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# Correlation of Fluconazole MICs with Clinical Outcome in Cryptococcal Infection

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**We have correlated the in vitro results of testing the susceptibility of *Cryptococcus neoformans* to fluconazole with the clinical outcome after fluconazole maintenance therapy in patients with AIDS-associated cryptococcal disease. A total of 28 isolates of *C. neoformans* from 25 patients (24 AIDS patients) were tested. The MICs were determined by the broth microdilution technique by following the modified guidelines described in National Committee for Clinical Standards (NCCLS) document M27-A, e.g., use of yeast nitrogen base medium and a final inoculum of $10^4$ CFU/ml. The fluconazole MIC at which 50% of isolates are inhibited ($MIC_{50}$) and $MIC_{90}$, obtained spectrophotometrically after 48 h of incubation, were 4 and 16 μg/ml, respectively. Of the 25 patients studied, 4 died of active cryptococcal disease and 2 died of other causes. Therapeutic failure was observed in five patients who were infected with isolates for which fluconazole MICs were ≥16 μg/ml. Four of these patients had previously had oropharyngeal candidiasis (OPC); three had previously had episodes of cryptococcal infection, and all five treatment failure patients had high cryptococcal antigen titers in either serum or cerebrospinal fluid (titers, >1:4,000). Although 14 of the 18 patients who responded to fluconazole therapy had previously had OPC infections, they each had only a single episode of cryptococcal infection. It appears that the clinical outcome after fluconazole maintenance therapy may be better when the infecting *C. neoformans* strain is inhibited by lower concentrations of fluconazole for eradication (MICs, <16 μg/ml) than when the patients are infected with strains that require higher fluconazole concentrations (MICs, ≥16 μg/ml). These findings also suggest that the MICs determined by the modified NCCLS microdilution method can be potential predictors of the clinical response to fluconazole therapy and may aid in the identification of patients who will not respond to fluconazole therapy.**

Cryptococcosis is an infection caused by the ubiquitous fungus *Cryptococcus neoformans*, an encapsulated yeast-like fungus. The most frequent clinical presentation of the disease is meningoencephalitis (5, 6, 12, 18, 19; M. S. Saag, Editorial Response, Clin. Infect. Dis. **21:**35–36, 1995); 75 to 90% of AIDS patients infected with *C. neoformans* will develop meningitis (5). Cryptococcosis in AIDS patients is seldom cured, and fluconazole is the drug of choice for the necessary lifelong suppressive (maintenance) therapy (17). Use of fluconazole for long-term suppressive therapy may be associated with the development of azole resistance in cryptococcal infections in AIDS patients (21).

Methods for testing the antifungal susceptibility of *C. neoformans* could become important tools in the selection and monitoring of an appropriate antifungal drug for the treatment and prophylaxis of cryptococcal infections. Ghannoum et al. (9) developed a broth microdilution method for measuring the susceptibility of cryptococci to fluconazole, which is described as an alternative approach in document M27-A of the National Committee for Clinical Laboratory Standards (NCCLS) (15). Witt et al. (22) explored the value of MICs obtained by this procedure (9) and other clinical variables as predictors of treatment failure in patients receiving fluconazole for acute AIDS-associated cryptococcal meningitis; the value of MICs determined by the macrodilution method described in document M27-A was also evaluated in that study.

The aim of our study was to correlate the MICs obtained by the modified microdilution method that is described in document M27-A (15) with the clinical response to fluconazole maintenance therapy in patients with AIDS-associated cryptococcal infection.

## MATERIALS AND METHODS

**Patient population and data collection.** We retrospectively reviewed 36 episodes of cryptococcal infection in 36 patients (34 with AIDS), who were treated from 1 January 1994 through 31 December 1996 at Christiana Care, Wilmington, Del. (19 patients), and the Valme University Hospital, Seville, Spain (17 patients). Of the 36 patients, 11 patients were excluded from this evaluation due to the lack of well-documented information regarding antifungal dosages, clinical and laboratory data, or information on the infecting isolates. The clinical evaluation included patient demographic features, the existence of previous episodes of oropharyngeal candidiasis (OPC), and the response to antifungal therapy for the cryptococcal infection. Results of cultures of clinical specimens (blood, cerebrospinal fluid [CSF], and urine) for fungi, $CD4^+$ T-cell counts, and cryptococcal antigen titers (in serum and CSF) were collected (Table 1). The measurement of cryptococcal antigen titers was performed by using the Crypto-LA latex agglutination procedure (Wampole Laboratories). The mean CD4 count was 33.5/μl, with a range of 0 to 139/μl.

