# CLINICAL MANAGEMENT AND OUTCOMES OF PATIENTS IN THE DUKE CRYPTOCOCCOSIS CLINICAL COHORT, 1996 - 2009

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## ABSTRACT

EMILY WENINK BRATTON: Clinical Management and Outcomes of Patients in the Duke Cryptococcosis Clinical Cohort, 1996–2009
(Under the direction of Charles Poole, PhD)

Using this single-center cohort of cryptococcosis patients, we characterized temporal trends among HIV-infected, transplant recipients and a third heterogeneous group of HIV-negative, non-transplant patients, over a 14-year period (1996 – 2009). We executed a comparison of clinical management and predictors of poor outcomes with respect to these three groups. Adherence to recommended treatment algorithms was an important research question in our study. The 2010 IDSA Guidelines for treatment of these three groups was created to better inform clinicians, yet supportive evidence from cohort studies is still lacking, particularly in the HIV-negative, non-transplant group.

All cryptococcosis patients diagnosed at Duke University Medical Center from 1996 – 2009 were included in our study (N=207). Although the total cases have remained steady (~15/year), there was a shift to a decreasing proportion of HIV-positive patients with a concomitant increase in HIV-negative cases, while transplant recipients remained steady. From the start of antifungal therapy overall mortality through one year was 25% (n=52). Cryptococcosis-attributable mortality through one year of follow-up was 15% (n=31); half of these deaths were among HIV-negative, non-transplant cases. Acute mortality was high, with 10% of severe disease patient deaths occurring during the first two weeks from the start of antifungal treatment.

As recommended, most patients with severe disease received amphotericin B for initial antifungal treatment and the majority of non-severe patients received fluconazole. Receiving a non-recommended antifungal regimen was associated with a higher relative risk of persistent infection at four weeks (RR1.9, 95%CI 0.9 – 4.3). The rate of attributable mortality among those not receiving the recommended dose of initial therapy was higher relative to those receiving recommended dosing (HR 2.3, 95%CI 1.0 – 5.0). Among severe disease patients, flucytosine exposure was associated with a lower overall mortality rate (HR 0.4, 95%CI 0.2 – 0.9) and attributable mortality (HR 0.5, 95%CI 0.2 – 1.2).

The future of cryptococcosis treatment and the development of new antifungal therapies should not only be informed by randomized trials, but also by key observational trends that help to identify problematic, real-world applications of drug regimens to diverse populations.

To my wonderful husband and loving family, always and in all ways

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## ABBREVIATIONS

| | |
|---|---|
| AmpB | Amphotericin B |
| AmpBd | Amphotericin B deoxycholate |
| ABLC | Amphotericin B Lipid Complex |
| ARDS | Acute respiratory distress syndrome |
| ARV | Antiretroviral |
| DCCC | Duke Cryptococcosis Clinical Cohort |
| DUMC | Duke University Medical Center |
| CFU | Colony forming units |
| CI | Confidence Interval |
| CL | Confidence Limits |
| CM | Cryptococcal meningitis |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| GVHD | Graft Versus Host Disease |
| HAART | Highly Active Antiretroviral Therapy |
| HIV | Human Immunodeficiency Virus |
| HR | Hazard Ratio |
| IDSA | Infectious Disease Society of America |
| LFAmpB | Lipid formulation amphotericin B |
| L-AmpB | Liposomal amphotericin B |
| LP | Lumbar puncture |
| OP | Opening pressure (relates to lumbar puncture procedure) |

| | |
|---|---|
| RR | Risk Ratio |
| SD | Standard deviation |
| SOT | Solid organ transplant |
| WBC | White Blood Cell |
| 5FC | 5-Fluorocytosine (Flucytosine) |

# CHAPTER I. SPECIFIC AIMS

The IDSA 2010 Clinical Practice Guidelines for the management of cryptococcosis [1] provide informative categories for analysis by classifying patient groups (HIV positive, solid organ transplant recipients, and HIV-negative/non-transplant) and severity of disease (meningeal, non-meningeal). Taking these categories into consideration, we examined the differences between the three different clinical groups and address the degree to which the various clinical manifestations of cryptococcosis and baseline patient immune status modify the effectiveness of antifungal treatment.

