ORIGINAL ARTICLE

Combination Antifungal Therapy for Cryptococcal Meningitis

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ABSTRACT

BACKGROUND

Combination antifungal therapy (amphotericin B deoxycholate and flucytosine) is the recommended treatment for cryptococcal meningitis but has not been shown to reduce mortality, as compared with amphotericin B alone. We performed a randomized, controlled trial to determine whether combining flucytosine or high-dose fluconazole with high-dose amphotericin B improved survival at 14 and 70 days.

METHODS

We conducted a randomized, three-group, open-label trial of induction therapy for cryptococcal meningitis in patients with human immunodeficiency virus infection. All patients received amphotericin B at a dose of 1 mg per kilogram of body weight per day; patients in group 1 were treated for 4 weeks, and those in groups 2 and 3 for 2 weeks. Patients in group 2 concurrently received flucytosine at a dose of 100 mg per kilogram per day for 2 weeks, and those in group 3 concurrently received fluconazole at a dose of 400 mg twice daily for 2 weeks.

RESULTS

A total of 299 patients were enrolled. Fewer deaths occurred by days 14 and 70 among patients receiving amphotericin B and flucytosine than among those receiving amphotericin B alone (15 vs. 25 deaths by day 14; hazard ratio, 0.57; 95% confidence interval [CI], 0.30 to 1.08; unadjusted P=0.08; and 30 vs. 44 deaths by day 70; hazard ratio, 0.61; 95% CI, 0.39 to 0.97; unadjusted P=0.04). Combination therapy with fluconazole had no significant effect on survival, as compared with monotherapy (hazard ratio for death by 14 days, 0.78; 95% CI, 0.44 to 1.41; P=0.42; hazard ratio for death by 70 days, 0.71; 95% CI, 0.45 to 1.11; P=0.13). Amphotericin B plus flucytosine was associated with significantly increased rates of yeast clearance from cerebrospinal fluid (−0.42 $\log_{10}$ colony-forming units [CFU] per milliliter per day vs. −0.31 and −0.32 $\log_{10}$ CFU per milliliter per day in groups 1 and 3, respectively; P<0.001 for both comparisons). Rates of adverse events were similar in all groups, although neutropenia was more frequent in patients receiving a combination therapy.

CONCLUSIONS

Amphotericin B plus flucytosine, as compared with amphotericin B alone, is associated with improved survival among patients with cryptococcal meningitis. A survival benefit of amphotericin B plus fluconazole was not found. (Funded by the Wellcome Trust and the British Infection Society; Controlled-Trials.com number, ISRCTN95123928.)

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There are approximately 1 million cases of cryptococcal meningitis annually and 625,000 deaths.[1] Treatment guidelines recommend induction therapy with amphotericin B deoxycholate (0.7 to 1 mg per kilogram of body weight per day) and flucytosine (100 mg per kilogram per day).[2] However, this treatment has not been shown to reduce mortality, as compared with amphotericin B monotherapy.[2,3] Flucytosine is frequently unavailable where the disease burden is greatest, and concerns about cost and side effects have limited its use in resource-poor settings.[4]

Fluconazole is readily available, is associated with low rates of adverse events, and has good penetration into cerebrospinal fluid (CSF), but it is associated with poor outcomes when used as monotherapy for cryptococcal meningitis.[2] Its safety profile, low cost, and availability make it an attractive alternative to flucytosine for combination therapy with amphotericin B, and it is recommended as an alternative in the guidelines.[2] However, when this combination was used in conventional doses (amphotericin B at a dose of 0.7 mg per kilogram per day and fluconazole at a dose of 400 mg per day), it did not improve the rate of yeast clearance from the CSF, in a study not powered for clinical end points.[5] Increased doses of amphotericin B (1 mg per kilogram per day) and fluconazole (800 to 1200 mg per day) independently result in improved rates of yeast clearance.[6,7] To our knowledge, these increased doses have not been tested in combination.[8]

In Asia, many patients receive treatment with amphotericin B monotherapy for 2 to 4 weeks, followed by fluconazole at a dose of 400 mg per day until the end of week 10. In view of the high mortality (55% in Asia and 70% in Africa[1]), we performed an open-label, randomized, controlled trial to determine whether combination therapy with either amphotericin B (at a dose of 1 mg per kilogram per day) and flucytosine (at a dose of 100 mg per kilogram per day) or amphotericin B and fluconazole (at a dose of 400 mg twice daily) offered a survival advantage, as compared with amphotericin B alone (at a dose of 1 mg per kilogram per day).

METHODS

STUDY DESIGN AND PARTICIPANTS

The study was designed as a randomized, three-group trial of induction therapy for cryptococcal meningitis in patients with human immunodeficiency virus (HIV) infection. Patients were recruited at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. Eligible patients had HIV infection, were more than 14 years old, and had symptoms and signs consistent with cryptococcal meningitis and one or more of the following: positive India ink staining of the CSF, a positive test for CSF cryptococcal-antigen, a positive CSF or blood culture for *Cryptococcus neoformans*, or a positive test for blood cryptococcal antigen (titer of >1:10). Patients could have normal or mildly elevated creatinine levels. Patients were excluded if they had received antifungal therapy for more than 3 days, had had cryptococcosis, were pregnant, had renal or liver failure, were receiving rifampin, or did not provide written informed consent. For details of the study design, see the study protocol, available with the full text of this article at NEJM.org.

