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MSG07: An International Cohort Study Comparing Epidemiology and Outcomes of Patients with *Cryptococcus neoformans* or *Cryptococcus gattii* infections

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Contributors Declaration of Interests

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Abstract

Background—Cryptococcosis due to *Cryptococcus neoformans* and *Cryptococcus gattii* varies with geographic region, populations affected, disease manifestations and severity of infection, which impact treatment.

Methods—We developed a retrospective cohort of patients diagnosed with culture-proven cryptococcosis during 1995–2013 from five centers in North America and Australia. We compared underlying diseases, clinical manifestations, treatment and outcomes in patients with *C. gattii* or *C. neoformans* infection.

Results—A total of 709 patients (452 *C. neoformans*; 257 *C. gattii*) were identified. Mean age was 50.2 years; 61.4% were male; and 52.3% were Caucasian. Time to diagnosis was prolonged in *C. gattii* patients compared with *C. neoformans* (mean 52.2 vs 36.0 days; p<0.003) and there was a higher proportion of *C. gattii* patients without underlying disease (40.5% vs 10.2%; p<0.0001). Overall, 59% had central nervous system (CNS) infection, with lung (42.5%) and blood (24.5%) being common sites. Pulmonary infection was more common in patients with *C. gattii* than those with *C. neoformans* (60.7% vs 32.1%; p<0.0001). CNS or blood infections were more common in *C. neoformans*-infected patients (p≤0.0001 for both). Treatment of CNS disease with induction therapy of amphotericin B and flucytosine occurred in 76.4% of patients. Crude 12-month mortality was higher in patients with *C neoformans* (28.4% vs 20.2%; Odds Ratio 1.56; 95% CI 1.08, 2.26).

Conclusions—This study emphasizes differences in species-specific epidemiology and outcomes of patients with cryptococcosis, including underlying diseases, site of infection and mortality. Species identification in patients with cryptococcosis is necessary to discern epidemiologic patterns, guide treatment regimens and predict clinical progression and outcomes.

Summary:

This large study emphasizes differences in species-specific epidemiology of patients with cryptococcosis, including underlying diseases, site of infection and mortality. Species identification in patients with cryptococcosis is important to discern epidemiologic patterns, guide treatment regimens and predict clinical progression and outcomes.

Keywords

Cryptococcosis; *Cryptococcus gattii* ; *Cryptococcus neoformans*

Introduction

Cryptococcus species are saprophytic yeast-like fungi responsible for a broad range of infections in humans. Pulmonary infection results from inhalation of the organism from an environmental source. Cryptococci have a propensity to infect the central nervous system (CNS) and meningoencephalitis is the most commonly recognized manifestation of severe disease[1].

The major species complexes that infect humans, *Cryptococcus neoformans* and *Cryptococcus gattii*, vary with regard to geographic distribution, populations affected, disease manifestations and severity[2, 3] *C. neoformans* is ubiquitous and abundant in soil contaminated by pigeon droppings. Most often it causes opportunistic infection, typically meningoencephalitis in patients with AIDS, malignancy, organ transplants and others receiving iatrogenic immunosuppression [1, 4, 5]. *C. gattii* was previously regarded as being limited to primarily tropical and subtropical regions, with a major endemic focus in Australia. It has now been described throughout the world with emergence in Vancouver Island and British Columbia, Canada, and the U.S. Pacific Northwest, where it is now endemic, in the late 1990s and early 2000s [6–13]. Ecologically, this species is found in soil and in association with certain species of *Eucalypt* [3] and other tree species around the world[14–16].

Small studies suggest that *C. gattii* tends to affect disproportionately patients with apparently normal immune systems compared with *C. neoformans*[9, 17–19]. Moreover, the frequency of clinical pulmonary disease in *C. gattii*-infected patients appears higher than in those infected with *C. neoformans* [19]. Development of intracranial and pulmonary cryptococcomas and need for ventricular shunting may be more frequent among patients with *C. gattii* infection, but the impact of species versus host characteristics on these disease manifestations and outcomes requires further elucidation[11, 19]. Data informing management and duration of antifungal therapy have either been generated from specific geographic regions, or involved only small patient cohorts, and are now dated[8, 10, 20]. Further, because standard culture methods do not differentiate the two species, identification to species level has not been performed in many areas, limiting comparative studies[12, 21].

Recently reported *C. gattii* infections in the U.S. Pacific Northwest, and identification of sporadic human cases in non-endemic areas has stimulated discussion regarding comparisons of these two distinct *Cryptococcus* species. As part of a multi-institutional initiative, we compared risk factors, clinical manifestations, treatment and outcomes of cryptococcosis among patients with *C. gattii* or *C. neoformans* infection in order to inform our understanding of the epidemiology and clinical progression and to guide patient management.

