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Approaches to Antifungal Therapies and Their Effectiveness among Patients with Cryptococcosis

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The goal of this study was to determine the degree to which the persistence of cryptococcosis, overall 1-year mortality, and 1-year mortality due to cryptococcosis were influenced by initial antifungal treatment regimen in a cohort of adults with cryptococcosis treated at a tertiary care medical center. Risk factors, underlying conditions, treatment, and mortality information were obtained for 204 adults with cryptococcosis from Duke University Medical Center (DUMC) from 1996 to 2009. Adjusted risk ratios (RR) for persistence and hazard ratios (HR) for mortality were estimated for each exposure. The all-cause mortality rate among patients with nonsevere disease (20%) was similar to that in the group with disease (26%). However, the rate of cryptococcosis-attributable mortality with nonsevere disease (5%) was much lower than with severe disease (20%). Flucytosine exposure was associated with a lower overall mortality rate (HR, 0.4; 95% confidence interval [CI], 0.2 to 0.9) and attributable mortality rate (HR, 0.5; 95% CI, 0.2 to 1.2). Receiving a nonrecommended antifungal regimen was associated with a higher relative risk of persistent infection at 4 weeks (RR, 1.9; 95% CI, 0.9 to 4.3), and the rate of attributable mortality among those not receiving the recommended dose of initial therapy was higher than that of those receiving recommended dosing (HR, 2.3; 95% CI, 1.0 to 5.0). Thus, the 2010 Infectious Diseases Society of America (IDSA) guidelines are supported by this retrospective review as a best-practice protocol for cryptococcal management. Future investigations should consider highlighting the distinction between all-cause mortality and attributable mortality so as not to overestimate the true effect of cryptococcosis on patient death.

The optimal antifungal treatment strategy for patients with cryptococcosis remains in question despite the 2010 Infectious Diseases Society of America (IDSA) guidelines (1). Treatment of cryptococcal meningoencephalitis is based on a small number of clinical trials, but most of the recent studies have been in resource-limited areas and may not reflect the situation with newer antifungals in advanced medical settings (2–5). Previous studies have suggested that treatment is generally 50 to 80% effective (6–10), that antifungal drugs cause toxicities in roughly one-third of cases, that the rate of mortality while on antifungal therapy remains high (approximately 20%), and that mortality varies considerably by the host underlying immune status (11–14).

There have been few comprehensive, comparative studies of cryptococcosis that encompass all three risk groups (HIV positive, solid-organ transplantation, and HIV negative/nontransplant) with and without meningeal involvement, identified by the 2010 IDSA guidelines in the era of lipid products of amphotericin B. Therefore, we examined the effectiveness of initial antifungal treatment among these three clinical groups within a single study center. The primary aim of this study was to determine the degree to which the risk for persistence of cryptococcosis and rates of 1-year mortality and mortality due to cryptococcosis were influenced by the initial antifungal treatment regimen in a cohort of patients with cryptococcosis treated at a tertiary care medical center. The advantage of this approach was the ability to observe real-world treatment strategies and compare risk groups where anticryptococcal drugs are available and the general care and management of this infection has been relatively consistent over the 14-year study period. Other treatment-related outcomes examined were (i) changes from initial therapy to definitive therapy, (ii) development of renal toxicity, (iii) development of immune reconstitution inflammatory syndrome (IRIS), and (iv) number of cases requiring multiple courses of induction therapy using amphotericin B.

MATERIALS AND METHODS

Study population. All consecutive 204 adults hospitalized with cryptococcosis and who were treated at Duke University Medical Center (DUMC) were enrolled in the cohort using International Classification of Diseases, ninth revision (ICD-9) (15), discharge codes of cryptococcosis (117.5) and cryptococcal meningitis (321.0) from 1996 to 2009. Three identified patients were excluded from this cohort because they had died prior to receiving any anticryptococcal therapy or refused treatment at DUMC. Risk factors, underlying conditions, treatment, and mortality information were obtained by chart review. Patients presented to DUMC, were diagnosed with cryptococcosis, and were assessed for severity of disease prior to starting treatment. "Severe" and "nonsevere" cryptococcosis disease categories divided patients who required induction therapy with amphotericin B (severe disease) and those for whom fluconazole was indicated as the primary therapy (nonsevere disease) based on the IDSA guidelines (1), whether or not the patients actually received the indicated treatment. To be defined as severe cases, patients had evidence of central

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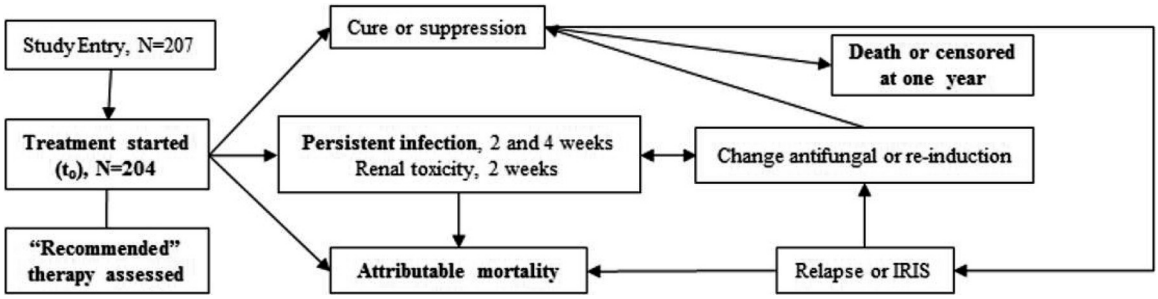

FIG 1 Diagram of patient flow scenarios from entry through 1 year of follow-up. Upon entry into the cohort, patient underlying condition(s) and severity of cryptococcosis were assessed. Three patients did not undergo antifungal therapy and were excluded from this treatment effectiveness study. Follow-up began at the start of therapy, and patients were subsequently evaluated for persistence, mortality rate, and secondary outcomes, such as renal toxicity (among severe disease patients taking amphotericin B) and IRIS, through 1 year.

nervous system (CNS) involvement with or without cryptococcemia or dissemination with evidence of high fungal burden based on serum cryptococcal antigen (CRAG) titers of ≥1:512). Nonsevere cases had no CNS involvement, evidence of pulmonary disease, or cryptococcemia or dissemination and CRAG titers of <1:512. For severe disease patients, flucytosine (5FC) combination therapy exposure was assessed separately from primary therapy for a more in-depth examination restricted to this severe group. Thus, "appropriate" or "recommended" initial treatment was amphotericin B based for severe disease patients and fluconazole based for nonsevere disease patients. Follow-up started when initial treatment was given, preceded by the occurrence of disease, patient admission, and assessment of cryptococcosis severity.