STUDY OVERSIGHT

The study was approved by the institutional review boards at the Hospital for Tropical Diseases and the Liverpool School of Tropical Medicine. Written informed consent was obtained from all patients or from a relative if the patient could not provide consent. An independent data and safety monitoring committee provided oversight. Interim analyses were performed after 12 months and after 200 patients had completed follow-up. All authors vouch for the completeness and accuracy of the data presented. Cipla and Ranbaxy Laboratories provided amphotericin B and fluconazole, respectively, at a reduced cost. Flucytosine (Valeant Pharmaceuticals France) was purchased at full cost from a pharmacy. None of the drug manufacturers or suppliers had any role in the study design, data accrual and analysis, or manuscript preparation.

LABORATORY INVESTIGATIONS

Lumbar punctures were performed weekly for the first month of treatment and as clinically indicated. Quantitative yeast counts were determined for all specimens.[5] All strains were confirmed as cryptococcus species. For details, see the Supplementary Appendix, available at NEJM.org.

TREATMENT

Patients were randomly assigned to one of three induction treatments. Patients in group 1 received intravenous amphotericin B at a dose of 1 mg per

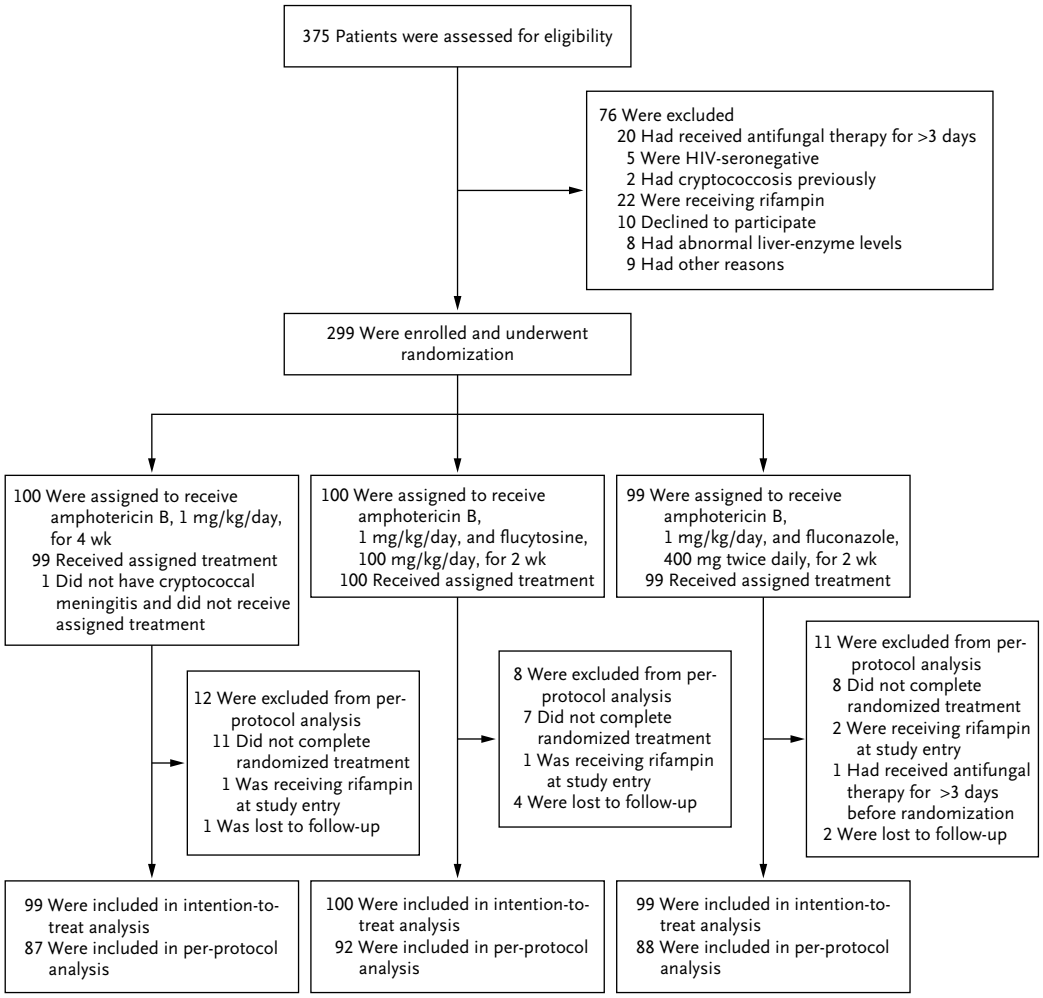


Figure 1. Study Enrollment, Treatment Assignments, and Analysis Populations.