Methods

Datasets and study population

This retrospective cohort study organized by the U.S. Centers for Disease Control and Prevention (CDC) and Mycoses Study Group (MSG) is based on a master dataset derived from five individual site datasets of patients with cryptococcosis. Contributing sites included the University of Alabama at Birmingham (1998–2010)[22]; Duke University Medical Center (1995–2009)[23]; British Columbia Centre for Disease Control (1999–2007)[8–10]; Washington State Department of Health and Oregon State Department of Health (2004–2011)[24]; Westmead Hospital, The University of Sydney, Australia (1999–2013). Case inclusion required a culture-positive result from cerebrospinal fluid (CSF), blood, other body fluid, tissue, or respiratory sample (sputum, bronchoalveolar lavage fluid) with a compatible

clinical and/or radiographic presentation. Species-specific culture methods were used to distinguish *C. gattii* from *C. neoformans* cases[25, 26].

Each site used a separate case report form for data collection. Australia included only patients initially diagnosed in the inpatient setting; all other sites included cases diagnosed in inpatient and outpatient settings. Prior to combining data from the five individual sites, similarity of case definitions, availability of comparable outcome information, structure of the different databases, and the nomenclature of the respective variables were examined. A final list of variables and definitions were agreed upon by the investigators. These included demographic characteristics, clinical presentation, underlying medical conditions, antifungal treatment, laboratory values including CSF test parameters, imaging studies and outcomes. To ensure consistency and accuracy in the process of master data set creation, individual level data for 20 variables from site files were reviewed before final formulation of the master data set. Mortality was defined as all-cause at 3- or 12-months post-diagnosis. Duration of induction therapy of one week was defined as 1–9 days; 2 weeks as 10–20 days; 4–6 weeks as 21–49 days; and 7 weeks or more, as 50–90 days.

We classified sites of involvement as follows: 1) CNS, which included meningeal and/or parenchymal brain involvement; 2) pulmonary, which included disease limited to the lungs, pleura, and/or pleural fluid; 3) bloodstream, which involved any isolation of *Cryptococcus* spp. in blood culture; and 4) other. Disseminated infection was defined as extra-pulmonary infection. Patients may have had more than one site of involvement. A patient was classified with no underlying disease if no evidence of HIV, transplantation, malignancy, neutropenia, chronic organ dysfunction, diabetes, corticosteroid use, immunosuppressant use or other immunodeficiency was identified. Malignancy was defined as cancer diagnosed or treated within 6 months prior to diagnosis of cryptococcosis or recurrent/metastatic cancer. Time to diagnosis was defined by the number of days between the date of symptom onset and confirmed date of diagnosis.

Statistical analysis

Characteristics for the overall cohort and for patients with infection due to *C. neoformans* or *C. gattii* were calculated using means and standard deviations, medians and interquartile ranges, or counts and percentages as appropriate. To analyze the relationship between independent variables and *Cryptococcus* species, univariate analyses were performed using chi-square or Fisher's exact test for categorical variables and analysis of variance (ANOVA) or Wilcoxon Rank Sum testing for continuous variables. Additional analyses of the relationship between lumbar puncture variables or sex and *Cryptococcus* species were adjusted by HIV infection. Two-sided p-values <0.05 were considered statistically significant. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between *Cryptococcus* species and outcomes of interest (e.g., mortality, complications) with and without adjustment for age, gender, race, time to diagnosis from symptom onset, HIV, chronic lung disease, and site of infection. SAS version 9.3 (SAS Institute Inc., Cary, North Carolina) was used for all analyses. The study was approved by the Institutional Review Boards (or equivalents) of all sites and CDC.

Role of the funding source

The funder had no role in study design, data collection or analysis, or data interpretation for drafting of the manuscript. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

In the master data set, 709 (452 *C. neoformans*; 257 *C. gattii*) patients had culture-positive cryptococcosis and were included in the final analyses (Table 1). Mean age of patients was 50.2 years; 61.4% were male and 59.0% were Caucasian. *C. gattii* patients were older and more frequently females ($p<0.01$ for both). After evaluating the relationship of sex and species and adjusting by HIV, female sex was associated with *C gattii* infection ($p=0.03$) Time to diagnosis of cryptococcosis was prolonged in *C. gattii*-infected patients (mean 52.2 for *C. gattii* vs 36.0 days for *C.neoformans*; $p<0.003$). Overall, most patients (59.1%) had CNS infections, with lung (42.5%) and blood (24.5%) the next most commonly affected sites (Table 1). Pulmonary infection was more commonly associated with *C. gattii* infection (60.7% vs 32.1% for *C. neoformans*; $p<0.0001$). In contrast, CNS, blood, or skin infections were more common in *C. neoformans*-infected patients ($p<0.01$ for all).

