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MEDICINE

Disseminated and Relapsing Cryptococcosis: What We Still Have to Learn—a Case Series and Review of Literature

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Abstract

Two cases of disseminated cryptococcosis are described. The first was an HIV-infected patient where cryptococcosis was diagnosed as "unmasking immune reconstitution syndrome"; the second was an immunosuppressed patient with multiple myeloma. In both cases, a definitive healing could not be reached despite long therapeutic approaches. This review summarizes both the most recent and relevant studies about disseminated and refractory form of cryptococcal infections and identifies research gaps. Given the limited data, we draw some conclusions with respect to management from literature: not clear and accepted indication are available regarding disseminated cryptococcosis, no specific schemes were identified, and the duration of therapy is usually decided case by case and supported only by case reports. In this perspective, usually standard therapeutic schemes and duration of induction depend on multiple factors (e.g., neurologic deficit, non-HIV/non transplant status, CSF culture positivity at 2 weeks, etc.). We found that there are no empiric and literature data that support a role of cryptococcal serum antigen (CRAG) in guiding the antifungal therapy; with the data collected, we think that although is possible, it is very rare to find disseminated cryptococcosis with negative CRAG. We looked also for the more important risk factor of recurrence. Some possible causes explored are risk of azole resistant strains, pre-existent conditions of patients that play a permissive role and the common situation where flucytosine is unavailable that led to suboptimal induction phase of therapy. Herein, we discuss disseminated cryptococcosis with a particular attention to antifungal therapy, role of cryptococcal antigen, and risk factors for recurrence of disease.

Keyword Cryptococcosis · Case series · Disseminated · Review · Antifungal therapy

Introduction

Cryptococcus neoformans is an encapsulated yeast, which is present in environment, causing life-threatening infections such as meningitis, pulmonary cryptococcosis, or disseminated form of the disease [1]. Predominantly, it affects patients with impairment of immune status such as human immunodeficiency virus (HIV) infection. Before the introduction of highly active antiretroviral therapy (HAART), as many as 5% of all HIV-infected persons developed cryptococcosis [2]. Since then, the incidence has decreased by approximately one-half [3]. With the improvement of antiretroviral therapy (ART) and reduction of AIDS diagnoses, more cases are related to other cause of immunosuppression, like solid organ transplantation (SOT), systemic lupus erythematosus (LES), malignancy, and sarcoidosis [4, 5, 6, 7, 8]. Nationally representative estimates for the incidence of cryptococcosis are difficult to obtain because cryptococcosis is only reportable in a few countries. More data are available regarding cryptococcal meningitis: the regions with the highest number of estimated cases were sub-Saharan Africa (162,500 yearly cases), followed by South and Southeast Asia (43,200 yearly cases), while Europe accounts for nearly 4,400 yearly cases [9].

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The diagnostic criteria of disseminated cryptococcosis have been defined as two or more non-adjacent organs being simultaneously affected with *Cryptococcus* spp. [10] and its management remains a challenge for doctors: persistence or recurrence of disease despite adequate therapy is indeed very common.

We present two cases of disseminated cryptococcosis focusing on clinical management and duration of therapy, infection control, and risk of relapse.

Case 1

HIV infection was diagnosed in a 49-year-old woman in September 2018 as a result of checks for weight loss and asthenia. Baseline CD4+ cells were 22/μL, HIV-RNA 1,360,000 copies/mL. Antiretroviral therapy (ART) with dolutegravir (DTG) + tenofovir disoproxil fumarate (TDF) + emtricitabine (FTC) has been started the same day when HIV was diagnosed according to the test and treat strategy [11]. After 1 month of therapy, the CD4 cell count was 182/μL and the viral load was of 478 copies/mL. In November 2018, she was hospitalized at our division for confusion, asthenia, and visual hallucination. Upon admission, she underwent lumbar puncture. Cerebrospinal fluid (CSF) was cloudy, white cells 240/μL (mainly monocytes), glucose 19 mg/dl, ratio blood glucose and CSF glucose 20 %, and protein 1.74 g/L; opening pressure was not reported during the procedure. Polymerase chain reaction (PCR) by FilmArray™ (Biomerieux, Ponte a Ema, Florence, Italy) for meningitis/encephalitis was positive for *Cryptococcus neoformans/gattii*; CrAg was positive (>1:512). Microscopic examination and CSF fungal culture were both negative.