The three-month mortality rate during management of acute cryptococcosis with CNS involvement is still nearly 20% despite medical advances and the advent of highly active antiretroviral therapy (HAART) [2,3,4]. In resource-limited settings, the two-week mortality rates after presenting to a health clinic have been reported as high as 100% [5]. With the high risk of mortality with severe disease, treatment and clinical management strategies are imperative to improving patient survival and preventing relapse or persistence of infection.

Definitions of treatment success varies based on immune status—particularly HIV status. HIV positive patients may be cured with initial therapy, with "cure" meaning the eradication of the fungal organism and elimination of symptoms, but early studies reveal rates of relapse nearing 50% [6,7,8,9]. Thus, commonly with this particular immunosupressed population, the goal of suppression of fungal disease without persistence or relapse is more realistic [3].

With the introduction of maintenance therapy, rates of relapse have dropped significantly to under 10% in geographic areas where induction therapy is the standard of care [3,10,11,12]. A study in France comparing cryptococcosis relapse rates among HIV-positive patients in the pre-HAART versus HAART eras found that relapse after initiating maintenance therapy was largely associated with a lower CD4 cell count at baseline, a slower rate of increase of CD4 cells in the first two weeks of HAART, as well as a serum cryptococcal antigen titer ≥1:512 at any time during follow-up [13]. Close monitoring of the patient for relapse may still be required despite this study showing that HIV patients with a history of cryptococcosis may be taken off maintenance therapy. Relapse rates among non-AIDS patients are still around 15-20% [3,14]. Commonly, HIV-positive patients with cryptococcosis are followed indefinitely with lifelong maintenance therapy and generally non-HIV patients are followed for at least one year [3].

Data is limited on predicting risk factors for failure of cryptococcal treatment. Based on previous studies [13,15,16,17,18,19,20,21], some risk factors that contribute to treatment failure for cryptococcosis, particularly cryptococcal meningitis (the most commonly studied), include: positive cultures (CSF, blood), high serum or CSF antigen titers, headache, and abnormal mental status at admission (Table 1.1). These suggested risk factors are not necessarily exhaustive, nor do they represent a consensus among the medical community.

Questions remain about optimal treatment strategy for patients given a particular set of risk factors. Previous studies have shown that treatment is generally 50-80% (median: 63%) effective, antifungal drugs cause toxicities in roughly one-third of cases, and mortality while on antifungal therapy still remains high (approximately 20%) [3,4,13,18,22]. This

mortality rate is not necessarily due to cryptococcosis, but can be a result of an underlying condition or co-infection.

**Study Rationale**

*Cryptococcus* is an invasive mycosis that causes considerable morbidity and mortality, but there are few larger cohort studies that focus on this disease, many of which are now more than a decade old [13,18,19]. With the global burden of cryptococcal infection reaching 1 million cases per year [23], many questions remain regarding the clinical management on of HIV-infected individuals, organ transplant recipients and more recently immunocompetent individuals or other non-HIV/non-transplant hosts.

Many HIV positive patients are on lifelong antifungal maintenance therapy with monthly monitoring. Therefore, identifying risk factors and treatment recommendations for HIV patients with cryptococcal disease is important not only for the potential prevention of immune reconstitution inflammatory syndrome, increased intracranial pressure, drug resistance and cryptococcomas, but also to identify the optimal treatment regimens of cryptococcal disease to reduce costs associated with treatment failure. This is particularly true in resource limited settings coping with the HIV epidemic without proper access to diagnostic tools or medications. Due to the absence of new drug development, there is much need for well-supported disease management strategies.

This is the first comprehensive, single-center observational cohort in the U.S. devoted to the in-depth examination of cryptococcosis among both the HIV-infected and non-HIV patient. It is the first database generated from a single medical center that will contain a

wealth of information on all three clinical groupings defined by the Infectious Disease Society of America (IDSA) [1] that include: 1) HIV-infected individuals; 2) solid organ transplant recipients; 3) non-HIV and non-transplant hosts. The third group is notably heterogeneous, but will most likely form the smallest proportion of patients in this database, although this group could prove of unique interest to clinicians and to serve as a comparison.

**Specific Aim 1.** How do the following three groups with cryptococcosis differ with respect to clinical presentation and management: HIV-positive, transplant recipients, and HIV-negative/non-transplant patients?