Most patients (78.8%) had underlying disease and in general, those with *C. gattii* were less immunocompromised; for example, HIV infection (3.5% vs 38.5%; $p<0.0001$) and solid organ transplantation (6.2% vs 25%; $p<0.001$) were less frequent among *C. gattii*-infected patients, and there was a higher proportion without underlying disease (40.5% vs 10.2%; $p<0.001$). Exceptions were malignancy (19.1% vs 11.7%; $p=0.007$) and chronic lung disease (22.2% vs 8.4%; $p=0.001$), which were more common in *C. gattii* patients. Among patients with malignancy, only non-hematologic malignancy was more common among *C. gattii* patients.

Among patients with CNS infection, headache (72.3%), vomiting (37.0%) and altered mental status (36.5%) were frequent clinical symptoms or signs (Table 2). Fever was present in 31% of patients. Symptoms and signs were similar among both patient groups, with the exceptions of altered mental status and nausea, which were significantly more common in *C neoformans*-infected patients. In contrast, headache was more commonly associated with *C gattii* infection (Table 2).

Computed tomography (CT) and magnetic resonance imaging (MRI) findings are summarized in Table 3. The most common lung abnormalities were mass lesions (56.9%), unilateral (38.3%), and bilateral nodules (22.8%). Multiple lobar infiltrates were more common among *C. neoformans*-infected patients (13.0% vs 1.1%; $p=0.002$). On baseline brain imaging (either CT or MRI) among 419 persons with CNS infection, 44 (10.1%) had hydrocephalus. Patients with *C. gattii* CNS disease were significantly more likely to present with brain mass lesions (cryptococcomas).

Lumbar puncture data were available for 419 patients with meningitis (Table 4). Mean or median CSF opening pressure was not significantly different, nor were baseline cryptococcal antigen titers (Table 4). However, mean CSF glucose (13.9 vs 43.5 mg/dl; $p<0.001$) was

lower, median WBCs were higher (119 vs 30/mm^3; p<0.001), and the percentage of patients with India Ink positivity (78.2% vs 71.2%; p=0.002) was greater in *C. gattii* vs *C. neoformans* infection. After adjusting for HIV there were no additional significant differences identified (Table 2). In an analysis of CNS infection in patients without HIV (Supplemental Table 1), patients with *C gattii* infection had lower mean CSF protein compared with *C. neoformans* patients (p=0.045).

The treatment of cryptococcosis frequently consisted of an amphotericin B (AmB) formulation plus flucytosine for induction therapy (Table 5). For patients with CNS diseases, an AmB formulation plus flucytosine as initial therapy was administered to 76.4% of patients and proportions were similar by cryptococcal species. Lipid formulations of AmB were more commonly used in *C gattii*-infected patients (50% vs 39.5%; p=0.046). For patients with data available on duration of induction therapy (n=473), duration was different by species, with a greater proportion of *C gattii*-infected patients receiving prolonged (>4 weeks) induction therapy (p<0.001).

All-cause mortality was 18.8% at 3 months and 25.5% at 12-months (Table 6). For the 12-month endpoint, mortality was higher among patients with *C. neoformans* (28.4% vs 20.2%; OR 1.56; 95% CI 1.08, 2.26). After adjustment for age, gender, race, time to diagnosis from symptom onset, HIV, chronic lung disease and site of infection, the strength of association was similar but not significant (OR 1.46; 95% CI 0.86, 2.46). In sub-group analyses of patients with CNS cryptococcosis, 12-month mortality was also greater among *C. neoformans*-infected patients in crude analysis (26.8% vs 15.9%; OR 1.94; 95% CI 1.13–3.34); but not significant after adjusted analysis (OR 1.99; 95% CI 0.91, 4.33). Among patients with pulmonary infection only, mortality was similar between cryptococcal species. The complication of immune reconstitution inflammatory syndrome (IRIS) was significantly less common among *C neoformans* patients in crude (3.6% vs 7.1%; OR 0.49, 95% CI 0.24–0.97) and adjusted analyses (OR 0.28; 95% CI 0.10–0.82). There was no significant difference in the proportion of patients with CNS disease receiving permanent ventricular shunt placement (*C. neoformans* 13.0%; *C gattii* 25%; OR 0.78; 95% CI 0.45–1.33).

