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Antifungal therapy and management of complications of cryptococcosis due to *Cryptococcus gattii*

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Key point summary

Induction amphotericin plus 5-flucytosine is indicated for *Cryptococcus gattii* lung (2-weeks) and neurological disease (6-weeks) followed by fluconazole (total course 6-12 and 18 months, respectively). Shunting for raised intracranial pressure is frequent. Immune reconstitution syndrome occurs in 9% of cases.

ABSTRACT

Background. We describe antifungal therapy and management of complications due to *Cryptococcus gattii* (CG) infection in 86 Australian patients followed for at least 12 months.

Methods. Patient data from culture-confirmed cases (2000-2007) were recorded at diagnosis, 6 weeks, 6 months and 12 months. Clinical, laboratory and treatment variables associated with raised intracranial pressure (ICP) and immune reconstitution syndrome (IRIS) syndrome were determined.

Results. Seven of 10 patients with lung infection received amphotericin B (AMB) induction therapy (6 with 5-flucytosine [5-FC], median 2 weeks); median duration of therapy including azole eradication therapy was 41 weeks, with a complete/partial clinical response in 78%. For neurological disease, 88% of patients received AMB, 78% with 5-FC, for a median 6 weeks. The median total course was 18 months. Nine receiving fluconazole induction therapy were re-induced with AMB plus 5-FC for clinical failure. Raised ICP (31 patients) was associated with initial abnormal neurology, and neurological sequelae and/or death at 12 months (both $P = .02$); cerebrospinal fluid drains/shunts were placed in 58% of cases and in 64% of 22 patients with hydrocephalus. IRIS developed 2-12 months after starting antifungals in 8 patients, who presented with new/enlarging brain lesions. Risk factors included female sex, brain involvement at presentation and higher median CD4 counts (all $P < .05$); corticosteroids reduced cryptococcoma-associated edema.

Conclusions. Induction AMB plus 5-FC is indicated for CG neurological cryptococcosis (6 weeks) and when localized to lung (2 weeks). Shunting was often required to control raised ICP. IRIS presents with cerebral manifestations.

INTRODUCTION

The epidemiology, clinical presentation, mortality and neurological sequelae of cryptococcosis due to *Cryptococcus gattii* (CG) differ from those of its sibling species, *Cryptococcus neoformans* (CN) [1-3]. Management principles are based on randomized clinical trials of CN disease in HIV-infected patients [4], case reports of CG infection [5-7] and expert opinion.

In a contemporary Australian series of 86 patients with CG infection, mortality was increased in immunocompromised patients whilst initial cerebrospinal fluid (CSF) cryptococcal antigen (CRAG) titres of ≥256 predicted death and/or neurological sequelae in central nervous system (CNS) disease [3]. Raised intracranial pressure (ICP), a predictor of mortality in CN meningo-encephalitis [4], was frequent at presentation (66% where measured) and 9% of patients developed immune reconstitution syndrome (IRIS) [3]. We now describe the management of cryptococcal infection and its complications in these patients, and compare this with the Infectious Diseases of America (IDSA) Clinical Practice Guidelines for CG cryptococcosis [4].

METHODS

Study Design

The Australia and New Zealand Mycoses Interest Group conducted a nationwide study of CG infection in adults (January 2000-December 2007) with approval from human ethics review committees [3]. Clinical, laboratory and radiological data, disease complications (raised ICP, hydrocephalus, IRIS, neurological sequelae) and patient outcomes were recorded. Treatment details included: antifungal therapy (type, daily dose, duration); use of corticosteroids or immune-modulators and surgery (excision, CSF shunt placement). Data were collected at diagnosis to 14 days after starting antifungal therapy and at 6 weeks, 6 months and 12 months [3]; total duration of antifungal therapy was also recorded.