Of 375 patients who underwent assessment, 299 were enrolled in the study. One patient, who underwent randomization but did not have cryptococcal meningitis, did not receive the assigned treatment and was excluded from the intention-to-treat analysis.

kilogram per day for 4 weeks, followed by oral fluconazole at a dose of 400 mg per day for 6 weeks, which was in line with local practice at the inception of the study. Patients in group 2 received amphotericin B deoxycholate at a dose of 1 mg per kilogram per day for 2 weeks, combined with oral flucytosine at a dose of 100 mg per kilogram per day in three to four divided doses. These patients then received fluconazole at a dose of 400 mg per day for 8 weeks. The patients in group 3 received amphotericin B deoxycholate at a dose of 1 mg per kilogram per day, combined with oral fluconazole at a dose of 400 mg twice daily, for 2 weeks. These patients then received fluconazole at a dose of 400 mg per day for 8 weeks. Details regarding drug administration are provided in the Supplementary Appendix.

A computer-generated sequence of random numbers was used to assign patients to treatment groups (for details, see the Supplementary Appendix). The attending physicians were responsible for enrolling participants and ensuring that the correct study drug was given. Daily monitoring of all inpatients by a member of the study team ensured uniform management and accurate recording of data. Increased intracranial pressure

was treated with therapeutic lumbar puncture. After discharge, patients were assessed monthly until 6 months after randomization.

All patients received *Pneumocystis jirovecii* pneumonia prophylaxis (co-trimoxazole at a dose of 960 mg per day). Antiretroviral therapy (ART) was prescribed according to national guidelines. Patients already receiving ART at the time of diagnosis continued the therapy. All patients who had not received ART were referred to the hospital ART clinic. The decision to initiate ART depended on the attending physician's assessment and the patient's preference and was independent of study participation.

ASSESSMENT OF OUTCOMES

The prespecified coprimary outcomes were all-cause mortality in the first 14 and 70 days after randomization. Prespecified secondary outcomes included mortality at 6 months, disability status at 70 days and at 6 months (defined as 182 days), changes in CSF fungal counts in the first 2 weeks after randomization, time to CSF sterilization, and adverse events during the first 10 weeks of the study. Disability status was assessed with the use of two simple questions ("Do you require help from anybody for everyday activities [e.g., eating, drinking, washing, brushing teeth, and going to the toilet]?" and "Has the illness left you with any

Table 1. Baseline Characteristics of the Study Participants.*

Characteristic	Group 1, Amphotericin B (N=99)	Group 2, Amphotericin B and Flucytosine (N=100)	Group 3, Amphotericin B and Fluconazole (N=99)
Age — yr†			
Median	28	28	27
Interquartile range	25–31	25–33	24–31
Male sex — no. (%)	81 (82)	80 (80)	84 (85)
Intravenous drug use — no./total no. (%)	51/90 (57)	49/94 (52)	53/97 (55)
Duration of symptoms — days‡			
Median	15	14	12
Interquartile range	7–22	8–18	7–20
Headache — no./total no. (%)	95/97 (98)	99/99 (100)	98/99 (99)
Fever — no./total no. (%)	75/97 (77)	75/98 (77)	72/98 (73)
Neck stiffness — no./total no. (%)	66/91 (73)	64/91 (70)	66/95 (69)
Seizure — no./total no. (%)	9/94 (10)	9/98 (9)	2/98 (2)
Glasgow Coma Scale score — no./total no. (%)§			
15	66/97 (68)	67/99 (68)	78/98 (80)
11–14	21/97 (22)	24/99 (24)	15/98 (15)
≤10	10/97 (10)	8/99 (8)	5/98 (5)
Cranial-nerve palsy — no./total no. (%)	27/97 (28)	22/98 (22)	18/98 (18)
Papilledema — no./total no. (%)	18/85 (21)	19/89 (21)	17/93 (18)
CSF opening pressure >18 cm of CSF — no./total no. (%)	56/83 (67)	61/80 (76)	55/81 (68)
CSF white-cell count — cells/ml¶			
Median	33	26	24
Interquartile range	7–76	8–61	7–83
CSF glucose level — mmol/liter‖			
Median	2.21	2.30	2.34
Interquartile range	1.50–3.00	1.70–2.98	1.70–2.99
Plasma glucose level — mmol/liter**			
Median	5.69	5.90	5.43
Interquartile range	4.84–6.50	4.88–6.90	4.80–6.20

Table 1. (Continued.)

