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Results Obtained with Various Antifungal Susceptibility Testing Methods Do Not Predict Early Clinical Outcome in Patients with Cryptococcosis

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The in vitro susceptibilities of *Cryptococcus neoformans* isolates from consecutive human immunodeficiency virus-positive and -negative patients to the antifungal agents fluconazole, amphotericin B, and flucytosine were determined by different techniques, including the CLSI method, Etest, and broth microdilution in yeast nitrogen base (YNB) medium, during a multicenter prospective study in France. The relationship between the in vitro data and the clinical outcome 2 weeks after the initiation of antifungal therapy was assessed. In addition, the correlation between the strain serotype and the in vitro activities of the antifungals was determined, and the susceptibility results obtained with the different techniques were also compared. Thirty-seven patients received a combination of amphotericin B with flucytosine as first-line therapy, 22 were treated with amphotericin B alone, and 15 received fluconazole alone. Whatever the antifungal tested, there was no trend toward higher MICs for strains isolated from patients who failed to respond to a given therapy compared to those from patients who did not with either the CLSI method, Etest, or broth microdilution in YNB medium. The MICs obtained by the CLSI or Etest method were significantly lower for serotype D strains than for serotype A strains for both fluconazole and amphotericin B, while flucytosine MICs were not different according to serotype. These findings suggest that the in vitro antifungal susceptibility of *C. neoformans*, as determined with the techniques used, is not able to predict the early clinical outcome in patients with cryptococcosis.

Cryptococcus neoformans is an encapsulated yeast responsible for severe infection, particularly in immunocompromised patients. Before the AIDS epidemic, cryptococcosis was a rare disease, but it became one of the major opportunistic infections in human immunodeficiency virus (HIV)-positive patients (6). Although the epidemiology of the disease has been largely modified with the introduction of highly active antiretroviral therapy in Western countries, with a dramatic decrease in the incidence, some factors, such as an African origin or heterosexually acquired HIV infection, are still associated with an increased risk of cryptococcosis (7, 17). In the absence of antifungal therapy, disseminated cryptococcosis is uniformly fatal. Treatment strategies have largely been evaluated, and standardized guidelines for management of the disease are available (22). Nevertheless, despite appropriate treatment, a persistent mortality rate as high as 17% has been evidenced during the first weeks after the initiation of antifungal therapy in HIV-infected patients (O. Lortholary, C. Droz, K. Sitbon, V. Zeller, S. Neuville, M. Alvarez, A. Boisbieux, F. Botterel, P. Dellamonica, F. Dromer, and G. Chêne, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1752, 2003).

Although the in vitro "resistance" of the infecting *C. neoformans* strain has been linked to clinical failure during the course of the disease in some case reports (18), only a limited number of studies have assessed the potential relationship between the in vitro susceptibility results obtained at the time of diagnosis and the clinical outcome of cryptococcosis (1, 11, 16, 20, 21, 30). Moreover, most of these studies have evaluated the clinical efficacy after 10 weeks of therapy, and it is still largely unknown if the MICs of the major antifungals used during the initial treatment of cryptococcosis could be used as predictors of the early clinical outcome. The difficulties of correlation of the in vitro susceptibility results with treatment efficacy are probably related, at least in part, to the absence of a well-standardized technique for the in vitro antifungal susceptibility testing of *C. neoformans* (19). Although the standardized CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS) methodology for susceptibility testing of *Candida* spp. has been shown to be reliable and clinically useful, at least for superficial infections in HIV-infected patients, there are still technical problems for *C. neoformans* (4).

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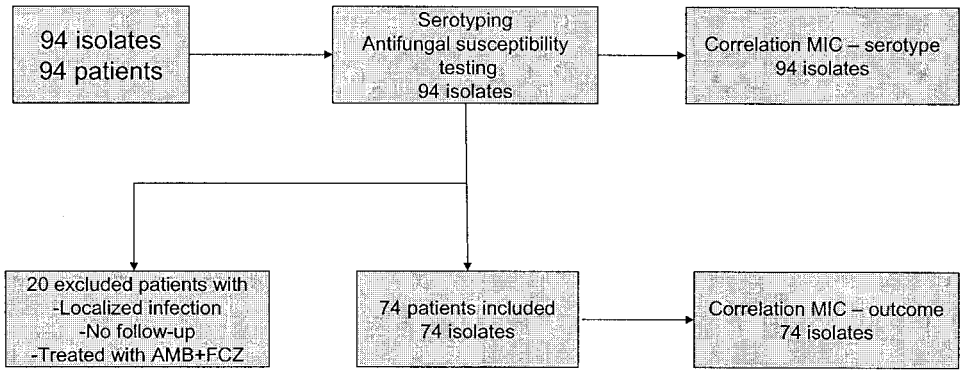

FIG. 1. Study design. AMB, amphotericin B; FCZ, fluconazole.

