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Cryptococcosis in non-HIV/non-transplant patients: A Brazilian case series

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Abstract

Cryptococcosis is a classical systemic opportunistic mycosis, primarily occurring among patients with significant immunologic impairment. However, this disease could also affect patients without any recognized immunologic defects, that is, phenotypically normal patients. The medical records of 29 non-HIV/nontransplant patients with cryptococcal disease during the period 2007–2014 were retrospectively reviewed. The most common site of infection was the central nervous system (n = 25, 86.2%), followed by the pulmonary system (n = 11, 37.9%) and blood (n = 2, 6.8%). Thoracic- and brain-computed tomography demonstrated abnormalities of 81.2% (n = 13) and 62.5% (n = 15), respectively. In sum, 22% (n = 6) of the patients experienced a significant underlying condition. More than one therapeutic regimen was used in 77.8% (n = 21) of the patients. The isolates were identified as being *Cryptococcus neoformans* species complex (n = 4, 36.4%) and *Cryptococcus gattii* species complex (n = 7, 63.6%). The overall mortality was 20.7% (n = 6). Herein, we presented the first case series of cryptococcosis in this specific population in São Paulo City, Brazil. The incidence of cryptococcosis in our hospital has not increased in recent years, and 77.8% (n = 21) of cases had no obvious predisposing factor. However, this disease remains associated with high mortality.

Key words: cryptococcosis, non-HIV/non-transplant patients, clinical manifestations, epidemiology.

Introduction

Cryptococcosis is among the most prevalent life-threatening mycoses and has a worldwide distribution. According to the recently revised classification the species complex comprise two species, namely, *Cryptococcus neoformans* and *C. gattii* with serotype A, D and AD for the former, and B and C for the latter species.[1,2] Previously, *C. neoformans* consisted of two varieties: *C. neoformans* variety *grubii* and *C. neoformans* variety *neoformans*. Recently, it has been proposed that *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* should be recognized as the following distinct species: *C. neoformans* sensu stricto (genotype amplified fragment length polymorphism (AFLP)1/VNI, AFLP1A/VNB/VNII, and AFLP1B/VNII) and

C. deneoformans (genotype AFLP2/VNIV). Additionally, *C. gattii* has been reclassified into five new species, including *C. bacillisporus* (genotype AFLP5/VGIII), *C. tetragattii* (genotype AFLP7/VGIV) and *C. decagattii* (genotype AFLP10/VGIV), *C. gattii* sensu stricto (genotype AFLP4/VGI), and *C. deuterogattii* (genotype AFLP6/VGII).[3]

Cryptococcosis is a classical systemic opportunistic mycosis primarily occurring among patients with significant immunologic impairment, including patients on chemotherapy and/or immunosuppressive therapy, individuals with end-organ dysfunction, and patients with innate or acquired immunodeficiencies.[4,5] Furthermore, cryptococcosis could also affect patients without any recognized immunologic defects, and in some clinical centres, up to 20% of cases of cryptococcosis occur in phenotypically "normal" hosts.[6] Although these patients are a homogeneous group, these individuals likely represent the congruence of subclinical immune injuries.[4]

Moreover, cryptococcosis in non-immunocompromised patients presents unique diagnostic and therapeutic challenges; however, detailed data addressing these aspects are lacking, leading to new insights regarding the pathogenesis, early diagnosis, and treatment of cryptococcal infection.[4] Reflecting the rare occurrence of this disease, many aspects of the clinical features and management principles are based on expert opinions, case series and retrospective cohort studies.

Therefore, in the present study, the first retrospective case series from Brazil in human immunodeficiency virus (HIV)-negative/non-transplant (NHNT) patients is presented. The aim was to provide information with as many details concerning the diagnosis and clinical management of cryptococcosis in this specific group to improve and contribute to a better understanding of this disease.

Materials and methods

A retrospective review was conducted of 29 NHNT patients with confirmed diagnoses of cryptococcal disease from January 2007 through December 2014, at Emílio Ribas Hospital, a tertiary public hospital and reference centre for infectious diseases in São Paulo City. This hospital has 199 beds divided into four wards for adults, a single children's ward, an intensive care unit (ICU), a one-day hospital facility, and an emergency room. These patients were drawn from all hospital wards. The study was approved through the Ethics in Research Committee (protocol 19/2014).

