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Failure of Early Mycological Clearance in HIV-Negative Cryptococcal Meningitis

Zhihui Su,²,³ Chongliang Luo,²,³ Kai Dai,²,³ Dasen Yuan,¹ Bang-e Qin,¹ Meifeng Gu,¹ Junyu Liu,¹ Yong Chen,³ Fuhua Peng,¹,² and Ying Jiang¹,³

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Background. Negative cerebrospinal fluid (CSF) cultures at 2 weeks after antifungal treatment (early mycological clearance [EMC]) should be a treatment goal of cryptococcal meningitis (CM). However, EMC in human immunodeficiency virus (HIV)–negative patients with CM is poorly understood.

Methods. We conducted a retrospective review of medical records and 1-year follow-up of 141 HIV-negative patients with CM with an initial positive CSF culture for Cryptococcus neoformans. Multivariate logistic regression was performed to analyze clinical features and laboratory and CSF findings of patients with CM with different EMC statuses. Random forest models were used to predict failure of EMC. All-cause mortality and clinical functional status were analyzed.

Results. Of 141 patients, 28 (19.9%) had EMC failure. The 1-year mortality rate was 5.7% (8/141). Multivariate analysis showed that non–amphotericin B (AmB)–based regimens, baseline log_{10} Cryptococcus count/mL, baseline CSF opening pressure (CSF-OP) >30 cm H₂O, and baseline serum creatinine were significantly associated with EMC failure. A parsimonious predictive rule given by the decision tree identified patients with CM with non-AmB-based therapy and baseline CSF-OP >30 cm H₂O as being at high risk of EMC failure. Incidence of all-cause mortality, the follow-up modified Rankin Scale, and Karnofsky performance status scores were not significantly related to EMC.

Conclusions. EMC failure in HIV-negative CM is attributed to non-AmB-based therapy and is associated with log_{10} Cryptococcus count/mL and CSF-OP >30 cm H₂O at baseline. Because of the small number of deaths, we are not able to comment on whether or not EMC is associated with mortality.

Keywords. amphotericin B; cryptococcal meningitis; early mycological clearance; HIV-negative; mortality.
patients treated with either FLU or VOR combined with AmB was not statistically different from that observed in patients treated with AmB plus 5-FC [12]. A recent study showed that the differences in CSF culture sterility at 2 weeks and mortality between the VOR + 5-FC group and the AmB + 5-FC group were not statistically significant [13]. However, the effect on EMC status by different regimen combinations based on AmB or non-AmB therapy for induction therapy in HIV-negative patients with CM is still unclear. To address this gap, we sought to investigate EMC status after antifungal treatment and explore factors associated with EMC status in HIV-negative patients with CM.

METHODS

Study Design
We analyzed data from 312 Chinese Han HIV-negative patients with CM who underwent fungal culture of CSF at the initial examination between January 2010 and December 2020 at the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Of these, 171 (54.8%) who had an initial positive CSF culture for C neoformans were enrolled, 20 lacked the clinical data or reports from the mycology laboratory at follow-up, 3 died prior to day 14 (their time to death was day 2, day 9, and day 12 from admission, respectively), and 7 did not have a lumbar puncture at 14 ± 2 days despite surviving to day 14. The remaining 141 (82.5%) survived to the end of 2 weeks of antifungal therapy and were followed up for 1 year. The flowchart of patient enrollment is shown in Figure 1.