**Demographic and clinical characteristics of the patient population.** Of the 25 patients included in the final evaluation (coded as 1 to 25 in Tables 1 and 2), 22 patients were men and 3 were women. The patients' ages ranged from 24 to 50 years, with a mean of 31.7 years. Twenty-four of the 25 patients were infected with human immunodeficiency virus (HIV), and 1 patient had a non-Hodgkin's lymphoma. The risk factors for HIV infection for the 25 patients were similar, either male homosexual contact (25%) or intravenous drug use (75%). Of the 25 patients, 16 had cryptococcal meningitis at the initial presentation. Eighteen of the 25 patients had prior episodes of OPC, and 9 of them were treated with fluconazole (100 to 200 mg/day orally [p.o.]) until all signs and symptoms of candidiasis had improved (approximately 7 to 10 days); data the therapeutic

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TABLE 1. Laboratory findings for 25 patients with cryptococcal infection

| Patient no. | CD4 cell count (no. of cells/μl) | Cryptococcal antigen titer | | Specimen positive by culture |
|---|---|---|---|---|
| | | CSF | Serum | |
| 1 | 12 | 1:1,024 | 1:512 | Blood, CSF |
| 2 | 124 | NT[a] | 1:4,096 | Blood, CSF, BAL[b] |
| 3 | 10 | NT | NT | Blood |
| 4 | 12 | NT | 1:16384 | Blood, CSF |
| 5 | NT | NT | 1:4,096 | Blood |
| 6 | 11 | NT | 1:2,048 | Blood, CSF |
| 7 | NT | NT | 1:2,048 | Blood |
| 8 | 9 | Negative | 1:1,024 | Blood, CSF |
| 9 | 30 | >1:4,000 | NT | Blood, CSF |
| 10 | NT | NT | 1:2,048 | CSF |
| 11 | 26 | 1:512 | NT | CSF |
| 12 | NT | NT | 1:2,2048 | Blood |
| 13 | NT | 1:2,048 | 1:2,048 | Blood, CSF |
| 14 | 140 | Negative | 1:256 | Blood |
| 15 | 7 | 1:1,024 | NT | Blood, CSF |
| 16 | 7 | 1:256 | Negative | Blood, CSF |
| 17 | 6 | 1:128 | NT | Blood, CSF |
| 18 | 29 | NT | NT | Blood, BAL |
| 19 | 130 | 1:2,048 | 1:2,048 | Blood, CSF |
| 20 | 37 | Negative | Negative | Blood |
| 21[c] | 0 | NT | >1:4,000 | Blood |
| 22 | 139 | >1:4,000 | NT | CSF |
| 23[c] | 2 | 1:1,024 | >1:4,000 | CSF |
| 24[c] | 61 | >1:4,000 | 1:1,024 | CSF |
| 25[c] | 4 | NT | >1:4,000 | Lung biopsy |

[a] NT; not tested.
[b] BAL, bronchoalveolar lavage.
[c] Death was attributed to cryptococcal infection.

regimens for the OPC infections were not available for the other patients. Three patients had previously had an episode of cryptococcal infection and relapsed while receiving maintenance therapy with oral fluconazole (200 mg/day).

**Clinical evaluation.** Patients were considered cured when they were free of symptoms, as well as when clinical findings and cultures of specimens from the affected site were negative at the end of 10 weeks of fluconazole therapy. Therapeutic failure was associated with either the persistence of symptoms and clinical findings and positive cultures after 10 weeks of fluconazole therapy or death caused by the cryptococcal infection before the end of 10 weeks of treatment. A patient was considered to have died of active cryptococcal disease during therapy if the cultures were positive at either death or autopsy. A patient was considered to have died of other causes during fluconazole therapy when the cultures were negative at the time of death or if clinical improvement was evident in the affected system before death. A relapse was defined as the recurrence of positive cultures of specimens from the original site or from a new site.

**Fungal strains.** A total of 28 strains of *C. neoformans* were studied. Twenty-five of these strains were isolated during the first episode of cryptococcal infection from each of the 25 patients, and the other 3 isolates were isolated from the 3 patients who had more than one episode of cryptococcal infection. The processing of the specimens and isolate identification were performed by conventional methods and with the YBC identification card (bioMerieux). All strains were stored at −70°C in cryogenic vials (Protect Technical Service Consultants Ltd., Heywood, United Kingdom) until they were ready for testing.