Characteristic	Group 1, Amphotericin B (N=99)	Group 2, Amphotericin B and Flucytosine (N=100)	Group 3, Amphotericin B and Fluconazole (N=99)
CSF yeast count — $\log_{10}$ CFU/ml††			
Median	5.91	5.81	5.74
Interquartile range	5.49–6.48	4.74–6.15	4.80–6.34
CD4 count — cells/mm^3‡‡			
Median	18	17	14
Interquartile range	8–37	9–28	8–41
Creatinine — μmol/liter§§			
Median	72.0	73.0	70.4
Interquartile range	61.0–93.5	60.0–86.0	61.1–88.5

* There were no significant between-group differences at baseline, with the exception of CSF yeast count (P=0.03 by the Kruskal–Wallis test). For additional details, see Table S1 in the Supplementary Appendix. CFU denotes colony-forming units, and CSF cerebrospinal fluid. To convert the values for glucose to milligrams per deciliter, divide by 0.05551. To convert the values for creatinine to milligrams per deciliter, divide by 88.4.
† Data were missing for 1 patient in group 3.
‡ Data were missing for 14 patients in group 1, for 6 in group 2, and for 8 in group 3.
§ Scores on the Glasgow Coma Scale range from 3 to 15, with lower scores indicating reduced levels of consciousness.
¶ Data were missing for 10 patients in group 1, for 12 in group 2, and for 12 in group 3.
‖ Data were missing for 6 patients in group 2, for 4 in group 2, and for 6 in group 3.
** Data were missing for 8 patients in group 1, for 8 in group 2, and for 6 in group 3.
†† Data were missing for 22 patients in group 1, for 20 in group 2, and for 20 in group 3.
‡‡ Data were missing for 28 patients in group 1, for 26 in group 2, and for 26 in group 3.
§§ Data were missing for 8 patients in group 1, for 3 in group 2, and for 4 in group 3.

other problems?") and the modified Rankin scale (scores range from 0 [no symptoms at all] to 6 [death]) and was classified as good (i.e., no disability), intermediate, severe, or death, as described elsewhere.[9]

STATISTICAL ANALYSIS

The trial was designed to detect, with 80% power, a difference in mortality of 45% versus 25% at 10 weeks between the group that received amphotericin B monotherapy and each group that received combination treatment, at a two-sided 5% significance level. The planned sample was 297 patients.

The primary aims of this study were the comparisons of survival at 14 and 70 days of the two combination treatments, respectively, with amphotericin B monotherapy. The time to death was compared between treatment groups at day 14, day 70, and day 182 with the use of a Cox regression model, with treatment indicators as the only covariates. Potential heterogeneity of the treatment effect depending on covariates was tested with the use of a likelihood-ratio test for interaction. For mortality at day 70 and at day 182, we also performed an adjusted Cox regression analysis with the following prespecified covariates (in addition to randomized treatment): age, sex, log-quantitative fungal count, Glasgow Coma Scale score (15 vs. <15, with scores ranging from 3 to 15, and lower scores indicating reduced levels of consciousness), CD4 cell count, hemoglobin level, serum sodium level, log CSF white-cell count, and CSF opening pressure.

The proportions of patients with good disability status on day 70 and on day 182 were compared among groups with the use of a logistic-regression model. The decline in the log CSF quantitative fungal count in the first 2 weeks was estimated by means of longitudinal measurements during that period and a linear mixed-effects model with an interaction term between the treatment group and study day. Time to fungal clearance was estimated with a cause-specific Cox regression model adjusted for baseline fungal count. The multivariate Cox regression analyses and the analysis of disability status were based on multiple imputation of missing covariates and disability outcomes.

The study had four prespecified primary analyses. There is no consensus in the literature re-

Table 2. Primary and Key Secondary Outcomes.*

Outcome	Group 1, Amphotericin B (N=99)	Group 2, Amphotericin B and Flucytosine (N=100)	Group 3, Amphotericin B and Fluconazole (N=99)
Coprimary outcomes			
Death by day 14			
No. of deaths	25	15	20
Probability of survival (95% CI)	0.75 (0.67 to 0.84)	0.85 (0.78 to 0.92)	0.80 (0.73 to 0.88)
Death by day 70‡			
No. of deaths	44	30	33
Probability of survival (95% CI)	0.56 (0.47 to 0.66)	0.69 (0.61 to 0.79)	0.67 (0.58 to 0.77)
Secondary outcomes			
Death by day 70 in the per-protocol population			
No. of deaths/no. of patients included in analysis	37/87	26/92	27/88
Probability of survival (95% CI)	0.58 (0.48 to 0.69)	0.71 (0.63 to 0.81)	0.69 (0.60 to 0.80)
Death by day 182§			
No. of deaths	53	34	45
Probability of survival (95% CI)	0.46 (0.37 to 0.57)	0.65 (0.56 to 0.75)	0.54 (0.45 to 0.65)
Estimated change in CSF fungal count in first 14 days (95% CI) — $\log_{10}$ CFU/ml/day	−0.31 (−0.34 to −0.29)	−0.42 (−0.44 to −0.40)	−0.32 (−0.34 to −0.29)
CSF fungal clearance			
No. of patients with documented clearance	52	74	63
Clearance rate per person-wk of follow-up (95% CI)	0.17 (0.13 to 0.23)	0.39 (0.31 to 0.50)	0.26 (0.20 to 0.34)

* Reported results are for the intention-to-treat population unless otherwise specified and were not adjusted for baseline covariates or multiple comparisons, except for time to CSF fungal clearance, which was adjusted for baseline fungal count. In the coprimary comparisons for death by 14 days and death by 70 days in group 2 versus group 1 and in group 3 versus group 1, conservative Bonferroni multiplicity adjustment would require doubling of the P values (adjustment for multiple primary end points) or would require the P value to be four times as large (adjustment for multiple primary end points and for multiple comparisons of the combination therapies versus a common control group). For disability outcomes, see Table S2 in the Supplementary Appendix.