Exposures. There were three main treatment exposures of interest: (i) appropriate initial treatment, (ii) appropriate initial treatment dose, and (iii) appropriate flucytosine use based on IDSA recommendations. Secondary treatment-related exposures of interest included the completion of at least 7 days of flucytosine combination antifungal therapy versus not receiving it or completing <7 days (confined to patients with severe disease), completion of at least 30 days of fluconazole therapy among surviving patients (nonsevere disease), and completion of at least 90 days of fluconazole therapy among surviving patients (nonsevere disease).

Patients were categorized by whether or not their initial antifungal drug and dosing were appropriate using the 2010 IDSA guidelines. Flucytosine exposure at the start of induction therapy was assessed among patients with severe disease. "Initial therapy" refers to the first antifungal drug administered at the start of induction treatment. This excludes subsequent switching from this initial drug to another formulation during the same induction period (e.g., deoxycholate to lipid amphotericin B). An exception to this definition was the use of fluconazole prior to confirmation of disease, in which case a patient was then placed on an amphotericin B regimen. Furthermore, fluconazole exposure was not considered as initial therapy if it was administered for ≤5 days after the first positive culture result before amphotericin B began, or else fluconazole would be considered as initial therapy. This exception was made for three patients with severe disease.

In order to account for initial dosing adjustments that can occur in the first few days of induction, the averaged dose of continuous antifungal therapy (no change of drug or interruption of treatment for ≥3 days) was used to define acceptable dosing of initial therapy. If there was a change from initial therapy (excluding flucytosine), then only the first drug and its corresponding average dosing were used to examine appropriate initial therapy dose. Acceptable dosing was defined as follows: 0.7 to 1.0 mg/kg of body weight/day amphotericin B deoxycholate (AmpBd), 3 to 6 mg/kg/day liposomal AmpB (L-AmpB), 4 to 6 mg/kg/day AmpB lipid complex (ABLC), and ≥400 mg/day fluconazole. Rounding to the nearest tenth for AmpBd and the nearest integer for AmpB lipid products was used to categorize appropriate dosing. Dosing of flucytosine was not examined in this study. Cumulative doses among patients who survived long enough to complete the recommended length of treatment (14 days for severe and 90 days for nonsevere) were summarized (see the supplemental material).

Outcomes. Follow-up time started when anticryptococcal therapy was initiated after patient admission, diagnosis, and severity evaluation were complete. We assessed persistent infection at 2 (severe disease only) and 4 weeks and cryptococcal-attributable mortality and all-cause mortality through 1 year of follow-up. Two and 4 weeks were chosen because severe-disease patients are recommended to receive at least 2 weeks of induction therapy and reliable follow-up and mortality information was available for the majority of surviving patients. Figure 1 illustrates overall patient flow. Follow-up for this study began at the start of anticryptococcal therapy and continued up to 4 weeks to evaluate persistence and up to 1 year for mortality outcomes.

Persistent cryptococcosis was defined as having a positive culture(s) 2 weeks after starting therapy (among the severe disease group only), and persistent cryptococcosis at 4 weeks was defined as having a positive culture(s) and/or positive indication of the presence of cryptococcus-related symptoms 4 weeks after starting therapy. Patients had to have survived until the time of measurement to be included in the analysis. Data were observational; measures for indicating persistent infection were not taken at exactly 2 weeks and 4 weeks to test for positive culture, CRAG, and/or infection-related symptoms. Acceptable values were used if they did not overlap the preceding measurement (e.g., a baseline culture could not be used for a 2-week test result) and did not extend beyond the designated time point (e.g., a 3-week measure would be counted not as a 2-week measure but instead as a 4-week measure if there was not an observation at 4 weeks). Persistence measures for 2 weeks had to have occurred at ≥1 week of therapy. Measures beyond the final time point (4 weeks) were accepted for that final time point if it was within 90 days since the start of therapy.

In order to assess 1-year mortality, we obtained data on survival and mortality up to 1 year after the date of cryptococcosis diagnosis from the Duke Data Support Repository (DSR), which uses the Social Security Administration death index, the Tumor Registry, and The Duke Information System for Cardiovascular Care death data to report mortality status. If the patient died beyond 1 year of follow-up or was alive or lost to follow-up at the end of the study period, they were censored subjects. Attributable mortality within 1 year from the start of anticryptococcal therapy was determined on the basis of the finding by a panel of experts that death was due to conditions related to at least one of the following: increased CNS pressure, persistent infection, relapse of infection while on treatment for cryptococcosis and an underlying disease, or organ failure while on antifungal treatment.

Additional outcomes for both severe and nonsevere cryptococcosis patients included frequency of reinduction(s) with amphotericin B, IRIS, and renal toxicity during initial therapy. Renal toxicity was defined as a >50% decrease in the glomerular filtration rate (GFR), also known as estimated creatinine clearance, during initial induction treatment. GFR

was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula (16). For patients with severe disease who received induction therapy with amphotericin B, creatinine values closest to day 0 and day 14 of treatment were used to determine renal toxicity. The definition for IRIS (adapted from reference 17) has been described elsewhere (18).

For severe and nonsevere disease groups, the dynamics of changing from one treatment regimen to another were compared to findings for patients who received uninterrupted antifungal therapy. Lastly, we estimated the association between patients who received 0 to 7 days of flucytosine and those who received more than 7 days of flucytosine with regard to the risks of persistence and mortality among 2-week survivors with severe disease.

Data analysis. To evaluate issues of confounding, we assessed the bivariate associations between all covariates and main exposures and outcomes. Minimum adjustment sets were determined using Directed Acyclic Graph (DAG) program (version 0.21) (19). However, the program resulted in 14 confounders in the minimum adjustment sets for each of our three chosen exposures (with slight variation; see Fig. S1 and S2 in the supplemental material), which our limited study size could not operably model. Based on previous studies that predicted poor outcomes (9, 10, 14, 20–24), we prioritized variables associated with severe underlying condition and high fungal burden from our minimal adjustment sets and proceeded with multivariate adjustment using a change-in-estimate approach with a 10% cutoff criterion (25), eliminating variables chosen by the DAG program that did not confound the association of effect estimates between the main exposures and outcomes. Changes in the precision of estimates were examined with the confidence limit ratio (CLR); covariates that improved precision were maintained in the final model. Effect measure modification by confounding variables was examined through the inclusion of interaction terms in the models and using the spreadsheet by Andersson et al. to determine the relative excess risk due to interaction (RERI) (26).