Demographic characteristics, clinical symptoms, comorbidities, blood and CSF findings, modified Rankin Scale (mRS) scores, Karnofsky performance status (KPS) scores, the events of postinfectious inflammatory response syndrome (PIIRS)/immune reconstitution inflammatory syndrome (IRIS)–like reconstitution syndrome, and treatments were recorded (Tables 1 and 2). PIIRS is defined in previously healthy patients with CM, paradoxically, as the presence of clinical deterioration during effective antifungal treatment due to an exuberant immune response; clinical manifestations include worsening or relapse of clinical symptoms and/or new magnetic resonance imaging, and continued negative fungal cultures [14]. Unlike PIIRS, which occurs in previously healthy patients, IRIS-like reconstitution syndrome occurs in patients who have received immunosuppressive therapy before bone marrow transplantation and other disease conditions or chemotherapy for malignancies. When immunosuppressive medications are reduced, the immune response is enhanced, which could result in the development of IRIS-like reconstitution syndrome [14, 15]. Altered mental status was defined as a Glasgow Coma Scale score of <15 [16].

Mycology Definitions
CSF clearance of cryptococci was defined as the initial positive CSF culture turning to negative for 2 consecutive CSF cultures after starting antifungal treatment. Failure of EMC was defined as having a positive cryptococcal culture in CSF sample 2 weeks after starting antifungal therapy [10]. Persistent infection was defined as a persistently positive cryptococcal culture in CSF sample 4 weeks after starting antifungal therapy [6]. Microbiological relapse was defined as recurrence of symptoms, with recovery of viable organisms from previously sterile CSF [6]. Cryptococcal cultures were considered negative if C neoformans was not isolated up to 5 weeks after incubation.

Treatment Strategies
Our treatment strategy in this study was based on 2010 Infectious Diseases Society of America guidelines [6] and the Chinese expert consensus [17]. The dose of AmB used was 0.5–0.7 mg/kg/day for induction therapy (ie, the dose recommended in current Chinese expert consensus [17]), which is lower than the recommended dose of 0.7–1.0 mg/kg/day used in current international guidelines [6]. The Chinese expert consensus also demonstrated that VOR could be used as an alternative to standard FLU treatment for patients with CM [17]. In some treatment combinations of this study, VOR was used to replace FLU. All enrolled patients were treated with intravenous AmB-based therapy or non-AmB-based therapy for 2–6 weeks initially (Table 2). The above treatment was combined with or without the use of 5-FC (100 mg/kg/day), FLU (600–800 mg/day), and VOR (400–600 mg/day), followed by FLU (400–600 mg/day) or VOR (400 mg/day) with or without 5-FC for 8 weeks as consolidation therapy. Maintenance therapy was given for >6 months and consisted of FLU (200 mg/day) or VOR (200 mg/day).

Follow-up
Follow-up time started when antifungal therapy was initiated after patient admission. We assessed all-cause mortality and functional status (mRS and KPS scores) of these patients at 1 year.

Statistical Analysis
The distributions of various factors at the baseline were univariately tested for differences between the 2 EMC status (success vs failure) groups. The χ² and Wilcoxon rank-sum tests were conducted for categorical and numerical variables, respectively. Significant variables from the univariate analysis were further analyzed by a multivariate logistic regression, with missing values imputed by the multiple imputation method. The final model was achieved by a stepwise forward model selection [18]. A sensitivity analysis was conducted for the effects of the second treatment agent (ie, VOR, FLU, or 5-FC) on EMC. The Kaplan-Meier curve for time to mycological clearance was stratified by AmB-based therapy and tested by log-rank test. The EMC status was further predicted by a random forest model. The prediction performance was measured by...
the area under the curve (AUC) predicting the testing set (randomly split 25%) using the model trained by the training set (75%). A parsimonious prediction rule was also given by fitting a decision tree using all the patients.

To test the possible difference of functional status change among the 2 EMC status groups, linear mixed-effect models are fitted by regression mRS or KPS scores on time after baseline (log-transformed days) and EMC status. Kaplan-Meier curve of time to death was stratified by EMC status, with the difference tested by log-rank test. All statistical analyses were performed using the R software and packages “mice,” “randomForest,” “party,” “lme4,” and “survival.”

Ethical Considerations
This study was approved by the medical ethics committee at the Third Affiliated Hospital of Sun Yat-sen University (ethics number 2021-02-123-01). For this type of retrospective study, the institutional review board approved the exemption of informed consent.

