Comparison of Clinical Features in Patients with Persistent and Nonpersistent Cryptococcal Meningitis: Twelve Years of Clinical Experience in Four Centers in China

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SUMMARY

Background and Aims: Cryptococcal meningitis (CM) has gradually increased in the recent 20 years in the whole world. Although the mortality decreased significantly in recent years, it was still high, especially in patients with persistent infection. Therefore, we compare differences of clinical features between persistent and nonpersistent CM patients.

Methods: We conducted a retrospective review of medical records of patients diagnosed with CM from January 2000 to December 2011 in four centers in China, including demographic features, underlying diseases, clinical presentations, laboratory data, and so on.

Results: Of 106 CM patients enrolled, 16 were identified as persistent cases. Among all variables, persistent CM patients were more like to be human immunodeficiency viruses (HIV) infection ($P < 0.05$), stiff neck ($P < 0.01$), a serum hemoglobin < 90 g/L ($P < 0.01$), a serum potassium concentration < 2.7 mg/L ($P < 0.01$), an intracranial pressure (ICP) > 400 mmH₂O ($P < 0.01$), and a latex agglutination cryptococcal antigen titer of cerebrospinal fluid (CSF LACT) $> 1:1024$ ($P < 0.01$) than nonpersistent ones. A multivariate analysis showed that HIV infection (OR 7.49), stiff neck (OR 11.7), a serum potassium < 2.7 mmol/L (OR 9.45), and an ICP > 400 mmH₂O (OR 6.83) were closely correlated with persistent CM.

Conclusions: Although it is difficult to deal with persistent CM nowadays, some cases could be predicted early enough in the future, so as to be treated appropriately and have relatively good outcomes.

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Introduction

Cryptococcosis, a potentially fatal infectious disease thought rare in the past, has gradually increased in the recent 20 years in the whole world, especially in China [1,2]. It is mainly caused by *Cryptococcus (C.) neoformans* and *C. gattii*, a kind of encapsulated yeast-like organisms. *C. neoformans* can be isolated from the environment of almost all areas of the world, while *C. gattii* can be isolated mostly from Eucalyptus trees. Cryptococcal meningitis (CM) is the most serious and deadly form of cryptococcosis. It is said that most cases of CM were caused by *C. neoformans* var. *grubii* [1]. This fungus can infect apparently normal hosts but much more frequently and with greater severity when it causes diseases in immunocompromised individuals, including patients with human immunodeficiency virus (HIV) infection, corticosteroids using, organ transplantations, chronic leukemia, lymphoma, rheumatic diseases, and diabetes mellitus [1,3–5]. Although the mortality decreased significantly in recent years, with the combination therapy of effective antifungal drugs such as amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole [6,7], it was still high, especially in patients with persistent CM.

The persistent infection in clinic may come from one or both of two aspects, that is, strains of *C. spp* and/or the hosts. Differences in characteristics between strains of *C.* isolated from persistent and nonpersistent infection patients were still under research, but our previous experiments demonstrated that strains of *C.* (whether *C. neoformans* or *C. gattii*) isolated from persistent infection patients showed no significant difference in susceptibility *in vivo* from those from nonpersistent infection patients (results not yet published). So, we hypothesize that besides characteristics of *C.* strains themselves, there might be of differences somewhere between these patients, and the characteristics and clinical features of patients with persistent infection might be different from those of patients with nonpersistent infection. In this retrospective study, we aimed to compare the demographic features, underlying diseases, clinical manifestations, laboratory data, radiographic findings, and outcomes between persistent and nonpersistent CM patients, who were admitted to four tertiary care hospitals from 2000 to 2011 in China.

Patients and Methods

Patients and Definitions

Cases of CM were identified from hospitalized patients of four tertiary hospitals, which were Changzheng hospital (Shanghai), Changhai hospital (Shanghai), Xijing hospital (Xi'an, Shaanxi Province), and Tangdu hospital (Xi'an, Shaanxi Province), from January 2000 to December 2011, no matter with or without a systemic infection. Patients were excluded if necessary information of their medical records were incomplete or unavailable, or their culture result showed negative during the course of hospitalization. The patients who died or were lost to follow up within 4 weeks after the starting of treatment were also excluded. All enrolled patients were divided into persistent CM group and nonpersistent CM group.

