients with acute/subacute onset suggest that the ratio of CSF glucose/blood glucose, impaired consciousness, and hospitalization length were indicated to be not only independent prognosis factors, but also factors significantly related to the survival time [43]. In a retrospective analysis of pulmonary cryptococcosis and extrapulmonary cryptococcosis, compared with pulmonary cryptococcosis, a larger proportion of extrapulmonary cryptococcosis patients were found to have immunocompromised conditions, which is consistent with the results of our study. Fever and headache were more common in extrapulmonary cryptococcosis patients [36]. And headache is an independent risk factor for poor prognosis in AIDS patients with cryptococcosis [44]. However, no similar phenomenon was found in our study, which may be for different groups of people, and the sample size can be further increased in the future to strengthen the robustness of the results.

In our study, multivariate analysis showed that extrapulmonary infection was a risk factor for the prognosis of cryptococcosis, and CM accounted for 86.7% of extrapulmonary infection. Breaking through the blood-brain barrier (BBB) is the most critical step in the pathogenesis of CM, current studies have found that *Cryptococcus* may have three potential traversal pathways to cross the BBB: Transcellular traversal, Trojan horse dissemination and the paracellular pathway [45, 46], and in which the transcellular traversal is primarily main pathway crossing the BBB [45, 47–49]. CD44 expressed on the surface of human brain microvascular endothelial cells (HBMEC) binds to hyaluronic acid in *Cryptococcus*, causing *Cryptococcus* to attach to HBMEC [50–53]. It has been shown that the cytoplasmic tail of CD44 interacts with HBMEC cytoskeletal linker proteins, which accumulate actin at the site of infection and promote cytoskeletal rearrangement [50]. Moreover, they found that Rac1 and other GTP-bound small Rho family proteins can regulate host cell actin cytoskeleton [54, 55]. *Cryptococcus* Plb1 helps fungi cross the BBB by activating host cell Rac1 and its association with STAT3 [56]. Although the mechanism of *Cryptococcus* entry into HBMEC remains further exploration, these related studies are based on the adhesion of *Cryptococcus* to HBMEC via hyaluronic acid and CD44, indicating the importance of CD44 in the invasion of *Cryptococcus* into the BBB. CD44 overexpression has been found in many diseases with abnormal immune status, such as HIV infection, tumors, and autoimmune diseases etc. Overexpression of the CD44 receptor results in heightened sensitivity to hyaluronic acid binding, which in turn promotes the invasiveness

of *Cryptococcus* through the BBB [57–62]. This study reveals that the incidence of abnormal immune status was greater in the group with extrapulmonary infections, particularly in patients with CM, suggesting that abnormal immune status may contribute to central nervous system involvement.

There are several limitations of this study that must be acknowledged. Firstly, Cryptococcal disease was classified as “proven” based on the presence of consistent radiographic abnormalities and culture positivity obtained from sterile fluid or confirmed by biopsy. Unfortunately, the pulmonary puncture technique was not utilized during the early clinical period, which resulted in a missed diagnosis of pulmonary cryptococcal infection. Additionally, not all patients were tested for cryptococcal podococcal antigen due to doctors’ empirical consultations, which may have resulted in a missed diagnosis of peripheral blood cryptococcal infection. Secondly, this study involved a limited number of cases, and a large prospective study is needed to further confirm our findings. Finally, since this was a retrospective study, medical records were limited, and some clinical information, such as inflammatory indicators, were missing. However, this did not affect our analysis or final conclusions.

## Conclusions

In conclusion, patients with abnormal immune status, decreased lymphocytes and monocytes, and increased neutrophils and NLR are more likely to develop extrapulmonary infection, whether novel exposure or through reactivation of a previously acquired latent infection. CM has a high mortality rate compared with pulmonary cryptococcosis. Timely identification of high-risk groups of CM and preventive antifungal treatment can reduce the incidence of CM. By delving into mechanisms of cryptococcal action that disrupt the BBB, inhibit receptor-ligand binding, and impede the transmission of signaling pathways, as well as by synergizing with antifungal medications, the objective of preventing and treating CM can be attained. This is poised to markedly elevate the prognostic outlook of cryptococcosis and mitigate its mortality.

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## Author contributions

ZWT, QQP designed this study. YLS and QQP performed the data acquisition. ZWT, CYW and ZH performed data analysis. ZWT, YLS, SCZ and YJ edited the manuscript. YLW and XWZ supervised the present study. All authors read and approved the final manuscript.

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## Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by The Ethics Committee of The First Affiliated Hospital with Nanjing Medical University. With the approval of the Ethics Committee (protocol 2021-SR-566), informed consent was waived because of the retrospective nature of the study, and the analysis used anonymous clinical data. Clinical trial number: not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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