Treatment with liposomal amphotericin B (L-Amb) 4 mg/kg/day plus fluconazole (200mg ×3/day) and dexamethasone 8mg/day was carried out for 2 weeks and ART stopped. 5-flucytosine was not used because it was not available. The symptoms disappeared and the woman was discharged after 2 weeks of therapy with home therapy based on fluconazole 400 mg/day prescribed for 8 weeks and with an indication to resume ART and follow fluconazole secondary prophylaxis (200mg/day orally).

In January 2019, under fluconazole prophylaxis, a magnetic resonance imaging (MRI) of the brain was performed and a 6-mm lesion in the *corpus striatum* was observed, compatible with cryptococcoma. A computerized tomography (CT) total body was therefore performed. It showed a subpleural nodular lesion in the left lung associated with enlarged node; both lesions were examined by positron-emission tomography (PET) revealing an intense metabolic activity. In the same month, a fine-needle biopsy was performed in the lung lesion with evidence of focal necrotic areas and a Gorcott-positive and PAS-positive microscopic formation, compatible with cryptococcosis.

In July 2019, a new CT scan showed new nodules in the left lung. Histological examination of the aspirate obtained by transbronchial needle aspiration (TBNA) showed a PAS-positive lesion suggesting a probable cryptococcal etiology.

Moreover, a new brain MRI showed enlargement of the aforementioned lesion and appearance of new lesions, so a new therapeutic attempt with L-Amb (at the dosage used previously) was started. Unfortunately, despite the maximal antifungal treatment with L-Amb 4 mg/kg and fluconazole 400mg administered periodically and ongoing at the time of the writing (September 2020), cerebral lesions are progressively worsening, and clinical conditions are deteriorated despite CD4+ cells increased at 270/μL, and HIV-RNA are <20 copies/mL.

Case 2

In July 2019, a 70-year-old man with multiple myeloma (MM) started having episodes of epilepsy treated with long-term levetiracetam.

Previously, in 2010, he was treated with an unsuccessful autologous hemopoietic stem cell transplantation (HSTC) followed by lenalidomide plus steroid treatment. In July 2019, a brain CT scan performed due to progressive headache was negative; conversely a concomitant chest X-ray showed opacity of the lower right lung lobe in the absence of pneumonia symptoms. In August 2019, the patient had new episodes of seizures, and a brain CT scan showed brain injuries (microangiopathic phenomena probably not related to the cryptococcal disease).

The patient, immediately hospitalized, underwent lumbar puncture: the cerebrospinal fluid was clear, proteins 0.30 g/l, leukocytes 9/μL and glucose 61 mg/dl, ratio blood glucose and CSF glucose 20 %, and India ink smear and cryptococcal antigen (CrAg) in CSF tested positive (>1:512); opening pressure was not reported during the procedure.

A prompt treatment with L-Amb (3.5 mg/kg/die) and fluconazole (200 mg × 3/die) for the first 2 weeks was started; as soon as flucytosine was available, we introduced it (dose 2.5 g × 2/die), while lenalidomide was stopped due to severe leukopenia. A new lumbar puncture was done 4 weeks apart and the culture for Cryptococcus spp. was negative; CrAg was still positive in CSF (titer >512).

After 60 days of treatment, an encephalic MRI did not show improvement. Moreover, a chest CT scan confirmed the previous right lung opacity, and through bronchoalveolar lavage and TBNA, *C. neoformans* infection was demonstrated (histologically). The hospital stays lasted 72 days, and during the antifungal treatment, several analyses of the cerebrospinal

fluid were repeated with persistently positive results of CrAg> 1: 512, PCR for C. neoformans and ink test.

As the clinical conditions improved, despite the failure to eradicate the Cryptococcus from the CSF, the patient was discharged with fluconazole at the 400-mg daily dose, but at the moment of writing this manuscript, he was lost at clinical follow-up.

Discussion

The diagnostic criteria of disseminated cryptococcosis have been defined as 2 or more non-adjacent organs being simultaneously affected with cryptococcosis [10]. Disseminated cryptococcosis is a real challenge for clinicians and its management continues to be unclear. Both cases show the paradigm that immune regulation is necessary to control the infection: in Case 1, the low CD4 +T cell count facilitated the spreading of infection. Moreover, it is a paradigmatic example of unmasking immune reconstitution syndrome (IRIS). In Case 2, the impairment of immune system made the patient prone to infection due to the underlying cellular and humoral immunodeficiency associated with multiple myeloma.