Definitions

Only culture-confirmed CG infections were included. Cryptococcal meningo-encephalitis was defined by CG isolation from CSF. Brain involvement was diagnosed by a radiologist's report of mass lesions (≥ 1cm diameter) or other parenchymal abnormalities e.g. vasculitic lesions, without an alternative diagnosis. Abnormal neurology was defined as previously described [8]. An opening CSF pressure ≥25 cm water was considered elevated [4]. Induction therapy was the initial (intensive) antifungal regimen given for ≥3 consecutive days [9]. Consolidation/maintenance therapy, subsequently referred to as eradication therapy (see discussion), was defined previously [4, 9]. Re-induction comprised intensified therapy following poor response to induction or eradication therapy, or worsening of symptoms [9]. IRIS was diagnosed when symptoms or radiological features consistent with inflammation worsened or appeared following a clinical and/or microbiological response to anti-cryptococcal therapy, and cultures were negative [3, 10, 11]. Clinical outcomes included all-cause mortality; progressive disease or failure (worsening clinical symptoms/signs); stable disease (no improvement in symptoms/signs); partial response (≤50% resolution of

symptoms/signs); or complete response (resolution of clinical symptoms/signs) [3]. Relapse of infection beyond 12 months was recorded.

Data Analysis

Data were analyzed using SPSS for Windows version 20 (SPSS, Chicago, IL). Two-tailed tests with a significance level of 5% were used. The median and lower to upper quartile range (LQ-UQ) were used to summarize continuous variables. Two-sample t tests or Mann-Whitney tests were used to compare the distribution of continuous variables whilst χ^2 or Fisher's exact tests were used to test for association between categorical variables. For analysis of the impact of raised ICP, only patients with CNS infection in whom CSF pressures were measured at diagnosis through to 14 days of therapy, were included.

RESULTS

Seventy-three of 86 patients had CNS infection, 10 had lung without CNS involvement (normal CSF examination *and* brain imaging findings) and 2, bloodstream infection only (Figure 1). One patient had biopsy-proven vertebral osteomyelitis and another, skin/subcutaneous disease (with meningitis).

Clinical responses at 12 months were complete in 33% of 85 patients, partial in 48%, and stable in 3.5%. Mortality was 13.6% in patients with CNS disease and 11% in those with isolated lung infection. There were no relapses; all patients with a partial response or stable disease at 12 months were subsequently cured.

Antifungal therapy

All patients were treated; induction regimens are summarized in Table 1. Overall, 73 of 86 patients (85%) received amphotericin B (AMB), 74% in combination with 5-flucytosine (5FC) and 15% received fluconazole monotherapy (Table 1).

One patient with isolated fungemia received cAMB for 3 days and the other, fluconazole for 8.5 months. The patient with osteomyelitis received cAMB plus 5-FC for 14 days followed by fluconazole for 6.5 months. All 3 had complete responses at 12 months.

Isolated pulmonary disease

Clinical data, treatment and outcomes of 10 patients with isolated lung disease are shown in Table 2. Seven had moderate-to-severe lobar/generalized consolidation with or without mass lesions; 2 had single large (5-6 cm diameter) cryptococcomas. Seven patients received induction therapy with an AMB formulation (median 2 weeks; mean 3.9; range 1-8), 6 in combination with 5-FC. Conventional AMB (cAMB; 0.7-1.0 mg/kg daily) was initiated in all 7 cases but 2 were switched to liposomal AMB (LAMB; 4 mg/kg daily) due to renal impairment after 2 and 16 days, respectively. Neither of 2 kidney transplant recipients (Table

2) treated with fluconazole monotherapy achieved a complete response at 12 months.

Azole eradication therapy was used in nine patients, typically fluconazole 400-800 mg daily (Table 2). Total duration of therapy was 24-208 weeks (mean 65.8; median 41). No lesions were resected surgically.

CNS disease

Of 73 patients, 25 (34%) achieved a complete clinical response, 33 (45%) a partial response, and 10 (13.6%) who failed therapy, died. AMB was initiated in 64 patients (88%), 57 (78%) in combination with 5-FC, for a median of 6 weeks (LQ-UQ 3-8; Table 3). All patients received cAMB initially but 31 (48.4%) were switched to L-AMB after a median of 8 days due to renal impairment.

