Symptomatic Relapse of HIV-Associated Cryptococcal Meningitis after Initial Fluconazole Monotherapy: The Role of Fluconazole Resistance and Immune Reconstitution

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(See the editorial commentary by Hamill on pages 1074–6)

Background. Cryptococcal meningitis (CM) in South Africa is often treated with fluconazole as initial therapy. Surveillance data suggest that the prevalence of fluconazole-resistant CM is increasing, and expanding access to antiretroviral therapy is resulting in increasing recognition of immune reconstitution inflammatory syndrome. Therefore, we conducted a study to assess the contribution of these factors to CM relapse in this context.

Methods. Patients with symptomatic relapse of CM were prospectively identified at 2 hospitals in Cape Town, South Africa, during the period of 2003–2005. Patients met the following criteria: (1) a previous laboratory-confirmed episode of CM, with resolution of symptoms after treatment; (2) reported adherence to fluconazole treatment; (3) recurrence of typical CM symptoms; (4) cerebrospinal fluid antigen test and/or culture positive for *Cryptococcus neoformans;* and (5) no alternative diagnosis. Data on patients' human immunodeficiency virus (HIV) and CM infections and treatment were collected and analyzed.

Results. Thirty-two episodes of relapse occurred among 27 patients. Episodes were classified into 3 groups: culture-positive episodes in antiretroviral therapy–naive patients (6 episodes), culture-positive episodes in patients receiving antiretroviral therapy (15 episodes), and culture-negative episodes in patients receiving antiretroviral therapy (11 episodes). Seventy-six percent of culture-positive relapses were associated with isolates that had reduced susceptibility to fluconazole. Drug-resistant cases required prolonged intravenous therapy with amphotericin B, and despite this treatment, the mortality rate was high (54% at a median of 6 months of follow-up). Despite a long interval between initiation of antifungal therapy and initiation of antiretroviral therapy (median interval, 144 days), immune reconstitution inflammatory syndrome contributed to at least one-third of relapses.

Conclusions. After initial treatment with fluconazole, relapses of symptomatic CM are often associated with fluconazole resistance and immune reconstitution inflammatory syndrome. These data add to concern about the efficacy of fluconazole, compared with amphotericin B, for initial treatment of HIV-associated CM.

As a consequence of the HIV epidemic in sub-Saharan Africa, *Cryptococcus neoformans* has become the leading cause of community-acquired meningitis in many areas [1], accounting for 13%–44% of all deaths in 3 HIV-seropositive cohorts [2–4]. At GF Jooste Hospital (Cape Town, South Africa), which is 1 of the 2 facilities where this study was conducted and which serves a population of 1.3 million people (seroprevalence of HIV infection, 11%) [5], cryptococcal meningitis (CM) is common, and the incidence is still increasing: in 2005, a mean of 12 India ink–positive cases were detected per month, compared with 9 cases per month in 2004 and 7 cases per month in 2003 (authors' unpublished data).

In much of the developing world, use of the fungicidal combination of amphotericin B and flucytosine is precluded by cost, availability, and difficulty of administration and monitoring [6]. Thus, during the pe-

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riod of this study (February 2003 through July 2005), fluconazole monotherapy (400 mg daily), although it takes longer to sterilize the CSF [7, 8], was the standard initial therapy for CM at the study sites, as it remains in many centers in Africa. Antiretroviral therapy (ART) became increasingly available through a public sector program starting in April 2004.

In the developed world, where amphotericin B–based regimens are the standard initial therapy, annual relapse rates for cryptococcal disease during maintenance fluconazole treatment are <5% [9–11], even in the absence of ART. There are few data on CM relapse in the era of ART or after initial therapy with fluconazole. Since 2000, when fluconazole became widely available in the public sector through the Diflucan donation program, we have noted symptomatic relapses of CM due to fluconazole-resistant strains of *C. neoformans.* Furthermore, surveillance data from South Africa have revealed an increase in the percentage of *C. neoformans* isolates with fluconazole resistance (MIC, $\geqslant$64 μg/mL), from 0% in 1999 (when 233 isolates were evaluated) to 12.7% in 2004 (when 63 isolates were evaluated) [12] (Pfizer, personal communication). Of concern, similar trends have been noted in some other developing countries [13, 14], although not on a worldwide basis [15].

Therefore, during the period from February 2003 through July 2005, we prospectively monitored all patients with CM admitted with symptomatic relapse, to determine the frequency of fluconazole resistance in such relapses and to explore risk factors for (and the outcome of) fluconazole-resistant *C. neoformans* infection. In addition, we noted the timing and culture results for symptomatic relapses as they related to the commencement of ART.