Binomial regression was used to estimate the risk ratio (RR) of the association between treatment exposures and these outcomes. Cox proportional-hazard models were used to estimate hazard ratios (HR) for the association between treatment exposures and mortality outcomes. Assessment of the proportional-hazard assumption (PHA) was performed using graphical methods (ln-ln survival plot) and by adding an interaction between each of the model predictors and (log) time. Corresponding 95% confidence intervals (CIs) were estimated to measure the precision for each estimate of exposure and outcome association.

Abstraction forms were entered into Microsoft Office Access (2007), and data analyses were performed using SAS, version 9.2 (SAS Institute, Cary, NC). Investigators recorded all information on a standardized abstraction form developed in collaboration with epidemiologists and clinicians.

Research ethics. This study was approved by both the Duke University Medical Institutional Review Board (IRB) and the University of Chapel Hill Biomedical IRB. Both named IRBs waived the need for informed consent for this study. This research met criteria for a waiver of informed consent according to 45 Code of Federal Regulations (CFR) 46.116(d).

RESULTS

Baseline characteristics. There were 204 patients with records describing their antifungal treatment; 129 (63%) patients had severe disease defined as requiring amphotericin B induction treatment, and there were 75 (37%) patients treated with fluconazole as recommended for nonsevere disease. Transplant recipients were the smallest group ($n = 42$ [21%]), and HIV-positive ($n = 85$ [42%]) and HIV-negative, nontransplant patients ($n = 77$ [38%]) composed the remainder of the cohort. The majority of patients presenting at DUMC had CNS disease ($n = 126$ [62%]), and pulmonary disease was seen in about a third of all cases ($n = 69$ [34%]). In addition to CNS disease patients, three patients from the "other" disease category ($n = 9$ [4%]) also fit the definition for severe disease requiring amphotericin B induction treatment. The average lengths of follow-up did not differ substantially between groups (mean, 254 days for severe disease and 273 days for nonsevere disease).

Patients differed considerably with regard to presenting symptoms and conditions based on severity of cryptococcosis, although the durations of symptoms prior to presentation were similar in range. As expected, patients with nonsevere cryptococcosis ($n = 75$) had fewer neurological symptoms than patients with cases involving the CNS. Diagnostically, among those who had a lumbar puncture (LP) procedure ($n = 176$), 70% of whom had at least one opening pressure measurement ($n = 99$ for severe disease and $n = 24$ for nonsevere disease), the proportion of patients with a maximum opening pressure of at least 20 cm H_2O was high for both severe (78%) and nonsevere (54%) cryptococcosis patients. Notably, among those who received an LP, opening pressure measurements were missing for 21% of severe disease patients and 53% of nonsevere disease patients.

Baseline covariate measures prior to starting therapy according to patient status regarding our four main treatment exposures are shown in Tables 1 and 2. Patients who received their recommended therapy and severe disease patients who received flucytosine combination therapy had higher frequencies of symptoms and diagnostics indicating CNS disease (headache, vomiting and nausea, altered mental status, high CRAG titers) than did patients who did not receive the recommended initial therapy. This trend was not seen when looking at initial treatment dose, which showed very few variations in covariate frequencies between exposure groups, except for altered mental status and high serum CRAG titer (Tables 1 and 2).

Severe disease patients who received flucytosine combination initial therapy had lower frequencies of pulmonary symptoms (shortness of breath and cough), but a higher proportion of patients had a longer duration of symptoms (≥14 days) and diagnostic evidence of CNS disease (India ink, high cerebrospinal fluid [CSF] CRAG titer) than did those who did not receive flucytosine (Table 1). Patients given a shorter length of flucytosine exposure (0 to 7 days) had a higher proportion taking corticosteroids at the time of diagnosis, with reported weight loss, and with highly active antiretroviral therapy (HAART) exposure (among HIV-positive patients) than for patients given ≥7 days of flucytosine, who had a higher proportion with positive blood and CNS cultures, India ink, and organ transplants (Tables 1 and Table 2).

Antifungal treatment. Initial antifungal therapy type was considered appropriate given the disease severity for the majority of patients (88% [Table 3]). Only 11 patients (9%) with severe disease ($n = 129$) were not given amphotericin B induction therapy within 5 days of starting therapy; 7 of these patients were eventually given induction treatment (Table 3). Fourteen patients (19%) that fulfilled the definition of nonsevere cryptococcosis did not receive fluconazole for initial antifungal therapy. Rather, amphotericin B was used. Given the severity of disease, appropriate therapy was similar in prevalence between underlying risk groups: 88% ($n = 75$) of HIV-positive patients received appropriate therapy, and 84% ($n = 39$) of transplant recipients and 93% ($n = 65$) of HIV-negative/nontransplant patients received appropriate antifungal therapy.

There were 192 patients (94%) with available initial treatment dose information (Table 4). The mean dose of AmpBd was 0.66

TABLE 1 Baseline covariates prior to starting antifungal therapy by exposure status (recommended therapy according to IDSA guidelines and flucytosine exposure among patients with severe disease)

Parameter	No. or % of patients with or without:															
	Recommended initial treatment				Recommended initial treatment dose				Flucytosine combination therapy, severe only				Flucytosine exposure days, severe 2-wk survivors			
	Yes (n = 179)	%	No (n = 25)	%	Yes (n = 126)	%	No (n = 66)	%	Yes (n = 101)	%	No (n = 28)	%	≤7 days (n = 35)	%	>7 days (n = 81)	%
Age > 44 yrs[a]	97	54	9	36	69	55	30	45	46	46	16	57	14	40	41	51
Symptom length ≥ 14 days[a]	70	39	12	48	53	42	27	41	48	48	7	25	11	31	40	49
Severe disease	118	66	11	44	75	60	42	64	100	100	0		35	100	NA[c]	
No symptoms	18	10	3	12	15	12	6	9	1	<1	0		1	<1	0	
Altered mental status	46	26	1	4	27	21	1	2	36	36	10	36	12	34	26	32
Headache	80	45	7	28	51	40	31	47	69	68	11	39	23	66	54	67
Cough	33	18	7	28	25	20	13	20	13	13	6	21	6	17	10	12
Shortness of breath	29	16	11	44	26	21	12	18	8	8	7	25	5	14	8	10
Night sweats	16	9	4	16	13	10	6	9	8	8	2	7	2	6	7	9
Fever	72	40	12	48	52	41	24	36	45	45	14	50	14	40	36	44
Nausea	57	32	5	20	38	30	19	29	39	39	11	39	15	43	33	41
Vomiting	47	26	3	12	29	23	16	24	32	32	9	32	12	34	27	33
Seizures	10	6	2	8	5	4	7	11	9	9	2	7	3	9	5	6
Weight loss	27	15	4	16	17	13	12	18	20	20	5	18	11	31	13	16
Renal insufficiency	28	16	4	16	21	17	8	12	14	14	3	11	4	11	13	16
Liver insufficiency	10	6	0		6	5	3	5	6	6	1	4	1	<1	5	6
Current steroid exposure	65	36	10	40	47	37	23	35	33	33	7	25	6	17	28	35
Hematologic malignancy	10	6	5	20	11	9	4	6	5	5	3	11	4	11	1	1
Nonhematologic malignancy	5	3	1	4	4	3	2	3	1	1	1	4	1	<1	1	1
Transplant recipient	39	22	3	12	30	24	9	14	15	15	3	11	3	9	15	19
HIV positive	76	42	10	40	53	42	29	44	59	58	15	54	23	66	45	56
Exposure to HAART[b]	30	39	6	60	22	42	12	41	20	34	11	73	16	70	14	31