Cryptococcus spp. isolates obtained from patients during their hospitalization were characterized at the species levels and were stored in glycerol 15% solutions under −20°C in our laboratory. During the study period, niger seed agar was used as the basal medium for growth. The *Cryptococcus* spp. isolates recovered were characterized at the molecular levels.[7–9] The molecular type standard strains, kindly provided from the mycology laboratory of the Institute of Clinical Research Evandro Chagas, Oswaldo Cruz Foundation, included the following strains: WM 148 (VNI), WM 626 (VNII), WM 628 (VNII), 629 (VNIV), WM 179 (VGI), WM 178 (VGII), WM 161 (VGIII), and WM 779 (VGIV). The *in vitro* susceptibility profiles of the etiologic agents against fluconazole (FLU) were determined using the reference broth microdilution method according to the M27-A3 document of the CLSI. The CLSI has not yet described the clinical breakpoints for *Cryptococcus* members; thus, we used epidemiologic cut-offs previously proposed.[10]

Cases of cryptococcal disease were defined based on the isolation of *Cryptococcus* spp. from at least one body site through culture, histopathological findings or molecular techniques. Positive cerebrospinal fluid (CSF) antigen detection using latex agglutination (LA) and direct examination (by Indian Ink) was also considered. All cases were tested for HIV, and all patients were observed to be HIV-negative. The demographics, presenting symptoms (including duration), underlying conditions at the time of diagnosis, systemic antifungal therapies and outcomes were collected. Glasgow Coma Scale (GCS) was obtained by assessing the following three parameters: eye opening, verbal, and motor response. The score varies between 3 and 15 points, and values of 8 or less correspond to serious conditions.[11] Increased intracranial pressure (ICP) was defined as an elevated opening pressure (OP) $\geq$ 25 cm H_2O. Laboratory data collected included CSF white blood cell count, protein, glucose levels, LA, direct examination, and fungal culture. Serum LA and results of blood fungal culture were also collected. Radiological findings of brain and thoracic imaging were assessed from the radiologist report at admission and during follow up. The data were collected three times: (1) upon admission, (2) at approximately 6 weeks, and (3) 12 months after the initiation of therapy. The data were entered into Excel Microsoft Office 2007 (Microsoft Corporation Version 12.0.6) and analyzed using STATA statistical software version 13.0 (Stata Corp LP, College Station, Texas, USA). For qualitative variables, the results are presented as frequencies, and for quantitative estimates, measures of central tendency and dispersion were used.

Results

Cryptococcal disease was diagnosed in 29 patients during the study period. Twenty-five patients had central nervous

Table 1. Body sites of infection in 29 HIV-negative/non-transplant patients with cryptococcal disease.

Site	No.	%
CNS	17	55.2
Lung	4	13.8
CNS and lung	5	20.7
CNS and blood	1	3.4
CNS, lung and blood	1	3.4
CNS, lung and lymph nodes	1	3.4
TOTAL	29	100

Note: CNS: central nervous system.

Table 2. Distribution of HIV-negative/non-transplant patients with positive cultures and species identified according to the minimum inhibitory concentration value for fluconazole (MIC-FLU).

MIC- FLU (μg/ml)	*Cryptococcus neoformans* (n = 2)		*Cryptococcus gattii* (n = 7)	
	no.	%	no.	%
0.12	–	–	1	14.3
1	1	50.0	–	–
2	–	–	1	14.3
4	1	50.0	–	–
16	–	–	1	14.3
32	–	–	2	28.5
64	–	–	1	14.3
>64	–	–	1	14.3

system (CNS) infection and four had pulmonary infection only. The disease was less frequently identified in other sites, such as the bloodstream and lymph nodes (Table 1). The majority of the patients were Caucasian (60.7%) males (55.2%) with a median age of 40 years (range, 3–73). Only two children (<18 years) were included in the sample. The average hospital stay was 3 months (SD ± 3.6 months). Among all patients, three had hospital stays longer than the overall average: a patient with pulmonary and central nervous system involvement, who remained hospitalized for 18.3 months due to a condition not related to the underlying disease; a patient with cryptococcal meningitis who remained hospitalized for 8.28 months due to slow improvements in focal inflammatory brain lesions; and a 12-year-old patient with central nervous system involvement, who showed relapse during consolidation therapy, thus requiring therapy with voriconazole (VOR). The first two patients received gamma interferon (IFN-γ) during hospitalization, and no patients evolved to death by the end of follow-up.

