

# Retrospective Study of Cryptococcal Meningitis With Elevated Minimum Inhibitory Concentration to Fluconazole in Immunocompromised Patients

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**Background.** Mortality for cryptococcal meningitis remains significant, in spite of available treatment. Resistance to first-line maintenance therapy, particularly fluconazole, has been reported.

**Methods.** A retrospective chart review was performed on immunocompromised patients with cryptococcal meningitis, who had susceptibility testing performed between January 2001 and December 2011, at 3 hospitals in Atlanta, Georgia.

**Results.** A total of 35 immunocompromised patients with cryptococcal meningitis were identified, 13 (37.1%) of whom had an elevated minimum inhibitory concentration (MIC) to fluconazole (MIC  $\geq 16$   $\mu\text{g}/\text{mL}$ ). Eighty percent of patients were males with African American predominance, the median age was 37 years, and 80% of the patients were human immunodeficiency virus (HIV) positive. Subsequent recurrence of cryptococcal meningitis was more likely in HIV patients compared with solid organ transplant patients ( $P = .0366$ ). Overall, there was a statistically significant increase in an elevated MIC to fluconazole in patients who had a history of prior azole use (odds ratio, 10.12; 95% confidence interval, 2.04–50.16). Patients with an elevated MIC to fluconazole and those with a high cerebrospinal fluid cryptococcal antigen load ( $\geq 1:512$ ) were more likely to have central nervous system complications ( $P = .0358$  and  $P = .023$ , respectively). Although no association was observed between an elevated MIC to fluconazole and mortality, those who received voriconazole or high-dose fluconazole ( $\geq 800$  mg) for maintenance therapy were more likely to survive ( $P = .0288$ ).

**Conclusions.** Additional studies are required to further investigate the morbidity and mortality associated with an elevated MIC to fluconazole in cryptococcal meningitis, to determine when it is appropriate to perform susceptibility testing, and to evaluate its cost effectiveness.

**Keywords.** azoles; *Cryptococcus*; elevated MIC; immunocompromised; meningitis.

Cryptococcal meningitis, caused by the environmental yeast *Cryptococcus neoformans*, occurs predominantly in immunocompromised patients, mainly those with acquired immune deficiency syndrome (AIDS) [1]. The incidence of cryptococcal meningitis has declined significantly with the use of early antiretroviral treatment (ART) and effective antifungal therapy [2]. However, it remains a major public health burden with an estimated 1 million cases leading to 600 000 deaths per year globally [3]. Active population-based surveillance in 2 US locations reports an annual incidence between 2 and 7 cases per 1000 individuals with AIDS and an incidence of 0.4–1.3 cases per 100 000 of the general population [4].

Resistance to first-line maintenance antifungal therapy, particularly fluconazole, has been reported [5], and the ARTEMIS DISK Global Antifungal Surveillance Study noted a progressive increase in *C neoformans* isolates with elevated minimum inhibitory concentration (MIC) to fluconazole between 1997 and 2007 (7.3%–11.7%) [6]. In vitro studies have shown that an elevated MIC to fluconazole occurs in a stepwise manner and is more evident with increased exposure to the azoles. The molecular basis of this resistance, although not entirely clear, involves multiple mechanisms such as multidrug efflux pump proteins, decreased affinity to target enzymes, or overall decreased drug uptake [7–10].

Although exposure to increased concentration of fluconazole results in more virulent strains in a murine model [11], data on the clinical outcomes of *C neoformans* meningitis in humans with elevated MIC to fluconazole are limited and contradictory [12–14]. The objective of our study is to better understand the predictors of an elevated MIC to fluconazole, in an immunocompromised population, and its effects on the clinical outcomes of cryptococcal meningitis.