[a] Median values for age and duration of symptoms were used to create binary categories.

[b] Among HIV-positive patients only. Percentages for this variable reflect the total number of HIV-positive patients in each column (refer to the row above for denominator).

[c] NA, not available.

mg/kg/day (interquartile range [IQR], 0.56 to 0.73 mg/kg/day). Mean doses for lipid products of amphotericin B were similar and generally within the recommended range (Table 4), but ABLC was used slightly more often and at slightly higher doses than L-AmpB. The fluconazole dose averaged 350 mg/day (IQR, 208 to 400 mg/day [Table 4]), which was close to the recommended ≥400 mg/day for primary therapy and was lowest for HIV-positive patients (data not shown).

Overall, 66% of patients received appropriate dosing of their initial therapy and the remainder were universally underdosed (Table 4). Dosing for severe patients was below the recommended range for 42 (36%) patients, and this was similar for nonsevere patients (n = 24 [32%)] Furthermore, 43% of patients who received AmpBd and 27% of patients who received fluconazole did not receive appropriate dosing of therapy, while approximately 13% of patients who received lipid formulation amphotericin B (LFAmpB) did not receiving appropriate dosing. Among patients with severe disease, AmpBd formulation was within the recommended range of 0.7 to 1.0 mg/kg/day for 50 patients (57%) (Table 4) and tended to be outside the recommended range for HIV-negative patients in particular (data not shown). Median cumulative dose of initial therapy for patients who did not switch therapy is shown in Table S1 in the supplemental material.

Treatment for severe disease. Flucytosine (5FC) was incorporated into initial therapy in 77% of patients with severe cryptococcosis (Fig. 2). In this group, 79% of HIV-positive patients received flucytosine with initial therapy; 83% of transplant patients and 70% of HIV-negative/nontransplant patients also received flucytosine in combination with amphotericin B. The mean duration of initial flucytosine was 11.8 days (standard deviation [SD], ±6.3 days). Only 37% of the 101 patients who received any flucytosine continued the drug for at least 14 days as recommended (Fig. 2).

Among patients who received any formulation of amphotericin B as initial therapy (n = 118) and survived for at least 14 days since the start of therapy (n = 106), 56 patients (53%) completed ≥14 days of amphotericin B treatment. Seventy-five patients (58%) did not switch from their initial amphotericin B formulation (Table 3). Among the 14-day survivors (n = 106), patients who switched from their initial amphotericin B treatment experienced significantly longer treatment exposure than those who did not (difference in means = 6 days; 95% CI, 3 to 9 days).

Treatment for nonsevere disease. Most nonsevere disease patients (81%) received fluconazole as the initial antifungal therapy (Table 3). There were 10 (13%) patients with nonsevere disease who later changed initial treatment, compared to 50 (39%) severe disease patients (Table 3). This continuity of initial therapy was significantly higher than that among patients with severe disease (RR_{crude}, 1.4; 95% CI, 1.2 to 1.7). Of the patients given fluconazole as the initial therapy, 33 patients (54%) completed 90 days of treatment, while prior to 90 days, 2 patients were lost to follow-up and 6 died (one death was attributable to cryptococcosis). Fifty-two patients (72%) completed 30 days of fluconazole treatment among those surviving at least that long (n = 72).

Persistence of cryptococcosis. Persistence of infection was common 2 weeks after the start of therapy (47% [Table 5]) among patients with severe disease. Overall persistence at 4 weeks after the start of therapy was 25%; the rates of persistence were 33% among severe and 11% among nonsevere patients.

Patient mortality. Mortality in the first year after the start of cryptococcosis treatment was high (Table 5), with an attributable mortality rate through 1 year of 15% (n = 30). The all-cause mortality rate through 1 year of follow-up was 25% (n = 52). Notably, half of attributable deaths (n = 15) were among HIV-negative/nontransplant cases. The acute mortality rate was high

TABLE 2 Patient diagnostics at baseline prior to starting antifungal therapy, stratified by to exposure status (recommended therapy according to IDSA Guidelines and flucytosine exposure among patients with severe disease)

Patient diagnostic	No. or % of patients with or without:															
	Recommended initial treatment				Recommended initial treatment dose				Flucytosine combination therapy, severe only				Flucytosine exposure days, severe 2-wk survivors			
	Yes ($n = 179$)	%	No ($n = 25$)	%	Yes ($n = 126$)	%	No ($n = 66$)	%	Yes ($n = 101$)	%	No ($n = 28$)	%	≤7 days ($n = 35$)	%	>7 days ($n = 81$)	%
Positive cultures																
CNS—first LP[a]	96	54	6	24	59	47	35	53	84	83	17	61	24	69	69	85
Blood	56	31	10	40	38	30	24	36	42	42	10	36	10	29	35	43
Pulmonary	39	22	7	28	29	23	15	23	7	7	3	11	3	9	8	10
Histological evidence of *Cryptococcus*	52	29	8	32	38	30	20	30	15	15	3	11	7	20	10	12
Serum antigen titer ≥ 1:1,024	78	44	10	40	58	46	26	21	64	51	11	39	21	60	49	60
First LP[b]																
CSF antigen titer ≥ 1:1,024	48	33	2	13	87	82	45	82	44	44	5	23	10	30	32	40
CSF/serum glucose ratio < 0.6	117	82	14	70	78	78	45	83	90	93	20	87	27	82	74	95
CSF glucose ≤ 40	69	45	6	30	47	44	20	36	58	58	16	67	18	53	52	64
CSF protein ≥ 45	143	86	14	70	31	32	17	33	91	94	23	96	29	88	77	97
Positive India ink stain	61	44	1	5	36	37	23	46	55	63	6	2	11	35	44	60
Peak LP OP ≥ 20 cm H_2O[b]	78	72	10	67	58	73	21	58	64	79	11	61	21	78	49	75