We managed both patients tailoring the antifungal therapy to the single patient, but such strategy has been ineffective and left us full of questions unsolved such as: How long the antifungal therapy should be prolonged? Is there a role for Cryptococcus antigen title in guiding the duration of therapy? Which are the main causes of relapsing infection? We reviewed what literature says focusing on these three aspects of management of disseminated cryptococcosis.

Antifungal Therapy

The optimal approach to antifungal therapy for *C. neoformans* meningoencephalitis and/or disseminated disease involves three phases: induction therapy with intravenous amphotericin B liposomal plus flucytosine (if available) or fluconazole for at least 2 weeks, followed by consolidative therapy for eight weeks, and then maintenance (i.e., suppressive) therapy for at least 1 year to decrease the risk of relapse with fluconazole. Table 1 and Table 2 summarize antifungal therapy both in HIV-infected individuals and transplant recipient ones.

Considering that no clinical trial has evaluated the optimal therapy of non-meningeal disease, the treatment regimen suggested is based on expert opinion, and induction phase should be prolonged for at least 6 weeks [13].

Treatment of disseminated cases of the disease should have an induction phase of four weeks at minimum (in HIV patients and non-HIV as well); this duration should be prolonged to 6 weeks if neurological forms are present.

In the studies found, these schemes appear to reduce mortality from 14–25% to 6% and relapses from 17–24% to 2–4% [14]. In all cases which flucytosine could not be used, it is suggested to add fluconazole amphotericin B. Although some articles of low quality revealed good outcomes for cryptococcal meningitis in HIV-negative patient treated with only fluconazole [2], this monotherapy remain suboptimal in HIV-positive patient [15].

In both presented cases, induction therapy with L-Amb was prolonged for more than 2 weeks: in Case 1, it was re-administered due to relapse of infection for further 2 months, while in Case 2, it was never stopped for 10 weeks due to the persistence of infection in the CSF until the discharge of the patient. Anyway, it is not always possible to prolong the antifungal therapy due to possible drug toxicity like nephrotoxicity, hypokalemia, and hypomagnesemia, so that infection remains uncontrolled.

It follows that regarding disseminated cryptococcosis, no specific schemes about induction, consolidation, and maintenance were identified by clinical trial, and duration of therapy is usually decided case by case and supported only by case report and case series [13]. A Chinese retrospective study made on 48 patients less than 18 years old with disseminated cryptococcosis showed good results (overall mortality rate of pediatric patients 11.5%) with amphotericin B deoxycholate (0.7–1.0 mg/kg/die), 5-flucytosine (100 mg/kg/die), and fluconazole (6 mg/kg/die) followed by fluconazole (6 mg/kg/die) for 6–12 months [16].

As seen in Table 3, different therapeutic schemes were used recently in disseminated cryptococcosis with heterogeneous results.

Never a doubt that amphotericin B is the milestone of anticryptococcal therapy, but half a century after the introduction of it, the management of cryptococcosis remains indeed unsatisfactory. Research about innovative drugs is warranted, with several antimicrobial agents showing in vitro activity against *Cryptococcus* [38] but such discussion is beyond the scope of this review.

Role of Cryptococcal Antigen

In both our cases, CrAg was useless as a follow-up marker of infection control despite the therapy: in Case 1, the titer (starting from >1:512) dropped in just one evaluation (1:128) before rising up again, while a titer of >1:512 was always found in Case 2.

CrAg level was demonstrated to be related to the presence of symptoms of cryptococcosis more often than asymptomatic form of the disease, which appears to anticipate death. The literature showed that several cohorts underline a connection among plasma titers and mortality: when the first increase, the latter is higher as well [39]. Another relevant point is that symptoms appear to be linked to survival only for certain titers of CrAg; in fact, titers <1:80 plus symptoms like headache did not correlate to survival, and on the other hand, titers between

Table 1 Antifungal therapy for cryptococcal meningoencephalitis in HIV-infected individuals [12]

Regimen	Duration
Induction therapy	/
Liposomal amphotericin B (3–4 mg/kg/die) plus flucytosine (100 mg/kg/die)	2 weeks
Amphotericin B deoxycholate (0.7–1.0 mg/kg/die) plus flucytosine (100 mg/kg/die)	2 weeks
Liposomal amphotericin B (3-4 mg/kg/die) OR amphotericin B deoxycholate (0.7–1.0 mg/kg/die) OR amphotericin B lipid complex (5 mg/kg/die) (for flucytosine-intolerant patients)	4–6 weeks
Alternative for induction therapy*	/
Amphotericin B deoxycholate (0.7–1.0 mg/kg/die) plus fluconazole	…
Fluconazole plus flucytosine	…
Consolidation therapy	/
Fluconazole 400 mg/die	8 weeks
Maintenance therapy	
Fluconazole 200 mg/die	≥1 year
Amphotericin B deoxycholate (1.0 mg/kg per week)	≥1 year

*Alternative treatment modalities have a suboptimal outcome and should be used only if the recommended treatment options are not feasible

1:160 and 1:320 in presence of symptoms correlate with lower survival more often than same titers but in asymptomatic patients. In case of very high titers (e.g., 1:640), survival appears to be poor independently of presence of symptoms [39].