**Antifungal susceptibility testing.** Fluconazole MICs were determined by a broth microdilution technique by following the modified guidelines for the susceptibility testing of *C. neoformans* (9) that are described in document M27-A (15). MICs for serial isolates were determined on the same day. Fluconazole (Pfizer Central Research, Sandwich, United Kingdom) was dissolved in 100% dimethyl sulfoxide, and drug dilutions (final range, 0.12 to 64 μg/ml) were prepared in the yeast nitrogen base (YNB) medium (Difco). The YNB broth was supplemented with 0.5% glucose and was buffered to pH 7.0 with 0.005 M MOPS (morpholinepropanesulfonic acid; Sigma) (9). The inoculum was prepared by picking five colonies of 1 mm in diameter from 48-h-old cultures and suspending the colonies in 5 ml of sterile saline (0.85%). The resulting suspension was adjusted with the aid of a spectrophotometer to a cell density of a 0.5% McFarland standard at a wavelength of 530 nm; the adjusted stock inoculum suspension was diluted 1:100 in the medium. The final inoculum was approximately $10^4$ CFU/ml (9, 15). MICs were determined visually and spectrophotometrically after 48 and 72 h of incubation at 35°C. The visually determined MICs were the lowest drug concentrations that resulted in a prominent inhibition of growth (15). The spectrophotometrically determined MICs were determined by using a microdilution automatic reader at a wavelength of 420 nm (9, 15). Before carrying out the spectrophotometric evaluation of growth inhibition, the plates were shaken for 5 min at 60 rpm. The spectrophotometrically determined MICs were the lowest drug concentrations that resulted in 80% growth inhibition (9, 10, 15).

**Quality control.** Two reference isolates (*C. neoformans* ATCC 90112 and ATCC 90113) and the quality control isolates recommended by NCCLS (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were used as control strains. The reproducibility of the in vitro results was assessed by determining fluconazole MICs for all strains twice on two different days in the two laboratories (Medical Mycology Research Laboratory, Richmond, Va., and Microbiology Laboratory, Valme University Hospital, Seville, Spain).

**Statistical analysis.** Statistical analysis was conducted by using the SPSS statistical analysis system. Continuous variables were compared with the Kruskal-Wallis test for two groups. When the expected number of patients in any cell was less than five, categorical variables were then compared by either the $\chi^2$ test or Fisher's exact test. Correlations between continuous variables were assessed by the Pearson's correlation coefficient method. When *C. neoformans* was isolated from both the patient's blood and CSF, the lowest MIC was included in the analysis. CSF and serum cryptococcal antigen titers were not included in the analysis because the small number of patients precluded a valid evaluation and these results were not available for each patient. All statistical tests were two tailed, and a maximum $P$ value of 0.05 was considered statistically significant.

## RESULTS

**In vitro susceptibilities of *C. neoformans* to fluconazole.** Overall, the MICs obtained by the microdilution method used in this study were highly reproducible; 100% of the MICs obtained on different days and by the two laboratories differed by no more than one- to twofold dilutions. Although there was also good agreement (±1 dilution) between the visually and the spectrophotometrically determined MICs, as well as between the MICs at 48 and 72 h, the spectrophotometrically determined MICs obtained at 48 h were the values used for the statistical analyses. The spectrophotometrically determined MIC at which 50% of isolates are inhibited ($MIC_{50}$) and $MIC_{90}$ endpoints at 48 h were 4 and 16 μg/ml, respectively (MIC range, 0.25 to 64 μg/ml) (Table 2).

**Clinical response.** Table 1 depicts the laboratory findings for the 25 patients evaluated, and Table 2 describes the antifungal dosages, the length of therapy, and the corresponding clinical responses. Of the 25 patients studied, 4 (patients 21, 23, 24, and 25) died of active cryptococcal disease and 2 (patients 4 and 6) died of other causes. Eighteen of the 25 patients were cured after receiving fluconazole maintenance therapy (400 mg/day p.o.) for the complete 8- to 10-week course. This treatment was followed either by oral fluconazole indefinitely (200 mg/day) or by oral fluconazole indefinitely after receiving 200 to 400 mg of fluconazole per day p.o. on discharge; the same regimen was used for the treatment failure patients (Table 2). *C. neoformans* was recovered from the blood of 18 of the 20 (85.7%) patients who did not fail fluconazole therapy or whose death was attributed to some other cause; 14 of these patients had OPC prior to the cryptococcal disease. The statistical analysis did not reveal a correlation between a positive blood culture and the mortality rate ($P = 0.06$).