Indeed, modifications of the CLSI technique (9) and alternative methods have been proposed (15), but the available data are still limited.

We designed a prospective study in France (Crypto A/D) that was conducted between 1997 and 2001 and that included all HIV-positive or HIV-negative patients with a first episode of culture-confirmed cryptococcosis at any clinical site. For all these patients, clinical information was available at the baseline and for up to 3 months after the initiation of antifungal therapy, and the infecting isolate(s) of *C. neoformans* was sent to the National Reference Center for Mycoses (NRCM).

The aim of the present study was to evaluate the relationship between the in vitro activities of amphotericin B, fluconazole, and flucytosine against *C. neoformans* isolates recovered before the initiation of antifungal therapy and the early clinical outcome in patients treated with these antifungal agents. Antifungal susceptibility testing was performed with different methods, including dilution and diffusion tests. The susceptibility results obtained with these different techniques were also compared, and the correlation between strain serotype and the in vitro activities of antifungals was determined.

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MATERIALS AND METHODS

Patients and study design. Ninety-four consecutive patients with culture-proven cryptococcosis were included in a national multicenter prospective study (Crypto A/D) conducted in France between 1997 and 2001 (12). For each patient, at least one *C. neoformans* isolate was recovered at the time of diagnosis. The isolates were sent to NRCM, where confirmation of the identification, serotyping (5), and antifungal susceptibility testing were performed for one strain per patient (Fig. 1). Among the 94 isolates, 65 isolates were serotype A, 28 isolates were serotype D, and 1 isolate was serotype B. Among the 94 patients, 20 were excluded (patients with localized infection, patients with no follow-up, and those who received amphotericin B in combination with fluconazole during the initial treatment course). Therefore, 74 patients were included in the analysis (Fig. 1) for the correlation between MICs and clinical outcome (74 isolates, 1 isolate per patient). Demographic information and clinical data, including antifungal treatment for the current episode were obtained. Meningitis was defined as positive cerebrospinal fluid (CSF) (i.e., a positive culture for *C. neoformans* and/or a positive India ink examination and/or antigen detection). Dissemination was defined as the isolation of *C. neoformans* from two or more noncontiguous sites. Follow-up of the patients, including cultures of specimens from all sites infected at the baseline, was conducted after 2 weeks (Wk2) to assess the early outcome of the disease and again after 3 months (M3). At first, treatment success was defined by sterilization at Wk2 of all initially infected body sites and failure was defined by death or the persistence of the organism with at least one positive culture at Wk2. A preliminary analysis of the results by use of this very stringent microbiological outcome failed to show any correlation between in vitro susceptibility data and the clinical outcome (data not shown). Due to the subacute nature of the infection and the immunocompromised status of the patients included (81% of patients were HIV positive), a more clinical outcome rather than a microbiological outcome was considered to be potentially more relevant. For this reason, treatment success was then defined as the absence of clinical signs of infection (fever, meningitis, neurological disorders, and abnormal mental status) and failure was defined by death or the persistence of clinical signs at Wk2. Failure at M3 was defined by death or the persistence of a positive culture. The demographic, clinical, and therapeutic data for the patients are presented in Table 1. Fifty percent of the patients received a combination of amphotericin B and flucytosine as first-line therapy. Among the other patients, 60% were treated with amphotericin B alone and 40% were treated with fluconazole alone. There were no differences between the treatment groups in terms of the sex ratio, clinical presentation, and the proportion of cases of meningitis (as determined by the chi-square test). Overall, the early outcome, 2 weeks after the initiation of therapy, was considered a success in 55% of the patients.