CM was defined on clinical manifestations and laboratory assessment including a positive mycological culture for *C. spp* or

positive findings of India ink stain together with cryptococcal antigen test of cerebrospinal fluid (CSF). Persistent CM was defined as a persistently positive cryptococcal culture of CSF after 4 weeks of standard antifungal treatment [7].

Database Reviewed

Database reviewed in this study was medical records including the admission history and physical examination results, progress notes, discharge summary, antifungal regimens, reports of computed tomography (CT), and magnetic resonance imaging (MRI) of head, and all laboratory data including microbiology reports. Both demographic data (including gender, age, residence, and job) and admission history (including dates of admission and discharge, diagnostic procedures, outcome of present hospitalization, and underlying diseases) were obtained. Initial symptoms and physical examinations were reviewed for the presence of fever, chills, headache, cough, chest pain, nausea, vomiting, seizure, apopsychia, dyspnea, stiff neck, visual and hearing disturbances, mental status, focal neurological deficits, meningismus, and so on.

Microbiology reports included the results of India ink stain and mycological cultures of CSF, and latex agglutination cryptococcal antigen titer (LACT) of CSF and serum were also collected, if any. All specimens for fungal culture were inoculated onto Sabouraud dextrose agar (SDA) containing chloromycetin and incubated at 30°C and were examined daily for 4 weeks.

Normal brain images on CT or MRI were defined as no obvious intracranial lesions demonstrated. Neuroimage findings included meningeal enhancement, cerebral edema, hydrocephalus, abscess, cerebral infarction, and other presentations such as encephalitis and hemorrhage.

Statistical Analysis

Data were presented as mean $\pm$ standard deviation (SD) or median with interquartile ranges. The standard two-sample *t*-test or rank sum test was used to test differences for numerical variable, while differences in proportions were tested using a Chi-squared test or Fisher's exact test if the expected values were below 10 in all collected variables. Risk factors for all-cause persistent CM were identified using a logistic regression model. All parameters were initially tested by univariate analysis, and those with a *P*-value < 0.05 were used for multivariate analysis. Statistical analysis was performed using SPSS 13.0 for Windows (The Second Military Medical University, Shanghai, China). A *P*-value < 0.05 was considered statistically significant. All tests were two-tailed.

Results

Patient Characteristics

From January 2000 to December 2011, a total of 324 CM patients were identified in the four centers, and 106 were enrolled in this retrospective study accordingly. Of these patients from 20 provinces of China, 16 (15.1%) were identified as persistent CM. There was no significant difference between persistent CM and nonpersistent CM patients in gender, age, residence, and job. By the way,

the highest age-specific rate was seen in people aged 21–60 years in both groups, as 68.8 and 78.9%, respectively.

Among nonpersistent CM patients, the most frequently underlying diseases and factors were corticosteroid medication (32.2%), hepatobiliary diseases (17.8%), renal diseases (10.0%), systemic lupus erythematosus (SLE; 10.0%), HIV infection (7.8%), and hypertension (7.8%; Figure 1). Fewer kinds of underlying diseases and factors were associated with persistent CM patients, and they were more likely to be of HIV infection as compared with nonpersistent CM patients, with 31.3% versus 7.8% ($P = 0.021$; Figure 1). And there were more than forty percents of patients without any apparent underlying disease and factors in each group (Figure 1), and 44/94 (46.8%, data not shown) in all patients with non-HIV infected.

The most commonly complained initial symptoms were headache (75/106, 70.8%), followed by fever (69/106, 65.1%), vomiting (54/106, 52.8%), and stiff neck (53/106, 50.0%; Figure 2).

Figure 1 Univariate comparison of underlying diseases and factors between persistent and nonpersistent cryptococcal meningitis (CM) patients. Underlying diseases and factors are ranked by the frequency for nonpersistent CM patients. Statistically significant differences between the two groups are displayed with a P -value in the figure. HIV, human immunodeficiency virus; SLE, systemic lupus erythematosus.