[a] A total of 176 patients had an LP performed. Denominators used for percentages according to exposure (across) were 145, 15, 106, 55, 101, 23, 33 and 81 (CSF antigen titer ≥ 1:1,024); 143, 20, 100, 54, 97, 23, 33, and 78 (CSF/serum glucose ratio < 0.6); 153, 20, 107, 55, 100, 24, 34, and 81 (CSF glucose ≤ 40); 166, 20, 98, 51, 97, 24, 33, and 79 (CSF protein ≥ 45); and 138, 19, 97, 50, 88, 23, 31, and 73 (positive India ink stain).

[b] In an effort to capture more opening pressure (OP) measurements, maximum LP OP was used for this variable, as the first LP did not always measure OP. There were 123 (70%) patients who had an LP OP reading among those receiving an LP ($n = 176$). Denominators used for percentages according to exposure (across) were 108, 15, 79, 36, 81, 18, 27, and 65.

among patients with severe disease. Twenty-six (20%) patients died due to cryptococcosis, and half of these deaths were during the first 2 weeks while patients received induction treatment; three additional patients died through 4 weeks of follow-up. Among patients with severe disease, 10 (10%) who did not complete full induction therapy died. Among those who did not switch from their initial amphotericin B therapy ($n = 75$), there were 12 deaths attributable to cryptococcosis (16%), which was similar to the 9 deaths among the 43 patients (21%) who switched their amphotericin B induction regimen. Nonsevere disease patients had lower mortality rates; three deaths (5%) occurred within the first month of follow-up from the start of induction therapy.

Appropriate initial treatment. The risk of persistence 2 weeks from starting therapy among surviving severe disease patients who did not receive recommended treatment was 1.4 (95% CI, 0.6 to 3.0) relative to those who received appropriate initial therapy (Table 6). The risk of persistence at 4 weeks out from treatment among all surviving patients who did not initially receive the recommended antifungal treatment was higher (RR, 1.9; 95% CI, 0.9 to 4.3 [Table 6]). If patient deaths during 2 and 4 weeks were considered persistent infection, the corresponding RRs were 1.2 (95% CI, 0.6 to 2.7) and 1.6 (95% CI, 0.7 to 3.3).

The association between appropriate initial treatment and patient mortality was weak (Table 6). The adjusted HR for cryptococcosis-attributable mortality through 1 year of follow-up for initial treatment was 0.8 (95% CI, 0.3 to 1.8). The hazard of overall mortality through 1 year of follow-up among patients who did not receive the recommended antifungal treatment was 1.1 times the hazard of those who received the recommended initial treatment (95% CI, 0.4 to 3.2), adjusted for underlying hematologic malignancy and severe disease.

Appropriate initial treatment dose. Treatment dose had no discernible association with the outcome of persistence (Table 6). There was no significant association between the relative risk of persistence at 4 weeks out from treatment among surviving patients who did not receive recommended antifungal treatment dosing compared to those who initially received the recommended dosing (RR, 1.1; 95% CI, 0.6 to 1.8). Among patients with severe disease, the adjusted RR of treatment doses outside the recommended range and 2-week persistence was also close to null (Table 6). If patient deaths during 2 and 4 weeks were considered persistent infection, the corresponding RRs were similar: 1.1 (95% CI, 0.9 to 1.4) and 1.2 (95% CI, 0.8 to 1.8).

The hazard rate of cryptococcosis-attributable mortality among patients who received treatment dosing outside what was recommended was 2.3 times the rate among patients who received the recommended dosing (95% CI, 1.0 to 5.0) after adjusting for underlying hematologic malignancy, severe disease, and positive blood culture (Table 6). The adjusted HR for overall mortality was 1.3 (95% CI, 0.7 to 2.4), adjusting for underlying hematologic malignancy and positive blood culture.

Flucytosine use among severe cases. The RR of 2-week persistence and receiving flucytosine was similar to the RR at 4 weeks (Table 6). Among patients with severe disease ($n = 129$) for which

TABLE 3 Initial antifungal regimen by baseline severity of disease

Treatment	Antifungal exposure	All		Severe disease		Nonsevere disease	
		No.	%	No.	%	No.	%
Initial therapy	AmpBd	18	9	13	10	5	7
	AmpBd + 5FC	88	43	82	64	6	8
	ABLC	5	2	2	2	3	4
	ABLC + 5FC	12	6	12	9	0	
	L-AmpB	2	<1	2	2	0	
	L-AmpB + 5FC	7	3	7	5	0	
	Fluconazole	71	34	10	7	61	81
	Other	1	<1	1	<1	0	
Total no. of patients		204		129		75	
Total no. or % of patients on appropriate therapy[a]		179	88	118	92	61	81
Initial dose[b]							
	AmpBd ($n = 98$)	54	55	50	57	4	36
	ABLC ($n = 15$)	13	87	10	9	3	4
	L-AmpB ($n = 8$)	7	88	7	6	0	
	Fluconazole ($n = 71$)	52	73	8	7	44	57
Total no. of patients		192		117[c]		75[c]	
Total no. or % of patients on appropriate dose		126	66	75	64	51	68
Therapy changes							
	Fluconazole only	60	29	3	2	57	76
	AmpBd only	60	29	53	41	7	9
	LFAmpB only	23	11	22	17	1	1
	Voriconazole only	1	<1	1	<1	0	
	Fluconazole to AmpBd	9	4	6	5	3	4
	Fluconazole to LFAmpB	2	1	1	<1	1	1
	AmpBd to LFAmpB	46	23	42	33	4	5
	LFAmpB to AmpBd	3	2	1	<1	2	3
Total no. of patients		204		129		75	
Total no. or % for whom therapy changed		60	29	50	39	10	13

[a] Therapy was appropriate (recommended) with respect to the 2010 IDSA guidelines (1).
[b] Initial therapy dose was within appropriate (recommended) range for each drug defined by the 2010 IDSA guidelines (1).
[c] Denominators for each drug by severity of disease are 87, 12, 8, and 10 patients for severe disease and 11, 3, 0, and 61 patients for nonsevere disease.

flucytosine in combination with amphotericin B treatment is recommended, the adjusted risk of persistence at 4 weeks from initiation of treatment among surviving patients who received flucytosine as part of initial therapy was 0.6 times the risk of those who did not receive any flucytosine (95% CI, 0.3 to 1.3 [Table 6]). If patient deaths during 2 and 4 weeks were considered persistent infection, the corresponding estimates were RRs of 0.8 (95% CI, 0.5 to 1.4) and 0.8 (95% CI, 0.4 to 1.4), respectively.