† Hazard ratios are shown for all outcomes except for the estimated change in CSF fungal count, for which the between-group difference in the estimated change is shown.

‡ When hazard ratios for death by day 70 were adjusted for baseline covariates, the results were as follows: for group 2 versus group 1, the hazard ratio was 0.62 (95% CI, 0.38 to 0.996), P=0.048; for group 3 versus group 1, the hazard ratio was 0.94 (95% CI, 0.58 to 1.51), P=0.80; and for group 2 versus group 3, the hazard ratio was 0.66 (95% CI, 0.39 to 1.10), P=0.11.

§ When hazard ratios for death by day 182 were adjusted for baseline covariates, the results were as follows: for group 2 versus group 1, the hazard ratio was 0.56 (95% CI, 0.36 to 0.87), P=0.01; for group 3 versus group 1, the hazard ratio was 1.01 (95% CI, 0.66 to 1.53), P=0.97; and for group 2 versus group 3, the hazard ratio was 0.55 (95% CI, 0.35 to 0.88), P=0.01.

garding whether statistical adjustment is needed for trials that use a common control group, because the addition of a group to a trial enhances rather than diminishes informativeness.[10,11] Schulz and Grimes argue that adjusting the analysis of related end points for multiple testing is not mandatory.[12] Our trial was not powered to account for multiplicity adjustment, and we report unadjusted P values for all comparisons. We present the Bonferroni-corrected P values as a supplementary analysis.

The primary analysis was performed with data from the intention-to-treat population, which included all patients who had undergone randomization. The analysis of mortality at 70 days was also performed with data from the per-protocol population, which excluded patients with major protocol violations. All analyses were performed

Hazard Ratio or Difference in Estimated Change (95% CI)†					
Group 2 vs. Group 1	P Value	Group 3 vs. Group 1	P Value	Group 2 vs. Group 3	P Value
0.57 (0.30 to 1.08)	0.08	0.78 (0.44 to 1.41)	0.42	0.72 (0.37 to 1.41)	0.34
0.61 (0.39 to 0.97)	0.04	0.71 (0.45 to 1.11)	0.13	0.87 (0.53 to 1.42)	0.57
0.60 (0.36 to 0.99)	0.04	0.68 (0.41 to 1.11)	0.12	0.88 (0.52 to 1.51)	0.65
0.56 (0.36 to 0.86)	0.01	0.78 (0.53 to 1.16)	0.23	0.72 (0.46 to 1.12)	0.14
–0.10 (–0.14 to –0.07)	<0.001	0.00 (–0.04 to 0.03)	0.83	–0.10 (–0.14 to –0.07)	<0.001
3.18 (2.17 to 4.66)	<0.001	1.39 (0.94 to 2.07)	0.10	2.29 (1.59 to 3.29)	<0.001

with the use of R software, version 2.13.1,[13] and the R software packages mice, version 2.8,[14] and multcomp, version 1.2-5.[15]

RESULTS

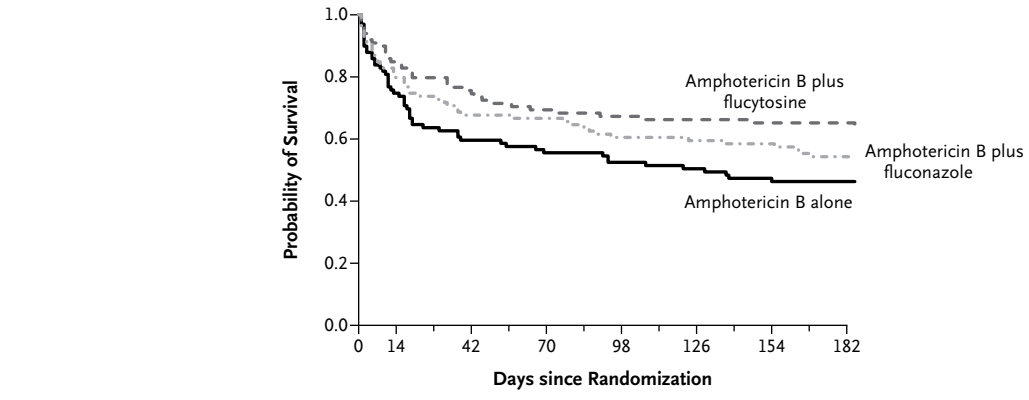
STUDY POPULATION

Figure 1 shows the numbers of patients who were enrolled, assigned to a treatment group, and included in the intention-to-treat and per-protocol analyses. A total of 299 patients were randomly assigned to induction antifungal therapy between April 2004 and September 2010. One patient, who underwent randomization but did not have cryptococcal meningitis, was excluded from the intention-to-treat analysis. An additional 31 patients were excluded from the per-protocol analysis: 26 patients withdrew before the completion of the randomly assigned treatment (11, 7, and 8 patients from groups 1, 2, and 3, respectively), 4 were subsequently found to be taking rifampin at randomization, and 1 had received antifungal therapy for more than 3 days. Survival status at 6 months was missing for 7 patients.