Nine patients (12%) received fluconazole induction therapy, 400-1600 mg daily; modal dose 400 mg (Tables 3, 4); 7 (78%) required re-induction with AMB plus 5-FC due to clinical failure after a median of 28 days though 1 patient was later diagnosed with IRIS; the daily fluconazole dose in these 7 patients was 400 mg (n=5), 1600 mg (n=1) and 800 mg (n=1). Five patients treated initially with AMB plus 5FC received re-induction therapy (Table 3) for presumed treatment failure, later confirmed as IRIS in 4 cases. Fluconazole- and AMB-treated patients were equally likely to manifest one or more of CSF, brain or lung infection (Table 4); 2 of the 9 (22.2%) fluconazole-treated patients were immunocompromised (vs. 16 of 51 [31.3%] prescribed AMB plus 5-FC) and 3 of 5 had CSF CRAG titres of ≥ 256 vs. 40 of 58 who received AMB (both $P = .7$).

Eradication therapy in patients surviving >6 weeks included fluconazole (n=62), posaconazole (n=1) and voriconazole (n=1). Total antifungal courses lasted a median of 18 months (LQ-UQ 14-22; range 8–60). Similar 12-month outcomes were seen in 18 patients who received a total of 9-12 months of therapy: 6 had a complete, and 9, a partial response (response unknown in 3). All 10 deaths occurred in patients treated with AMB with/without

5-FC; 7 of them were immunocompromised. Proportionately more patients with CNS infection than with isolated lung disease were treated initially with AMB plus 5-FC (78% vs. 60%) but these differences were not significant ($P = .2$).

Raised Intracranial Pressure

CSF pressures, measured in 48 patients, were elevated in 31 (66%), 23 at diagnosis and a further 8, at 14 days; 8 patients (26%) had ICPs >35 cm water. Median time from onset of symptoms to CG diagnosis was 26 days (LQ-UQ 21-42) in patients with raised ICP and 29 days, [LQ-UQ 14-120], in those with normal ICP ($P > 0.05$); in all cases it was <48 hours from presentation to medical care.. Mortality was 19%. Raised ICP was associated with abnormal neurology at presentation and neurological sequelae (4 patients had visual loss) and/or death at 12 months but not with concomitant/late hydrocephalus or serum CRAG titers of ≥256 (Table 5).

Elevated ICP was managed with daily/second daily lumbar punctures (LPs) in 13 patients, 6 of whom later received a CSF drain or shunt. Drains/shunts were inserted as primary ICP management in 11 (data unavailable for 7). Overall lumbar or extraventricular (EVD) drains were placed in 9 patients (median 27 days; range 4-36) for raised ICP and lumbar-peritoneal or ventriculo-peritoneal shunts in 13 (median 42 days; range 13-124 days) including 5 who had both procedures. Shunts were inserted in an additional 5 cases to treat concomitant hydrocephalus. The regimens and durations of induction and total antifungal therapy were similar in patients with/without raised ICP (Table 5). Eight patients received corticosteroids to treat raised ICP *per se* and 2, to reduce cryptoccocoma-associated edema.

Hydrocephalus

Obstructive hydrocephalus was identified by cerebral imaging in 22 (30%) patients with CNS cryptococcosis, 18 within 2 months and 4, 2-12 months after presentation. Brain cryptococcomas were present in 9 cases (5 had large single lesions and 3, multiple lesions). Ten patients with early hydrocephalus also had raised ICP. Fourteen (64%) patients had CSF drains (n=7) or shunts (n=10) placed a median of 14 days (range 3-504) after diagnosis with antifungal drugs being introduced at time of placement. Shunts remained *in situ* long-term and in most cases, indefinitely. Twenty patients received induction therapy with AMB with (n=17) or without (n=3) 5-FC. The remaining 2 failed fluconazole therapy and were reinduced with AMB and 5-FC. Thirteen patients were treated with corticosteroids, 5 for cryptococcoma-associated edema, 4 for concomitant raised ICP and in 4 the indication was uncertain. Three patients (13.6%) died.