RESULTS
Baseline Characteristics
The distribution of baseline clinical characteristics, laboratory, and CSF findings is shown in Tables 1 and 2. The median age of the patients was 47 years (range, 14–79 years), and 70.9% (100/141) were male. India ink stain of CSF was carried out in 95.7% (135/141) of cases, and 13.3% (18/135) of CSF specimens were negative by India ink staining.

Risk Factors Associated With Failure of EMC
The EMC failure rate was 19.9% (28/141). In univariate analysis (Table 1), failure of EMC was associated with non-AmB-based regimens, comorbidities, number of serial lumbar punctures, Cryptococcus count/mL, higher CSF opening pressure (CSF-OP), CSF white blood cell count ≥20 cells/μL, higher CSF total protein, serum uric acid, serum creatinine, serum sodium, and the positive rate of India ink staining at baseline.
Table 1. Clinical Features and Laboratory and Cerebrospinal Fluid Findings of Patients With Cryptococcal Meningitis With Success or Failure of Early Mycological Clearance at Baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients (N = 141)</th>
<th>Early Mycological Clearance</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, median (range)</td>
<td>47.0 (14.0–79.0)</td>
<td>46.5 (14.0–71.0)</td>
<td>.568</td>
</tr>
<tr>
<td>Sex, male</td>
<td>100/141 (70.9)</td>
<td>20 (71.4)</td>
<td>1</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>119/141 (84.4)</td>
<td>88 (85.0)</td>
<td>.939</td>
</tr>
<tr>
<td>Fever</td>
<td>100/141 (70.9)</td>
<td>96 (70.8)</td>
<td></td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>73/141 (51.8)</td>
<td>9 (32.1)</td>
<td>.035</td>
</tr>
<tr>
<td>Vision impairment</td>
<td>34/141 (24.1)</td>
<td>4 (14.3)</td>
<td>.266</td>
</tr>
<tr>
<td>Hearing impairment</td>
<td>18/141 (12.8)</td>
<td>3 (10.7)</td>
<td>.962</td>
</tr>
<tr>
<td>Incontinence</td>
<td>5/141 (3.5)</td>
<td>1 (3.6)</td>
<td>1</td>
</tr>
<tr>
<td>With VFS</td>
<td>36/1412 (25.5)</td>
<td>4 (14.3)</td>
<td>.200</td>
</tr>
<tr>
<td>Initial mRIS, median (range)</td>
<td>2.00 (0–5.00)</td>
<td>2.00 (0–5.00)</td>
<td>.480</td>
</tr>
<tr>
<td>Initial KPS, median (range)</td>
<td>80.0 (10.0–90.0)</td>
<td>80.0 (10.0–90.0)</td>
<td>.306</td>
</tr>
<tr>
<td>Initial GCS score &lt;15</td>
<td>23/141 (16.3)</td>
<td>19 (16.8)</td>
<td>.969</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>63/141 (44.7)</td>
<td>44 (31.9)</td>
<td>.011</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-based initial therapy</td>
<td>87/141 (61.7)</td>
<td>81 (71.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Non-AmB-based initial therapy</td>