Baseline characteristics of the patients are shown in Table 1. *C. neoformans* was cultured from the CSF of 291 of 298 patients (97.7%) and from the blood of 122 of 168 patients (72.6%). All infections were *C. neoformans* var. *grubii* molecular type VNI. Seven patients had mildly elevated creatinine levels (range, 145 to 188 μmol per liter).

PRIMARY OUTCOMES

Key outcomes are summarized in Table 2. By day 70, a total of 44 patients treated with amphotericin B monotherapy had died, as compared with 30 patients treated with amphotericin B and flucytosine and 33 patients treated with amphotericin B and fluconazole (Fig. 2A). Treatment with



No. at Risk								
Amphotericin B alone	99	74	59	54	51	49	46	30
Amphotericin B plus flucytosine	100	84	73	67	64	63	62	46
Amphotericin B plus fluconazole	99	79	67	65	59	58	57	39

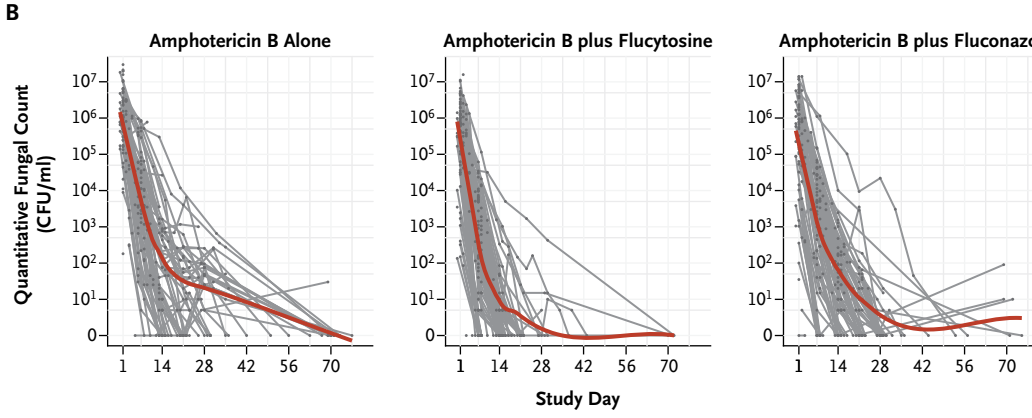


Figure 2. Kaplan–Meier Survival Estimates and Cerebrospinal Fluid (CSF) Fungal Counts, According to Treatment Group.

Panel A shows the Kaplan–Meier survival estimates according to treatment group. For mortality at 70 days, P=0.04 for the comparison of amphotericin B plus flucytosine with amphotericin B monotherapy, and P=0.13 for the comparison of amphotericin B plus fluconazole with amphotericin B monotherapy. Panel B shows the CSF quantitative fungal counts over time, according to treatment group. Study day 1 corresponds to the day of randomization. All recorded CSF quantitative counts are shown, including those in patients who subsequently died. CSF fungal decline in the first 14 days and time to clearance were significantly faster among patients receiving amphotericin B plus flucytosine than among patients in the other treatment groups (P<0.001 for all comparisons). In each graph, gray lines indicate data for individual patients; the red line indicates a loess scatterplot smoother calculated with the use of local regression. CFU denotes colony-forming units.

amphotericin B and flucytosine was associated with a significantly reduced hazard of death by day 70 in the intention-to-treat analysis (hazard ratio, 0.61; 95% confidence interval [CI], 0.39 to 0.97; P=0.04); this benefit was maintained in the per-protocol analysis and after adjustment for predefined baseline covariates. Fewer patients receiving combination therapy with high-dose fluconazole died, as compared with those treated with amphotericin B monotherapy, but this finding was not significant (hazard ratio, 0.71; 95% CI, 0.45 to 1.11; P=0.13).

No evidence of heterogeneity in treatment effects was detected for CD4 count, intravenous drug use, baseline log fungal count, or score on the Glasgow Coma Scale (P>0.10 for all tests).

Between-group differences in survival rates at day 14 were not significant (15 deaths in group 2 vs. 25 deaths in group 1; P=0.08).