Discussion

Cryptococcosis remains an important cause of morbidity and mortality among many patient populations, especially the immunocompromised. Because routine culture methods and cryptococcal antigen testing cannot distinguish species, it is very likely that cases have been misclassified as *C. neoformans* in prior epidemiologic studies. This study evaluated a cohort of patients with species-specific culture-positive cryptococcosis in order to compare characteristics of patients with *C. gattii* or *C. neoformans* infection. We identified several differences in the epidemiology and outcomes in the two groups: specifically, among *C. gattii*-infected patients symptom duration prior to diagnosis was prolonged, patients were less immunocompromised, pulmonary disease was more common, all-cause 12-month mortality was lower and IRIS more common.

There has been an increasing interest in *C. gattii* infections over the past two decades due to the emergence of *C gattii* in the Pacific Northwest of the US and western Canada

and the identification of sporadic cases across Canada and the United States[8, 10–13, 27, 28]. Moreover, previous comparisons of *C. gattii* and *C. neoformans* have described that *C. gattii* infection is more likely to occur in non-immunocompromised hosts and typically with pulmonary rather than CNS disease[17–19]. It is important to note that *C. neoformans* and *C. gattii* produce diseases in both immunocompromised and immunocompetent hosts although species frequency is different in the two general risk groups.

An increased proportion of *C. neoformans* patients were of male gender. When adjusted by HIV infection, this association was attenuated, but remained significant. Patients differed in frequency of site of disease, with *C. gattii* patients having a greater proportion of patients with pulmonary disease, and fewer CNS or bloodstream infections. These differences are influenced by underlying diseases in our cohort [3, 17]. HIV-infected patients with *C. neoformans* infection when compared to other host groups are more likely to have CNS or bloodstream infection, but fewer pulmonary infections [22, 23, 29]. HIV-infected patients in this cohort were much more common among *C. neoformans* cases.

An important differentiating finding was the prolonged duration of symptoms prior to diagnosis among *C. gattii* cases. This may reflect a lower diagnostic suspicion among HIV-negative cases, where cancer may have been considered first; closer monitoring of patients with HIV who may be more involved in the healthcare system; or higher proportion of meningitis among HIV cases, leading to more acute presentations. Even though the overall proportion of *C. gattii* patients that were immunocompromised was less, 60% had an immunocompromising condition. *C. gattii* cases also had fewer CNS and bloodstream infections, and a greater proportion of pulmonary infections. Given the higher proportion of pulmonary-only disease among *C. gattii* patients and the associated non-specific pulmonary symptoms, cryptococcosis may have been lower in the differential diagnosis and testing for cryptococcosis may have been delayed. *C. gattii*-infected patients were more likely to have underlying chronic lung disease. Perhaps in some cases, cryptococcal infection may have been attributed to chronic lung diseases, delaying diagnosis. A difference in underlying diseases may also affect duration of symptoms, as prior studies have reported a longer duration of symptoms in non-HIV patients with *C. neoformans* infection[22, 23].

Clinical features among patients with CNS disease were similar. Headache was more common in patients infected with *C. gattii*, whereas altered mental status and nausea were more common among *C. neoformans*-infected patients. These differences are not easily explained by the lumbar puncture results, where most CSF parameters were similar, including opening pressure at baseline. However, CSF white blood cell count was significantly higher and CSF glucose correspondingly lower among *C. gattii* patients. When adjusted for HIV infection, these species-specific differences did not change significantly. Furthermore, there were a greater number of cases of IRIS in *C. gattii* patients. Elevated white blood cells could reflect a unique feature of the host-pathogen interaction, or simply a greater proportion of non-immunocompromised hosts with relatively intact inflammatory responses among *C. gattii* patients. However, among patients without HIV infection elevated CSF white blood cell counts remained significantly greater among *C. gattii* patients.

Previous studies have described imaging findings that may help to differentiate *C. neoformans* or *C. gattii* infection[10, 18, 30]. Phillips et al. studied 152 patients with *C. gattii* infection, 66% of whom had lung cryptococcomas. Among the 43 patients with CNS disease and available imaging studies, brain cryptococcomas were present in 18.6% and hydrocephalus in 6.9% [10]. Chen et al. noted that brain cryptococcomas were more common among *C. gattii* than *C neoformans*-infected patients[2]. Hydrocephalus is described in up to 50% of *C gattii* infections [2, 11, 17–19]. Our findings confirm these observations: first, on brain imaging with MRI or CT, *C. gattii*-infected patients were more likely to have mass lesions (cryptococcomas) and "normal" imaging was less common. In contrast, on lung imaging, mass lesions were more frequent in *C. neoformans* patients.