<td>54/141 (38.3)</td>
<td>32 (28.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Baseline blood chemistry, median (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>20.0 (6.00–76.0) (n = 136)</td>
<td>20.0 (6.00–76.0)</td>
<td>.251</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>28.0 (5.00–343) (n = 136)</td>
<td>27.0 (5.00–343)</td>
<td>.165</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>65.5 (38.2–85.7) (n = 135)</td>
<td>65.5 (38.2–81.9)</td>
<td>.846</td>
</tr>
<tr>
<td>ALB, g/L</td>
<td>39.4 (18.2–51.4) (n = 136)</td>
<td>39.3 (18.2–51.4)</td>
<td>.909</td>
</tr>
<tr>
<td>Tbil, µmol/L</td>
<td>11.1 (1.90–66.4) (n = 125)</td>
<td>12.0 (6.00–66.4)</td>
<td>.171</td>
</tr>
<tr>
<td>Dbil, µmol/L</td>
<td>4.30 (0.40–47.0) (n = 125)</td>
<td>4.70 (0.77–45.5)</td>
<td>.202</td>
</tr>
<tr>
<td>Uric acid, µmol/L</td>
<td>194 (35.0–687) (n = 122)</td>
<td>201 (35.0–687)</td>
<td>.015</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>64.3 (30.0–399)</td>
<td>67.0 (30.0–399)</td>
<td>.011</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.69 (2.17–6.26)</td>
<td>3.68 (2.17–6.26)</td>
<td>.548</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>134 (121–149)</td>
<td>135 (121–149)</td>
<td>.028</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>95.8 (84.9–115)</td>
<td>95.9 (84.9–115)</td>
<td>.545</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>9.25 (0.500–296) (n = 105)</td>
<td>9.60 (0.700–84.1)</td>
<td>.866</td>
</tr>
<tr>
<td>WBC count, 10³/L</td>
<td>9.00 (1.38–30.4) (n = 140)</td>
<td>8.75 (2.14–30.4)</td>
<td>.650</td>
</tr>
<tr>
<td>RBC count, 10¹²/L</td>
<td>4.31 (1.89–6.72) (n = 140)</td>
<td>4.38 (2.21–5.34)</td>
<td>.684</td>
</tr>
<tr>
<td>Hemoglobin ≥ 100 g/L</td>
<td>123 (89.4)</td>
<td>1240 (0–132 000)</td>
<td>.477</td>
</tr>
<tr>
<td>Platelet count, g/L</td>
<td>246 (1.96–751) (n = 140)</td>
<td>248 (1.96–488)</td>
<td>.483</td>
</tr>
<tr>
<td>Neutrophil ratio</td>
<td>0.808 (0.373–3.50) (n = 139)</td>
<td>0.802 (0.495–0.918)</td>
<td>.703</td>
</tr>
<tr>
<td>CSF opening pressure (cm H₂O), mm Hg</td>
<td>117/135 (86.7)</td>
<td>115 (84.0–137)</td>
<td>.018</td>
</tr>
<tr>
<td>Positive rate of India ink staining</td>
<td>28/28 (100)</td>
<td>89/107 (83.2)</td>
<td>.02</td>
</tr>
</tbody>
</table>
| Neutrophil ratio, percentage of neutrophils in the total number of white blood cells; PIIRS, postinfectious inflammatory response syndrome; RBC, red blood cell; Tbil, total bilirubin; TP, total protein; VPS, ventriculoperitoneal shunt; WBC, white blood cell.