The adjusted hazard of overall mortality through 1 year of follow-up among patients who received flucytosine with their initial antifungal therapy was 0.4 times the hazard of those who did not receive flucytosine (95% CI, 0.2 to 0.9) (Table 6). The HR of attributable mortality through 1 year of follow-up for flucytosine exposure was similar though less precise (HR, 0.5; 95% CI, 0.2 to 1.2).

There was no clear association with risk of persistence (2 or 4 weeks) between patients who received >7 days of flucytosine in combination with their primary antifungal therapy and those who received ≤7 days (Table 6). Receiving >7 days of flucytosine was protective of cryptococcosis-attributable mortality hazard compared to the risk for those who received ≤7 days of flucytosine, though the association was not significant (Table 6). Notably, full flucytosine exposure was not possible for those that succumbed to acute mortality within 14 days of starting treatment ($n = 13$; 50% of all attributable deaths).

Additional outcomes. Reinduction with amphotericin B treatment was performed in 25% ($n = 29$) of severe disease patients and 7% ($n = 5$) of nonsevere disease patients (Table 5). IRIS was diagnosed rarely ($n = 7$ [3%]) among all patients surviving through the end of initial therapy, and most of these diagnoses were among patients with severe disease ($n = 6$).

Pertaining to severe disease patients, renal toxicity during initial treatment occurred in approximately one-third of cases ($n = 33$), and 26 of these patients did not complete 14 days of induction

TABLE 4 Dosing of initial induction therapy and baseline disease severity among patients receiving antifungal therapy and with dosing information

Antifungal therapy	Dosage[a] for patient group					
	Total ($n = 192$)[b]		Severe ($n = 117$)[c]		Nonsevere ($n = 75$)[c]	
	Mean	IQR	Mean	IQR	Mean	IQR
Amphotericin B ($n = 121$)						
AmpBd	0.66	0.56–0.73	0.66	0.57–0.73	0.65	0.48–0.84
ABLC	4.90	4.62–5.20	4.86	4.54–5.14	5.06	4.80–5.47
L-AmpB	4.28	3.29–5.05	4.28	3.29–5.05	NA[d]	
Fluconazole ($n = 71$)	350	208–400	367	400–400	346	208–400

[a] Fluconazole dosing is in mg/day. All other medications are in mg/kg/day.
[b] Twelve patients were missing information on initial treatment dose (either dosing or body weight information).
[c] Denominators for each antifungal drug (top to bottom) are 87, 12, 8, and 10 patients for severe disease and 11, 3, 0, and 61 patients for nonsevere disease.
[d] NA, not available.

treatment. Out of the 33 cases with renal toxicity, 19 (58%) patients switched from their initial therapy. Fifteen of these 19 patients who experienced renal toxicity and switched initial therapy did not complete 14 days of induction treatment. Of the remaining 14 patients who experienced renal toxicity but did not switch from their initial therapy, 11 did not complete 14 days of induction treatment. Among the patients surviving at least 14 days (eligible to switch therapy within recommended treatment length), evidence of renal toxicity was associated with a higher relative risk of switching initial treatment (RR_{crude}, 1.9; 95% CI, 1.0 to 3.3).

DISCUSSION

This study of cryptococcal management provides important insights into the disease in an advanced treatment center and presents additional data and support for the current IDSA guidelines. Retrospective reviews have limitations. However, they can provide crucial knowledge about "real-world" effectiveness of therapy that is not captured in randomized controlled studies. The outcomes of individual cases are subject to underlying disease, toxicity of medications, and difficulties in understanding complications, such as IRIS and increased intracranial pressure. By studying the effectiveness of therapy, we can evaluate the current standards of care and their real-world outcomes and identify obstacles for improving that care.

There were important differences in symptoms, underlying conditions, and diagnostics based on cryptococcosis disease severity. Of note, elevated cerebrospinal fluid opening pressure was prevalent in two divergent groups (one with and one without indicators of central nervous system disease). This result was interesting knowing that the nonsevere group was negative for central nervous system cryptococcosis, yet the high frequency of missing values hinders interpretation. It demonstrates that an elevated cerebrospinal fluid opening pressure in patients with neurological symptoms can be an indicator for intracranial management (27), but the potentially low specificity observed in our study suggests that it may not serve as a precise diagnostic tool, as it is presently measured at the bedside with a manometer. However, in clinical practice, intracranial pressure measurements are importantly linked to other neurological symptoms of disease such as headache or altered mental status. All of these factors remain essential for treatment and resolution of cryptococcal meningitis (28), but better and more precise measurement technology may be warranted.

Overall, appropriate initial treatment of cryptococcosis was high over the 14-year study period, with a recommended therapeutic regimen being used for 88% of patients and the recommended dosing being followed in 66% of patients. Despite small numbers and the retrospective nature of this study, there was favorable evidence for compliance and clinical results supporting the IDSA guidelines, but increased adherence may improve clinical outcome. For instance, patients not receiving initial recommended treatment regimens had a higher risk of persistent infection at 4 weeks (RR, 1.9) and a higher attributable mortality (RR, 2.3) in those not receiving the recommended dose. However, over

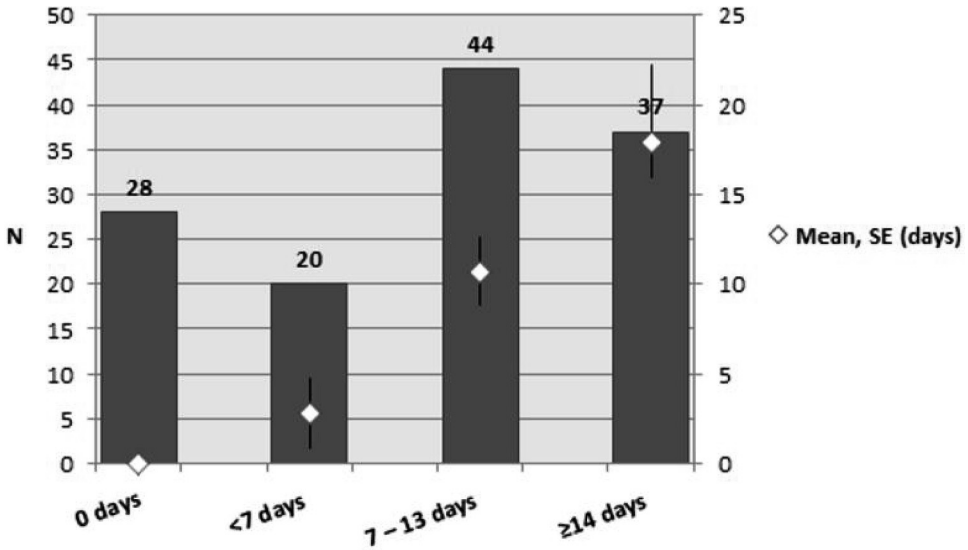

FIG 2 Duration of flucytosine combination treatment with initial primary therapy (severe disease; $n = 129$).