Antifungal susceptibility testing. Testing of the susceptibilities of 94 strains of *C. neoformans* to fluconazole, amphotericin B, and flucytosine was performed at

TABLE 1. Demographic, clinical, and therapeutic data for 74 patients with a first episode of culture-confirmed cryptococcosis

Characteristic	No. (%) of patients in the different treatment groups[c]			
	AMB alone (n = 22)	FCZ alone (n = 15)	AMB + 5FC (n = 37)	All (n = 74)
Sex				
Male	21 (95)	12 (80)	31 (84)	64 (86)
Female	1 (5)	3 (20)	6 (16)	10 (14)
HIV infected	20 (91)	10 (67)	30 (81)	60 (81)
Fungal infection[a]				
Meningitis	21 (95)	12 (80)	36 (97)	69 (93)
Dissemination	15 (68)	11 (73)	27 (73)	53 (72)
Treatment outcome[b]				
Success	11 (50)	11 (73)	19 (51)	41 (55)
Failure	11 (50)	4 (27)	18 (49)	33 (45)

[a] Meningitis was defined as positive CSF, as detected by a positive culture of *C. neoformans*, positive India ink examination, and/or positive antigen detection. Dissemination was defined as the isolation of *C. neoformans* from two or more noncontiguous sites.

[b] Treatment success was defined as the absence of clinical signs of infection (fever, meningitis, neurological disorders, and abnormal mental status) and failure by death or persistence of clinical signs at Wk2.

[c] AMB, amphotericin B; FCZ, fluconazole; 5FC, flucytosine.

TABLE 2. Susceptibilities of 94 clinical strains of *C. neoformans* to fluconazole, amphotericin B, and flucytosine determined by CLSI methodology and Etest[a]

Parameter	FCZ[b]		AMB[b]		5FC	
	CLSI	Etest	CLSI	Etest	CLSI	Etest
Minimum MIC (μg/ml)	0.12	0.015	0.03	0.002	0.12	0.03
Maximum MIC (μg/ml)	4	256	2	0.25	64	64
Geometric mean MIC (μg/ml)	0.7	9.5	0.26	0.044	2.4	0.58
MIC_{50} (μg/ml)	1	8	0.25	0.06	2	0.5
MIC_{90} (μg/ml)	2	64	1	0.125	8	4

[a] Etest was performed on RPMI medium for fluconazole and amphotericin B and on semisynthetic medium for flucytosine. Etest MICs between twofold dilutions were converted to the next higher concentration. AMB, amphotericin B; FCZ, fluconazole; 5FC, flucytosine.

[b] Fluconazole MICs were lower with the CLSI methodology than with Etest on RPMI medium ($P < 0.001$), and amphotericin B MICs obtained with the CLSI technique were higher than those obtained with Etest on RPMI medium ($P < 0.001$).

three centers. The 94 strains were tested at NRCM (center A) by a broth microdilution technique (9), in Lyon (center B) by the CLSI methodology, and in Marseille (center C) by Etest. All strains were then tested in the same center by the given technique.

Broth microdilution tests. In center B, the standardized CLSI technique was used (4). Briefly, RPMI (Gibco BRL, Cergy Pontoise, France) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid was used with a final inoculum size of 0.5×10^3 to 2.5×10^3 CFU/ml. The final concentrations of the drugs were 0.12 to 64 μg/ml for fluconazole and flucytosine and 0.03 to 16 μg/ml for amphotericin B. Microplates were incubated at 35°C for 72 h, and the MICs were determined visually as complete inhibition for amphotericin B and as a prominent decrease in turbidity compared to that for the control growth for fluconazole and flucytosine.

In center A, a broth microdilution technique described previously (9) was used for fluconazole and amphotericin B. Microplates containing yeast nitrogen base (YNB; Difco, Detroit, MI) medium adjusted to pH 7.0 with a final inoculum size of 1×10^4 CFU/ml were incubated at 35°C, and the MICs were determined spectrophotometrically after 48 h of incubation. The final concentrations of the drugs were 0.12 to 64 μg/ml for fluconazole and 0.015 to 8 μg/ml for amphotericin B. The MIC endpoints that were used were defined as the lowest drug concentrations that gave 50% inhibition of growth compared to the growth for the drug-free control.