Data are presented as No. (%), unless otherwise indicated. P values are 2-sided and obtained from the Mann-Whitney test for numerical variables and χ² test for categorical variables.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AmB, amphotericin B; AST, aspartate aminotransferase; CL, chloride; CRP, C-reactive protein; CSF, cerebrospinal fluid; Dbl, direct bilirubin; GCS, Glasgow Coma Scale; IRIS, immune reconstitution inflammatory syndrome; KPS, Karnofsky performance status; LP, lumbar puncture; mRIS, modified Rankin Scale; Neutrophil ratio, percentage of neutrophils in the total number of white blood cells; PIIRS, postinfectious inflammatory response syndrome; RBC, red blood cell; Tbil, total bilirubin; TP, total protein; VPS, ventriculoperitoneal shunt; WBC, white blood cell.

*See Table 2 for detailed data.
Table 2. Therapy and Comorbidities of Patients With Cryptococcal Meningitis With Success or Failure of Early Mycological Clearance

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Early Mycological Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N = 141)</td>
</tr>
<tr>
<td>AmB-based initial therapy</td>
<td>87</td>
</tr>
<tr>
<td>AmB + VOR + 5-FC</td>
<td>20</td>
</tr>
<tr>
<td>AmB + FLU + 5-FC</td>
<td>63</td>
</tr>
<tr>
<td>AmB + 5-FC</td>
<td>3</td>
</tr>
<tr>
<td>AmB + VOR</td>
<td>1</td>
</tr>
<tr>
<td>Non-AmB-based initial therapy</td>
<td>54</td>
</tr>
<tr>
<td>VOR + 5-FC</td>
<td>1</td>
</tr>
<tr>
<td>FLU + 5-FC</td>
<td>46</td>
</tr>
<tr>
<td>FLU</td>
<td>7</td>
</tr>
<tr>
<td>Comorbidities*</td>
<td>63</td>
</tr>
<tr>
<td>Systemic lupus erythematous</td>
<td>10</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>1</td>
</tr>
<tr>
<td>Behcet disease</td>
<td>1</td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>1</td>
</tr>
<tr>
<td>Allergic purpura</td>
<td>3</td>
</tr>
<tr>
<td>Wegener granulomatosis</td>
<td>1</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>4</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>3</td>
</tr>
<tr>
<td>Myositis</td>
<td>3</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>32</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>16</td>
</tr>
<tr>
<td>Malignant tumor</td>
<td>3</td>
</tr>
<tr>
<td>Solid organ transplant</td>
<td>4</td>
</tr>
</tbody>
</table>

Characteristics: AmB, amphotericin B; CI, confidence interval; CSF, cerebrospinal fluid; FLU, fluconazole; OR, odds ratio; VOR, voriconazole. *One patient may have multiple comorbidities.

Multivariate analysis demonstrated that among these significant differential factors listed above, AmB-based regimens (odds ratio [OR], 1.436 [95% confidence interval [CI], 1.282–1.609]; P < .001), low baseline Cryptococcus counts (OR, 1.065 for every unit decrease of log₁₀ count/mL [95% CI, 1.026–1.105]; P = .001), low baseline CSF-OP <20 cm H₂O (compared to OP >30 cm H₂O: OR, 1.176 [95% CI, 1.030–1.344]; P = .017), and high baseline serum creatinine (OR, 1.002 [95% CI, 1–1.004]; P = .037) were associated with the success of EMC (Table 3).

Prediction of Failure of EMC by Random Forests

The candidate predictor variables were ranked by their importance as shown in Supplementary Figure 1. AmB-based therapy and baseline CSF Cryptococcus counts were the most important predictors. The random forest achieved an accuracy of 0.807 (95% CI, .686–.929) and AUC of 0.793 (95% CI, .621–.950) by 200 replicates of random splitting. A parsimonious predictive rule given by the decision tree is presented in Supplementary Figure 2. Non-AmB-based therapy and high baseline CSF-OP (>30 cm H₂O) were identified as predictive of EMC failure.

Table 3. Multivariate Logistic Regression Results of Patients With Cryptococcal Meningitis With Success of Early Mycological Clearance

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB-based therapy</td>
<td>1.436 (1.282–1.609)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Baseline log₁₀ Cryptococcus count/mL</td>
<td>0.939 (.905–.975)</td>
<td>.001</td>
</tr>
<tr>
<td>Baseline CSF-OP &gt;20–30 cm H₂O</td>
<td>1.018 (.877–1.182)</td>
<td>.817</td>
</tr>
<tr>
<td>Baseline CSF-OP &gt;30 cm H₂O</td>
<td>0.85 (.744–.971)</td>
<td>.017</td>
</tr>
<tr>
<td>Baseline serum creatinine</td>
<td>1.002 (1–1.004)</td>
<td>.037</td>
</tr>
</tbody>
</table>

Abbreviations: AmB, amphotericin B; CI, confidence interval; CSF-OP, cerebrospinal fluid opening pressure; OR, odds ratio.