## METHODS

Between January 2001 and December 2011, patients with antifungal susceptibility testing performed on positive cerebrospinal fluid

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(CSF) cultures for *C neoformans* at Emory University-affiliated hospitals (Emory University Hospital and Emory University Hospital Midtown, Atlanta, GA) and Grady Health System (Atlanta, GA) were identified and included in this study. Susceptibility testing was determined using Sensititre YeastOne (Thermo Fisher Scientific, formerly Trek Diagnostic Systems) at all our laboratories. Susceptibility testing is not routinely done on all positive *C neoformans* cultures, it is requested at the discretion of the treating physician, and the reason for the request was not available for our chart review. The medical records of all eligible patients were reviewed, and data regarding demographics, medical history, clinical and laboratory characteristics, treatment regimens, and clinical outcomes were collected. Patients were observed for recurrence and mortality for as long as data were available.

Patient characteristics and risk factors associated with an elevated MIC to fluconazole (age, race, gender, human immunodeficiency virus [HIV] status, prior azole exposure, cryptococcal antigen titer, prior occurrence) were reported. Prior azole exposure was defined as any exposure to azole (as treatment or prophylaxis) within 1 year of disease onset. High-dose azole maintenance therapy was defined as treatment with  $\geq 800$  mg of daily fluconazole. Recurrence of infection was defined as any microbiologic recurrence during the follow-up period. Elevated MIC to fluconazole was defined as an MIC  $\geq 16$   $\mu\text{g}/\text{mL}$  as supported by the data in the literature, although studies were limited [12, 15]. A sensitivity analysis was also performed using 8 and 32  $\mu\text{g}/\text{mL}$  as a cutoff for an elevated MIC. Main outcomes evaluated were mortality (early 14-day mortality and at any time over the follow-up period) and central nervous system (CNS) complications (ie, requiring ventriculoperitoneal shunt insertion, deafness, blindness or other vision loss, or seizures). Comparisons were done using  $\chi^2$  testing or the Fisher exact test for categorical variables and the Wilcoxon Mann-Whitney *U* test for continuous variables. All reported *P* values were 2 sided, and  $\alpha < 0.1$  was considered significant. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC). This study was approved by the Emory University Institutional Review Board and Grady Research Oversight Committee.

## RESULTS

Over a 10-year time period, 35 patients had a positive CSF culture for *C neoformans*, and antifungal susceptibility testing was performed. This represented approximately 9.3% of all positive CSF cultures for *Cryptococcus* performed at our 3 hospitals (35 of 377). The median follow-up time was 4 years, with an interquartile range (IQR) of 1–7 years. Males accounted for 80% of cases, 82.9% were African American, and the median age was 37 years (IQR, 30–45). Central nervous system symptoms (primarily headache) were reported in 88.6% of patients, and approximately half of the patients had nausea and vomiting (45.7%) (Table 1).

In this cohort of cryptococcal meningitis patients who had antifungal susceptibility testing done, 13 (37.1%) were found to

**Table 1. Clinical Characteristics of Patients With Cryptococcal Meningitis<sup>a</sup>**

Clinical Characteristics	N = 35 (Median)	% (IQR)
Age (years)	37	(30–45)
Black Race	29	(82.9)
Male	28	(80)
Symptoms at presentation <sup>a</sup>		
CNS	31	(88.6)
Headache	24	(68.6)
Abnormal mental Status	8	(22.9)
Gastrointestinal	16	(45.7)
Systemic	12	(34.3)
Respiratory	3	(8.6)
HIV positive	28	(80)
HIV infection diagnosed on presentation	12	(50)
CD4 count (cells/mL)	16	(9–42)
Viral load ( $\times 10^3$ copies/mL)	61	(31–317)
On antiretroviral medications	7	(25)
Solid organ Transplant	6	(17.1)
Time from transplant (years)	4.5	(1–6)
Fluconazole MIC, $\geq 16$ $\mu\text{g}/\text{mL}$	13	(37.1)
Cryptococcal antigen		
Serum	512	(128–512)
CSF	512	(32–1024)
CSF WBC	44	(6–100)
Recurrence	10	(35.7)
CNS complications	7	(20)
Crude mortality	10	(32.3)
Mortality at 14 d	3	(13.6)

Abbreviations: CNS, central nervous system; CRAG cryptococcal antigen CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range; MIC, minimal inhibitory concentration; VP, ventriculoperitoneal; WBC, white blood cells.

