Comparisons of cryptococcal meningitis caused by Cryptococcus neoformans between HIV-negative patients with and without lung infection in two Chinese university hospitals

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Research article

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Abstract

Background Lung infection may cause many symptoms, such as fever and headache, that may be confused with cryptococcal meningitis (CM) symptoms. This study aimed to investigate the discrepancy in clinical features and outcomes of CM between HIV-negative patients with and without lung infection.

Methods We retrospectively reviewed the medical records of patients with CM admitted to two hospitals in Southwest China from 1 January 2014 to 31 December 2018.

Results A total of 71 patients was included during the 5 years, among which 35 (49.3%) patients had lung disease. CM occurred more frequently in male (62.9% vs. 44.4%, P=0.12) and young (≤30 years, 31.4% vs. 16.7%, P=0.30) patients with lung infection than in the patients without lung infection, with more fever (77.1% vs. 30.6%, P=0.001) and less central nervous system symptoms (5.7% vs. 16.7%, P=0.28) and vomiting (25.0% vs. 14.3%, P=0.26). In addition, patients with lung infection presented higher percentages of white blood cell (WBC) counts 20×106/L (45.7% vs. 22.2%, P=0.036) and lower percentages of ethmoid sinusitis, maxillary sinusitis, paranasal sinusitis, and otitis media than patients without lung infection (8.6% vs. 30.6%, P=0.02). The Cryptococcus neoformans isolates were sensitive to itraconazole, voriconazole, fluconazole, and amphotericin B but resistant to flucytosine. Patients with lung infection had higher mortality at discharge compared with patients without lung infection (8.6% vs. 0, P=0.12). Multivariable analyses showed that WBC counts 20×106/L was significantly associated with treatment outcome (OR=0.01, 95% CI=0-0.833, P=0.041).

Conclusions There were significant discrepancies in clinical features between CM patients with and without lung infection. Clinicians must consider the divergences in the diagnosis of CM in patients with lung infection.

Background

Cryptococcal meningitis (CM) caused by *Cryptococcus neoformans* is a common opportunistic infection, the main cause of mortality in human immunodeficiency virus (HIV)-infected patients, and increasingly observed among patients with non-HIV immunosuppression [1, 2]. Mortality outcomes for CM in HIV-negative individuals seem to be no better than those in HIV-positive patients [3–5]. Lung infection is a common disease that can be caused by many pathogens, such as *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Mycoplasma pneumonia,* and fungi; lung infection may cause many symptoms, such as fever and headache, that may be confused with central nervous system (CNS) infection. In immunosuppressed individuals, infection begins in the lung after inhalation of fungal spores and often spreads to other organs, particularly the brain [6]. Many CM patients also have concomitant lung involvement, which is often overlooked or misdiagnosed as tuberculosis [7].

The clinical characteristics and outcomes in CM have been shown to vary depending on the underlying condition [8–14]. The HBV-positive CM patients presented with lower initial complaints of visual

symptoms, lower cerebrospinal fluid (CSF) white blood cell (WBC) counts, lower percentages of the total protein in the CSF exceeding 0.45 g/L, higher glucose levels in the CSF, higher percentage of positive results for *Cryptococcus* culture in the CSF, more extraneural involvement sites, and a higher proportion of normal brain images compared with the HBV-negative CM patients [14]. Compared with the immunocompromised patients, CM was present in a younger population, with higher initial complaints of visual and auditory symptoms, higher CSF WBC counts, higher proportion of normal brain images in the immunocompetent patients [11]. In addition, the elderly patients (≥65 years) were more vulnerable to CM than adults aged <65 years, and had female predominance, higher rates of altered consciousness and recent cerebral infarction [15]. Therefore, HIV-negative patients with lung infection but with CM tend to present with atypical features, which can lead to significant delays in their diagnosis and poorer outcomes.

There are many HIV-negative CM patients with and without lung cryptococcosis in clinics, but few epidemiological study has been explored in this region.Here, we retrospectively reviewed the medical records of patients with CM who were admitted to two Chinese university hospitals in Southwest China in the past 5 years to investigate the discrepancies.

Methods

Study population and definition

Inclusion criteria were the following: (1) hospitalized at the First Affiliated Hospital of Army Medical University in Chongqing and the Affiliated Hospital of North Sichuan Medical College in Nanchong City of Sichuan Province, China, from January 2014 to December 2018; (2) a positive isolate of *Cryptococcus neoformans* from cerebrospinal fluid (CSF) culture; and (3) diagnosed with CM.