AmB-Based Regimens Used Among the Cases

Of patients who received non-AmB-based regimens, 22 of 54 (40.7% [95% CI, 27.6%–53.8%]) failed to achieve a negative CSF culture by week 2 compared with 6 of 87 (6.9% [95% CI, 1.6%–12.2%]) of those who received AmB-based regimens. The AmB-based regimens were 33.8% more effective than non-AmB-based regimens (95% CI, 18.2%–49.5%; P < .001) for mycological clearance (Figure 2). The median length of time to the last positive CSF culture was 1 day in the AmB-based regimen group (95% CI, 1–1 day) and 6 days in the non-AmB-based regimen group (95% CI, 1–15 days) (log-rank P < .001). The sensitivity analysis for the second treatment agent showed no association between VOR, FLU, or 5-FC and EMC.

All-Cause Mortality and Clinical Functional Status

Among patients surviving to the end of 2 weeks of antifungal therapy, 4.3% (6/141) died within 10 weeks, and 5.7% (8/141) died within 1 year (Table 1). Among the 8 cases who died within 1 year, 5 had no comorbidities and 3 had diabetes mellitus (1 of them had both diabetes mellitus and hepatitis B). In addition, of these 8 deaths, 3 were in the AmB-based regimen group and 5 were in the non-AmB-based regimen group. Surprisingly, none of those with EMC failure died. Primary causes for death included CM (n = 7) and severe pneumonia (n = 1). The median time to death was 41 days from diagnosis (interquartile range [IQR], 37–69.5 days). As to the all-cause mortality in 1 year, there was no significant difference (log-rank test P = .152; Supplementary Figure 3) between the 2 statuses of EMC (success vs failure).

We also analyzed the changes of clinical functional status in 1 year. At any given visit in 1 year, there were no significant differences in mRS (–0.10 [95% CI, −.56 to .36]; P = .667) or KPS (1.55 [95% CI, −.606 to 9.16]; P = .690) between the 2 statuses of EMC (Supplementary Figure 4).

Cryptococcal PIIRS/IRIS-Like Reconstitution Syndrome, Persistent Infection, and Microbiological Relapse

Overall, 23 of 141 (16.3%) cryptococcal-related PIIRS/IRIS-like reconstitution syndrome events occurred: 19 of 113 (16.8%) among those with success of EMC and 4 of 28 (14.3%) among

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those with failure of EMC ($P = .746$). The median time to PIIRS/IRIS-like reconstitution syndrome was 44 days from diagnosis (IQR, 26–82.5 days). The median length of time to PIIRS/IRIS-like reconstitution syndrome was 69.5 days in the failure of EMC group (95% CI, 28–71 days) and 44 days in the success of EMC group (95% CI, 53–67 days) ($P = .199$). In addition, overall persistence at 4 weeks was 5.7% (8/141); the rate of persistence was 0% (0/87) among the AmB-based regimen group and 14.8% (8/54) among the non-AmB-based regimen group. Only 2 of 141 patients (1.4%) had microbiological relapse, which occurred in those with the failure of EMC (2/28 [7.1%]) and non-AmB-based regimen group (2/54 [3.7%]).

**DISCUSSION**

To the best of our knowledge, this is the first study to investigate EMC status in HIV-negative CM after antifungal treatment. Of the 141 HIV-negative patients with CM surviving to 2 weeks, 28 (19.9%) failed to achieve a negative CSF culture by week 2. The 1-year mortality rate of these 141 patients was 5.7% (8/141). Non-AmB-based initial therapy, baseline log$_{10}$ *Cryptococcus* count/mL, baseline CSF-OP $> 30$ cm H$_2$O, and baseline serum creatinine were the factors related to failure of EMC. The decision tree identified that patients with CM with non-AmB-based initial therapy and high baseline CSF-OP had high risk of EMC failure. Further analysis revealed that EMC may not be related to mortality and clinical functional status.