Current treatment guidelines for CNS cryptococcosis recommend AmB formulations plus 5-flucytosine, with fluconazole recommended for mild to moderate pulmonary disease[1]. In our cohort, patients with *C gattii* were more likely to receive lipid preparations of AmB and induction therapy was longer. This may be a result of institutional-specific practice guidelines, where local experience in at least one site supports prolonged induction (6–8 weeks) for eradication of disease. In addition, treatment guidelines for non-immunocompromised patients recommend longer courses of induction therapy and suppression for *C. gattii* infection, especially those who have large and multiple pulmonary or CNS cryptococcomas [1]. The specific preciseness of recommendations for treatment between *C. neoformans* and *C gattii* cannot be improved by this retrospective, uncontrolled study. However, it supports present recommendations that induction, consolidation, and maintenance therapies are similar for both species and local experience may help guide lengths of this therapy.

Mortality in patients with cryptococcosis ranges from 8–50% and is related to underlying disease[2, 22, 31–37]. Typically, immunocompromised HIV-negative patients are older with a variety of underlying diseases and have increased mortality[22, 23, 35, 37]. In this study, *C. neoformans* was associated with a higher all-cause 12-month mortality. After adjusted analyses, the strength of association remained consistent, but the findings were no longer significant. This is likely related to a decrease in power after multivariable analysis; however, we were not able to collect and analyze data on mortality attributable to cryptococcosis or treatment course, which may further inform the association. The presence of IRIS was significantly less common among *C. neoformans* patients, even after adjustment for potential confounders.

Our findings should be interpreted in light of several limitations. Our master dataset was derived from several datasets from which patients had been enrolled in overlapping time periods and from different settings (i.e., hospitalized, outpatients). Treatment regimens were not standardized, which may have affected overall outcomes. Although we only included variables in the master data set that were defined similarly in the individual data sets, there is a risk of misclassification. Some data (chest imaging, duration of therapy, clinical response, lumbar punctures) were not available from all patients or sites. Finally, for this study we have not considered the newest proposals relating to the further division of *Cryptococcus* into multiple species, as this discussion has developed since these data were collected[38]. However, a large dataset such as we present herein will be necessary if we are to discern

relevant clinical differences, if any, among the newly proposed species classifications for cryptococci.[11]

In summary, this description of a large collection of cases highlights differences in epidemiology and clinical outcomes of patients with cryptococcosis due to *C. neoformans* or *C. gattii*. In addition, we have identified that cryptoccocal species may be associated with mortality. Our work supports the need for species-specific testing in patients with cryptococcosis to better define epidemiologic and clinical patterns, to guide appropriate treatment regimens and to predict clinical progression and outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of patients with cryptococcosis

Characteristic	Total (N=709)	C. *neoformans* (N=452)	C. *gattii* (N=257)	P-value
Location				
Australia	82 (11.9)	15 (3.3)	67 (26.1)	<0.0001
British Columbia	111 (16.2)	0 (0.0)	111 (46.2)	
Duke	178 (25.9)	178 (39.4)	0 (0.0)	
Oregon/Washington	112 (16.3)	34 (7.5)	78 (30.4)	
University of Alabama	226 (31.9)	225 (49.8)	1 (0.4)	
Age (years), mean ± SD	50.2 ± 16.2	49.0 ± 15.7	52.5 ± 16.9	0.0064
Mean time to diagnosis from symptom onset (mean days ± SD)	41.7 ± 65.5	36.0 ± 68.7	52.2 ± 57.7	0.0032
Sex				
Male	432 (61.4)	295 (66.0)	137 (53.3)	0.0009
Female	272 (38.6)	152 (34.0)	120 (46.7)	
Race				
African American	197 (27.8)	192 (42.5)	5 (2.0)	<0.0001
Caucasian	371 (52.3)	233 (51.6)	138 (53.7)	
Asian/Pacific Islander	25 (3.5)	7 (1.6)	18 (7.0)	
Aboriginal	20 (2.8)	0 (0.0)	20 (7.8)	
Other	16 (2.3)	13 (2.9)	3 (1.2)	
Unknown	80 (11.3)	7 (1.6)	73 (28.4)	
Site of Infection[1]				
Central Nervous System (CNS)	419 (59.1)	291 (64.4)	128 (49.8)	0.0001
Pulmonary	301 (42.5)	145 (32.1)	156 (60.7)	<0.0001
Blood	174 (24.5)	154 (34.1)	20 (7.8)	<0.0001
Skin	18 (2.5)	18 (4.0)	0 (0.0)	0.0012
Bone or joint	11 (1.6)	9 (2.0)	2 (0.8)	0.34
Soft tissue	13 (1.8)	12 (2.7)	1 (0.4)	0.039
Prostate	2 (0.3)	2 (0.4)	0 (0.0)	0.54
Disseminated[2]	493 (69.5)	353 (78.1)	140 (54.5)	<0.0001
Other	21 (3.0)	18 (4.0)	3 (1.2)	0.034
Underlying Diseases				
None	150 (21.2)	46 (10.2)	104 (40.5)	<0.0001
Corticosteroids	242 (34.1)	174 (38.5)	68 (26.5)	0.0012
HIV	183 (25.8)	174 (38.5)	9 (3.5)	<0.0001
CD4, mean ± SD	29.2 ± 44.3	25.3 ± 39.3	93.7 ± 71.8	<0.0001
CD4, median (IQR)	11(5–32)	10 (5–27)	80 (40–160)	<0.0018
Solid organ transplant	129 (18.2)	113 (25.0)	16 (6.2)	<0.0001