Patients with pneumonia and tuberculosis were classified as having lung infection. Sex, age, length of hospital stay, CSF profiles, brain images, underlying diseases, initial presentation, drug resistance of *Cryptococcus neoformans,* antifungal therapy and outcomes were recorded. The outcomes were classified as “cured”, “improved”, “other”, “untreated”, “invalid”, and “death” at discharge and further classified into satisfactory (cured or improved) and unsatisfactory (the others) outcomes in accordance with previous studies [11, 16]. (1) “Cured,” no reappearance of symptoms, and 2 sequential negative CSF cultures for C. neoformans with at least a 2-week interval; (2) “Improved,” no reappearance of symptoms, but without sequential negative CSF cultures for C. neoformans with at least a 2-week interval; (3) “Invalid”, the symptoms were not improved.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL) was used to analyze the data. Data are presented as the mean ± SD or median and range. Independent Student’s t-test and Mann-Whitney U test were used to compare

parametric and nonparametric continuous variables, respectively. Categorical variables were compared using the Chi-squared test or Fisher's exact test. Then the regression equations for predicting the probability of poor prognosis of CM were established. A P-value of <0.05 was considered statistically significant. All analyses were conducted as 2-sided tests.

Results

Demographic data

From January 2014 to December 2018, a total of 58 patients from Southwest Hospital were included during the 5 years, and 14 (24.1%) patients were HIV positive. A total of 40 patients from North Sichuan Medical College were included in the study, and 13 (32.5%) patients were HIV positive. Among the 71 non-HIV CM patients, 35 (49.3%) patients had lung disease.

Compared with that in the patients without lung infection, CM occurred more frequently in male (62.9% vs. 44.4%) and young (aged under 30 years, 31.4% vs. 16.7%) patients with lung infection. Among CM patients without lung infection, the common initial symptoms were headache (25/36, 69.4%), followed by fever (11/36, 30.6%), vomiting (9/36, 25.0%), dizziness (6/36, 16.7%), ventosity (6/36, 16.7%), progressive disturbance of consciousness (4/36, 11.1%), vague speech, memory deterioration, and walking lability (4/36, 11.1%). Among CM patients with lung infection, the common initial symptoms were fever (77.1%), headache (77.1%), dizziness (14.3%), vomiting (14.3%), progressive disturbance of consciousness (17.1%), edema of lower extremities (28.6%), and cough and expectoration (28.6%). Compared with CM patients without lung infection, CM patients with lung infection presented more fever (P□0.01), more edema of lower extremities and cough and expectoration (P□0.01), less vomiting (14.3% vs. 25.0%), less central nervous system symptoms and less ventosity (both 5.7% vs. 16.7%).

The common underlying diseases, factors or complications for CM patients without lung infection were decompensated hepatic cirrhosis of hepatitis B (11.1%), sepsis (11.1%), epilepsy (11.1%), hypertension (11.1%), intracranial infection (16.7%), and hydrocephalus (13.9%). The common underlying diseases, factors or complications for CM patients with lung infection were intracranial infection (17.1%), sepsis (14.3%), nephrotic syndrome (14.3%), systemic lupus erythematosus (17.1%), and type II diabetes mellitus (11.4%). There were no significant differences in underlying diseases, factors or complications between CM patients with and without lung infections (P□0.05) (table 1).

Laboratory data

The positive rates of the Pandy test of CSF in CM patients with and without lung infection were both high (77.1% vs. 72.2%), and the chloride ion and glucose levels were both decreased, while the total protein was increased in both. However, the percentage of CM patients with lung infection with WBC counts □20×10^6/L was higher than that of patients without lung infection (45.7% vs. 22.2%, P□0.05). The brain

images detected by CT or MRI showed that the percentages of ethmoid sinusitis, maxillary sinusitis, paranasal sinusitis, and otitis media in CM patients without lung function were higher than those in patients with lung infection (30.6% vs. 8.6%, P□0.05) (table 2).

Antifungal therapy and outcome

Most of the isolated *Cryptococcus neoformans* from CSF in patients with and without lung infection were sensitive to itraconazole, voriconazole, fluconazole, and amphotericin B but resistance to flucytosine (25.7% vs. 19.4%) (table 3). More CM patients with lung infection used fluconazole+amphotericin B than patients without lung infection (74.3% vs. 50.0%, P□0.05). The symptoms of CM of most patients with and without lung infection were improved at discharge (65.7% vs. 61.1%) (table 4).