SECONDARY OUTCOMES

The survival benefit seen for patients receiving amphotericin B and flucytosine, as compared with those receiving amphotericin B monotherapy, was more marked at 6 months (hazard ratio, 0.56; 95% CI, 0.36 to 0.86; P=0.01). Treatment with amphotericin B and fluconazole did not confer a survival advantage, as compared with monotherapy. There was no significant difference in survival between the two combination-treatment groups. However, after adjustment for baseline covariates, combination therapy with flucytosine was associated with a reduced hazard of death, as compared with amphotericin B alone (hazard ratio, 0.56; 95% CI, 0.36 to 0.87; P=0.01) or with amphotericin B plus fluconazole (hazard ratio, 0.55; 95% CI, 0.35 to 0.88; P=0.01). The multivariable Cox regression identified the following independent predictors of 6-month survival: baseline fungal count (hazard ratio for each increase of 1 $\log_{10}$ colony-forming unit [CFU] per milliliter, 1.33; 95% CI, 1.08 to 1.65; P=0.01) and a score on the Glasgow Coma Scale of less than 15 (hazard ratio, 2.30; 95% CI, 1.57 to 3.36; P<0.001).

Patients receiving amphotericin B and flucytosine had a significantly higher chance of being free of disability at 6 months, as compared with those receiving monotherapy (odds ratio, 2.01; 95% CI, 1.04 to 3.88; P=0.04). At day 70, a visual deficit was present in 16 of 46 assessed patients treated with amphotericin B, as compared with 9 of 54 patients treated with amphotericin B and flucytosine and 8 of 48 patients treated with amphotericin B and high-dose fluconazole. A total of 8 patients had complete visual loss (no light perception).

The time to fungal clearance was significantly shorter in patients receiving amphotericin B plus flucytosine than in those receiving amphotericin B alone or in combination with fluconazole, with more rapid rates of decline in the colony count (−0.42 $\log_{10}$ CFU per day vs. −0.31 $\log_{10}$ CFU per day and −0.32 $\log_{10}$ CFU per day, respectively; P<0.001 for both comparisons) (Fig. 2B).

EFFECT OF ANTIRETROVIRAL THERAPY

A total of 89 patients were receiving or started receiving ART during the 6-month follow-up: 27 patients in the group receiving amphotericin B alone, 32 in the group receiving amphotericin B plus flucytosine, and 30 in the group receiving amphotericin B plus fluconazole. A total of 2, 5, and 3 patients in the three groups, respectively, were receiving ART at study entry; 2, 2, and 4 patients started receiving ART within 2 weeks after randomization; and 17, 15, and 17 patients started receiving ART between day 14 and day 70. Because ART was started after enrollment for most patients and was conditional on survival, this study cannot determine whether ART improved survival, although studies to assess this effect are under way.

ADVERSE EVENTS

Adverse events occurred with similar frequency among all the treatment groups (Table 3). The most frequent adverse events were anemia, hypokalemia, elevated aminotransferase levels, neutropenia, hypercreatinemia, and opportunistic infection. Neutropenia was more frequent among patients receiving amphotericin B with either flucytosine or fluconazole than among those receiving amphotericin B monotherapy (34% and 32%, respectively, vs. 19%; P=0.04 for overall comparison). Fewer patients had severe anemia in the group receiving amphotericin B with fluconazole (29% of patients) than in the group receiving amphotericin B monotherapy (46%) and the group receiving amphotericin B with flucytosine (35%). Modification or interruption of treatment with the study drug occurred in eight patients in each group.

DISCUSSION

Our study population was characterized by high CSF fungal burdens and a high proportion of patients (28%) with a Glasgow Coma Scale score of less than 15 at presentation, which are variables that are recognized to be important predictors of a poor outcome.[3,16-22] The results of this study suggest that in such patient populations, combination therapy with amphotericin B and flucytosine is associated with improved survival, as compared with amphotericin B monotherapy. The survival benefit was apparent 10 weeks after randomization and was sustained for at least 6 months. Moreover, amphotericin B plus flucytosine was associated with a higher likelihood of survival without disability than was amphotericin B monotherapy.