Underlying conditions considered as risk factors for cryptococcosis were obtained in 93.1% (n = 27) of the records, including diabetes mellitus (4 cases), solid organ neoplasm (1 case), and hyper IgE syndrome with DOCK 8 deficiency (1 case), comprising a total of six patients (22.2%). Moreover, six patients in the series were cigarette smokers.

Among all patients, 27 subjects underwent lumbar puncture to examine CNS involvement through cerebrospinal fluid (CSF) analysis, observing cryptococcal meningitis in 25 patients. Regarding the clinical aspects, the major symptoms of patients with cryptococcal meningitis included headache (n = 24, 96.0%), fever (n = 10, 40.0%), altered mental status (n = 9, 36.0%), motor deficit (n = 7, 28.0%), nausea or vomiting (n = 15, 60.0%), paraesthesia (n = 1, 4.0%), hearing and visual deficit (n = 6, 24.0% and n = 8, 32.0%, respectively), and seizures (n = 8, 32.0%). The median duration of symptoms was 30 days (range 8–270 days). The majority of the patients presented GCS values equal to 15 (n = 18, 72.0%), and the minimum value observed was 8 (n = 1, 4.0%).

Cytological analyses of CSF revealed median levels of cellularity, protein and glucose at 32 cells/mm^3 (0–635 cells), 57 g/l (17–335), and 45 mg/dl (12–88), respectively. Moreover, the CSF analysis showed positive cultures in 52% (13/25) of the patients. Eleven isolates of *Cryptococcus* spp. obtained from patients during their hospitalization were characterized at the species level and during the study period, eight isolates were viable for molecular analysis; showing four isolates (36.4%) being *C. neoformans* species complex (two isolates were AFLP1/VNI and two isolates were not typed) and seven isolates (63.6%) were *C. gattii* species complex (six isolates were AFLP6/VGII and one isolate was not typed). The distribution of minimum inhibitory concentration (MIC) according to the species for FLU was observed for 9 of the 11 isolates (Table 2). Among patients with cryptococcal meningitis and negative CSF cultures (n = 12, 48.0%), five patients (41.7%), presented positive result in CSF (by Indian Ink), three patients (25.0%) presented positive CSF antigen detection using LA, two patients (17.0%) presented positive results using both methods, and two patients (16.7%) presented positive results in blood cultures. Increased intracranial pressure (ICP) was observed in 19 patients (76.0%), ranging from 20 to 176 cmH_2O. The median time to control increased ICP was 18 days (range, 1–142 days), and lumbar-peritoneal or ventriculo-peritoneal drains were placed in 17 patients due ICP (n = 15, 88.2%) and hydrocephalus (n = 2, 11.8%). Complications were observed in five patients (29.4%), with infection presenting as the most frequent complication. Forty-two days after admission, CSF analysis was conducted in 21 patients, and among

Table 3. Distribution of HIV-negative/non-transplant patients with cryptococcal meningitis according to the results of brain-computed tomography (CT) obtained during follow-up.

Brian CT findings	Admission	(%)	42 days	(%)	1 year	(%)
Single lesion	–		–		–	
Multiple lesion	5	(33.3)	8	(61.5)	2	(33.3)
Edema	3	(20.0)	5	(38.5)	–	–
Dilatation	5	(33.3)	5	(38.5)	–	–
Atrophy	3	(20.0)	5	(38.5)	3	(50.0)
Herniation	1	(6.7)	–	–	–	–
Other anomalies	6	(40.0)	6	(46.2)	2	(33.3)

these individuals, 38.1% (n = 8) of the patients showed a positive direct examination, but all samples examined in culture were negative. Of note, one patient underwent lymph node biopsy with suggestive histopathological examination for *Cryptococcus* spp.