TABLE 5 Patient outcomes after antifungal therapy, according to treatment exposure status

Outcome	No. or % of patients with or without:															
	Recommended initial treatment				Recommended initial treatment dose				Flucytosine combination therapy, severe only				Flucytosine exposure (days), severe 2-wk survivors			
	Yes	%	No	%	Yes	%	No	%	Yes	%	No	%	≤7 days	%	>7 days	%
Persistence, 2 wks[a]																
Yes	52	50	4	40	33	42	17	47	46	50	10	43	16	46	40	50
No	53	50	6	60	37	47	19	53	46	50	13	57	19	54	40	50
Persistence, 4 wks																
Yes	39	24	6	27	26	25	15	26	29	33	8	33	11	34	26	33
No	121	76	16	73	89	85	43	74	60	67	14	67	21	66	53	67
Cryptococcosis-attributable mortality																
Yes	26	15	4	16	12	10	14	21	18	11	8	29	6	17	7	9
No	153	85	21	84	114	90	52	79	83	89	20	71	29	83	74	91
1-yr mortality																
Yes	41	23	8	32	26	21	18	27	22	22	12	43	7	20	14	17
No	138	77	17	68	100	79	48	73	79	78	16	57	28	80	67	83
Patient reinduced[b]																
Yes	29	18	5	23	20	17	11	19	22	19	7	30	8	23	21	26
No	132	82	17	77	95	83	48	81	71	81	16	70	27	77	60	74
IRIS[c]																
Yes	6	4	1	5	4	3	3	5	6	6	0	0	1	3	5	6
No	155	96	21	95	111	97	57	95	87	94	23	100	34	97	76	94
Renal toxicity[d]																
Yes	30	31	3	43	23	35	9	28	27	32	6	30	10	32	23	32
No	67	69	4	57	42	65	23	72	57	68	14	70	21	68	50	68

[a] Among severe cases only: 2-week persistence, $n = 115$ (13 deaths; 1 patient with missing information); for 4-week persistence, $n = 182$ (5 additional deaths since 2 weeks and 3 patients with missing information).
[b] Patient had to survive through 2 weeks for severe disease and 90 days for nonsevere disease (end of recommended treatment duration) to be eligible for denominator of reinduction status.
[c] Patient had to survive through 2 weeks for severe disease and 90 days for nonsevere disease (end of recommended treatment duration) to be eligible for IRIS diagnosis.
[d] Drop in glomerular filtration rate of >50% (among severe disease only)—measured from the date closest to the start of therapy (week 0) to the date closest to 2 weeks after starting therapy (week 2). Patient had to survive through 2 weeks (end of recommended treatment duration) to be eligible for renal toxicity outcome.

one-third of patients did change from their initial therapy; 28% of these patients were switched from a nonrecommended to a recommended therapy given their disease severity. The consequences of this change in therapy resulted in patients who experienced an overall longer duration of induction than did those who did not change therapy.

Flucytosine was used in 78% of severe disease patients for initial therapy, but only 37% of these recipients continued combination treatment for at least 14 days. High acute mortality (prior to completion of induction) and renal toxicity among severe disease patients likely contributed to this high incompletion rate. Nonetheless, patients receiving flucytosine combination therapy experienced lower rates of overall and attributable mortality than those patients who did not receive any flucytosine. However, receiving more than 7 days of flucytosine was not significantly associated with lower mortality rates than for those who received at most 7 days, suggesting that early acute mortality may not be preventable with flucytosine use but is a consequence of other underlying factors, such as malignancy or AIDS, contributing to poor patient outcome. On the other hand, the observed protective effects of flucytosine support a growing body of evidence that combination therapy is important to a positive outcome (8, 10, 29). The polyene and flucytosine combination has consistently demonstrated its superior success, with retrospective data identifying better outcomes at 2 weeks (8), prospective randomized trial data on fungicidal activity in the CSF (29), and correlation with this fungicidal activity and outcome (30, 31). Thus, our study highlights the importance of increased use of flucytosine. This may be improved with rapid-access flucytosine levels and/or close follow-up of complete blood counts—elements needed to increase the likelihood patients will receive at least 2 weeks of flucytosine.

By our definition persistence at 2 weeks was a common outcome, but 75% of patients through 4 weeks did have documented resolution of symptoms and microbiological signs of disease. The percentage of patients with disease resolution through 4 weeks falls into the same range of outcomes as previously published cohorts (6, 30, 32, 33). This rate of persistence of infection likely reflects the high burden of *Cryptococcus* in the CSF of these patients and challenges us to be more aggressive about measuring how our treatment regimens impact the killing of yeasts during induction therapy.

The rate of all-cause mortality of nonsevere disease patients

TABLE 6 Final adjusted models of the influences of treatment type, dosing, and flucytosine use (severe disease only) on the risk of persistence of disease at 2 weeks (severe disease only) and 4 weeks (both groups) and hazard of mortality

Risk assessment	Outcome	Initial therapy type[a]		Initial therapy dose[b]		Flucytosine[c]		Flucytosine[d]	
		Ratio	95% CI	Ratio	95% CI	Ratio	95% CI	Ratio	95% CI
RR	Persistence at 2 wks (severe only)[e]	1.4	0.6–3.0	1.1	0.8–1.4	0.8	0.5–1.4	0.9	0.7–1.2
	Persistence at 4 wks[e]	1.9	0.9–4.3	1.1	0.6–1.8	0.6	0.3–1.3	0.8	0.4–1.3
HR	Cryptococcosis-attributable mortality	1.1	0.4–3.2	2.3	1.0–5.0	0.5	0.2–1.2	0.6	0.2–1.8
	1-yr all-cause mortality	0.8	0.3–1.8	1.3	0.7–2.4	0.4	0.2–0.9	1.1	0.4–2.7

[a] Patient not given recommended antifungal therapy regimen initially (1) or received recommended therapy (0). Persistence at 2 weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1,024). Persistence at 4 weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1,024) and severe disease. Cryptococcosis-attributable mortality was adjusted for underlying hematologic malignancy and positive blood culture. One-year all-cause mortality was adjusted for confounding by severe disease and underlying hematologic malignancy.