Other surgery

Lung lobectomy was performed at presentation for suspected malignancy in 3 of 9 patients with CNS plus lung infection, and in 2, at 8 and 30 months, to remove large lung lesions unresponsive to therapy. Resection of brain lesions (all cerebellar) was performed at diagnosis in 2 patients for suspected malignancy and at 12 months for therapeutic failure in a further 2; one of these was diagnosed with IRIS-like syndrome.

IRIS

Eight patients developed an IRIS after 6 weeks-12 months, while receiving azole eradication therapy. Two were immunocompromised and 1 was pregnant (Table 6). New and/or enlarging brain lesions with surrounding edema were universally present and 4 patients developed new neurological deficits, including blindness. One individual developed a concomitantly

enlarging lung mass and new sub-carinal lymphadenopathy. Risk factors for IRIS included female sex, brain involvement at presentation, concurrent brain, CSF and lung disease, and higher median CD4 counts. CSF mononuclear leukocyte counts, and protein and glucose concentrations at diagnosis of cryptococcosis were similar in patients with and without IRIS (Table 6).

Five patients were re-induced with AMB with/without 5-FC; 3 also received interferon-gamma for presumed therapeutic failure and in 5, corticosteroids were either commenced or doses were increased to reduce cerebral edema. Cerebral imaging abnormalities returned to pre-IRIS appearances within 4-6 months. No patient died but 4 had persistent neurological sequelae at 12 months including the 3 who received interferon-gamma.

DISCUSSION

This large study of therapy and long-term outcomes of CG cryptococcosis is unique and shows that despite antifungal and supportive therapy, CG-associated morbidity is substantial. To eradicate infection, longer antifungal treatment courses than those used in CN cryptococcosis [4] are required. Our data suggest that in both isolated lung and CNS cryptococcosis, induction therapy with AMB plus 5-FC leads to better outcomes than with fluconazole.

Lung disease: Based primarily on case reports, IDSA guidelines recommend 4-6 weeks of induction therapy with AMB plus 5-FC for large/multiple CG cryptococcomas [4, 7, 12-14] although fluconazole has been effective in mild-to-moderate CG lung disease [13] and HIV-negative patients with CN lung infection [9, 15]. In our series, initial selection of AMB +/- 5-FC was likely driven by typical, radiologically-extensive, disease (80% patients) though not by specific radiological abnormalities or host immunocompromise. Our data suggest that 2 weeks induction therapy is sufficient in isolated lung disease. Fluconazole eradication therapy was continued, with a total treatment duration of 12 months, similar to that recommended for CN infection [4]. No patients relapsed although 4 required > 12 months' therapy to achieve cure. Fluconazole induction therapy was given to 2 kidney transplant recipients to avoid AMB-nephrotoxicity and consequent poor outcomes in such patients [16].

CNS disease The IDSA guidelines, informed by small series [2, 6, 7, 17], recommend induction, consolidation and suppressive treatment for CNS disease due to CG [4]. A key finding of this study is that initial AMB plus 5-FC is essential in patients with CG: 80% of patients receiving AMB had a complete/partial clinical response at 12 months. Conversely, 78% of those receiving primary fluconazole failed therapy (although 1 had IRIS); Noting that the number of patients was small (n=7), therapeutic failure was not correlated with

fluconazole dose. Optimal duration of induction therapy for CG meningitis is uncertain; in our study, 6 weeks was used most commonly, consistent with recommendations for CN infection in immunocompetent hosts (4-6 weeks) [4, 18]. We have substituted the term "eradication therapy" for "consolidation/maintenance therapy" because fluconazole (typically 400 mg/day) was continued throughout the post-induction treatment period, "maintenance" therapy is not needed in the absence of ongoing immunosuppression and there were no late relapses (5-7 years) in this or a previous study [17]. A total of 6-12 months of therapy is recommended in CN patients [4] yet >70% of our patients received much longer courses (IQR 14-22 months). Healthy hosts with CG infection have historically received longer courses of antifungals than immunosuppressed patients with CN disease [2]. The optimal duration of therapy is also uncertain. Details of antifungal therapy [6, 19, 20] are absent from previous reports, or therapy was not stratified by site of infection [7]. Although the 10 deaths occurred in AMB-treated patients, all were severely ill and 7, immunocompromised; host immunocompromise is a risk factor for mortality [3]. We recommend a single treatment regimen in CNS infection due to CG, independent of host immune status.