Risk factors for poor treatment outcome of HIV negative CM

We included eight factors lung infection, sex, age, fever, WBC counts□20×10^6/L, chloride ion, protein, and glucose that may impacting the prognosis of CM patients in the regression. Multiple regression analysis showed that WBC counts □20×10^6/L was significantly associated with treatment outcome (OR = 0.01, 95% CI = 0–0.833, P = 0.041), and lung infection showed a tendency of association with treatment outcome (OR = 0.026, 95% CI = 0.001–1.173, P = 0.06) (table 5).

Discussion

It is generally known that CM is an opportunistic infection in HIV-positive patients, but it also occurs in HIV-negative patients. In a population-based study in the United States, the incidence of cryptococcosis among HIV-negative patients was close to half of the overall cases reported [8]. In the current study, 72.4% (71/98) of the patients were HIV negative, which may be because the patients were transferred to a professional hospital once HIV was discovered.

Lung infection affects many people in China, and our study showed a high proportion (49.3%) of CM patients with lung infection. The clinical characteristics of CM varied depending on the underlying conditions, such as virus infection, immune state, fungal species or lineage differences, and age [15, 17]. In the current studies, the overall sex and age in CM patients with and without lung infection were not significantly different; however, CM occurred more frequently in male and younger patients (aged ≤30 years) with lung infection than in patients without lung infection. In a United States series of over 300 HIV-negative patients with cryptococcal infection, half had CNS involvement, and of these, 24% had chronic liver, kidney or lung disease; 16% had a malignancy; and 15% had received a solid organ transplant [18]. However, in our data, among 71 HIV-negative patients with CM, half had lung infection, followed by intracranial infection (16.9%), systemic lupus erythematosus (12.7%), sepsis (12.7%), nephrotic syndrome (9.9%), type II diabetes mellitus (9.9%), hypertension (9.9%), epilepsy (8.5%), and

decompensated hepatic cirrhosis of hepatitis B (8.5%), which were different from the previous studies. Patients with CM presented with neurological symptoms, most typically headache and altered mental status, as well as with fever, nausea and vomiting. In the current studies, the main symptoms of patients were neurological symptoms, such as headache, followed by fever, vomiting, dizziness, ventosity, progressive disturbance of consciousness, vague speech, memory deterioration, and walking lability, and the symptoms in the two groups had no significant differences.

A large proportion of HIV-negative patients may have a marked systemic inflammatory response and hydrocephalus [17]. In our data, patients with lung infection presented with a higher proportion of fever, cough and expectoration, a lower proportion of noncentral nervous system symptoms, and a higher percentage of WBC counts in CSF ≥20×10^6/L than patients without lung infection, which may suggest a higher inflammatory response of the brain in patients with lung infection than in patients without lung infection. However, in the current studies, only 9.9% of patients had hydrocephalus. In addition, patients with lung infection had less vomiting, ventosity, ethmoid sinusitis, maxillary sinusitis, paranasal sinusitis, and otitis media than patients without lung infection, and 28.6% of patients had edema of lower extremities; clinicians should pay attention to this symptom.

In contrast to the rare resistance of *Cryptococcus neoformans* to flucytosine that showed a baseline fluconazole resistance rate of 12% in previous studies [19, 20], our results showed that *Cryptococcus neoformans* isolates from CSF were sensitive to fluconazole but resistant to flucytosine, with a resistance rate of 22.5%. Most of the recommendations for the management of non-HIV CM patients are extrapolated from HIV studies, among which the combination therapy of amphotericin B and flucytosine for the treatment of CM was the most commonly used in clinical trials [21, 22]. However, only 11.3% of patients were treated with amphotericin B + flucytosine in our data; most (62.0%) CM patients used fluconazole + amphotericin B due to the drug resistance rate of *Cryptococcus neoformans* to flucytosine. Outcomes for patients with HIV-associated cryptococcal meningitis in Africa suggested a 3-month mortality of 70% [17]. In prospective research studies, for patients treated with fluconazole, mortality at 10 weeks was 50–60% [23, 24]. In the current studies, 67.6% of patients had satisfactory results at discharge, which was consistent with previous studies. In HIV-negative individuals, altered mental status, markers of a poor inflammatory response, and low CSF white cell count have been linked with poor prognosis [25]. According to our results, the mortality of patients in the lung infection group was increased, which was consistent with the results showing a reduced CSF white cell count, furthermore, multivariable analyses showed that WBC counts ≥20×10^6/L was the risk factor of treatment outcome.