Agar diffusion test. In center C, Etest was performed according to the instructions of the manufacturer, but alternative culture media were also tested. Briefly, agar plates were inoculated with an inoculum adjusted to a no. 1 McFarland turbidity and were incubated at 35°C for 48 h; for flucytosine, however, the incubation was extended to 72 h. Amphotericin B and fluconazole were tested on RPMI agar plates (American Bioorganics, Buffalo, NY) at pH 7.0 and on Casitone agar (Bio-Rad, Marnes-la-Coquette, France) at pH 6.6. Flucytosine was tested on plates containing solidified RPMI at pH 7.0 or semisynthetic medium (SSM) at pH 5.6 (Bio-Rad). MICs were read at the point of 100%, 95%, and ca. 80% inhibition for amphotericin B, flucytosine, and fluconazole, respectively.

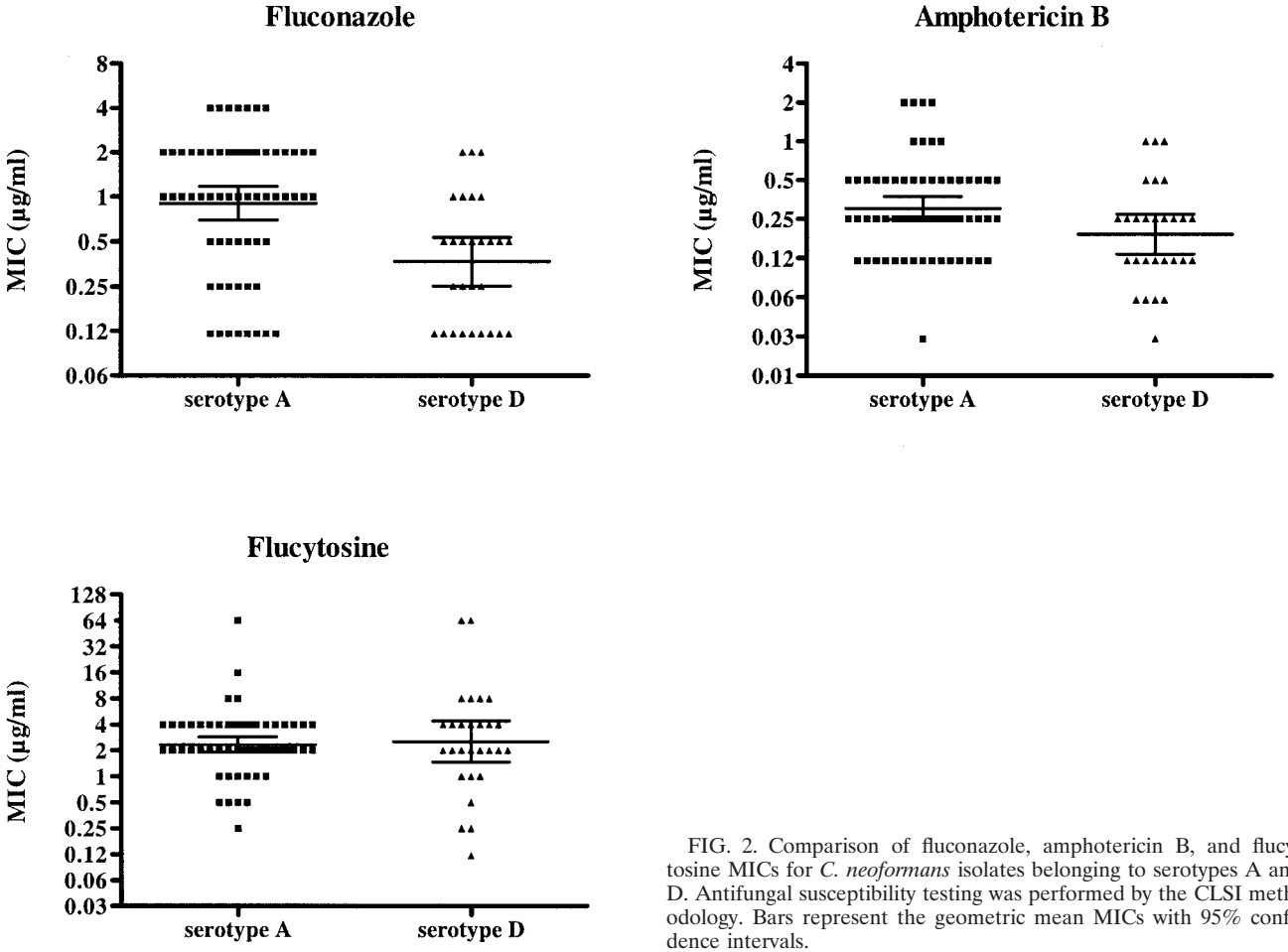

FIG. 2. Comparison of fluconazole, amphotericin B, and flucytosine MICs for *C. neoformans* isolates belonging to serotypes A and D. Antifungal susceptibility testing was performed by the CLSI methodology. Bars represent the geometric mean MICs with 95% confidence intervals.