Table 3. Adverse Events.*

Event	Group 1, Amphotericin B (N=99)	Group 2, Amphotericin B and Flucytosine (N=100)	Group 3, Amphotericin B and Fluconazole (N=99)	P Value†
Any event				
At least one event — no. of patients (%)	82 (83)	85 (85)	85 (86)	0.85
No. of events	338	376	362	
Hypokalemia — no. of patients (%)				
All grades	54 (55)	56 (56)	54 (55)	0.98
Grades 3 and 4	20 (20)	22 (22)	13 (13)	0.24
Anemia — no. of patients (%)				
All grades	62 (63)	63 (63)	57 (58)	0.71
Grades 3 and 4	46 (46)	35 (35)	29 (29)	0.04
Neutropenia — no. of patients (%)				
All grades	19 (19)	34 (34)	32 (32)	0.04
Grades 3 and 4	2 (2)	9 (9)	9 (9)	0.07
Thrombocytopenia — no. of patients (%)				
All grades	8 (8)	15 (15)	11 (11)	0.32
Grades 3 and 4	2 (2)	4 (4)	3 (3)	0.91
Rigor — no. of patients (%)	13 (13)	7 (7)	6 (6)	0.18
Opportunistic infection — no. of patients (%)	32 (32)	32 (32)	28 (28)	0.79
Rash — no. of patients (%)	5 (5)	7 (7)	5 (5)	0.86
New neurologic sign or symptom — no. of patients (%)	11 (11)	12 (12)	10 (10)	0.97
Seizure — no. of patients (%)	2 (2)	0	2 (2)	0.4
Elevated aminotransferase level — no. of patients (%)				
All grades	38 (38)	44 (44)	42 (42)	0.72
Grades 3 and 4	11 (11)	6 (6)	14 (14)	0.14
Hyponatremia — no. of patients (%)				
All grades	28 (28)	33 (33)	33 (33)	0.71
Grades 3 and 4	3 (3)	8 (8)	9 (9)	0.19
Hypercreatinemia — no. of patients (%)				
All grades	34 (34)	41 (41)	46 (46)	0.22
Grades 3 and 4	2 (2)	2 (2)	2 (2)	1.00
Other — no. of patients (%)‡	28 (28)	23 (23)	31 (31)	0.41

* All opportunistic infections and all events of grade 3 or 4 were classified as severe adverse events. For details, see Table S3 in the Supplementary Appendix.
† P values correspond to overall comparisons among the three groups with the use of Fisher's exact test.
‡ Other adverse events occurred with a frequency of 0.3%, except nausea and vomiting (in 2.7% of all patients), sepsis (in 1.7%), and thrombophlebitis, hematemesis, diarrhea, and urinary retention (each in 0.7%).

Our primary comparisons did not account for multiplicity. As noted above, there is no agreement on whether such an adjustment is mandatory or even helpful.

We did not find a significant difference in survival between patients receiving amphotericin B combined with high-dose fluconazole and those receiving amphotericin B monotherapy, although fewer deaths had occurred in the former group at 10 weeks.

The comparison between combination treatments was secondary and did not reach statistical significance for most of the outcomes. However, in an exploratory comparison of survival at

6 months that was adjusted for predefined baseline factors, mortality was significantly higher among patients receiving amphotericin B plus fluconazole than among those receiving amphotericin B plus flucytosine and did not differ significantly from mortality among those treated with amphotericin B monotherapy.

The survival benefit with amphotericin B plus flucytosine that we observed in this study is in contrast to the results of a trial in North America.[3] However, that study analyzed the effect of combination therapy at 2 weeks, included few patients with impaired consciousness, had a low overall death rate, and may not have had sufficient power to show a survival effect. The survival benefit seen in our study is biologically plausible, being associated with significantly increased rates of yeast clearance. These clearance data are consistent with smaller studies showing that flucytosine combined with amphotericin B resulted in faster CSF yeast clearance than did amphotericin B monotherapy or amphotericin B plus fluconazole at a daily dose of 400 mg (i.e., a lower dose than was used in our study). A subsequent analysis of raw data collated from randomized, controlled trials and cohorts has suggested that the rate of fungal clearance from CSF is associated with the outcome.[5,6,16] Conversely, a recent study from South Africa did not show a significant difference in rates of CSF yeast clearance between amphotericin B plus flucytosine and amphotericin B plus fluconazole, but the study was limited by its small size and the lower fungal burden in the patients, as compared with the patients in our study.[23] The association among antifungal-treatment combination, rate of clearance of yeast from CSF, and mortality shown in our study is evidence that optimizing antifungal therapy is an important factor in improving outcomes of cryptococcal meningitis. The rate of decline of CSF yeast counts is a potential marker of survival in the evaluation of antifungal-treatment regimens, although the usefulness of measuring rates of fungal decline in the treatment of individual patients is not clear. However, our study shows the feasibility of designing trials of treatment for cryptococcal meningitis that are powered to assess mortality end points, and such studies seem appropriate for a disease with high mortality.

We found that a difference in antifungal therapy during the first 2 weeks of a 10-week treatment was associated with a survival benefit at 6 months. Between 10 weeks and 6 months, 4 additional deaths occurred in patients receiving amphotericin B plus flucytosine, versus 9 and 12 deaths in patients receiving amphotericin B alone and those receiving amphotericin B plus fluconazole, respectively. The causes of death in these patients were unclear, since many patients had returned to their home provinces. The lower death rate among patients receiving flucytosine, as compared with the rate among those receiving amphotericin B monotherapy, may have been due to lower rates of disability in these patients, which protected them from further complications, or lower rates of disease relapse, an association that has been previously identified.[24,25]

In conclusion, the results of this study suggest that initial combination therapy with amphotericin B and flucytosine for 2 weeks in our setting was associated with reduced mortality among patients with HIV-associated cryptococcal meningitis, as compared with 4 weeks of amphotericin B monotherapy. Combination therapy with fluconazole for 2 weeks was not found to offer a benefit. Improving access to flucytosine has the potential to reduce the number of deaths from this disease.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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