This study has some limitations. First, the study was performed in only two hospitals in Southwest China. Second, we only investigated the recent 5-year clinical records of CM patients, and the number of patients was relatively small. Third, this investigation is a retrospective study, some *Cryptococcus neoformans* isolates with unique genotype have higher virulence or azole-resistance, we could not provide genotype data of the related *Cryptococcus neoformans* isolates in the study, and we could not obtain the long-term outcome of patients. Further multicenter studies are needed to confirm our results and investigate more significant factors to improve the diagnosis and treatment of CM patients with lung infection.

Conclusions

In conclusion, we found that compared with the patients without lung infection, male and younger patients with lung infection presented with CM more frequently, with more fever, edema of lower extremities and cough and expectoration; less central nervous system symptoms, vomiting and ventosity; higher percentage of WBC counts ≥20×10^6/L; and lower percentage of ethmoid sinusitis, maxillary sinusitis, paranasal sinusitis, and otitis media. Clinicians must consider the divergences in the diagnosis of CM in patients with lung infection.

Abbreviations

CM: cryptococcal meningitis; CNS: central nervous system; CSF: cerebrospinal fluid; HIV: human immunodeficiency virus; WBC: white blood cell

Declarations

Acknowledgments

Not applicable.

Author Contributions

L. C. and P. X. conceived the study; M. Y., F. S., F. L., W. F., Q. Y., and Y. W. searched the data, M. Y. and L. C. performed the analyses, L. C. prepared the first manuscript draft, all authors reviewed and revised the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Army Medical University and was in compliance with the Declaration of Helsinki. No administrative permission were

required to access data.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Tables

Table 1 Demographic and clinical features in HIV-negative cryptococcal meningitis patients with and without lung diseases

Variables	Without lung infection (n=36)	With lung infection (n=35)	*P*-value
Sex			0.12
Male	16 (44.4%)	22 (62.9%)	
Female	20 (55.6%)	13 (37.1%)	
Age (years)			0.30
≤30	6 (16.7%)	11 (31.4%)	
31-60	22 (61.1%)	16 (45.7%)	
60	8 (22.2%)	8 (22.9%)	
Length of hospital stay (d)	32.7 (18.6, 46.7)	36.2 (22.8, 49.7)	0.49
Presenting symptoms and signs			
Fever	11 (30.6%)	27 (77.1%)	0.001
Headache	25 (69.4%)	27 (77.1%)	0.46
Dizziness	6 (16.7%)	5 (14.3%)	0.78
Vomiting	9 (25.0%)	5 (14.3%)	0.26
Vague speech, memory deterioration, and walking lability	4 (11.1%)	2 (5.7%)	0.70
Progressive disturbance of consciousness	4 (11.1%)	6 (17.1%)	0.70
No central nervous system symptoms	6 (16.7%)	2 (5.7%)	0.28
Ventosity	6 (16.7%)	2 (5.7%)	0.28
Edema of lower extremities	0	10 (28.6%)	0.001
Cough and expectoration	0	10 (28.6%)	0.001
Underlying diseases, factors or complications			
Decompensated hepatic cirrhosis of hepatitis B	4 (11.1%)	2 (5.7%)	0.70
Severe chronic hepatitis B	2 (5.6%)	0	0.49
Sepsis	4 (11.1%)	5 (14.3%)	0.96
Epilepsy	4 (11.1%)	2 (5.7%)	0.70
Hypertension	4 (11.1%)	3 (8.6%)	1.00
Upper gastrointestinal bleeding	2 (5.6%)	0	0.49
Hypokalemia	3 (8.3%)	2 (5.7%)	1.00
Intracranial infection	6 (16.7%)	6 (17.1%)	0.96
Nephrotic syndrome	2 (5.6%)	5 (14.3%)	0.40
Systemic lupus erythematosus	3 (8.3%)	6 (17.1%)	0.45
Chronic transplantation kidney disease	2 (5.6%)	0	0.49
Cerebral infarction	3 (8.3%)	3 (8.6%)	1.00
Ventriculoperitoneal shunt	3 (8.3%)	2 (5.7%)	1.00
Hypoproteinemia	2 (5.6%)	3 (8.6%)	0.97
Myasthenia gravis	2 (5.6%)	0	0.49
Rheumatic arthritis	2 (5.6%)	0	0.49
Type II diabetes mellitus	3 (8.3%)	4 (11.4%)	0.97
Thrombocytopenic purpura	2 (5.6%)	0	0.49
Herpes zoster	2 (5.6%)	0	0.49
Gallbladder carcinoma with liver metastasis	0	2 (5.7%)	0.46
Non-Hodgkin's lymphoma	1 (2.8%)	0	1.00