Other symptoms such as cough, chest pain, dyspnea, seizure, apoplechia, altered mental status, abnormal vision, and hearing were complained too. There was no significant difference in initial presentations between the two groups, except stiff neck, which was more frequent in persistent CM group, as 87.5% versus 43.3% ($P = 0.001$; Figure 2).

Diagnosis, Treatment, and Outcome

CM was diagnosed by clinical manifestations and laboratory assessment including a positive mycological culture for *C. spp* or positive findings of India ink stain together with cryptococcal antigen test in CSF. India ink stain of CSF was carried out in 83 cases, and the positive rates in persistent CM and nonpersistent CM groups were 75.0% (9/12) and 64.8% (46/71), respectively, with no difference between the two groups. All enrolled patients were treated with intravenous amphotericin B (or amphotericin B liposomes)-based therapy or voriconazole-based regime for 8–12 weeks initially, no matter with or without the use of flucytosine and fluconazole, followed by oral fluconazole or itraconazole for more than 6 months as maintenance therapy. And intrathecal amphotericin B was used for those with intracranial pressure (ICP) >400 mmH₂O. Only 6.3% and 13.3% of the patients were diagnosed in <1 week from the initial onset of symptoms in persistent CM and nonpersistent CM groups, respectively. And the proportion of patients with a duration of more than 1 month was higher in the former group than the latter one (43.8% vs. 16.7%, $P = 0.033$). About 92.2% of nonpersistent CM patients were hospitalized for <12 weeks, and none of them were hospitalized for more than 16 weeks, while 56.3% of persistent CM patients were hospitalized for more than 16 weeks ($P < 0.000$). As to the in-hospital mortality, there was no difference between the two groups, with 12.5 and 5.6%, respectively.

Laboratory Data and Radiological Findings

The results of laboratory data and radiological findings are shown in Table 1. Although the median CSF white blood cell count (WBC) was 13 cells/mm³ in persistent CM patients, which seemed lower than that of nonpersistent CM group (34 cells/mm³), there was no statistically significant difference between the two groups. There was also no statistical difference in CSF total protein, glucose, and chloride level between the two groups. Although the mean ICP in persistent CM group was 343 ± 116 mmH₂O, which showed no difference with that in nonpersistent CM group (265 ± 138 mmH₂O), the proportion of patients with ICP >400 mmH₂O was higher than that in the latter group (81.3% vs. 26.7%, $P = 0.000$). CSF samples for cryptococcal antigen test were collected from all 106 patients, while serum cryptococcal antigen test were not carried out in all patients. A higher proportion of the patients with CSF LACT >1:1024 in the former group was demonstrated with 68.8% than in the latter one with 21.1% ($P = 0.000$).

There was no difference between the two groups in serum WBC, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sodium levels. While serum hemoglobin (Hb) level was lower in persistent CM group than that in nonpersistent CM

Figure 2 Univariate comparison of initial symptoms between persistent and nonpersistent cryptococcal meningitis (CM) patients. Initial symptoms are ranked by the frequency for nonpersistent CM patients. Statistically significant differences between the two groups are displayed with a *P*-value in the figure.

group, as 93.5 ± 23.2 g/L versus 112 ± 17.9 g/L ($P=0.045$), the number of patients with Hb level below 90 g/L in the two groups was 8/16 (50.0%) and 8/90 (8.9%), respectively ($P=0.000$). Serum potassium level, also presented lower in the former group than in the latter, as 2.7 ± 0.9 mmol/L versus 3.1 ± 1.0 mmol/L ($P=0.037$) and the proportion of patients with potassium level <2.7 mmol/L in the two groups were 7/16 (43.8%) and 9/90 (10.0%), respectively ($P=0.002$).

Brain images in either CT or MRI were performed in 80 patients, 15 of persistent CM, and 65 of nonpersistent CM. There was no difference between the two groups in various kinds of brain images. The most common manifestation was meningeal enhancement both in persistent CM group and nonpersistent CM group, with incidence rate of 7/15 (46.7%) and 26/65 (40.0%), respectively, followed by cerebral edema (22/80, 27.5%), hydrocephalus (14/80, 17.5%), and cerebral infarction (9/80, 11.3%). Seven (8.8%) patients had other presentations such as encephalitis and hemorrhage. Even, 2/15 (13.3%) patients in persistent CM group and 13/65 (20.0%) in nonpersistent CM group showed normal image.