Therapeutic failure was observed in 5 of the 24 patients with AIDS (classification C3 of the Centers for Disease Control and Prevention; (patients 21 to 25, Tables 2 and 3); 4 of them had had OPC 1 year (patients 21, 22, and 24) or 4 months (patient 24) prior to the cryptococcal infection. Three of these five patients also had previously had cryptococcal infections and relapses while receiving fluconazole maintenance therapy (200 mg/day p.o.) for either meningitis (patients 23 and 24) or a pulmonary infection (patient 25). These three patients were treated for the first episode of cryptococcal infection until a final dose of 1 to 1.5 g of amphotericin B (0.5 mg/kg of body weight/day) was achieved. This regimen was followed by oral fluconazole treatment (at 400 mg/day for 8 to 10 weeks and then at 200 mg/day for 15 and 23 months for patients 23 and 25,

TABLE 2. In vitro susceptibilities of *C. neoformans* to fluconazole and details of treatments and clinical outcomes for 25 patients

| Patient no. | Fluconazole MIC (μg/ml) | Antifungal therapy[a] | Outcome of therapy |
|---|---|---|---|
| 1 | 0.25 | A + FL for 38 days and then FZ at 400 mg day on discharge (day 44) | Cure |
| 2 | 0.5 | A + FL for 7 days, then FZ at 400 mg/day for 7 days and then FZ at 400 mg on discharge | Cure |
| 3 | 1 | A + FL for 4 wk and then on day 7 FZ at 200 mg/day on discharge | Cure |
| 4 | 1 | A + FL for 7 days and then on day 7 FZ at 400 mg intravenously | Died on day 11 |
| 5 | 1 | A for 29 days and then FZ at 400 mg/day on discharge | Cure |
| 6 | 1 | A + FL for 24 h and then FZ at 400 mg/day | Died on day 3 |
| 7 | 2 | A for 10 days and then on day 10 FZ at 400 mg/day | Cure |
| 8 | 2 & 4[b] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 9 | 4 | A until a total dose of 1 g and then FZ at 400 mg/day[a] | Cure |
| 10 | 4 | A + FL for 8 days and then FZ at 400 mg/day on discharge | Cure |
| 11 | 4 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 12 | 4 | A + FL for 19 days and then FZ at 400 mg/day | Cure |
| 13 | 4 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 14 | 4 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 15 | 4 & 8[b] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 16 | 4 & 8[b] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 17 | 8 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 18 | 8 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 19 | 8 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 20 | 8 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Cure |
| 21 | 16 | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Failure (died, day 9) |
| 22[d] | 32 | A until a total dose of 1 g and then FZ at 400 mg/day | Failure |
| 23 | 8→32[e] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Failure (died, wk 6) |
| 24 | 8→64[e] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Failure (died, wk 10) |
| 25 | 4→64[e] | A until a total dose of 1 g and then FZ at 400 mg/day[c] | Failure (died, wk 5) |

[a] A, amphotericin B; FL, flucytosine; FZ, fluconazole.
[b] MICs for isolates from blood and CSF, respectively.
[c] Patients were treated until the total dose of amphotericin B was achieved; treatment with fluconazole at 400 mg/day was continued for 8 to 10 weeks, and then the patient was maintained on fluconazole at 200 mg/day p.o.
[d] Currently treated with itraconazole (600 mg/day p.o.).
[e] Fluconazole MIC for initial and relapse isolates, respectively.

respectively). Although good compliance was observed in patients 23 and 25, patient 24 had repeatedly stopped fluconazole therapy (200 mg/day p.o.) during six previous episodes of cryptococcal infection over 17 months. The CSF or serum cryptococcal antigen titers were >1:4,000 for these five treatment failure patients. *C. neoformans* was recovered from either blood (patient 21), lung tissue (patient 25), or CSF (patients 23 and 24) at the onset of the relapse episode and at autopsy. Patient 21 died in the first 2 weeks of fluconazole maintenance therapy, while patients 23, 24, and 25 died after 1 month. The other patient, who failed fluconazole maintenance therapy (patient 22), was treated with intravenous amphotericin B for 4 weeks, followed by oral fluconazole (400 mg/day) for 15 days. Because symptomatology persisted, this patient was switched to maintenance therapy with itraconazole (600 mg/day p.o.). The symptoms resolved and the patient remained well after 40 months. None of the patients were receiving other drugs known to modify the pharmacokinetics of fluconazole (e.g., rifampin or rifabutin).