Voriconazole or posaconazole were rarely used as induction or eradication therapy. In 2 patients, fluconazole caused alopecia, and voriconazole was substituted with good outcomes. Fluconazole eradication therapy remains appropriate in Australia; notably CG isolates with minimum inhibitory concentrations (MICs) of >8 μg/ml are rare (modal, range and geometric mean MICs of 4, 0.75-8 and 2.98 μg/ml, respectively; supplementary Table S1 and unpublished data). Surveillance of azole susceptibility is ongoing, given reports of some CG isolates with fluconazole MICs >8 μg/ml [14, 21, 22] and a reported association with genotypes not common in Australia, (eg. VGII and its subtypes) [21, 22] though the clinical significance of these elevated MICs is unknown.

Although c-AMB was the AMB formulation prescribed initially, 46.5% of patients were switched to LAMB (4 mg/kg daily) due to AMB-induced nephrotoxicity. Lipid AMB formulations and cAMB appear to be of equivalent efficacy in cryptococcal meningitis associated with HIV/AIDS and organ transplantation, [4]. Our data suggest that this is also true of CG cryptococcosis, although there are no comparative efficacy studies of c-AMB with lipid formulations in non-HIV, non-transplant populations.

Raised ICP was more common in patients presenting with abnormal neurological features but unlike in HIV/AIDS patients, not those with CSF CRAG titres (≥256) [23]. CSF pressure control is a critical determinant of outcome [4]; in our study, raised ICP was associated with neurological sequelae and/or death at 12 months with 25% of such patients sustaining visual loss. Management of raised ICP in CG infection has not been investigated previously [6, 24, 25]. Raised ICP was controlled by daily/second daily LPs in only 45% with the remainder requiring insertion of CSF drains/shunts, at a median of 4-6 weeks (range 0.6-17). We cannot readily explain the apparent lag of 4-6 weeks prior to surgical correction of raised ICP, but recommend early neurosurgical review in all cases of raised ICP if frequent LPs fail to control CSF pressure after 1-2 weeks. Corticosteroids were used in 8 patients. However, this approach is reportedly of no benefit in HIV/AIDS patients and may increase mortality [4].

Hydrocephalus was frequent (30%) and can occur late. Shunt insertion was usually required and should be considered early. Shunts may be placed during active infection as long as effective antifungal therapy has been introduced [4, this study]. Resection of extensive pulmonary or cerebellar cryptococcomas was undertaken after 8-30 months in 4 patients with progressive disease; post resection, one was diagnosed with a cerebellar IRIS-like syndrome. The impact of early resection on duration of antifungal therapy is unknown.

That 9.4% of patients (5.9% immunocompetent) developed IRIS is noteworthy. IRIS has occasionally been described in CG infection including in association with pregnancy [26-28]; 1 of our patients presented post-partum. Risk factors included female sex, initial presentation with brain, or brain, meningeal and lung, involvement, and a higher median CD4 count; enlarging/new brain lesions were the predominant presenting feature. Interestingly, initial CSF leukocyte counts, protein and glucose, and high cryptococcal loads (positive India Ink stain and CSF CRAG titres of ≥256) were similar in patients with and without IRIS, contrasting with IRIS in HIV-associated cryptococcal meningitis, where low initial CSF leukocyte counts and protein levels were risk factors [27]. In HIV/AIDS and organ transplant patients, IRIS correlates with recovery of immune function and reduction of immune suppression, respectively [4, 29]. It is proposed that in healthy hosts, cryptococcal capsular polysaccharide induces a Th2 anti-inflammatory cytokine response, which is then replaced by pro-inflammatory Th1 responses following response to antifungal therapy; in some cases the latter are sufficiently robust to cause IRIS [30].