Among all patients, twenty-four (82.8%) underwent brain-computed tomography (CT) upon admission, and three patients had previously undergone a cranial nuclear magnetic resonance (NMR) in another service prior to being admitted to our center.

Abnormalities were observed in 15 patients (62.5%), including multiple lesions in five patients (33.3%), edema in three patients (20.0%), ventricular dilation in five patients (33.3%), atrophy in three patients (20.0%), herniation in one patient (6.7%), and other changes in 6 patients (40.0%), including calcification (n = 2), ventricular compression (n = 2), midline deviation (n = 1), and nonspecific subcortical lesions (n = 1). All patients (n = 15) underwent brain CT within 42 days of admission. Among these, 13 patients (87.0%) showed abnormalities, including multiple lesions in eight patients (61.5%), edema in five patients (38.5%), ventricular dilation in five patients (38.5%), atrophy in five patients (38.5%), and other changes in six patients (46.2%), including unspecific lesions in three patients, gyral enhancement in two patients, and calcification in one patient. Only six patients underwent brain CT at one year after admission, of which two patients (33.3%), still presented abnormalities, and two patients (33.3%) had other findings, including calcification, ischemia, and unspecified findings (Table 3).

Furthermore, all patients (n = 16, 55.2%) who underwent cranial nuclear magnetic resonance (NMR) at admission had previously shown lesions in the brain CT. The described abnormalities included single (n = 2, 12.5%) and multiple lesions (n = 6, 37.5%), pseudocysts (n = 3, 18.8%), edema (n = 5, 31.3%), and ventricular dilatation (n = 3, 18.8%). Additional findings in 14 patients (87.5%) included ventricular compression (n = 1), midline deviation (n = 1), calcification (n = 1), meningeal thickening (n = 1), gyral enhancement (n = 1), ventriculitis signals (n = 1), areas of diffuse ischaemic stroke, meningeal enhancement (n = 3) enlargement of the perivascular space (n = 2), and unspecified lesions (n = 5). After 42 days, 13 patients underwent NMR, and the major findings included gyral enhancement (n = 1, 7.7%), optic nerve myelitis (n = 1, 7.7%), ischaemia (n = 1, 7.7%), meningeal enhancement (n = 2, 15.4%), and nonspecific injury (n = 5, 38.5%). Only six patients underwent NMR examinations at the 1-year follow-up examination, and the findings were characterized as nonspecific lesions.

Sixteen patients (55.2%) underwent lung CT upon admission, and abnormalities were observed in 13 patients (81.2%), including nodules (n = 8, 61.5%), mass (n = 4, 30.8%), ground glass opacity (n = 3, 23.0%), consolidation (n = 3, 23.0%), lymphadenopathy (n = 2, 15.4%), and cavitation (n = 1, 7.7%), followed by other anomalies, such as reticular patterns (n = 1, 7.7%), pleural thickening (n = 1, 7.7%), bronchiectasis (n = 2, 15.4%), and fibrosis (n = 2, 15.4%). The invasive investigation of pulmonary cryptococcosis was performed in 12 patients (41.4%), and the results were confirmed through culture in one patient (8.3%), histopathological examination in nine patients (75.0%), and polymerase chain reaction (PCR) analysis in one patient (8.3%). These isolates were not characterized at the species and molecular levels.

Interesting, only nine of the eleven patients (81.8%) with confirmed diagnoses of pulmonary cryptococcosis had symptoms, such as cough, chest pain, dyspnea, and haemoptysis. The duration of symptoms ranged from 30 days to 10 years. Both asymptomatic patients had a previous diagnosis of cryptococcal meningitis, and lung lesions were observed at the time of disease investigation using thoracic-computed tomography.