**In vitro versus in vivo correlations.** Table 4 describes the clinical response to maintenance fluconazole therapy and its relation to MICs. Fluconazole MICs increased from 4 to >16 μg/ml for the *C. neoformans* isolate pairs (initial and relapse episode isolates) recovered from the three patients who relapsed (patients 23, 24, and 25); these values were obtained simultaneously (Table 2). When failure of fluconazole therapy was observed, the fluconazole MIC for the corresponding *C. neoformans* isolate was ≥16 μg/ml. On the other hand, MICs were <16 μg/ml for the isolates obtained from the 20 patients who either responded to fluconazole maintenance therapy (18 patients) or died of other causes (2 patients). There was a significant correlation between high MICs (≥16 μg/ml), the mortality rate ($P = 0.0009$), and the fluconazole treatment failure ($P = 0.000003$). However, there was no significant correlation between high MICs and the CD4 counts ($P = 0.31$). For isolates from patients with negative blood cultures, fluconazole MICs were higher than those for isolates from patients with positive blood cultures; that is, a correlation was found between the high fluconazole MICs and the negative blood culture ($P = 0.009$). We were unable to establish a significant difference between fluconazole MICs and treatment with amphotericin B either alone or in combination with flucytosine ($P = 0.16$).

## DISCUSSION

In the present study, we correlated the in vitro activity of *C. neoformans* with the clinical outcome after fluconazole maintenance therapy. Different approaches for the in vitro measurement of *C. neoformans* susceptibility to antifungal agents have been reported. Anaissie et al. (1) evaluated YNB, RPMI 1640 medium broth, and Eagle's minimum essential medium broth and found that RPMI 1640 broth was a suitable medium for susceptibility testing of *C. neoformans*. Franzot and Hamdan (8) corroborated these findings. RPMI 1640 medium is the standard broth recommended by NCCLS (15), and with this medium, MIC determinations require 72 h of incubation. Ghannoum et al. (9) tested 21 strains of *C. neoformans* by a microdilution technique with four broths, RPMI 1640, YNB (pH 7), synthetic amino acid medium-fungal (SAAMF), and YNB (pH 5.4), and three antifungal agents, amphotericin B, fluconazole, and flucytosine. Those investigators concluded

TABLE 3. Characteristics of the five patients with fluconazole maintenance therapy failure

| Characteristic | Value |
|---|---|
| Mean age (yr [range]) | 32 (22–47) |
| No. (%) of males | 4 (80) |
| Risk factor for HIV infection (no. [%] of patients) | |
| Intravenous drug use | 5 (100) |
| Male homosexual activity | 0 |
| Mean CD4 cell count (no. of cells/μl [range]) | 41.4 (139–0) |
| CDC[a] stage of AIDS (no. [%] of patients) | |
| B3 | 1 (20) |
| C3 | 4 (80) |
| Previous OPC (no. [%] of patients) | |
| Total | 4 (80) |
| Fluconazole therapy | 4 (80) |
| Previous CI[b] | |
| Total | 3 (60) |
| Fluconazole therapy | 3 (60) |
| Clinical outcome (no. [%] of patients) | |
| Death | 4 (80) |
| Survival | 1 (20) |

[a] CDC, Centers for Disease Control and Prevention.
[b] CI, cryptococcal infection.

that YNB (pH 5.4) enhanced the growth of *C. neoformans*, and because of that, spectrophotometrically determined MICs could be obtained at 48 h. These finding were confirmed by Jessup et al. (13). On the basis of these findings, we evaluated the in vitro activities of *C. neoformans* by this modified broth microdilution method, which is described in NCCLS document M27-A (15).