Risk Factors Associated with Persistent CM

The multivariate analysis demonstrated that among these significant differential factors listed above, HIV infection (OR 7.49, 95% CI: 1.24–45.3, $P=0.028$), stiff neck (OR 11.7, 95% CI: 1.24–110, $P=0.032$), a potassium <2.7 mmol/L (OR 9.45, 95% CI: 1.77–

50.5, $P=0.009$), and an ICP >400 mmH₂O (OR 6.83, 95% CI: 1.40–33.3, $P=0.018$) were independent risk factors for persistent CM, as shown in Table 2.

Discussion

Although CM is often described as an opportunistic infection in immunocompromised patients [8–11], it is also known to affect apparently healthy individuals. In a series of 306 HIV-negative patients with cryptococcosis, the predisposing conditions were glucocorticosteroid (28%), chronic organ failure (liver or kidney; 18%), solid-organ transplant (18%), malignancy (18%), rheumatological diseases (13%), and chronic lung disease (12%) [4]. Chen *et al.* [5] reviewed 3698 cryptococcosis cases that had clear description of the underlying diseases reported in China between 1985 and 2010 and found that the most common underlying disease was HIV infection (16%), followed by tuberculosis (14%), liver disease (12%), SLE (9%), and DM (6%), and 17% patients occurred without underlying diseases. Of the 106 patients identified as CM in this study, corticosteroid medication, hepatobiliary diseases, renal diseases, SLE, HIV infection, and hypertension occupied the top six underlying diseases and factors. Among these diseases and factors, there was no significant difference between persistent CM group and nonpersistent CM group, except HIV infection, which was higher in the former than the latter group and might be a predictor of persistent CM. Unlike the study of Lee *et al.* [12], the present study showed that the number of patients without any identified

Table 1 Laboratory data and radiographic findings in patients with persistent and nonpersistent cryptococcal meningitis (CM)

Variables	Persistent CM N = 16	Nonpersistent CM N = 90	P-value
CSF	16	90	
WBC, median (range), cells/mm ³	13 (0–240)	34 (0–400)	0.471
<20	8 (50.0%)	32 (35.6%)	0.187
Total protein, median (range), mg/L	485 (278–978)	396 (259–775)	0.573
>600	5 (31.3%)	16 (17.8%)	0.061
Glucose, median (range), mmol/L	4.4 (2.1–5.6)	4.7 (3.0–6.1)	0.575
<4.0	6 (37.5%)	31 (34.4%)	0.842
Chloride (mean ± SD), mmol/L	127 ± 43.8	123 ± 31.6	0.929
<115.0	4 (25.0%)	18 (20.0%)	0.978
ICP (mean ± SD), mmH ₂ O	343 ± 116	265 ± 138	0.124
>400	13 (81.3%)	24 (26.7%)	0.000
Positive India ink	9/12 (75.0%)	46/71 (64.8%)	0.780
LACT >1:1024	11 (68.8%)	19 (21.1%)	0.000
Serum	16	90	
WBC (mean ± SD)/μL	7037 ± 668	6374 ± 787	0.656
>10,000	5 (31.3%)	28 (31.1%)	0.957
Hb (mean ± SD), g/L	93.5 ± 23.2	112 ± 17.9	0.045
<90	8 (50.0%)	8 (8.9%)	0.000
ALT (mean ± SD), U/L	37.5 ± 21.5	40.4 ± 17.5	0.345
AST (mean ± SD), U/L	45.7 ± 17.2	39.9 ± 15.2	0.444
Sodium (mean ± SD), mmol/L	136 ± 7.0	134 ± 8.1	0.974
Potassium (mean ± SD), mmol/L	2.7 ± 0.9	3.1 ± 1.0	0.037
<2.7	7 (43.8%)	9 (10.0%)	0.002
Brain images (CT or MRI)	15	65	
Meningeal enhancement	7 (46.7%)	26 (40.0%)	0.762
Cerebral edema	5 (33.3%)	17 (26.2%)	0.157
Hydrocephalus	4 (26.7%)	10 (15.4%)	0.509
Cerebral infarction	2 (13.3%)	7 (10.8%)	0.678
Abscess	1 (6.7%)	2 (3.1%)	0.998
Others*	2 (13.3%)	5 (7.7%)	0.849
Normal	2 (13.3%)	13 (20.0%)	0.819