METHODS

The study was approved by the University of Cape Town Research Ethics Committee. During the period from February 2003 through July 2005, all cases of cryptococcal meningitis with symptomatic relapse were prospectively recorded at 2 Cape Town referral hospitals: Groote Schuur Hospital and GF Jooste Hospital. Cases of relapse were defined as occurring in patients with the following characteristics: (1) a previous laboratory-confirmed episode of CM, with resolution of symptoms for $\geqslant$1 month after treatment; (2) reported adherence to fluconazole treatment; (3) recurrence of typical CM symptoms; (4) CSF antigen test and/or culture positive for *C. neoformans;* and (5) no alternative diagnosis.

For identified cases, data were collected on aspects of HIV infection (date of commencement of ART and CD4 cell count and viral load before the commencement of ART and during the relapse) and on the first and relapse episodes of CM (date of the episode, symptoms and signs of relapse, CSF microscopy findings, culture results, susceptibility of isolates to fluconazole, management of the case [admission duration, drug treatment, concomitant medication, and treatment with steroids], and clinical outcome).

Relapses were classified into episodes that occurred before the commencement of ART ("ART-naive," all of which occurred in patients with positive culture results) and episodes that occurred after the commencement of ART ("on-ART"), with the second group subdivided into culture-positive and culture-negative. The latter were separately categorized according to whether they conformed to a definition of cryptococcal meningitis immune reconstitution inflammatory syndrome (CM-IRIS) [16]. A patient could be classified as having >1 relapse episode, provided that the 2 episodes belonged to separate relapse categories and that there had been a complete resolution of symptoms for at least 30 days in the interim period. For patients who experienced a relapse before receiving ART, the time intervals from the first episode to the relapse episode, as well as from the relapse episode to the commencement of ART, were calculated. For those patients who experienced relapse while receiving ART, the intervals from the first episode to the commencement of ART, as well as from the commencement of ART to the relapse episode, were calculated.

During the duration of this study, fluconazole MICs were determined for all *C. neoformans* isolates obtained at Groote Schuur Hospital and all relapse isolates from GF Jooste Hospital at the Groote Schuur laboratory using Etests (AB Biodisk). The laboratory is an accredited laboratory, was a participating ARTEMIS study site during 1999–2003 [12], and is subject to regular internal and external quality control. Etests have been shown to yield results in good agreement with the broth microdilution method [17]. Plates were incubated at 35°C for 48 h, and MICs were the lowest concentration at which the elliptical inhibition zone intercepted the scale on the strip. Results were interpreted on the basis of the following criteria, keeping in mind that cutoff values for *C. neoformans* have not yet been established [18–20]: susceptible, MIC of $\leqslant$8 μg/mL; dose-dependent susceptibility, MIC of 16–32 μg/mL; and resistant, MIC of $\geqslant$64 μg/mL.

Data were analyzed using Stata software, version 8 (StataCorp). Continuous variables were reported as median values with interquartile ranges, and differences between such variables were tested using a 2-sample rank sum test. Differences in proportion were tested using Fisher's exact test.

RESULTS

During the 30 months of surveillance, a total of 27 patients fitted the case definition, among whom there were 32 distinct relapse episodes: 6 episodes in the ART-naive, culture-positive group; 15 in the on-ART, culture-positive group; and 11 in the on-ART, culture-negative group. Five patients had 2 relapse episodes each: 4 had relapse episodes both before and on ART,

and 1 patient had distinct culture-negative followed by culture-positive relapse episodes on ART.

Two of the 27 patients were receiving ART at the time of their initial CM diagnosis; the other 25 started ART subsequently. The first episode of CM had been treated with fluconazole in 26 of 27 patients. For the 25 patients who were not already receiving ART, the median interval from the first episode to the commencement of ART was 146 days. For all patients, the median CD4 cell count at the commencement of ART was 27 cells/μL (interquartile range, 14–44 cells/μL), and the median viral load was 271,000 copies/mL (interquartile range, 59,000–455,000 copies/mL). Response to ART was good for 14 of 15 patients tested, who achieved a viral load of <50 copies/mL and an increase in the CD4 cell count to a median of 108 cells/μL after 6 months of therapy.

For 16 of 21 culture-positive relapse episodes, *C. neoformans* isolates were shown to have dose-dependent susceptibility (2 isolates) or resistance (14 isolates) to fluconazole. Twelve isolates (recovered from 10 patients) had MICs $\geqslant$256 μg/mL (figure 1). Seven (44%) of 16 patients who experienced relapses involving dose-dependent susceptible or resistant isolates had been receiving concomitant rifampicin as part of tuberculosis treatment, compared with none of the 4 patients who experienced relapses involving susceptible isolates, although this did not reach statistical significance ($P = .15$, by Fisher's exact test). Most of the patients with culture-positive relapses received prolonged daily treatment with amphotericin B (up to 42 days), followed by weekly doses of amphotericin B, unless testing confirmed susceptibility to fluconazole. Patients with culture-negative cases continued to receive fluconazole, the dosage of which was sometimes increased to 800 mg daily.