Table 2 Cerebrospinal fluid characteristics and brain images of patients with CM in HIV-negative cryptococcal meningitis patients with and without lung diseases

Variables	Without lung infection (n=36)	With lung infection (n=35)	*P*-value
Cerebrospinal fluid profiles			
Pandy test (positive)	26 (72.2%)	27 (77.1%)	0.63
WBC counts ($\times 10^9$/L)	0.12 (0.05, 0.18)	0.105 (-0.001, 0.211)	0.30
≥20×10^6/L	8 (22.2%)	16 (45.7%)	0.036
Chloride ion (120-130 mmol/L)	115.6±5.5	118.1±5.4	0.19
Protein (0.15-0.45 g/L)	1.17 (0.55, 1.78)	1.04 (0.39, 1.69)	0.71
≥0.45 g/L	28 (77.8%)	22 (62.9%)	0.17
Glucose (2.5-4.5 mmol/L)	1.74±1.09	1.72±1.27	0.66
≤2.5 mmol/L	30 (83.3%)	28 (80.0%)	0.72
Adenylate deaminase (0-15 U/L)	2.31(1.34, 3.28)	2.18(1.58, 2.78)	0.63
Brain images (CT/MRI)			
Cerebral ischemia/infarction	16 (44.4%)	14 (40.0%)	0.71
Meningeal enhancement	3 (8.3%)	0	0.25
Hydrocephalus	5 (13.9%)	2 (5.7%)	0.45
Ethmoid sinusitis, maxillary sinusitis, paranasal sinusitis, otitis media	11 (30.6%)	3 (8.6)	0.02
Normal	8 (22.2%)	7 (20.0%)	0.82

WBC, white blood cell

Table 3 Drug resistance of *Cryptococcus neoformans*

Antifungal agent	Without lung infection (n=36)				With lung infection (n=35)				*P*-value
	Sensitive	Intermediary	Resistance	Not available	Sensitive	Intermediary	Resistance	Not available	
Itraconazole	11 (30.6%)	1 (2.8%)	0	24 (66.7%)	12 (34.3%)	0	0	23 (65.7%)	0.59
Voriconazole	12 (33.3%)	0	0	24 (66.7%)	12 (34.3%)	0	0	23 (65.7%)	0.93
Fluconazole	11 (30.6%)	1 (2.8%)	0	24 (66.7%)	14 (40.0%)	0	0	21 (60.0%)	0.46
flucytosine	4 (11.1%)	3 (8.3%)	7 (19.4%)	22 (61.1%)	5 (14.3%)	0	9 (25.7%)	21 (60.0%)	0.34
Amphotericin B	12 (33.3%)	0	0	24 (66.7%)	11 (31.4%)	0	0	24 (68.6%)	0.86

Table 4 Antifungal therapy and outcome

Treatment	Without lung infection (n=36)	With lung infection (n=35)	*P*-value
Fluconazole alone	4 (11.1%)	4 (11.4%)	1.00
Amphotericin B alone	10 (27.8%)	4 (11.4%)	0.08
Fluconazole+amphotericin B	18 (50.0%)	26 (74.3%)	0.035
Amphotericin B+flucytosine	6 (16.7%)	2 (5.7%)	0.28
Fluconazole+flucytosine	1 (2.8%)	0	1.00
Did not receive antifungal therapy	12 (33.3%)	10 (28.6%)	0.66
Outcome			
Improved	19 (52.8%)	21 (60.0%)	0.54
Cured	3 (8.3%)	2 (5.7%)	1.00
Invalid	6 (16.7%)	4 (11.4%)	0.77
Others	8 (22.2%)	5 (14.3%)	0.39
Death	0	3 (8.6%)	0.12

Table 5 Risk factors associated with poor prognosis of HIV negative cryptococcal meningitis (CM)

Variables	Odds ratio	95% CI	*P*-value
Lung infection	0.026	0.001-1.173	0.06
Male	4.939	0.368-66.343	0.228
Age	0.922	0.841-1.01	0.081
Fever	3.663	0.26-51.674	0.336
WBC counts≥20×10^6/L	0.01	0-0.833	0.041
Chloride ion	1.266	0.888-1.806	0.193
Protein	0.946	0.345-2.593	0.915
Glucose	0.395	0.096-1.633	0.2

WBC, white blood cell