<sup>a</sup> CNS (headache, parasthesia, seizures, weakness, neck stiffness, ataxia, slurred speech); gastrointestinal (nausea, vomiting, diarrhea, abdominal pain); systemic (fever, fatigue, weight loss, chills, night sweats); respiratory (cough, shortness of breath, acute respiratory distress syndrome, sore throat); CNS complications (VP shunt, blindness or other vision loss, deafness, seizures); cryptococcal antigen.

have an elevated MIC to fluconazole (MIC  $\geq 16$   $\mu\text{g}/\text{mL}$ ). Exposure to azole before the diagnosis of cryptococcal meningitis was a significant risk factor for elevated MIC to fluconazole (odds ratio [OR], 10.12; 95% confidence interval [CI], 2.04–50.16). The indications for prior azole exposure were as follows: cryptococcal meningitis (n = 5), oropharyngeal candidiasis (n = 1), and *Candida* esophagitis (n = 1). Overall, 10 patients died (32.3%), 3 (13.6%) within 14 days of admission, and increased MIC to fluconazole was not associated with increasing mortality in our study. Patients with a high CSF cryptococcal antigen load ( $\geq 1:512$ ) and those with an elevated MIC to fluconazole were more likely to have CNS complications (*P* = .0312 and *P* = .0754, respectively) (Table 2). No significant correlation with mortality or CNS complications was found when sensitivity analysis was performed using MIC cutoff as  $\geq 8$  or  $\geq 32$   $\mu\text{g}/\text{mL}$ . Of note, of the 8 patients who received voriconazole or high-dose fluconazole ( $\geq 800$  mg) for maintenance therapy, none died despite the high MIC to fluconazole (*P* = .0288).

**Table 2. Univariate Analysis of Cases With Different Fluconazole MICa**

Clinical Characteristics	Fluconazole MIC $\geq 16$ $\mu\text{g/mL}$ N = 13	Fluconazole $< 16$ $\mu\text{g/mL}$ N = 22	P Value	OR (95% CI)
Age (years)	42 (34–52)	35 (29–44)	.2746	
Black Race	11 (84.6)	18 (81.8)	1	1.22 (.19–7.82)
Male	10 (76.9)	18 (81.8)	1	0.74 (.14–3.99)
HIV positive	11 (84.6)	17 (77.3)	.6889	1.62 (.26–9.85)
Prior azole	9 (69.2)	4 (18.2)	.0042* <sup>b</sup>	10.12 (2.04–50.16)
Recurrence	4 (36.4)	6 (35.3)	1	1.05 (.22–5.09)
CNS complications	5 (38.5)	2 (9.1)	.0754*	6.25 (1–39.09)
Crude mortality	4 (36.4)	6 (30)	1	1.33 (.28–6.32)

Abbreviations: CI, confidence interval; CNS, central nervous system; HIV, human immunodeficiency virus; MIC, minimal inhibitory concentration; OR, odds ratio; VP, ventriculoperitoneal.

<sup>a</sup> CNS complications (VP shunt, blindness or other vision loss, deafness, seizures).

<sup>b</sup>  $\chi^2$  tests or Fisher exact test,  $\alpha = 0.1$ ; significant P values marked with an asterisk (\*).

The majority of the patients had AIDS (28); 6 patients were solid organ transplant recipients; and 1 patient had non-Hodgkin's lymphoma and was getting chemotherapy treatment. Cryptococcal meningitis was the AIDS-defining illness in 50% of patients in whom the HIV status was unknown at time of presentation. In addition, HIV-positive patients were slightly younger than transplant patients (median of 36 vs 43 years, not statistically significant) and more likely to be African American (96.4% vs 33.3%,  $P = .0015$ ). Human immunodeficiency virus-positive patients were also more likely to have a recurrence ( $P = .057$ ) and a trend towards lower CNS complications (Table 3). Median time to onset of cryptococcal meningitis was 4.5 years (IQR, 1–6 years) from HIV diagnosis. Recurrent cryptococcal meningitis, defined as microbiologic recurrence, occurred only in HIV-positive patients (47.6% vs 0%,  $P = .057$ ). However, there was no difference in crude mortality between HIV-positive and transplant patients (29.2% vs 33.3%) with a median follow-up period of 4 years (IQR, 1–7 years) (Table 3).