For patients who experienced relapse while receiving ART, the median interval from the start of ART to relapse was <2 months (27 days for culture-negative cases and 55 days for culture-positive cases; the difference was not significant) (table 1). CSF total WBC count, lymphocyte count, polymorph count, protein level, and glucose level did not differ significantly between first episodes and on-ART relapse episodes. Five of 15 culture-positive relapses and 6 of 11 culture-negative relapses during ART occurred in patients who had received steroid treatment because of the severity of symptoms and the presumption that IRIS was playing a role. There was no difference in mortality between patients treated with steroids (4 [36%] of 11 died) and those who were not so treated (4 [27%] of 15 died).

Relapse episodes, especially when they were culture positive, required prolonged hospitalization (the median duration of stay was 5 days for the first episode versus 28 days for all relapses; $P = .002$, by 2-sample rank sum test) (table 1). The median duration of follow-up was 6 months (interquartile range, 4–10 months). Nine (33%) of 27 patients died. Almost all of the deaths (7 of 9) occurred in the fluconazole-resistant group, and most (7 of 9) were directly attributable to CM. Seven (54%) of 13 patients relapsing with fluconazole-resistant isolates died.

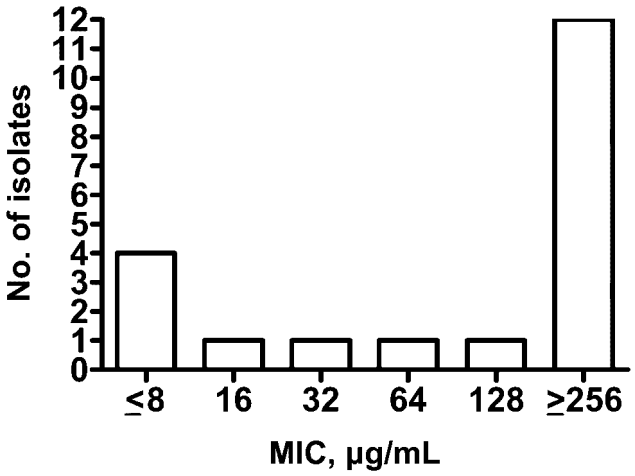

Figure 1. Graph showing MICs of fluconazole for 20 *Cryptococcus neoformans* isolates recovered from patients with culture-positive relapse of cryptococcal meningitis. For 1 isolate from a patient with culture-positive relapse, sensitivity data were not available.

DISCUSSION

Symptomatic relapse of CM prompting referral to the hospital occurred at a rate of ~1 case per month at our health care centers, even when relapses clearly associated with nonadherence to maintenance fluconazole therapy were excluded. In a setting where initial therapy consisted of fluconazole and where ART was increasingly available, we found that approximately two-thirds of such relapses were culture positive, and 76% of these were associated with *C. neoformans* isolates with reduced susceptibility to fluconazole. At least one-third of relapses were consistent with culture-negative CM-IRIS.

To our knowledge, this is the largest study to date of fluconazole-resistant cases of CM [21–25], and the results are consistent with our clinical impression that such cases were increasing in South Africa, as well as with national fungal susceptibility surveillance data. In 3 patients who were infected with isolates that had reduced susceptibility, for whom susceptibility testing was performed on the initial isolate (a practice at Groote Schuur Hospital), all 3 initial isolates were fully susceptible to fluconazole, demonstrating that the resistance was secondary. Rifampicin induces fluconazole metabolism [26–28], and 44% of patients infected with fluconazole-resistant isolates had been receiving concurrent rifampicin without adjustment of fluconazole dose. However, tuberculosis very frequently precedes CM, such that up to one-third of all patients with HIV-associated CM in South Africa are receiving antituberculous medication when CM is diagnosed [29] (authors' unpublished data). Thus, how important a role this played in the development of resistance in our study

Table 1. Time intervals and outcomes for episodes of symptomatic cryptococcal meningitis (CM) relapse.

Relapse group	No. of episodes	Median duration (interquartile range)					No. of patients	
		First CM episode to relapse CM days	First CM to ART, days	ART to relapse, days	Hospitalization, days	Follow-up, months	Alive with neurological sequelae[a]	Died
ART-naive, culture positive	6	96 (83–148)	182 (148–222)	…	68 (39–92)	9 (6–12)	0	5
ART recipients								
Culture negative	11[b]	…	144 (86–389)	27 (14–50)	21 (13–28)	5 (2–10)	2	3
Culture positive	15[c]	…	107 (52–218)	55 (14–121)	40 (18–58)	6 (4–9)	0	5

NOTE. ART, antiretroviral therapy.