**Objectives:**

1. Examine the changing populations for acquiring Cryptococcus (HIV positive, transplant recipients, HIV-negative/non-transplant)

2. Summarize temporal trends in diagnosis and amphotericin B formulations as initial therapy

3. Describe and assess differences and similarities of underlying three groups with respect to initial presentation, diagnostics and approaches to clinical management
    a. Examine prevalence and type of immunosuppression in the three groups—HAART exposure in HIV-infected patients, corticosteroid use and immunosuppressive agents among transplant and HIV-negative/non-transplant, as well as prevalence of patients appearing "immunocompetent."

**Specific Aim 2.** How does initial antifungal therapy for cryptococcosis influence patient outcomes of persistence of infection, attributable mortality and overall mortality?

**Objectives:**

1. Determine the association of aspects of initial treatment on poor patient outcomes that include the risk of persistent infection and rates mortality due to cryptococcosis and overall 1-year mortality
    a. Estimate the effect of *initial antifungal treatment type* on persistence and mortality
    b. Estimate the effect of *initial treatment dosing* on persistence and mortality
    c. Estimate the effect of *flucytosine exposure* among patients with severe cryptococcosis disease on persistence and mortality
        i. Estimate the effect of ≤ 7 days or >7 days of flucytosine on the risk of persistence and mortality rate among patients surviving at least 14 days from the time of cryptococcosis diagnosis.

2. Assess other strong predictors for the above outcomes in (1).

3. Report the frequency of secondary outcomes of Immune Reconstitution Inflammatory Syndrome (IRIS), renal toxicity measured by at least a 50% decline in Glomerular Filtration Rate (GFR), re-induction of antifungal therapy, and changing antifungal therapy during initial induction.

**Table**

Table 1.1. Indicators of risk for failure (including mortality) of cryptococcosis antifungal treatment.

| **Non-HIV Infected** | **HIV-infected** |
|---|---|
| Positive India ink | Positive culture at 2 weeks |
| High LP OP | Positive blood and urine cultures |
| Low CSF glucose | Treatment without flucytosine |
| Extra-neural sites | High serum or CSF antigen titer |
| CSF antigen titer >1:32 | Age >30 years |
| CSF antigen titer ≥1:1024 | Low CSF glucose |
| Steroid therapy | Intravenous drug use[a] |
| Lymphoreticular malignancy | High MIC to fluconazole[b] |
| Hematologic malignancy | Mechanical ventilation[a] |
| No headache | ICU admission[a] |
| Organ failure | Organ failure |
| Abnormal mental status | |
| Low CSF leukocyte count[a] | |
| Absent antibody[a] | |
| Age ≥60 | |
| Male gender | |

[a] These risk factors were not measured in our study and will be uncontrolled in our analyses; CSF nucleated host cell count was used as a proxy for CSF leukocyte count

[b] High MIC to fluconazole or other antifungal drugs were measured very infrequently and will unlikely be included in our study as a risk factor for treatment failure.

## CHAPTER TWO - BACKGROUND AND SIGNIFICANCE

*Cryptococcus spp., an encapsulated yeast,* is an opportunistic human fungal pathogen isolated from the environment worldwide, particularly in urban areas [24,25]. Humans are thought to be exposed by inhaling the fungal basidiospores, which are small enough to establish in the alveoli of the lung [26]. Pigeon guano is a common source for infectious propagules of *C. neoformans* and is postulated to play a central role in transmission from the environment to humans [3,24,27,28,29,30,31,32,33]. As the spores are inhaled, the lungs are the portal of entry and pneumonia is a common manifestation of cryptococcal diseases, however, the most common manifestation of disease is when the pathogen spreads to the central nervous system (CNS) and causes meningoencephalitis. This is more common among immunocompromised individuals who are unable to contain the infection at the primary site (the lung) and from the advent of the HIV epidemic, *C. neoformans* has become an increased global concern among those who are HIV infected [34]. Furthermore, cryptococcal meningitis is uniformly fatal without treatment [3,24,35,36,37].

Cryptococcosis refers to invasive *C. neoformans* infections. *C. neoformans* exists in two varieties— *grubii* (serotype A) and *neoformans* (serotype D) [36]. Both of these *C. neoformans* varieties cause disease predominantly in immunocompromised individuals [36]. However, serotype A is identified in more than 95% of clinical cryptococcosis isolates from immunocompromised patients [3,24,25,35]. In the late 1990's *Cryptococcus gattii* emerged in Vancouver Island, British Columbia, Canada, resulting in an outbreak of infections in both

immunosuppressed, and more often than *C. neoformans*, immunocompetent humans and animals [38,39]. *C.gattii* is rare in the Southeastern U.S. with only one identified clinical case in an immunocompromised adult [40], hence this species was not the focus of our study.