Characteristic	Total (N=709)	C. *neoformans* (N=452)	C. *gattii* (N=257)	P-value
Lung	19 (2.7)	15 (3.3)	4 (1.6)	0.16
Heart	23 (3.2)	22 (4.9)	1 (0.4)	0.0012
Liver	20 (2.8)	19 (4.2)	1 (0.4)	0.0032
Kidney	70 (9.9)	60 (13.3)	10 (3.9)	<0.0001
Pancreas	6 (0.9)	6 (1.3)	0 (0.0)	0.092
Stem cell transplant	3 (0.4)	2 (0.4)	1 (0.4)	>0.99
Malignancy[3]	102 (14.4)	53 (11.7)	49 (19.1)	0.0074
Hematologic	49 (6.9)	31 (6.9)	18 (7.0)	0.94
Non-hematologic	61 (8.6)	26 (5.8)	35 (13.6)	0.0003
Lymphoma	27 (3.8)	16 (3.5)	11 (4.3)	0.62
Leukemia	20 (2.8)	11 (2.4)	9 (3.5)	0.41
Neutropenia	9 (1.3)	2 (0.4)	7 (2.7)	0.013
Rheumatologic disease	31 (4.4)	18 (4.0)	13 (5.1)	0.50
Lupus	7 (1.0)	3 (0.7)	4 (1.6)	0.25
End Stage Renal Disease	19 (2.7)	16 (3.5)	3 (1.2)	0.060
Renal insufficiency	102 (14.4)	82 (18.1)	20 (7.8)	0.0002
Diabetes	116 (16.4)	78 (17.3)	38 (14.8)	0.39
Sarcoidosis	14 (2.0)	11 (2.4)	3 (1.2)	0.24
Liver disease	53 (7.5)	32 (7.1)	21 (8.2)	0.60
Cirrhosis	32 (4.5)	23 (5.1)	9 (3.5)	0.33
Chronic lung disease[4]	95 (13.4)	38 (8.4)	57 (22.2)	<0.0001
COPD	27 (3.8)	7 (1.6)	20 (7.8)	<0.0001
Pregnancy (among females)	8 (2.9)	4 (2.6)	4 (3.3)	0.73
Idiopathic CD4 lymphopenia	3 (0.4)	1 (0.2)	2 (0.8)	0.30

[1]Represents number of patients who had infection at that the site. Patients may have involvement of more than one site and may be represented more than once.

[2]Patients may have more than one extra-pulmonary site

[3]Malignancy was defined as cancer diagnosed or treated within 6 months prior to diagnosis of cryptococcosis or recurrent/metastatic cancer

[4]Includes asthma, COPD, sarcoidosis, bronchiectasis, bronchiolitis, chronic bronchitis, chronic lung disease, cystic fibrosis, end stage lung disease, emphysema, pulmonary fibrosis, pulmonary alveolar proteinosis, sarcoidosis, reactive airway disease.

Note: The values represent n (%) unless otherwise specified. Analysis of variance (ANOVA) or Wilcoxon Rank Sum testing and chi-square or Fisher's exact tests used to compare continuous and categorical variables, respectively.

COPD=chronic obstructive pulmonary disease; SD=standard deviation; IQR=interquartile range

Table 2.

Baseline clinical findings in patients with any CNS cryptococcosis

Characteristic	Total (N=419)	C. *neoformans* (N=291)	C. *gattii* (N=128)	P-value
Clinical Presentation				
Headache	303 (72.3)	199 (68.4)	104 (81.3)	0.0067
Vomiting	155 (37.0)	108 (37.1)	47 (36.7)	0.94
Altered mental status	153 (36.5)	121 (41.6)	32 (25.0)	0.0012
Nausea	145 (34.6)	119 (40.9)	26 (20.3)	<0.0001
Fever	130 (31.0)	86 (29.6)	44 (34.4)	0.33
Visual impairment	109 (26.0)	68 (23.4)	41 (32.0)	0.063
Malaise/weakness	92 (22.0)	69 (23.7)	23 (18.0)	0.19
Focal neurologic impairment	50 (11.9)	29 (10.0)	21 (16.41)	0.061
Seizures	41 (9.8)	32 (11.0)	9 (7.0)	0.21
Cranial nerve palsy	35 (8.4)	27 (9.3)	8 (6.3)	0.30
Myalgias	26 (6.2)	14 (4.8)	19 (7.4)	0.075

Note: The values represent n (%) unless otherwise specified. Chi-square or Fisher's exact tests were used to compare categorical variables.