Based on reported cases and the present study, IRIS develops 4 weeks to as long as 12 months after initiating antifungal drugs. Its recognition is important as it can be misdiagnosed as clinical failure; indeed 5 of our 8 patients received unnecessary re-induction AMB and 5FC. Further, 3 also received interferon-gamma, the use of which carries a theoretical risk of exacerbating IRIS due its pro-inflammatory effect. Prognosis is good. Corticosteroids were used successfully to control symptoms and cryptocccoma-associated edema.

In summary, key points highlighted by this CG study are: induction therapy with AMB plus 5-FC is indicated in patients with CNS disease; 6 weeks is standard. In lung disease, 2 weeks of induction AMB plus 5-FC is likely sufficient. Eradication therapy with fluconazole is appropriate to complete total therapy of 18 months in CNS disease and 12 months in lung infection, longer than that recommended for CN infection. Routine

identification of *Cryptococcus* isolates should distinguish between CG and CN. Antifungal susceptibility is recommended in patients not responding to therapy, for tracking MICs and for correlating them with clinical outcomes. Surgical relief of CSF pressure is often needed in patients with persistently raised pressures.

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CONFLICTS OF INTEREST

All authors: no conflicts of interest in relation to this article.

Sharon Chen, Monica Slavin, Christopher Heath, E. Geoffrey Playford, Deborah Marriott and Tania Sorrell are on the Antifungal Advisory Boards of Gilead Sciences Inc, Merck, and Pfizer Australia. Sarah Kidd is on the Antifungal Advisory Board of Pfizer Australia. Tania Sorrell, E. Geoffrey Playford, Deborah Marriott, Monica Slavin, Sharon Chen, Christopher Heath, Narin Bak and Sarah Kidd have received funding in the form of untied grants from Gilead Sciences Inc., Merck and Pfizer Australia. Tony Korman is on the Antibacterial Advisory Board of Pfizer Australia. He has received monetary reimbursement for service on speakers bureaus for Novartis (2010) and Merck (2011).

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Figure Legend

Figure 1

Bar chart showing body sites of infection in 86 patients with *Cryptococcus gattii* infection. Abbreviations: CNS, central nervous system.

Key: Lung – CNS = lung infection in the absence of CNS infection; CNS - lung = CNS infection in the absence of lung infection; CNS + skin = both CNS and skin infection; blood + other = bloodstream infection plus at least one other site of infection.

TABLES

Table 1: Induction antifungal therapy regimens in the first 14 days of treatment by site of infection[a].

Induction therapy (total no. patients)	Lung without CNS (no.)	CNS (no.)	Bone only (no.)	Blood only (no.)
AMB plus 5-FC (64)	6	57	1	-
AMB only (7)	-	6	-	1
AMB plus Fluconazole[b] (2)	1	1	-	-
Fluconazole[c] only (12)	2	9	-	1
Fluconazole[d] 4 days then voriconazole (1)	1		-	-
Total	10	73	1	2

Abbreviations: AMB, amphotericin B; CNS, central nervous system; 5-FC, 5-flucytosine.

[a] mutually exclusive categories

[b] Fluconazole 400 mg daily in both cases

[c] Fluconazole doses ranged from 200-1600 mg daily. Mode 400 mg daily (n=8); 200 mg daily adjusted for creatinine clearance, n=1; 800 mg daily n=2; 1600 mg daily n=1)

[d] Fluconazole 400 mg daily

Table 2: Antifungal treatment and features of *Cryptococcus gattii* infection in patients with lung, but not central nervous system, disease.