Statistical analysis of results. Scattergrams of the MICs for both the treatment success and the treatment failure groups were generated, and the distributions of the MICs were compared by a nonparametric test (the Mann-Whitney test).

Statistical analyses were performed with GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, Calif.). Statistical significance was defined as a P value ≤ 0.05.

RESULTS

In vitro susceptibility. The susceptibilities of the 94 strains of *C. neoformans* to fluconazole, amphotericin B, and flucytosine determined by the CLSI M-27 methodology and Etest are presented in Table 2.

For fluconazole, the MIC_{90} was 5 $\log_2$ dilutions lower with the CLSI methodology than with Etest on RPMI medium ($P < 0.001$). In contrast, for amphotericin B, the MIC_{90} obtained with the CLSI technique was 3 $\log_2$ dilutions higher than the MIC_{90} obtained with Etest on RPMI medium ($P < 0.001$). Interestingly, for flucytosine, MICs determined by Etest were strikingly different depending on the medium used. While on SSM agar medium (pH 5.6) 90% of the isolates were inhibited by a concentration of 4 μg/ml (Table 2), 87% of the isolates had MICs >32 μg/ml when RPMI agar (pH 7.0), which is the medium recommended by the manufacturer, was used as the test medium (data not shown).

Relationship with serotype. The distributions of the MICs obtained by the CLSI method and Etest for the three antifungal drugs were also compared according to the serotypes of the isolates (Fig. 2). MICs obtained by the CLSI method were significantly lower for serotype D than for serotype A for both fluconazole (geometric mean MICs, 0.37 versus 0.90 μg/ml; $P = 0.0003$) and amphotericin B (geometric mean MICs, 0.19 versus 0.30 μg/ml; $P < 0.03$). The flucytosine MICs were not different according to the serotype. Similar results were observed when the MICs obtained by Etest were compared (data not shown).

Relationship with clinical outcome. The data for 74 patients were used to assess the relationship between MICs and early clinical outcome (Fig. 1). Table 3 summarizes the susceptibility testing results (geometric mean MICs) obtained by the CLSI method according to the clinical response at Wk2 for the three treatment groups. For none of the three drugs was there a relationship between the MIC distribution and the response to treatment. Likewise, analysis of the MIC distribution according to outcome was also performed for the other techniques. For fluconazole, although the MICs ranged over a large interval of $\log_2$ dilutions, there were no trends with CLSI (Table 3), Etest, or broth microdilution in YNB medium (Fig. 3) toward higher MICs for the strains isolated from 11 patients who responded to fluconazole therapy compared to those for the strains from 4 patients who did not. The MIC results for amphotericin B, obtained by the same three techniques, for the strains isolated from 59 patients treated with this antifungal drug either alone (22 patients) or in combination with flucytosine (37 patients) are shown in Table 3 and Fig. 4. There was no relationship between the in vitro susceptibilities of the strains and the clinical outcome. Among the 37 patients who were treated with flucytosine (in combination with amphotericin B), 18 failed to respond to therapy, but none of the methods used for susceptibility testing yielded results that predicted the clinical outcome (data not shown).

TABLE 3. Susceptibility testing results by CLSI method for *Cryptococcus neoformans* isolates from patients with subsequent success or failure of antifungal treatment after 2 weeks of antifungal therapy (74 strains)

Treatment and outcome[a]	No. of strains	Geometric mean MIC (μg/ml)[b]		
		AMB	FCZ	5FC
AMB alone				
Success	11	0.22		
Failure	11	0.39		
FCZ alone				
Success	11		0.56	
Failure	4		0.21	
AMB + 5FC				
Success	19	0.27		2.24
Failure	18	0.22		2.87

[a] Treatment success was defined as the absence of clinical signs of infection (fever, meningitis, neurological disorders, and abnormal mental status), and failure was defined by death or persistence of clinical signs at Wk2.
[b] AMB, amphotericin B; FCZ, fluconazole; 5FC, flucytosine.