In HIV-infected patients with OPC, the clinical response to treatment with fluconazole therapy has been correlated with MIC results (15). However, studies that establish the value of MICs as predictors of the clinical response to therapy in patients with *C. neoformans* infections are scarce. Because of that, the establishment of interpretive breakpoints for this pathogen are not available. Casadevall et al. (4) found an increase in the fluconazole MICs for serial *C. neoformans* isolates that were recovered from five patients with recurrent cryptococcal meningitis. Those investigators, however, neither described their clinical findings nor followed a standardized procedure. Paugam et al. (16) and Birley et al. (3) reported clinical and in vitro fluconazole resistance in three AIDS patients with recurrent cryptococcal meningitis (increases in MICs from 4 to 64, 16 to 128, and 0.25 to 16 μg/ml, respectively). Armengou et al. (2) described another possible case of fluconazole resistance development (an increase in the MIC to 64 μg/ml) during suppressive therapy in patients with AIDS-associated cryptococcal meningitis. Most MICs were determined by microdilution methods (7, 11, 14) in those studies (2, 3, 13, 16), as they were in the present study. However, although we used different testing parameters, an association of high fluconazole MICs (≥16 μg/ml) with a lack of response to therapy was as evident in our study as it has been in those previous studies (2, 3, 13, 16).

Witt et al. (22) determined the role of the in vitro susceptibility of cryptococci to fluconazole and other clinical variables as predictors of treatment failure in 76 patients with acute AIDS-associated cryptococcal meningitis. These patients received initial therapy with fluconazole alone or in combination with flucytosine. The mean log fluconazole MIC for the isolates associated with failure was significantly higher ($P = 0.012$) when MICs were determined with YNB medium than when MICs were determined by the NCCLS macrodilution method with RPMI 1640 medium (22). Witt et al. (22) concluded that MIC data can predict the clinical outcome only when the patient's clinical data are also incorporated into the overall strategic analysis. Therefore, in addition to high fluconazole MICs, those investigators associated clinical failure with initial positive blood and urine cultures, high serum and CSF cryptococcal antigen titers, and the use of oral fluconazole therapy alone (or without concomitant flucytosine therapy).

In our study, CSF and serum cryptococcal antigen levels were higher for the 5 patients with treatment failure than for the other 20 patients. The fluconazole MICs for the infecting isolates from the five treatment failure patients were ≥16 μg/ml, while the fluconazole MICs for the other patients' isolates were lower (<16 μg/ml). However, only one of these five patients had a positive blood culture. Witt et al. (22) found that the probability of treatment failure could be >40% for a patient who has a negative blood culture, who does not receive flucytosine therapy, and for whose infecting isolate the MIC is ≥16 μg/ml. In our study, the probability of treatment failure was higher (100%). These discrepant results could be due to the smaller number of patients in our study: 25 versus the 76 patients evaluated by Witt et al. (22); also, the initial therapy used in both studies was different. In addition, our conclusions are based on a retrospective analysis of the data, which precluded the measurement of fluconazole levels in serum and CSF and thus limited our ability to delineate the relationship between fluconazole dosage, the resulting drug levels in serum and CSF, and treatment outcome.

In conclusion, it appears that a more positive clinical response to fluconazole maintenance therapy could be expected when the fluconazole MIC is <16 μg/ml than when the MIC is ≥16 μg/ml for the infecting *C. neoformans* strain. These findings also suggest that MICs determined by the modified NCCLS M27-A microdilution method (15) evaluated in our study can potentially predict the clinical response to fluconazole maintenance therapy and may identify the potential clinical failures of fluconazole therapy. However, host factors, pharmacokinetic data, patient compliance, and other factors may also influence the clinical outcome of antimicrobial therapy (20). Our data indicate that fluconazole MICs should be determined for *C. neoformans* isolates recovered prior to and during fluconazole therapy, as well as when the patient is failing therapy. More studies are warranted to further deter-

TABLE 4. Summary of patient's response to fluconazole and corresponding MIC data

| No. of patients | MIC (μg/ml)[a] | No. of patients | | |
|---|---|---|---|---|
| | | Cured | Failed | Died |
| 6 | ≤1 | 4 | 0 | 2[b] |
| 2 | 2 | 2 | 0 | 0 |
| 9 | 4 | 9 | 0 | 0 |
| 6 | 8 | 6 | 0 | 0 |
| 5 | ≥16 | 0 | 1 | 4[c] |

[a] The MICs were obtained by the microdilution method with YNB and spectrophotometric reading at 48 h.
[b] These death's were not attributed to cryptococcal infection.
[c] Patients who died within first 10 weeks of therapy with positive cultures.

mine the relationship between this in vitro result and the clinical outcome of therapy.

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