The importance of asymptomatic CrAg as a precursor to symptomatic meningitis and death has also already well been defined [40]. In fact, CrAg is detectable in blood weeks to months before onset of meningitis symptoms [41], and long-term maintenance therapy with fluconazole is indicated to prevent relapse after an episode of cryptococcal meningitis in patients with advanced HIV infection and asymptomatic presence of cryptococcal antigen [42].

Moreover, is not well established if there is a value of CrAg titer that can predict the disseminated form of the disease. As is possible to see in Table 4, among the most recent case report, the titer had different values (mean 858, median 384). There was also a report of a very high titer (>1:100.000) [43]. These data suggest that although is possible, it is very rare to find disseminated form with negative CrAg.

Among asymptomatic CrAg-positive persons, CrAg titers of ≥1:160 are associated with increased mortality despite receiving fluconazole pre-emptive therapy [48].

The WHO, indeed, recommends routine serum or plasma CrAg screening in ART (antiretroviral therapy)-naïve adults a CD4 counts < 100 cells/μL, followed by pre-emptive anti-fungal therapy if CrAg positive, to reduce the development of cryptococcal disease. It is proved that this strategy can prevent clinical disease, avoid hospitalization, and improve long-term survival to be equivalent to

Table 2 Antifungal therapy for cryptococcal meningoencephalitis in transplant recipients [12]

Regimen	Duration
Induction therapy	/
Liposomal amphotericin B (3–4 mg/kg/die) plus flucytosine (100 mg/kg/die)	2 weeks
Amphotericin B lipid complex (5 mg/kg/die) plus flucytosine (100 mg/kg/die)	2 weeks
Alternative for induction therapy*	/
Liposomal amphotericin B (6 mg/kg/die)	4–6 weeks
Amphotericin B lipid complex (5 mg/kg/die)	4–6 weeks
Amphotericin B deoxycholate (0.7 mg/kg/die)	4–6 weeks
Consolidation therapy	/
Fluconazole 400–800 mg/die	8 weeks
Maintenance therapy	
Fluconazole 200–400 mg/die	6 months to 1 year

*Alternative treatment modalities have a suboptimal outcome and should be used only if the recommended treatment options are not feasible

Table 3 Therapeutic schemes in case reports of disseminated cryptococcosis reported in literature from 2010 to 2020