Additionally, for all the patients, the correlation between the in vitro fluconazole MIC and the outcome at M3 was assessed. Fluconazole MICs, as determined by any of the three methods, were not significantly different for isolates from patients who had treatment failure at M3 compared to those for isolates from patients who were successfully treated.

DISCUSSION

The three drugs that are commonly used for the treatment of cases of cryptococcosis in humans are fluconazole, amphotericin B, and flucytosine. Moreover, flucytosine is never used as monotherapy because of the rapid emergence of resistance during therapy (10) and thus is used in combination, mostly with amphotericin B (22). For in vitro antifungal susceptibility testing of *C. neoformans*, no breakpoint is currently available for any drug, and the role of the susceptibility of the initial isolate as a predictor of both early and late clinical failure is, in fact, poorly understood. This study was designed to look for in vitro methods, including commercialized and reference techniques, able to show a relationship between in vitro results and clinical outcome. Indeed, it should be noted that several technical problems remain for the in vitro antifungal susceptibility testing of *C. neoformans*. Thus, modifications of the CLSI standardized method for yeasts have been proposed. In particular, the use of YNB medium instead of RPMI as the test medium may promote the growth of *C. neoformans* and improve the clinical relevance of MICs (9, 11, 30). Moreover, the CLSI methodology poorly identifies putatively amphotericin B-resistant strains, and breakpoints for this antifungal are not yet available (4). In a comparative study of different methods for the detection of strains with elevated amphotericin B MICs, it has been shown that Etest performed either on RPMI agar or on antibiotic medium 3 agar was the best technique for discrimination between amphotericin B-susceptible and -resistant clinical strains of *C. neoformans* (15). In the present study the MICs determined with YNB medium did not predict the early clinical outcome better than the MICs obtained with RPMI did. Agar-based techniques, such as Etest, have also

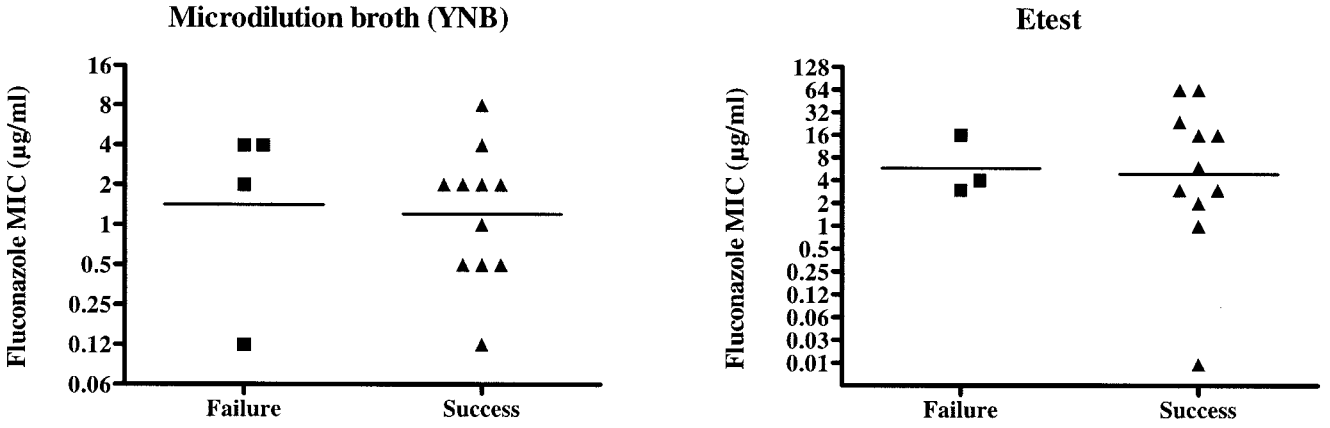

FIG. 3. Relationship between fluconazole MICs and early outcome in 15 patients treated with fluconazole alone according to the in vitro method used: the broth microdilution test in YNB medium or the agar diffusion test (Etest) on RPMI agar. The incubation time was 48 h, and the MIC endpoints were 50 and 80% of inhibition for the microdilution tests and Etest, respectively. The bars represent the geometric mean MICs.