## DISCUSSION

Our study showed that immunocompromised patients, mostly those with AIDS, who had a history of prior azole exposure were more likely to develop an elevated MIC to fluconazole

and subsequent CNS complications on univariate analysis. Although the fluconazole MIC did not correlate with a difference in mortality in our study, patients who were subsequently on voriconazole or high-dose fluconazole maintenance therapy were more likely to survive.

In a landmark study published in 1996, Witt et al [16] found that MIC to fluconazole was an important and independent predictor of treatment success. Since then, there has been conflicting data from published papers on the relationship between fluconazole MIC and clinical outcomes. Initially, Aller et al [12] noted that a fluconazole MIC of  $< 16$   $\mu\text{g/mL}$  was predictive of a positive clinical response, with a statistically significant association between MIC  $\geq 16$   $\mu\text{g/mL}$  and mortality. However, the sample size in that study was small with only 5 (4 of whom died) of their 25 patients having elevated MIC to fluconazole and none of them having received a 5-fluorocytosine (5FC)-based regimen. We found no correlation between MIC and increasing mortality in our study. On the other hand, Dannaoui et al [14] reported that in vitro antifungal susceptibility testing of *C neoformans* is not a predictor of clinical outcomes in patients with cryptococcal meningitis, where clinical outcome was defined as death, sterilization of all initially infected body sites, or persistence of the organism in cultures after 2 and 12 weeks.

**Table 3. Selected Characteristics of HIV and Solid Organ Transplant Patientsa**

Clinical Characteristics	HIV Positive N = 28(%)	Solid Organ Transplant N = 6(%)	P Value	OR (95% CI)
Age (years)	36 (31–45)	43 (28–56)	.3822	
Black race	27 (96.4)	2 (33.3)	.0015* <sup>b</sup>	54 (3.93–741.79)
Male	24 (85.7)	3 (50)	.0531*	6 (.88–40.87)
CSF CRAG, $\geq 16$ $\mu\text{g/mL}$	20 (71.4)	2 (33.3)	.0809*	5 (.76–32.93)
CSF WBC, $< 20$	11 (39.3)	1 (16.7)	.3	3.24 (.33–31.54)
Recurrence	10 (47.6)	0	.057*	—
CNS complications	7 (25)	0	.3058	—
Crude mortality	7 (29.2)	2 (33.3)	1	0.82 (.12–5.57)

Abbreviations: CI, confidence interval; CNS, central nervous system; CRAG, cryptococcal antigen; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; OR, odds ratio; WBC, white blood cells.

<sup>a</sup> Age median/interquartile range.

<sup>b</sup>  $\chi^2$  tests or Fisher exact test,  $\alpha = 0.1$ ; significant P values marked with an asterisk (\*).

However, most MICs in the latter were  $\leq 4$   $\mu\text{g}/\text{mL}$ , and the study only enrolled patients who presented with the first episode of cryptococcal meningitis and were likely azole naive. In 2012, Lee et al [17] performed a retrospective analysis of 46 patients with cryptococcal meningitis and demonstrated an association between fluconazole MIC  $\geq 8$   $\mu\text{g}/\text{mL}$  and lack of therapeutic cure. However, only 6 of their 46 patients were HIV positive, and they only compared cure versus noncure rates, defined as resolution of symptoms, disappearance of negative clinical findings (eg, fever, abnormal mental status, neurologic deficits), or sterilization of CSF cultures.