By our definition, failure of EMC was a common outcome (19.9%), but most patients (98.6%) achieved CSF microbiological sterilization at 4 weeks. Moreover, we found that a high baseline log$_{10}$ *Cryptococcus* count/mL was related to failure of EMC, which is similar to results of a previous study [10] suggesting that a high baseline fungal burden was related to failure in mycological clearance at 10 weeks in HIV-associated CM.
The failure of EMC likely reflects the high burden of *C. neoformans* in the CSF of these patients and requires us to select how our treatment regimens impact the killing of yeasts during initial antifungal treatment. Current options for treating CM include AmB alone, AmB plus 5-FC, and azoles such as FLU and VOR. None of these treatments is entirely satisfactory. Compared to AmB plus FLU and to AmB monotherapy, AmB plus 5-FC is superior for early cryptococcal clearance and survival [22, 23]. Though FLU combined with 5-FC is recommended as second-line treatment, AmB-sparing combinations consistently show lower rates of yeast clearance from CSF than those containing AmB [11]. As suggested by our findings, we identified that non-AmB-based regimens had the strongest association with failure to achieve CSF culture sterilization at 2 weeks, such that more effective AmB-based initial therapy strategy should be selected. Although our results show that FLU or VOR may not be related to EMC status in combination therapy, our study cannot completely rule out the relationship between FLU or VOR and fungal clearance. In addition, a negative CSF culture at the end of the induction phase is an important predictor of fungal clearance at 10 weeks [9]. In our study, regardless of EMC status at 2 weeks, all of the CSF cultures except 1 were sterile before 10 weeks; moreover, we also found that the population with EMC failure had the possibility of persistent infection at 4 weeks and microbiological relapse.

As previously reported, there was a positive correlation between high CSF-OP with higher fungal burden [24], higher baseline CSF cryptococcal antigen titer, headache, meningeal symptoms, papilledema, hearing loss [25], and poorer short-term survival [26]. We found that high baseline CSF-OP (>30 cm H2O) was associated with failure of EMC, which is similar to a previous study in HIV-associated CM [10]. However, this association between raised CSF-OP and failure of EMC is not likely to be causal, but rather linked to the role of high fungal burden. Moreover, the findings of the decision tree also identified patients with CM with non-AmB-based therapy and high baseline CSF-OP (>30 cm H2O) as being at high risk of EMC failure in our study. Interestingly, our study found that baseline serum creatinine was related to EMC status. However, the association is unlikely to be on causal pathways. At present, there is no relevant literature showing that baseline serum creatinine level was correlated with the probability of EMC failure. It was not clearly known why these patients with EMC failure had lower baseline serum creatinine level, but decreased serum creatinine at baseline may indicate poor nutrition, which was likely to trigger other balances and mechanisms leading to the failure of EMC. However, baseline serum albumin and hemoglobin were not significant in this study. Therefore, further study is needed.

Ten-week mortality of AIDS-associated CM in developed country settings is as low as 10%–15% with AmB-based treatment [27–29]. Increased risk of death has been observed in persons with AIDS-associated CM with nonsterile CSF [30, 31]. However, the mortality rate in our cohort was very low, with 5.7% of patients dying within 1 year; the mortality rate was not statistically different between the 2 statuses of EMC. However, all of the deaths after 2 weeks were in those who had EMC success. The lack of death in patients with EMC failure may be related to the relatively small number. This study evaluating EMC status did not include all participants, wherein we restricted analysis to those who survived to 2 weeks of antifungal therapy. It is possible that patients with EMC failure were more likely to die early (3 deaths occurred within the early 2-week period), which leads to mortality bias of patients with EMC failure in our cohort. In addition, because this study is retrospective, there were 20 patients with incomplete follow-up data. Among these 20 patients, there were those who failed in EMC, and some of them may have died within 1 year, leading to mortality bias. Furthermore, this finding provides a potential insight into the differences in pathology between HIV-associated CM and CM in HIV-negative individuals where the host response and resultant inflammation may be more important in driving pathology. A previous study [32] reported that persons with sterile CSF cultures after 14 days of induction AmB therapy had worse neurocognitive outcomes than those still culture positive at 14 days (P = .002). However, no association between EMC status and the follow-up mRS and KPS scores was found in our study.