Patient no.	Ethnicity	Immuno-compromise	Induction therapy (duration in weeks)	Consolidation/ Maintenance therapy	Therapeutic course	Chest imaging (diagnosis)	Clinical response (12 months)
29	Caucasian	No	AMB + 5-FC (4)	FLU	> 4 weeks[a]	Single cryptococcoma	Unknown[a]
35	Aborigine	Yes	FLU[b] (unknown)	FLU	24 weeks	Single cryptococcoma[c]	PR
43	Aborigine	Yes	AMB (8) + 5-FC (1)	None	8 weeks	Lobar Consolation	Died (10 weeks)
47	Aborigine	No	AMB + 5FC (1)	FLU	20 weeks	Multiple small cryptococcomas	CR
49	Aborigine	No	AMB + 5-FC (2)	FLU	30 weeks	Generalised consolidation	CR
74	Aborigine	Yes	FLU[b] (6)	FLU	24 weeks	Lobar consolidation	PR
75	Caucasian	Yes	AMB + FLU (2)	FLU	>52 weeks	Multiple small cryptococcomas, generalised	CR

						consolidation	
84	Aborigine	No	AMB + 5-FC (2)	FLU	208 weeks	Single[c] cryptococcoma, lobar consolidation	CR
85	Caucasian	No	AMB + 5-FC (8)	FLU	104 weeks	Single[c] cryptococcoma, lobar consolidation	CR
86	Caucasian	No	FLU[b] (4 days) then VOR[d] (unknown)	VOR, then FLU	64 weeks	Generalized consolidation	SD

Abbreviations: AMB, amphotericin B; CR, Complete response; 5-FC, 5-flucytosine' FLU, fluconazole; PR, partial response; SD, stable disease; VOR, voriconazole patients 35 and 74 were the kidney transplants

[a] no data

[b] Fluconazole dose refers to dose in induction therapy -Patient 35, 200 mg daily as adjusted for creatinine clearance; Patient 74, fluconazole 800 mg daily; Patient 75, fluconazole 400 mg daily; Patient 86, fluconazole 400 mg daily

[c] Cryptococcomas ≥ 8 cm diameter in both cases [d] dose of 6 mg/kg daily on day 1 and then 4 mg/kg/ daily

Table 3. Induction antifungal therapy in patients with central nervous system disease, and outcomes.

Antifungal regimen	Duration induction therapy (weeks)			Re-induction therapy (no. patients)	No. raised ICP	No. normal ICP	No. deaths (%)	No. (%) complete clinical response (12 months)
	Median	LQ, UQ	Range					
AMB +5FC (n=57)	6	3, 8	2-16	5[a]	29	13	9 (16)	22 (39)
AMB only (n=6)	2	-	0.14 - 8	-	-	3	1 (17)	1 (17)
AMB + fluconazole[b] (n=1)	-	-	8	-	-	1	-	-
Fluconazole[c] (n=9)	4	4, 4	2-16	7	2	-	-	2 (22)
Total (n=73)	6	-	0.14 – 16	16	31	17	10 (13.6)	25 (34)

Abbreviations: ICP, intracranial pressure; IRIS, immune reconstitution syndrome; LQ, lower quartile; UQ, upper quartile.

[a]4 patients had re-induction to treat IRIS, 1 for worsening of disease.

b fluconazole dose, 400 mg daily

[C] fluconazole dose: 400 mg daily n=7; 800 mg daily, n=1; 1600 mg daily, n=1.

Table 4. Patients who received fluconazole monotherapy in central nervous system infection.

Patient no.	Immune-compomise	Infection site	Initial CSF CRAG ≥256	ICP values (cm water)	Duration induction therapy	Reinduction therapy	Total duration therapy	12-month clinical response
9	No	CSF	Yes	NA	4 weeks	Yes (AMB, 5-FC)	17 months	CR
16	No	CSF, lung	No	NA	8 weeks	No	9 months	PR
17[a]	Yes	Brain	NA	NA	4 weeks	Yes (AMB, 5FC)	>14 months	SD
18	No	CSF, Brain	No	28	4 weeks	Yes (AMB, 5-FC)	9 months	PR
25	Yes	CSF, brain, lung	NA	NA	4 weeks	Yes (AMB, 5-FC)	Not known	PR
28	No	Brain lung	NA	NA	16 weeks	Yes (AMB, 5-FC)	24 months	CR
34	No	CSF, brain, lung	Yes	36	2 weeks	Yes (AMB, 5-FC)	>12 months	PR
64	No	Brain, lung	NA	NA	4 weeks	No	7 months	PR
65	No	CSF, lung	Yes	NA	4 weeks	Yes (AMB, 5-FC)	36 months	PD