Up to 60% (n = 7) of the patients underwent lung CT after 42 days, and other findings were identified, including pleural thickening (n = 1, 14.3%), subpleural opacity (n = 1, 14.3%), emphysema (n = 1, 14.3%), fibrosis (n = 2, 28.6%), and atelectasis (n = 2, 28.6%). After 1 year, the identified anomalies included atelectasis (n = 1, 14.3%), bronchiectasis (n = 1, 14.3%), emphysema (n = 1, 14.3%), fibrosis (n = 3, 42.9%), and pleural thickening (n = 3, 42.9%).

Regarding the therapeutic scheme, 14 patients (48.3%) received treatment prior to admission. Among these, 13 patients (92.9%) showed impairments in the central nervous system. Treatment with combination therapy was conducted in 27 patients (93.1%). The median number of days of the induction phase was 69 days (range, 1–517 days),

Table 4. Induction of antifungal treatment in HIV-negative/non-transplant patients with cryptococcal disease.

	Induction phase (n = 27)	
Therapeutic regimen	no.	%
Amphotericin (mg/kg/day)		
none	0	0.0
0,7 to 1 mg/kg deoxycholate or 5 mg/kg phospholipid complex or 3 mg/kg liposomal	11	40.7
> 1 mg/kg deoxycholate or > 5 mg/kg phospholipid complex or > 3 mg/kg liposomal	7	25.9
< 0,7 to 1 mg/kg deoxycholate or 5 mg/kg phospholipid complex or 3 mg/kg liposomal	9	33.3
Fluconazole (mg/day)		
None	6	22.2
< 800	4	14.8
800 to 1200	16	59.3
> 1200	1	3.7
Flucytosine (mg/kg/day)		
None	15	55.6
< 100	9	33.3
≥ 100	3	11.1
Voriconazole		
None	22	88.0
Yes	3	12.0

and the therapeutic predominantly prescribed included amphotericin B (AMB) deoxycholate (0.7 to 1 mg/kg), amphotericin B lipid complex (5 mg/kg), or liposomal amphotericin B (>3 mg/kg) in 40.7% (n = 11) of patients associated with FLU (800 to 1200 mg/day) in 59.3% (n = 16) of patients. More than half of the treated patients did not receive 5-flucytosine (5-FC) (n = 16, 55.6%), and two patients (6.9%) received therapy with only FLU (Table 4).

IFN-γ, as an adjuvant therapy, was administered to three patients. These patients had brain lesions without radiological responses despite treatment; however, these patients showed improvement after INF-γ treatment.

In the consolidation phase, 22 patients (75.9%) were treated for a median period of 354 days (range 6–2.424 days). The majority of the patients received FLU, however different therapeutic schemes in combination with 5-FC or AMB were also observed and only one patient was treated with VOR.

Moreover, six deaths (20.7%) occurred during the hospital stay: four deaths resulted from cryptococcosis, and two deaths resulted from nosocomial infections.

Discussion

In the present study, we conducted a retrospective study on the clinical presentation, laboratory findings and outcomes in NHNT patients from 2007 to 2014. Based on this population, cryptococcosis was more prevalent in men, and the most common species isolated from culture was *C. gattii* species complex. Different from other retrospective studies involving 14-year observations the NHNT cases showed a slight increase with time.[12] The overall annual case frequencies of cryptococcosis in this group did not significantly change during the study period, with an average of three NHNT patients per year (range, 0–8 cases/year).

In our case series, most of the patients had no underlying conditions considered as risk factors associated with cryptococcal disease. This large number of "apparently normal hosts," compared with other studies describing the percentage of NHNT patients, between 16.9% and 27.7%,[4,13,14] might reflect the fact that our hospital is a infectious diseases specialized unit and primarily receives patients with HIV infections with no other underlying conditions, such as solid-organ transplantation, rheumatologic disorders, and hematological malignancies.

Primary immunodeficiency (DOCK-8 deficiency) was only documented in one patient exhibiting cryptococcal meningitis resulting from an infection with *C. neoformans*. The precise incidence of primary immunodeficiencies in the general population is uncertain, reflecting diagnostic difficulties. However, considering the findings of this disease in the studied population, the investigation of primary immunodeficiencies should be recommended in particular cases. [15] In addition, it was possible to identify other predisposing factors in only five patients. This finding is not surprising, and the largest series of cryptococcosis in HIV-negative patients demonstrated no significant predisposing conditions in 22% of 66 patients.[6]

Interestingly, consistent with other studies, with percentages ranging from 36% to 51%, in the study population examined herein, up to 22% of patients were cigarette smokers.[14,15] The association between smoking and cryptococcosis was also suggested in a case-control study conducted during an outbreak in British Columbia.[16] However, further studies are warranted to validate these findings.