been used for susceptibility testing of *C. neoformans*. In the present study, Etest on RPMI medium yielded higher MICs for fluconazole and lower MICs for amphotericin B than the CLSI method, and this is in agreement with the findings presented in previous reports (8, 29). In our hands, RPMI buffered to pH 7.0 was not a suitable medium for the testing of susceptibility to flucytosine with Etest, as the majority (87%) of the strains showed abnormally high MICs (>32 μg/ml) to this antifungal. Similar discrepancies between Etest and the broth microdilution technique have been reported previously, such as in a multicenter study that reported that Etest performed on RPMI misclassified *C. neoformans* as resistant to flucytosine in 22.5 to 60% of the cases (29). It seems that pH is a critical parameter for flucytosine susceptibility testing with both yeasts and some molds (26). In a recent study, the median MICs of flucytosine determined by Etest against 16 strains of *C. neoformans* were >32 μg/ml at pH 7.0 and 0.12 μg/ml at pH 5.4 (28).

Among *C. neoformans* strains, four main serotypes (serotypes A, B, C, and D) are recognized; and there are differences between serotypes A/D and serotypes B/C in terms of epidemiology, pathogenicity, and clinical manifestations. Nevertheless, few studies have evaluated the potential correlation between antifungal susceptibility and serotype. Overall, serotypes B and C have been reported to be less susceptible than serotypes A and D to amphotericin B (3), flucytosine (3, 25), and possibly, the azoles (2); but direct comparison of the susceptibilities between serotypes A and D has seldom been reported (27). In the present study flucytosine susceptibility was similar for serotypes A and D, but we found that serotype A was significantly less susceptible to amphotericin B and fluconazole than serotype D both by the CLSI method and by the Etest methodology. However, the clinical relevance of these findings remains to be determined.

Up to now, few studies have evaluated the relationship between the in vitro susceptibility of *C. neoformans* and the clinical outcome. In the present work, we used different formats, including commercialized techniques, such as Etest, to test isolate susceptibilities to amphotericin B, fluconazole, and flucytosine. Nevertheless, no relationship between in vitro data and early outcome was found for any of the three drugs.

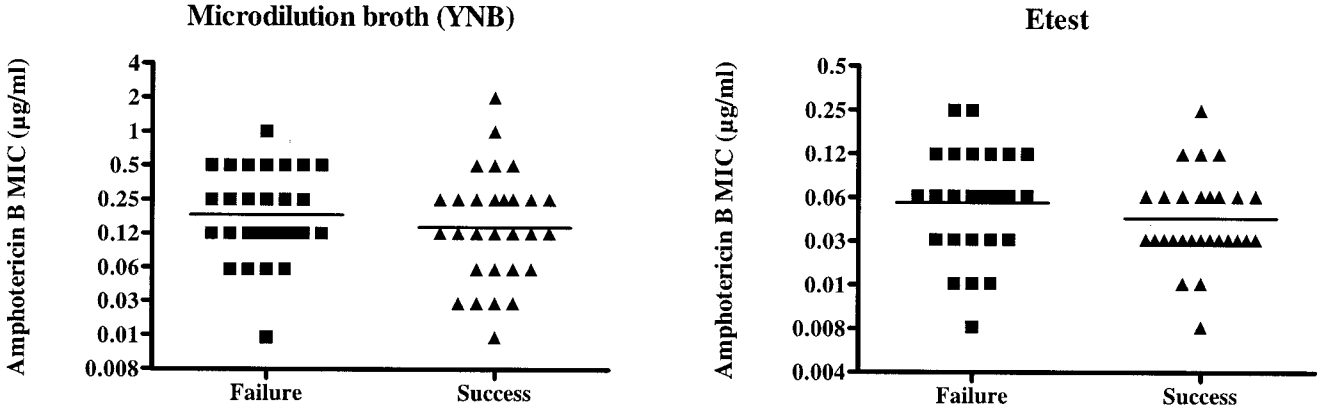

FIG. 4. Relationship between amphotericin B MICs and early outcome in 56 patients treated with amphotericin B alone or in combination with flucytosine according to the in vitro method used: broth microdilution test in YNB medium or agar diffusion test (Etest) on RPMI agar. The incubation time was 48 h, and the MIC endpoints were 50 and 100% inhibition for the microdilution test and Etest, respectively. The bars represent the geometric mean MICs.