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSF, cerebrospinal fluid; CT, computed tomography; Hb, hemoglobin; ICP, intracranial pressure; LACT, latex agglutination cryptococcal antigen titer; MRI, magnetic resonance imaging; SD, standard deviation; WBC, white blood cell count. Variables that are statistically discernibly different at the 5% level are displayed in bold font. *Others included presentations such as encephalitis and hemorrhage.

underlying disease accounted for 41.5% of all patients and 46.8% of non-HIV-infected ones, close to the result in the report of Zhu *et al.* [13], which was 66.9% of 154 non-HIV-infected patients.

Many cases were often misdiagnosed or delayed in diagnosis and treatment because of lack of specificity of clinical manifestations. In a statistical report of 7315 cases by Chen *et al.* [5], headache (87%), fever (74%), meningeal irritation (67%), and

Table 2 Factors associated with persistent cryptococcal meningitis (CM)

Variables	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P	Odds ratio	95% CI	P
HIV infection	5.39	1.46–19.9	0.021	7.49	1.24–45.3	0.028
Stiff neck	9.15	1.96–42.7	0.001	11.7	1.24–110	0.032
>1 month for diagnosis	3.89	1.25–12.1	0.033			
Hb <90 g/L	10.3	3.03–34.7	0.000			
Potassium <2.7 mmol/L	7.00	2.10–23.3	0.002	9.45	1.77–50.5	0.009
ICP >400 mmH ₂ O	11.9	3.12–45.5	0.000	6.83	1.40–33.3	0.018
LACT >1:1024	8.22	2.55–26.5	0.000			

Hb, hemoglobin; HIV, human immunodeficiency virus; ICP, intracranial pressure; LACT, latex agglutination cryptococcal antigen titer.

vomiting (61%) were the most common clinical manifestations of CM, while mental status was abnormal in only 26% of the cases. They usually had no pathognomonic brain image of CM in either CT or MRI scans, but might show normal or meningeal enhancement, single or multiple nodules, cerebral edema, or hydrocephalus instead [7,14]. In the present study, headache, fever, vomiting, and stiff neck were the most commonly complained initial presentations out of various symptoms, which was partly consistent to the earlier reports [1,5,12,14]. Meanwhile, the most common presentations of brain images in CT or MRI scan were meningeal enhancement, cerebral edema, hydrocephalus, and cerebral infarction, and 15 patients had normal image findings, which was also consistent with the previous reports [7,14]. Among all the presentations, only stiff neck occurred more frequently in persistent CM patients than in nonpersistent CM ones. Only 6.3% and 13.3% of the patients were diagnosed with CM in <1 week from the initial onset of symptoms in persistent CM and nonpersistent CM groups, respectively. And the proportion of those with the duration of more than 1 month were higher in the former group than in the latter one, indicating that a delayed diagnosis and subsequent treatment might be associated with persistent infection later. And obviously, a state of persistent infection resulted in a longer hospitalization. About 81.3% of patients in persistent CM group were hospitalized for more than 9 weeks and 56.3% for more than 16 weeks, while the corresponding proportion in nonpersistent CM group were 37.8 and 0%, respectively.

Lee *et al.* [12] listed seven poor prognostic factors for CM reported by prior studies, which included (1) cryptococcus growth from sites other than CSF, (2) alteration in mental status, (3) elevated opening pressure >200 mmH₂O, (4) CSF glucose <40 mg/dL, (5) CSF cell count <20 cells/mm³, (6) high CSF LACT, and (7) positive India ink smear. No publications have ever indicated any associated factor for persistent CM till now. In our study, a multivariate analysis showed that HIV infection, stiff neck, a serum potassium <2.7 mmol/L, and an ICP >400 mmH₂O were closely correlated with persistent CM.