[b] Patient dose for initial antifungal therapy was outside the range recommended (1) or dosing was within the recommended range (0). Persistence at 2 weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1,024). Persistence at 4 weeks was adjusted for severe disease. Cryptococcosis-attributable mortality was adjusted for underlying hematologic malignancy, positive blood culture, and severe disease. One-year all-cause mortality was adjusted for confounding by positive blood culture and underlying hematologic malignancy.

[c] Flucytosine was used with initial therapy among severe disease patients only ($n = 129$). Persistence at 2 weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1,024). Persistence at 4 weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1,024) and ≥14 days of symptoms prior to presentation; both cryptococcosis-attributable mortality and overall mortality rates were adjusted for underlying hematologic malignancy and receipt of amphotericin B deoxycholate.

[d] Flucytosine exposure categories were >7 days (referent) and ≤7 days of exposure. Two-week persistence was adjusted for high CSF cryptococcal antigen titer (≥1:1,024) at first LP; 4-week persistence was adjusted for ≥14 days of symptoms prior to presentation and high initial CSF cryptococcal antigen titer (≥1:1,024) at first LP; attributable mortality and overall mortality rates were adjusted for underlying hematologic malignancy.

[e] Persistence outcomes were contingent on survival through 2 weeks and 4 weeks since the date of starting antifungal therapy.

(20%) was similar to that in the severe disease group (26%). When comparing cryptococcosis-attributable mortality, the mortality rate in the nonsevere disease patient group was found to be much lower than the mortality rate in the severe disease patient group (5% and 20%, respectively). Future investigation should consider highlighting the distinction of these two mortality outcomes so as to not overestimate the true effect of cryptococcosis on patient death. This also serves to show that nonsevere disease also does identify a patient with a serious underlying disease in many cases.

This difference between attributable and overall mortality rates emphasizes how the specific underlying disease was a risk factor for poor outcomes. For example, a hematologic malignancy was a strong independent predictor of patient mortality in our cohort and likely reflects the end stage of the underlying disease when cryptococcosis appears (34, 35). Fourteen out of 15 patients with hematologic malignancy were HIV negative and had not received a solid-organ transplant. In fact, 50% of cryptococcosis-attributable deaths were in HIV-negative, nontransplant patients with a variety of underlying diseases. Thus, identifying background rates of cryptococcosis and baseline risk factors for earlier diagnosis and assessing present treatment strategies in relationship to how they are efficiently handling the fungal burden could be very useful in reducing morbidity and mortality rates for this group.

Four major randomized treatment trials served as important comparators to this study and informed the treatment recommendations in the current IDSA guidelines (6, 9, 10, 22). The key difference between these studies and this cohort was that this study was observational (nonrandomized) and used a single center instead of multiple sites for patient recruitment. Only one study (10) used a larger patient group for analysis, and all but one study (22) recruited only cryptococcal meningitis patients. Our study spanned a much longer period (14 years, compared to ≤5 years for the randomized trials). The continuity of care at a single center allowed for such an extended period of in-depth retrospective observation. However, there were striking similarities between results in our study and those of these randomized trials. First, we observed a prevalence of cure or improvement through 4 weeks of 75%, and Dismukes et al. observed 80% cure through 4 weeks (22). Second, van der Horst et al. reported that through 2 weeks of combination induction therapy, 60% patients were culture negative (10). In our study, 42% of patients with severe disease had signs of persistent infection through 2 weeks. Third, attributable mortality and overall mortality rates were consistent with those found in three other randomized trials (6, 9, 33). Among patients with severe disease in our study, 10% died in the first 2 weeks. This sobering figure demonstrates that acute mortality continues to be a serious problem despite over 3 decades of clinical study and experience. Fourth, since timing of relapse was difficult to categorize in this observational study, reinduction might be considered a proxy for relapse—19% of our patients were reinduced. This finding was similar to the prevalence of relapse (16% in a 6-week arm and 27% in a 4-week arm) reported by Dismukes et al. (22). This relapse rate may be artificially high secondary to lack of IRIS appreciation, although in retrospect in our review, we found a relatively low incidence of this condition in our cohort.

Although an observational study design was used, we obtained results similar to those in previous randomized trials. Taking into consideration our single-center attribute, the observed consistency in treatment may have had the unintended consequence of revealing underlying factors contributing to patient failure rather than determining whether variations in initial treatment were contributing to persistence of cryptococcosis or its attributable mortality rate. In fact, positive CSF cultures, high CSF antigenemia, absence of headache, and long duration of symptoms prior to admission continue to emerge in our analyses as strong predictors of patient failure rather than treatment regimens. Within these risk factors are likely buried important features of outcome regarding high burden of yeasts and poor host inflammatory re-

sponses within the CNS which must be carefully defined and monitored in relationship to therapy (32).

Strengths and limitations. Our results may not be applicable to all centers in the United States, as approaches for ancillary care may differ. Furthermore, using hospital records favors cases with severe disease and results in selection bias against asymptomatic or mild disease. The total population at risk was not estimable in this study, and the underlying source population and referral patterns could shift over time.

In order to obtain a reportable picture of various outcomes, we created definitions of severe versus nonsevere, persistence of infection (2 and 4 weeks), and attributable mortality, and we used the IDSA guidelines to define appropriate therapy. Regardless, the overall mortality rate in patients with severe cryptococcosis remains high, at almost 25% within 1 year.

This study was limited to a single tertiary care center and teaching hospital. Although this could be considered a limitation, evaluating patients at a single center allowed us to examine a broader time period with higher uniformity of data availability, continuity of care, and treatment consistency. Because cryptococcosis is a rare disease with a high acute mortality rate, smaller case numbers limited the ability to adjust for all confounders identified in minimum adjustment sets and prevented robust statistical analyses of treatment effects, but to our knowledge this study is the largest single-center cryptococcosis cohort study, and it provides in-depth information on a heterogeneous group of patients. It represents an important insight into how this infection is being managed and what the outcomes have been. Future studies combining our cohort with additional patient groups from other institutions would provide beneficial robustness for treatment effectiveness analyses.

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