Although there was no detected difference in mortality in our study, the lack of effect on mortality, especially early mortality (at 14 days), could be explained by the fact that none of our cases were treated with fluconazole alone in the induction phase. All of our cases were treated with either an amphotericin B-based regimen (94.3%) or a 5FC-based regimen (82.9%) as part of their induction therapy, or they were treated with a combination of both amphotericin B and 5FC (77.1%) in accordance with clinical guidelines [1]. It is interesting that no patient died if they received high-dose fluconazole (800 mg) or voriconazole for maintenance therapy. A recent study that evaluated the pharmacokinetics and pharmacodynamics of fluconazole in murine models that extrapolated to humans found that clinical breakpoints for fluconazole are likely to be lower than epidemiological cutoff values [18]. Although they were discussing fluconazole for induction therapy, they recommended that the highest possible doses be used. This could be considered in maintenance therapy, because fluconazole is not typically used for induction in the treatment of cryptococcal meningitis in the United States, especially in cases with resistance or recurrence.

Cheong et al [19] also published a case series looking at 20 patients with treatment failure after fluconazole for cryptococcal meningitis, and 30% of these patients had fluconazole strains with MIC  $\geq 8$   $\mu\text{g}/\text{mL}$  despite no history of exposure to antifungals; however, there was no control group. In our study, this was not the case: we found prior azole use to be a significant risk factor for developing fluconazole resistance (OR, 10.12; 95% CI, 2.04–50.16).

Although we had a small sample size, we compared the characteristics and outcomes of HIV-positive and transplant patients. Davis et al [20] evaluated patient data in The Prospective Antifungal Therapy Alliance and compared HIV and transplant patients with cryptococcal disease. Although their study did not focus specifically on cryptococcal meningitis, they report an increased age in the transplant group and lower predominance of black race, which likely reflects the overall patient population as seen in our population. The overall 12-week mortality in that study was 22.6%, similar to our 14-day mortality of 13.6%.

Our study has several limitations, mainly due to its retrospective design and small number of cases where susceptibility

testing was performed. It is unclear why the susceptibility testing was initially performed and varied based on physician's request. We are limited unfortunately by the fact that no rationale was needed for a physician to order the antimicrobial susceptibility testing (AST), and the AST request was not limited to the Infectious Diseases consultant. It is interesting to note that only 5 of the 35 cases were relapses and that the majority of AST were performed on the fungal culture from initial lumbar puncture; therefore, most of the requests for AST were not based on prior medical history or on patient's response to induction therapy. Few patients were on prior fluconazole (13 total), and it is interesting to note that participants who were listed as on ART were likely noncompliant with medications in general (because all patients had AIDS and a detectable viral load) and therefore were unlikely to be on any other medications (such as azoles). We believe the request for AST was random, and the population reported here reflects the general population of cryptococcal meningitis with immunocompromised states in our hospitals.

The data on type, timing and adherence to ART could not be consistently obtained and controlled for; though none of the AIDS patients had an undetectable HIV viral load. Both the Clinical and Laboratory Standards Institute and the European Committee for Antimicrobial Susceptibility Testing have not established breakpoints for *C. neoformans* tested against antifungal agents in general and fluconazole in particular. We selected an MIC  $\geq 16$   $\mu\text{g}/\text{mL}$  as the cutoff for an elevated MIC to fluconazole as supported by clinical (with statistically significant correlation between MIC  $\geq 16$   $\mu\text{g}/\text{mL}$  and mortality) [12] and epidemiological data from the literature [15]. We performed a sensitivity analysis and looked at the correlation of mortality with different MIC cutoff points (4 and 8  $\mu\text{g}/\text{mL}$ ); no significant correlations were found with any of the risk factors evaluated.

In addition, it is difficult to predict the clinical outcome of patients with cryptococcal meningitis based solely on antifungal susceptibility testing because the prognosis of the disease itself depends on several factors (degree of immune suppression, severity of illness, adherence to therapy, etc), and that could not be accounted for due to the retrospective nature of our study and the availability of that data. Considering the limitations listed, no firm conclusion can be made from our data for the effect of elevated fluconazole MIC on patient outcomes.

## CONCLUSIONS

In summary, it may be reasonable to consider susceptibility testing in patients who have been exposed to azoles in the past. In such cases, early recognition of resistance could help guide therapy, specifically maintenance therapy. High-dose fluconazole or voriconazole should be considered in cases with elevated fluconazole MIC, in an attempt to decrease CNS complications.

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