Abbreviations: AMB, amphotericin B; CR, complete response; CSF, 5-FC, 5-flucytosine; NA, no data available; PD, PR, SD alater developed IRIS-like syndrome

Table 5. Features of *C. gattii* infection in patients with and without elevated intracranial pressure.

Characteristic	Raised ICP no./total no. (%)	Without raised ICP no./total no. (%)	*P* (Fisher's exact or χ^2)
Brain disease	20/31 (65)	9/17 (53)	0.63
Abnormal neurology (presentation)	24/31 (77)	6/17 (35)	0.02
Hydrocephalus	10/31 (32)	6/17 (353)	0.91
Cryptococcoma (single or multiple)	15/31 (48)	7/17 (41)	0.86
Serum CRAG titre ≥ 512	17/28 (61)	10/15 (67)	0.22
CSF CRAG titre ≥ 256	25/31 (77)	9/16 (63)	0.09
Death at 12 months	6/31 (19)	1/17 (6)	0.39
Death/neurological sequelae at 12 months (20)	16/31 (52)	2/17 (12)	0.02
Duration induction antifungal therapy in weeks (median, LQ-UQ	6 ((3-6)	6 (2-6)	0.74
Total duration antifungal therapy in survivors (median, LQ-UQ)	12 (6.5-17.5)	13.5 (9.2-20.5)	0.51

Abbreviations: CRAG, cryptococcal antigen; CSF, cerebrospinal fluid; ICP, intracranial pressure; LQ-UQ, lower quartile to upper quartile.

Table 6. Features of patients with, and without, IRIS[a].

	IRIS (n=8)	Without IRIS (n=77)	P (Fisher's exact or χ^2)
Initial presentation	No. (%)	No. (%)	
Males/females (%)	1/8 (13%)	49/77 (64%)	0.007
Caucasian (%)	6/8 (75%)	43/75 (57%)	0.46
Underlying immunosuppression	2/8 (25%)	22/77 (29%)	1.0
Post-pregnancy	1/8 (13%)	2/77 (2.6%)	0.26
Other comorbidity	-	5/77 (7%)	-
CD4 count[b] (median, lower quartile, upper quartile, [range])	900, 750, 1009, [397-1199]	343, 179, 540, [12-1930])	0.002
Neurological abnormality at presentation	5/8 (63%)	38/77 (49%)	0.71
Sites of involvement Brain Brain, meninges, lung	 8/8 (100%) 6/8 (75%)	 37/77 (48%) 17/66 (26%)	 0.01 0.003
CSF OP >=25 cm water	4/5 (80%)	19/34 (56%)	0.63
CSF CRAG >= 256	4/6 (67%)	38/59 (64%)	1.0
India Ink pos	6/6 (100%)	56/59 (95%)	1.0
CSF mononuclear in cells/uL median, lower quartile, upper quartile)	108, 59, 118	31, 5, 140	0.34
CSF protein in g/dL (median,	0.91, 0.79, 1.0	0.75, 0.47, 1.02	0.53

lower quartile, upper quartile)			
CSF glucose mmol/L (median, lower quartile, upper quartile)	2.1, 1.8, 2.8	2.4, 1.8, 3.2	0.49
Two weeks			
Culture positive	1/6 (17%)	11/45 (24%)	1.0
India Ink positive	5/5 (100%)	36/43 (84%)	1.0

[a]Data available for 85 patients
[b]Data available for 7 patients with IRIS and for 34 without IRIS.