Table 3.

Baseline imaging results among patients with pulmonary or CNS cryptococcosis

Chest Imaging in Pulmonary Cryptococcosis				
Computed Tomography (CT) Imaging	**Total (N=167)**	**C. *neoformans* (N=77)**	**C. *gattii* (N=90)**	**P-value**
Normal	3 (1.8)	2 (2.6)	1 (1.1)	0.60
Single lobar infiltrate	20 (12.0)	9 (11.7)	11 (12.2)	0.92
Multiple lobar infiltrate	11 (6.6)	10 (13.0)	1 (1.1)	0.0020
Mass lesion	95 (56.9)	50 (64.9)	45 (50.0)	0.052
Unilateral nodule	64 (38.3)	29 (37.7)	35 (38.9)	0.87
Bilateral nodules	38 (22.8)	15 (19.5)	23 (25.6)	0.35
Cavitation	9 (5.4)	4 (5.2)	5 (5.6)	>0.99
Diffuse/Interstitial	7 (4.2)	5 (6.5)	2 (2.2)	0.25
Pleural effusion	22 (13.2)	11 (14.3)	11 (12.2)	0.69
Brain Imaging in CNS Cryptococcosis[1]				
Computed Tomography (CT) Imaging	**Total (N=325)**	**C. *neoformans* (N=219)**	**C. *gattii* (N=106)**	**P-value**
Normal	192 (59.1)	152 (69.4)	40 (37.7)	<0.0001
Hydrocephalus	36 (11.1)	17 (7.8)	19 (17.9)	0.0062
Mass lesion	32 (9.9)	7 (3.2)	25 (23.6)	<0.0001
Magnetic Resonance Imaging (MRI)	**Total (N=127)**	**C. *neoformans* (N=97)**	**C. *gattii* (N=30)**	**P-value**
Normal	63 (49.6)	56 (57.7)	7 (23.3)	0.0010
Hydrocephalus	10 (7.9)	9 (9.3)	1 (3.3)	0.45
Mass lesion	23 (18.1)	12 (12.4)	11 (36.7)	0.0025

Note: The values represent n (%) unless otherwise specified. Chi-square or Fisher's exact tests were used to compare categorical variables. Chest imaging data were available from all sites except Australia

Table 4.

Baseline lumbar puncture results among patients with any CNS Cryptococcus

Lumbar Puncture[1]	**N**	**Total (N=419)**	**C. *neoformans* (N=291)**	**C. *gattii* (N=128)**	**P-value**	**P-value**[2]
Opening pressure, mmH20						
Mean ± SD	237	312 ± 149	314 ± 152	306 ± 138	0.72	0.90
Median (IQR)	237	300 (190–420)	280 (190–430)	310 (180–400)	0.90	NA
WBCs/mm^3, mean ± SD	369	158 ± 339	115 ± 265	267 ± 460	<0.0001	0.014
WBCs/mm^3, median(IQR)	369	46 (9–150)	30 (6–104)	119 (44–360)	<0.001	NA
% Polys	136	21 ± 27	18 ± 26	37 ± 29	0.002	0.003
% Lymphocytes	164	67 ± 27	70 ± 26	59 ± 27	0.02	0.032
Protein, mg/L, mean ± SD	372	112 ± 137	116 ± 133	100 ± 146	0.31	0.067
Glucose, mg/dl mean ± SD	374	35 ± 30	44 ± 28	14 ± 23	<0.0001	<0.001
% India Ink positive	269	197/269 (73.2)	136/191 (71.2)	61/78 (78.2)	0.002	0.009
% CSF culture positive	399	389/399 (97.5)	272/280 (97.1)	117/119 (98.3)	0.73	0.72
CSF CRAG, median (IQR)[2]	329	512 (64–1024)	512 (64–1024)	256 (128–1050)	0.80	NA
CSF CRAG positive	329	319 (97.0_	225/235(95.7)	94/94(100)	0.07	
Serum CRAG, median (IQR)[2]	254	512 (128–1024)	1024 (64–2048)	512 (128–1024)	0.63	NA
Serum CRAG positive	254	250/254 (98.4)	169/173(97.7)	81/81 (100)	0.17	

[1]Baseline lumbar puncture defined as up to 14 days before and six days after the date of diagnosis.

[2]Adjusted for HIV

[3]Cryptococcal antigen (CRAG) testing performed by latex agglutination

Note: The values represent n (%) unless otherwise specified. Analysis of variance (ANOVA) or Wilcoxon Rank Sum testing and chi-square used to compare continuous and categorical variables, respectively.