Study (author, year)	Therapeutic regimen	Duration (weeks)	HIV	Outcome
Chavapradit, 2018 [17]	I: amphotericin B deoxycholate (0.7 mg/kg/die); C: fluconazole 800 mg/die M: fluconazole 200 mg/die	I=6 C=8 M=52	No	Improvement of clinical status
Han, 2017 [18]	I: amphotericin B liposomal (4 mg/kg/die); C: fluconazole 400 mg/die M: fluconazole 200 mg/die	I=4 C=8 M=38	No	Healing
Vechi, 2019 [19]	I: amphotericin B deoxycholate (1mg/kg/die) + fluconazole 800 mg/die then fluconazole 1200 mg/die; C: fluconazole 800 mg/die M: fluconazole 450 mg/die	I=1 + 2 C=8 M= NR	Yes	Improvement of clinical status
Sato, 2019 [20]	I: amphotericin B liposomal 250 mg/die + flucytosine 1500 mg/die	I=2	No	Died
Ito, 2017 [21]	I: amphotericin B liposomal 200 mg + flucytosine 7000 mg C: fluconazole 800 mg/die M= fluconazole 800 mg/die	I=2 C=21 M=ongoing	No	Improvement of clinical status
Pal, 2015 [22]	I: Amphotericin B deoxycholate (0.5mg/kg/die) C: fluconazole 400 mg/die	I=3 C=25	No	Healing
Sacht, 2016 [23]	I: amphotericin B deoxycholate 50mg/die + fluconazole 900mg/die C: fluconazole 300mg/die	I=4 C=8	No	Healing
Huang, 2015 [24]	I: amphotericin B liposomal + flucytosine M: fluconazole	I= 10 M=ongoing	No	Healing
Beji; 2017 [4]	I: voriconazole 800mg/die M: fluconazole 400mg/die	I=1 M=1	No	Died
Slawinska, 2017 [25]	I: amphotericin B liposomal + flucytosine C= fluconazole 400mg/die M=fluconazole 200mg/die	I=2 C=8 M= NR	Yes	Relapsing
Qu, 2020 [26]	I= amphotericin B liposomal 150mg/die + 0.01 mg intrathecal + fluconazole 400mg/die M=fluconazole	I=2 M=NR	No	Healing
Inaba, 2017 [27]	I= amphotericin B liposomal 3mg/kg/die	I=7	No	Died
Ni, 2013 [28]	I= fluconazole 400mg/die	I=1	No	Died
Adzic-Vukicevic, 2020 [29]	I= amphotericin B deoxycholate 1mg/kg/die + fluconazole 800 mg/die C= fluconazole 800 mg/die M= fluconazole 400 mg/die	I=1 C=4 M= 24	No	Healing
Suner, 2014 [30]	I= amphotericin B liposomal + flucytosine	I= 1.5	No	Died
Sciaudone, 2010 [31]	I= fluconazole 400mg/die C= fluconazole 200mg/die	I=1 C=5	No	Healing
Cian, 2017 [32]	I= amphotericin B liposomal + flucytosine C= Fluconazole	I= 6 C= NR	No	Healing
Hirai, 2011 [33]	I= Amphotericin B deoxycholate 1mg/kg/die + flucytosine M= fluconazole 200mg	I= 8 M= ongoing	No	Improvement of clinical status
Matsuda, 2011 [34]	I= fluconazole 400mg/die then amphotericin B liposomal 150mg/die	I= 16 + 6	No	Relapsing
Friedman, 2012 [35]	I= amphotericin B deoxycholate 1mg/kg/die C= fluconazole 12mg/kg/die	I= 2 C= 8	Yes	Improvement of clinical status
Chaya, 2013 [36]	I= amphotericin B lipid complex 6mg/kg/die C= fluconazole 400mg/die M= fluconazole	I= 2 C= 12 M= lifelong	No	Healing
Nankeu, 2012 [37]	I= amphotericin B liposomal + flucytosine M= fluconazole	I= 4 M= 72	No	Healing

I induction; *C* consolidation; *M* maintenance; *NR* not reported

CRAG-negative persons, and it is also regarded as cost effective for specific groups [49, 50]. In a cluster randomized study that enrolled ART-naive participants with CD4 ≤100x106/L, asymptomatic CrAg + participants received pre-emptive fluconazole therapy; the results showed that 6-month mortality of participants with CrAg titers ≤1:160 and CRAG-negative patients did not differ, but patients with CrAg titers >1:160 had 2.6-fold higher 6-month mortality than patients with titers ≤1:160 suggesting that pre-emptive antifungal therapy for asymptomatic

Table 4 CRAG titer in case reports of newly diagnosed disseminated cryptococcosis from 2010 to 2020

Study (author, year)	CRAG titer	HIV
Chavapradit, 2018 [17]	1:128	No
Saini, 2018 [44]	1:1024	No
Ito, 2017 [21]	1:4096	No
Chen, 2015 [45]	1:1280	No
Haraga, 2018 [46]	1:256	No
Huang, 2015 [24]	1:128	No
Beji, 2017 [4]	1:1600	No
Ni, 2013 [28]	1:1024	No
Adzic-Vukicevic, 2020 [29]	Negative	No
Sciaudone, 2010 [31]	1:256	No
Matsuda, 2011 [34]	1:256	No
Hung, 2010 [47]	1:512	No

CRAG titer is intended at the moment of diagnosis

cryptococcosis seemed to be effective in patients with CrAg titer ≤1:160 and a more aggressive approach is required for persons with CrAg titer >1:160 [51].

A Cochrane systematic review showed that antifungal prophylaxis reduced the risk of developing and dying from cryptococcal disease. Therefore, where CrAg screening is not available, antifungal prophylaxis may be used in patients with low CD4 counts at diagnosis and who are at risk of developing cryptococcal disease [52].

There are no empiric and literature data that support a role of cryptococcal serum antigen (CRAG) in guiding the antifungal therapy. Future areas of research should include evaluation of customized therapy according to titer in persons with cryptococcal infection.