Although adjuvant therapy with IFN-γ is controversial because of the theoretical risk of exacerbating immune reconstitution inflammatory syndrome (IRIS) due to the proinflammatory effects of this cytokine.[17] In our study, improvements were observed after treatment with IFN-γ in all patients that had inflammatory brain lesions without radiological improvement after the appropriate therapy. Furthermore, corticosteroids were not administered to any of the patients. However, reported cases have suggested

a favourable clinical response to corticosteroids in selected patients with *C. gattii* species complex CNS infections who did not clinically respond to appropriate treatments associated with both CSF sterilization and the development of new or worsening focal inflammatory brain lesions.[18]

Regarding the clinical presentation, the percentage of patients with cryptococcal meningitis was slightly higher compared with the percentage of patients reported in previous studies, ranging from 43% to 63%.[12,19] However, the prevalence of pulmonary cryptococcosis was similar to other studies, ranging from 9% to 63%.[12,20,21] Notably, in our case series, 25% of patients with pulmonary cryptococcosis were asymptomatic at the time of diagnosis. Consistently, previous studies have described pulmonary cryptococcosis patients that present only mild symptoms (e.g., cough) or might be asymptomatic, observed in approximately 23% of patients with NHNT.[6,12]

Although CSF LA was positive in most patients; due to the high cost, quantitative latex test to determine the antigen titre in CSF is not performed in our hospital, although it has been suggested that high titres ($\geq$1:512) are associated with severe disease and increased mortality, warranting a more aggressive antifungal therapy in these patients.[14,22]

In the current study, the patients presented an equally high percentage of inflammatory lesions compared with two other studies (71.9% and 87%).[22,23] In our opinion the high number of "apparently immunocompetent" patients, capable of mounting a more effective immune response with an intense inflammatory response, in part, reflects the high number of abnormalities observed among the study population. In addition, consistent with previous studies, patients without HIV infections have a longer duration of symptoms and delays in diagnosis and the institution of therapy, consequently resulting in a more advanced disease.[6,12,22]

Few retrospective studies have described the use of CT or MRI of the brain, and no one have clearly addressed the relationship between cerebral lesions and the precise duration of treatment in such cases. In the present study, when comparing the images obtained upon admission, at 42 days and after 1 year, persistent abnormalities on brain CT/MRI was observed. The persistence of these findings, particularly in the CNS, might warrant a more prolonged induction treatment phase in these patients, as radiological improvement is important when deciding to change the treatment for the consolidation phase.

The high abnormalities observed in the chest CT compared with other studies, ranging from 55.1% to 63%[14,20] might reflect radiological changes unrelated to cryptococcosis, which also explains the large number of radiological abnormalities persisting even after one year of follow-up. Furthermore, only a few studies with radiological reports have attempted to interpret or compare the described images.

In our hospital, a systematically aggressive treatment for ICP and/or hydrocephalus is performed, which is of paramount importance, as the largest numbers of neurological sequels and deaths have been associated with the inadequate management of ICP.[14,24] Our data showed that the majority of the patients had increased ICP, which is consistent with the percentage of patients with ICP reported in previous studies, ranging from approximately 52% to 82.1%.[12,22]

Interestingly, compared with HIV patients, uninfected patients did not show a higher percentage of ICP or higher average of OP, despite the increased cellularity in the CSF.[12,23] Neurosurgery was required in nearly 80% of the patients examined in the present study, and this number was significantly higher than the average number of cases with similar percentages of NHNT patients, ranging from 17.7% to 35%.[17,21,22]