SD=standard deviation; IQR=interquartile range; WBC=white blood cell; CSF=cerebrospinal fluid; NA=not applicable

Table 5.

Induction antifungal therapy among patients with cryptococcosis

Antifungal therapy (all sites)	**Total (N=709)**	**C. *neoformans* (N=452)**	**C. *gattii* (N=257)**	**P-value**
Any AmBd	291 (41.0)	201 (44.5)	90 (35.0)	0.0014
Any LAmB	227 (32.0)	144 (31.9)	83 (32.3)	0.90
Any flucytosine	361 (50.9)	250 (55.3)	111 (43.2)	0.0019
Any AmB plus flucytosine	358 (50.5)	248 (54.9)	110 (42.8)	0.0020
AmBd plus flucytosine	224 (31.6)	160 (35.4)	64 (24.9)	0.0039
LAmB plus flucytosine	156 (22.0)	90 (19.9)	66 (25.7)	0.075
Any CNS cryptococcosis	**Total (N=419)**	**C. *neoformans* (N=291)**	**C. *gattii* (N=128)**	**P-value**
Any AmBd	251 (59.9)	175 (60.1)	76 (59.4)	0.88
Any LAmB	179 (42.7)	115 (39.5)	64 (50)	0.046
Any flucytosine	320 (76.4)	226 (77.7)	94 (73.4)	0.35
Any AmB plus flucytosine	320 (76.4)	226 (77.7)	94 (73.4)	0.35
AmBd plus flucytosine	203 (48.5)	147 (50.5)	56 (43.8)	0.20
LAmB plus flucytosine	137 (32.7)	81 (27.8)	56 (43.8)	0.0014
Duration of Induction (all sites)[1]	**Total (N=473)**	**C. *neoformans* (N=331)**	**C. *gattii* (N=142)**	**P-value**
1 week	100 (21.1)	76 (23.0)	24 (16.9)	<0.0001
2 weeks	240 (50.7)	186 (56.2)	54 (38.0)	
4–6 weeks	107 (22.6)	62 (18.7)	45 (31.7)	
≥7 weeks	26 (5.5)	7 (2.1)	19 (13.4)	

[1] Duration of induction of 1 week defined as 1–9 days; 2 weeks defined as 10–20 days; 4–6 weeks defined as 21–49 days; 7 weeks or more defined as 50–90 days.

Note: The values represent n (%) unless otherwise specified. Analysis of variance (ANOVA) or Wilcoxon Rank Sum testing and chi-square or Fisher's exact tests used to compare continuous and categorical variables, respectively.

AmBd=amphotericin B deoxycholate; LAmB- lipid formulation of amphotericin B; AmB=amphotericin B (includes deoxycholate and lipid formulations); CNS=central nervous system

Table 6.

Outcomes of patients with cryptococcosis

Outcomes	**Total (N=709)**	**C. *neoformans* (N=452)**	**C. *gattii* (N=257)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**
Mortality at 3-months	131 (18.8)	89 (19.7)	42 (17.0)	1.20 (0.80–1.80)	0.95 (0.53–1.70)
Mortality at 12-months	178 (25.5)	128 (28.4)	50 (20.2)	1.56 (1.08–2.26)	1.46 (0.86–2.46)
IRIS present	34 (4.8)	16 (3.6)	18 (7.1)	0.49 (0.24–0.97)	0.28 (0.10–0.82)
Any CNS infection	**Total (N=419)**	**C. *neoformans* (N=291)**	**C. *gattii* (N=128)**		
Shunt placement	70 (22.4)	38 (13.0)	32 (25.0)	0.78 (0.45–1.33)	1.26 (0.58–2.72)
Mortality at 3-months	69 (16.6)	54 (18.6)	15 (11.9)	1.69 (0.91–3.12)	1.40 (0.58–3.37)
Mortality at 12-months	98 (23.5)	78 (26.8)	20 (15.9)	1.94 (1.13–3.34)	1.99 (0.91–4.33)
Pulmonary infection only	**Total (N=204)**	**C. *neoformans* (N=104)**	**C. *gattii* (N=100)**		
Mortality at 3-months	38 (18.6)	17 (16.4)	21 (21.0)	0.74 (0.36–1.49)	0.46 (0.17–1.29)
Mortality at 12-months	50 (24.5)	27 (26.0)	23 (23.0)	1.17 (0.62–2.23)	1.12 (0.46–2.70)

Chi-square or Fisher's exact tests were used to compare categorical variables.

*Adjusted for age, gender, race, time to diagnosis from symptom onset, HIV, chronic lung disease, and site of infection.