Several studies have demonstrated that a higher risk of CM-IRIS occurs with a high initial organism load and persistently positive CSF culture results at 2 weeks [33, 34]. However, no association between EMC status and CM-PIIRS/IRIS-like reconstitution syndrome was found in our study. Given the relatively low mortality within 1 year, our results suggest that CM-PIIRS/IRIS-like reconstitution syndrome is not uncommon (16.3%) and can usually be managed by appropriate treatment (such as corticosteroid) with a good prognosis. A Chinese study involving patients with AIDS showed that 15.1% (16/106) were identified as having persistent CM [35]. In our cohort, the overall persistence at 4 weeks (5.7%) was obviously lower than the above result, which may be related to the fact that all the patients we included were HIV-negative. However, in the non-AmB-based therapy group, we still need to pay attention to persistent infection (14.8%). In the developed world, where AmB-based regimens are the standard initial therapy, annual relapse rates for cryptococcal disease during maintenance FLU treatment are <5% [36–38]. Azoles such as FLU and VOR are used as maintenance therapy in our setting, where we have noted that the rate of microbiological relapse is very low (1.4%) in HIV-negative CM, especially in the AmB-based regimen group. However, we still need to pay attention to the possibility of microbiological relapse (7.1%) in the population with failure of EMC, which also has
been reported in prior studies, positive cultures being associated with nearly 2-fold higher odds of treatment failure or relapse later during consolidation therapy [9, 27].

Antifungal resistance is an important issue for the treatment of CM. According to our results of drug susceptibility testing, most of the C neoformans isolates from the CSF of patients were sensitive to Amb, VOR, 5-FC, and FLU, whereas itraconazole is not a good antifungal regimen choice for CM. In addition, we found that antifungal susceptibility of baseline isolates of C neoformans did not correlate with mycological clearance, which is similar to findings of a recent study [39]. There are several limitations to this study. It was a retrospective study from a single center and a single ethnic population. Some variables (such as serum cryptococcal antigen titer and body mass index) were incomplete or not available in our study. Most patients did not have extraneural cultures assessed. Besides, quantitative culture for calculating EFA was not able to be performed; we used a quantitative CSF cryptococcal count by microscopy (not colony-forming units) as a quantitative assessment of fungal burden. In addition, considering that it is necessary to meet the inclusion criteria, we excluded 30 patients and only kept 141 patients for the final analysis. The fairly substantial proportion (30/171 [17.5%]) who were not included may bias the study. Moreover, this study only analyzed and described 141 patients in a cohort of 312 individuals (less than 50%). This may lead to a high likelihood of some sort of selection bias.

CONCLUSIONS
This study, with a complete follow-up of 141 patients to 1 year posttherapy, extends the understanding of EMC in HIV-negative CM. EMC failure in HIV-negative CM is attributed to non-AmB-based initial therapy and is associated with log$_{10}$ Cryptococcus count/mL and CSF-OP >30 cm H$_2$O at baseline. Because of the small number of deaths, we are not able to comment on whether or not EMC is associated with mortality. These results underscore the need for a therapeutic strategy of AmB-based initial regimens for treatment of CM in HIV-negative individuals.

Supplementary Data
Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Author contributions. Y. J. and F. P. contributed to the conception and design of this study. Z. S., K. D., D. Y., B. Q., M. G., and J. L. collected and organized the data. C. L., Z. S., Y. J., and Y. C. analyzed the data. All authors drafted the manuscript. Y. J., F. P., and Y. C. reviewed the manuscript, figures, and tables. All authors read and approved the final manuscript.

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

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