Notably, cryptococcal disease in NHNT patients has often been associated with the *C. gattii* species complex, which is recognized as a more virulent and resistant species in comparison with the sibling *C. neoformans* species complex. Evidence suggests that in some settings and depending on the host factors and strain, *C. gattii* species complex tend to produce more severe CNS manifestations compared with *C. neoformans* species complex characterized by high intracranial pressure complications, cryptococcomas and poor prognoses in terms of neurological sequelae.[25,26]

Based on our population, *C. gattii* species complex-infected patients presented with the same rate of increased ICP, although more patients required shunt (85% *vs.* 50%) during hospitalization. Moreover, regarding the presence of cryptococcomas, higher percentage of inflammatory lesions (71% *vs.* 25%) was observed in this group of patients. One patient died from each group, and poor prognoses in terms of neurological sequelae was uncovered in our study. Our data suggest that differences were far less striking than those reported between *C. gattii* species complex and non-*C. gattii* species complex-infected patients probably because of the relative small number of patients. In fact, although only a small number of isolates were obtained to be properly evaluated in the present study, there were more *C. neoformans* species complex-infected patient with a recognized predisposing factor (50% *vs.* 28%), and the majority of the cases with no known predisposing factors were infected by *C. gattii* species complex as expected.[27,28]

Additionally, the identified isolates were evaluated for the MIC of FLU, and, consistent with previous studies, higher MIC values were observed for *C. gattii* species complex samples, confirming the lower susceptibility of this

species in comparison with its sibling *C. neoformans* species complex.[10]

One of the greatest challenges in cryptococcosis is the lack of a fully established consensus for the treatment of this disease in individuals not infected with HIV therefore significantly reflecting in patient care. In the present study, the therapeutic schemes were clearly not uniform and varied among patients and during the treatment of the same patient, using either doses or schemes. Different treatment regimens used in the induction phase were AMB, AMB + FLU, AMB + 5-FC, AMB + FLU + 5-FC, AMB + 5-FC + VOR, and FLU. Twenty-two percent of the patients used only one scheme in the induction phase, while 55.6% used two schemes, and 22.2% used three schemes. This different approach during the induction phase might reflect the lack of 5-FC, available only for patients who filed a lawsuit to receive this drug; more patients with comorbidities require adjustments in the treatment regimen, and because of the severity of the symptoms, the clinician might only typically use two or three drugs. Interestingly, Bratton et al. (2012) classified 79 NHNT patients according to the severity of the disease in severe and nonsevere conditions. The use of AMB monotherapy was similar among groups. However, the use of AMB + 5-FC was higher among patients with severe conditions. Similarly, the use of FLU in monotherapy was higher in patients with nonsevere conditions, reflecting clinical treatment using the most effective antifungal therapy according to the severity of the disease.[12]

Furthermore, other studies support the variety of therapeutic regimens used in NHNT patients, showing that schemes based on FLU, compared with schemes based on AMB, were associated with increased mortality.[21] Moreover, most deaths associated with cryptococcal disease occur earlier compared with overall mortality. In the present study, mortality was 20%, consistent with previous studies, which reported 11.5% to 35% mortality.[14,19]

In summary, cryptococcosis is an important infection in NHNT patients, particularly because there has been less focus on treatment of this specific population, and yet this group suffered the highest mortality.[12] The result from our study found important clinical aspects and uniquely describes the realities in the management of this disease at one institution. First, the prolonged duration of symptoms in this group prior to diagnosis deserves greater attention. Indeed, as this group of patients have no evident risk factors, the possibility of cryptococcosis is not thought in the first medical visits and the diagnosis is delayed. In our opinion, this delay may contribute to a higher percentage of parenchymal brain involvement and to a poorer outcome. Furthermore, our study supports the finding that healthy hosts with cryptococcal disease have historically received longer courses of antifungals than immunosuppressed patients.[17] Therefore, although more clinical studies are necessary to define optimal treatment guidelines, we suggest that on the basis of existing recommendations in NHNT patients,[24] induction therapy should be recommended for at lest 6 weeks and after clinical, mycological (CSF culture sterilization) response and radiological improvement. Beyond this observation, the consolidation therapy with fluconazole should be continued throughout the treatment period recommended until complete clinical and radiological response.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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