Recurrence of Disease

Increasing concerns are spreading about high level of persistence and frequency of relapse after a case of cryptococcal meningitis or disseminated cryptococcosis [12].

We summarized three aspects that may explain recurrence of disease.

On the one hand, the widespread use of fluconazole for long-term suppressive therapy of cryptococcal infection may cause the development of fluconazole resistance, especially among the relapsing patients [53].

The importance of susceptibility testing of *C. neoformans* isolates in all cases of meningeal cryptococcosis, even without fluconazole exposure, has been stressed out in several works and case reports, but in real-life scenarios, the methods are not always feasible. In both our cases, unfortunately, it has not been carried out because of the absence of growth of the microorganism, and we cannot exclude a fluconazole resistance as trigger of relapsing infection (especially in Case 1).

However, the unavailability of MIC interpretive breakpoints for any antifungal against Cryptococcus spp. together with discrepancies between the available methods makes it difficult to correlate in vitro MICs and clinical outcome when a single episode is tested [53].

In the literature, this problem was already took in account: a systematic review with a collection of 4995 isolates of Cryptococcus from 3210 patients, with 248 (5%) of the isolates from relapsing episode, showed resistance level of 12.1% (95% CI: 5.5–15.6) in whom had no relapse and 24.1% (95% CI: 3.1–51–2) in relapsing case [54]. A gradual increase in fluconazole resistance appeared over the years in USA: data reported in 1993 and 2001 demonstrated zero (0%) and 1.1% resistance, respectively [55, 56]. However, data are uncertain since some studies suggest that *C. neoformans* appeared not to increase the risk of failure or relapse during treatment [21]. Notably, some serotype of the fungus shows less susceptibility to azoles (serotype A is less susceptible compared to serotype D) [57].

The second point regards the importance of combining flucytosine to amphotericin B in the treatment strategy. Some authors stressed that without flucytosine as backbone, patients did not negativize cultures easily with the possibility that the pathogen may be still isolated in different samples after 2 or more months of adequate therapy (amphotericin B and fluconazole). Probably, the absence of flucytosine may cause the development of resistance in course of therapy: a study tried to demonstrate this possibility, and it was found that in a group with relapses or those who did not negativize cultures, one isolate (out of 256 strains) became resistant after therapy (MIC ≤64 mg/ml) and other four showed dose-dependent susceptibility (MIC 16–32 mg/ml) [58].

A third element that could determine the risk of relapse or treatment failure is the underlying condition that plays a role in the onset of the disease. Beyond the typical subset of people living with HIV and transplant recipients, it has been pointed out that even non-HIV non-transplant (NHNT) patients suffer from a high mortality form of this disease suggesting that cryptococcosis in NHNT patients appears to be a distinct entity that needs further study and requires a higher level of clinical suspicion than it currently receives [59].

Obviously, in transplant recipients with cryptococcosis, the outcome appears to be influenced by the type of immunosuppressive agent employed [60].

A possible and much rare cause could be the presence of sanctuary district for Cryptococcus where level of drugs remains too low to eradicate the fungus.[21 36]

Conclusion

Definitive treatment recommendations for disseminated cryptococcosis are hampered by the absence of prospective, randomized controlled trials or prospective cohort studies of patient outcomes. Treatment guidelines are based on retrospective case series, expert opinion, and are inferred from studies of CNS cryptococcosis, especially those of cryptococcal meningitis in HIV-infected patients.

There is also an urgent need to establish antifungal breakpoints for *Cryptococcus* spp. For the moment, it could be reasonable to obtain new specimens at each relapse and retest the MIC of the isolate to identify treatment failure early and reduce the risks of further relapses; in this setting, measurement of antifungal drug levels could also help.

The use of non-azole therapy early in the course of the disease for such patients could potentially improve clinical outcome and prognosis.

Given the importance of CRAG titer in predicting meningitis and/or death, CRAG titer will likely be used in the future to customize therapy both for prevention and treatment of cryptococcal meningitis.

Availability of Data and Material Not applicable.
Code Availability Not applicable.

Author Contribution A.B. and M.P. had the idea for the article, performed the literature search, and drafted the work. F.L., L.Z., B.B., and G.S. critically revised the work. A.B. provided academic leadership.

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Declarations

Ethical Approval and Informed Consent The study does not need ethics committee approval because all data were collected for clinical purpose in adherence with ethical standards. We obtained the patient informed consent for publication in anonymous form.

Consent to Participate and Publication Both patients gave consent to participate and make a publication regarding their clinical data.

Conflict of Interest The authors declare no competing interests.

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