[a] Visual impairment and cerebellar dysfunction.

[b] Includes 2 patients who experienced prior relapses before commencing ART, 1 of whom died.

[c] Includes 2 patients who experienced prior relapses before commencing ART, both of whom died, and 1 patient who experienced a previous culture-negative relapse while receiving ART and who died.

is unclear. More than one-half of fluconazole-resistant isolates developed in patients who had never received rifampicin. Fluconazole resistance resulted in the necessity for costly and nephrotoxic, prolonged intravenous therapy with amphotericin B; furthermore, despite the administration of amphotericin B therapy, the mortality rate was high (>50% at a median of 6 months of follow-up).

The defining features of IRIS are a temporal relationship to the commencement of ART and evidence of immune restoration and viral suppression. The 11 culture-negative relapses that occurred during ART would fit this definition: all were associated with viral suppression and an increase in the CD4 cell count. CSF parameters did not differ significantly in episodes of CM-IRIS from those in the first episodes of CM. Some previous reports [30], but not all [16], have found a significant increase in the CSF WBC count in patients with CM-IRIS. During ART, relapses occurred early (a median of 1–2 months after the commencement of ART), which is in agreement with the findings of Shelburne et al. [30].

Immune reconstitution may also be a contributing factor in the pathogenesis and presentation of culture-positive relapses during ART [31]. All patients who experienced culture-positive relapse while receiving ART were asymptomatic when they started ART but experienced relapse soon thereafter, coincident with immune restoration and viral suppression. The overlap of positive culture results with drug resistance and IRIS has been described previously for tuberculosis [32].

Fluconazole, an essentially fungistatic agent, takes longer to sterilize the CSF in CM than do amphotericin B–based regimens [7, 8]. In an ongoing prospective study, we have found that the number of *C. neoformans* colony-forming units at baseline in CSF specimens obtained from patients with CM in South Africa is similar to the high counts previously reported in patients in Thailand [33], and the rate of decrease in the number of colony-forming units over the first 2 weeks of treatment with fluconazole is much slower than with amphotericin B (authors' unpublished data). This combination of factors, perhaps exacerbated by frequent concurrent rifampicin treatment, favors the development of drug resistance, and it may also predispose patients to a high rate of IRIS because of ongoing active infection or a high residual antigen load at the time that ART is started. Studies have demonstrated that a higher risk of CM-IRIS occurs with a high initial organism load, as assessed by CSF antigen titer, and persistently positive CSF culture results at 2 weeks [16, 30]. Four of the 6 patients who experienced culture-positive relapses before they started ART had a second relapse (2 culture-positive relapses and 2 culture-negative relapses) after the initiation of ART, perhaps as a consequence of prolonged high organism load. The study period spanned the introduction of ART to the public sector in South Africa, explaining the relatively long delay between the onset of CM and the commencement of ART. Risk of CM-IRIS is also increased when ART is started within 1 month of antifungal therapy [16, 30]. In our series, despite the delay in ART, IRIS reactions appeared to be common.

Since the time of this study, aided by successful lobbying to reduce the cost of amphotericin B in South Africa [6], a policy of administering amphotericin B (1 mg/kg per day for 7–14 days or until clinical response) has been adopted as initial therapy for CM at GF Jooste Hospital. In addition, awareness of the need to increase the fluconazole dose when rifampicin treatment is being administered concurrently has been raised in the primary HIV clinics. It is hoped that the use of initial amphotericin B therapy will reduce the incidence of fluconazole-resistant CM. (At the time of writing, 1 year after implementation of this policy, we have not seen secondary fluconazole resistance in any patients receiving initial therapy with amphotericin B). Higher-dose fluconazole for initial therapy may be another means of reducing secondary fluconazole resistance in health care centers where amphotericin B is not available. Given the inconvenience and high relapse rates associated with weekly administration of amphotericin B [10], research into the ef-

fectiveness of voriconazole for maintenance treatment of patients who receive a diagnosis of fluconazole-resistant CM is needed, as are pharmacokinetic studies to explore the feasibility of the use of voriconazole with selected ART regimens, although concurrent rifampicin treatment would preclude use of voriconazole in some patients. The risk of fluconazole resistance and possible predisposition to IRIS add to concerns about the efficacy of fluconazole monotherapy, compared with amphotericin B therapy, if this is locally feasible for initial therapy for HIV-associated CM.

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