For fluconazole, a recent experimental study showed a correspondence of in vitro and in vivo dose-response curves for one isolate of *C. neoformans* (13). Nevertheless, it has also been shown in an experimental model of disseminated cryptococcosis that factors other than the MIC, such as the size of the fungal burden in the brain, influenced the in vivo efficacy of fluconazole (14). In clinical studies, conflicting results have been obtained. In one study there was no relationship between MICs and the time to sterilization of the CSF culture in patients treated with fluconazole, although there was a trends toward a more rapid sterilization of CSF when the fluconazole MIC of the infecting strain was <4 μg/ml (16). In another study (30), although fluconazole MICs were not predictive of the outcome when the CLSI technique was used, a relationship was observed when MICs, determined in YNB medium, were used in the analysis along with clinical data. Similarly, it was shown (11) that MICs determined by the CLSI method were not predictive of the clinical outcome after 10 weeks of fluconazole therapy, but there was a trend toward a better outcome in patients infected with strains exhibiting low MICs by the YNB medium-based method. More recently, a better clinical outcome was observed after 10 weeks of maintenance therapy with fluconazole when the fluconazole MIC of the initial strain was <16 μg/ml, as determined in YNB medium (1). Here we evaluated the clinical outcome (2 weeks after the initiation of therapy) and showed that there was no relationship between the in vitro activity of fluconazole, as determined by different techniques, and its therapeutic efficacy. Patients with treatment failure received fluconazole dosage (between the initiation of therapy and Wk2) similar to those received by patients with favorable outcomes. There are several potential reasons for this lack of a correlation. First, the sample was probably too small for evaluation, as only 15 patients were treated with fluconazole alone, and this limited the power of the study. Second, the endpoint was earlier than that used in other studies. Although two different endpoints were used (microbiological and clinical), the microbiological clearance at 2 weeks might be too stringent for this population of immunosuppressed patients, especially for a static agent such as fluconazole. In addition, clinical improvement of cryptococcal meningitis is not solely due to microbiologic clearance. Other entities such as management of intracranial pressure might also be important to the clinical outcome. Nevertheless, the clinical outcome was also assessed 3 months after the initiation of therapy for all 74 patients (as they all received fluconazole after the initial course of antifungal therapy), but there was still no in vitro-in vivo correlation by any of the techniques used. Finally, although the MIC distribution was broad, most of the isolates could be considered susceptible, and then the lack of a correlation could be related to the few isolates with high MICs.

For amphotericin B, studies of relationships between in vitro susceptibility and clinical outcome are very scarce. Determination of minimum fungicidal concentrations and results from time-kill curves have been shown to be better predictors of the clinical outcome than MICs (20, 21). In the present study, although a large number of patients were included, the amphotericin B MICs determined by Etest or by the other techniques were not predictive of the early clinical outcome.

In the present study we also did not observe a relationship between flucytosine MICs, mostly evaluated at pH 7.0, and the early clinical outcome; and similar results were reported for 38 patients treated with flucytosine in combination with fluconazole (30). Nevertheless, it must be noted that evaluation of the in vitro-in vivo relationship is more difficult to assess when a drug is given in combination because a synergistic interaction could be observed even when the strain has an elevated MIC for one of the antifungals. In particular, it has previously been shown that the same pattern of in vitro interaction between flucytosine and amphotericin B was observed for flucytosine-susceptible and -resistant strains of *C. neoformans* (24). In addition, experimental data obtained in studies with mice with disseminated cryptococcosis showed that a synergistic effect, even in the brain, could be obtained when amphotericin B and flucytosine were combined in the case of flucytosine resistance (23). If these in vitro and in vivo results obtained with experimentally infected animals are clinically relevant, it means that flucytosine susceptibility is not likely to influence the clinical outcome in patients treated with this drug in combination with amphotericin B.

In conclusion, the data obtained in the present study suggest (i) that for amphotericin B, fluconazole, and flucytosine, primary resistance is uncommon and that susceptibility testing performed at the time of diagnosis of cryptococcosis, at least with the techniques used, is not relevant, as it is not predictive of the outcome. It is likely that other factors, not only the appropriate management of patients with meningitis and elevated intracranial pressure but also attempts to control the underlying immune deficiency, are more important than the susceptibility of the infecting strain as a predictor of the therapeutic efficacy in cryptococcosis. (ii) RPMI is not a suitable medium for testing of flucytosine susceptibility of *C. neoformans* with Etest. (iii) Strains belonging to serotype A are less susceptible to fluconazole and amphotericin B, but not to flucytosine, than strains of serotype D.

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