It was said that CM patients with HIV usually had higher LACT of CSF than patients without HIV [15], and LACT appeared to

have both diagnostic and prognostic value [16–18]. In this study, CSF LACT were collected from all of the patients, and the proportion of patients with CSF LACT of more than 1:1024 in the persistent CM group was higher than that of nonpersistent CM group. And 11 of 12 patients with HIV infection presented CSF LACT of more than 1:1024 in either group, which was consistent with previous reports. Besides, 6/11 (54.6%) of patients without HIV infection in the former group presented CSF LACT of more than 1:1024, while 12/83 (14.5%) in the latter one, indicating that a high CSF LACT of more than 1:1024 might be closely related to persistent CM. But a multivariate analysis did not support the presumption. Maybe it was really not related to persistent CM, or maybe it was a misunderstanding due to the smaller number of cases or the statistical distribution bias, which needed more cases to confirm in the future.

There was no relevant literature showing that abnormal serum potassium level was correlated with the susceptibility to cryptococcosis and its mortality, but it was clear that the use of amphotericin B or amphotericin B liposomes did cause hypokalemia in most of the cryptococcal patients [19,20]. Herein, patients in the persistent CM group had a lower serum potassium level than those in the nonpersistent CM group, and the proportion of patients with serum potassium level <2.7 mmol/L was higher in the former group than in the latter group, which might be closely related to the use of amphotericin B and amphotericin B liposomes. Although it was not clearly known why these patients had more severe hypokalemia, serious hypokalemia might lead to serious disorders of electrolyte balance in the host, which was likely to pull the trigger of other balances and mechanisms, adding to the emergence of persistent infection.

Although Ganiem et al. [21] reported that 1-month mortality and death during extended follow-up were strongly associated with HIV infection in adult CM in Indonesia, some more studies [12,22,23] showed that HIV infection did not play a role in mortality within a certain period mainly due to the management of repeated lumbar puncture to relieve increased ICP. In the present study, those patients with HIV infection usually had an ICP higher than 400 mmH₂O, which was difficult to be relieved even though the patients received repeated lumbar punctures and intrathecal amphotericin B. Likewise, some HIV-negative patients also experienced similar process. Meanwhile, a state of stiff neck also was the reflection of a high ICP and severe infection in CSF. And, most of those patients, in fact, were persistent CM patients.

Usually, the outcome of persistent CM patients was worse than that of nonpersistent CM ones, including death. But the existing retrospective records in this study showed that persistent infection had no direct correlation with high in-hospital mortality. We believed that it was a false appearance indeed. Some inpatients in critical condition were automatically discharged because of some special reasons (such as the economy, the folk custom, etc.) in

China and thus were lost to follow-up. As a result, most of these patients died soon after discharge, resulting in the existing data biased.

As our study was a retrospective research using secondary data source, that is, patients' medical records, there were limitations in this study such as the absence of serum LACT records, the lack of genotyping of *C.*, the absence of information about susceptibility testing, inconsistency in testing schedule, and the false appearance of in-hospital mortality in this study. These limitations of missing data and misclassification are quite common in this kind of study. But on the other side, these factors would sometimes lead to more conservative and credible results.

In summary, this study, scilicet twelve years of clinical experience in the four centers in China, showed that persistent CM patients were more likely to be of HIV infection, more likely to have stiff neck, an ICP >400 mmH₂O, a CSF LACT >1:1024, a serum Hb level <90 g/L, a serum potassium level <2.7 mmol/L, and more likely to have a duration of more than 1 month from initial symptoms to diagnosis than those of nonpersistent CM. And a multivariate analysis showed that only HIV infection, stiff neck, a serum potassium <2.7 mmol/L, and an ICP >400 mmH₂O were independent risk factors for persistent CM. Could these clinical features, besides characteristics of *C.* strains, predict persistent CM effectively and exactly? Retrospective and prospective studies in more cases are needed to confirm it in the future. Although it is still very difficult to deal with persistent CM nowadays, some cases could be predicted early enough in the future, so as to be treated appropriately and have relatively good outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

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