Diagnosis without laboratory capabilities is difficult because clinical presentation of human cryptococcosis can be very nonspecific. However, the central nervous system (CNS) and respiratory tract are the most common organs involved in cryptococcal infection [34]. Clinical manifestations have a wide range of severity from an asymptomatic nodule in the lung to sudden death from septic shock with acute respiratory distress syndrome. Clinical signs can be seen in the CNS, lung, skin, eye, genitourinary tract, bone and joints, muscle, heart, gastrointestinal tract, breast, lymph nodes, thyroid, adrenal gland, and head and neck [3].

Cryptococcosis can occur at any age. Being at least 30 years of age is reported as a risk factor for cryptococcosis treatment failure among HIV positive individuals [41,42], while cases that are ≥60 years are reported among HIV-negative patients [21]. Age may have an independent impact on survival or incidence of treatment failure. A male predominance exists in both HIV positive and HIV negative cryptococcosis patients [4,41,43,44].

**Disease populations—the three groups**

The 2010 IDSA Cryptococcal Guidelines defined three distinct risk groups for induction treatment of cryptococcosis [1]: (1) HIV-positive; (2) transplant recipients; and (3) a heterogeneous group with neither of these conditions (i.e., HIV-negative/non-transplant).

*HIV Positive*

Cryptococcal infections in HIV-positive patients almost always occur in advanced stages of the disease, and are for the most part, incurable but treatable— individuals who survive initial illness require lifelong maintenance therapy and close monitoring [3]. Symptoms and clinical findings common in AIDS patients include: headaches, fever, shorter duration of symptoms than non-AIDS patients, positive India ink examination, cerebrospinal fluid (CSF) antigen titer ≥1:1024, CSF pleocytosis <20/μl, CD4 counts <100 cells/μl, serum antigen positive, cryptococcemia and increased intracranial pressure (Table 2.1) [3,6,7,8,9,45,46]. There is less knowledge on common symptoms among HIV-negative patients, but similar to HIV-positive patients headache, fever and abnormal CSF chemistry (glucose <40, protein >45) are considered most common (Table 2.1) [45].

Parallel with the rise of the AIDS epidemic in 1981, the incidence of cryptococcosis increased [3]. Before the HIV epidemic, cryptococcosis was much more uncommon and occurred predominantly among patients with compromised immunity, such as those with hematologic malignancy or had undergone solid organ transplantation at a rate of 0.2 – 0.8 per 100,000 depending on geographic area [34,47]. In more recent decades, HIV-associated cryptococcal infection is estimated to account for up to 80% of all cases [34,47,48]. It is estimated the current rate of HIV-associated cryptococcosis is approximately 1 million cases per year worldwide [23]. Despite this high estimate of disease, the incidence among HIV/AIDS patients has fallen since the early 1990's in the United States, with two cities (Houston, TX and Atlanta, GA) reporting drops in incidence from 24 per 1000 and 66 per 1000 in 1993/1992 to 2 per 1000 and 7 per 1000 in 2000, respectively [42]. This pattern of

observation was also made in a large retrospective study in France, with a 46% decrease in cryptococcosis incidence between pre-HAART and post-HAART eras [41].

Incidence rates of cryptococcosis remain the highest in areas where HIV disease is high and availability of highly-active antiretroviral therapy (HAART) is limited [1,23]. About 5-10% of patients with AIDS in the United States will develop cryptococcosis (pulmonary and/or meningeal) [1,3]. Prevalence estimates among HIV patients in the U.S. range from 5-13% depending on region and study year (1985-1997) [3,6,7,8,9,49,50,51]. In an eight-year surveillance study in the United States, under one-third of the HIV-infected cryptococcosis cases had been placed on HAART before diagnosis [42], emphasizing the importance of access and adherence to HAART in prevention of opportunistic disease, not to mention being tested for HIV infection. Though progress has been made, the 3-month mortality rates from acute cryptococcal meningitis continue to hover around 20% [2,4].

*Solid Organ Transplant*

The risk of cryptococcosis has not vanished in developed countries because of the increased use of immunosuppressive therapy and continued progress of transplant medicine [34]. Cryptococcosis is the third most common invasive fungal infection affecting solid organ transplant recipients, with a mortality rate of 10-20% and near 40% with CNS involvement [43,52,53,54]. With the advancement in solid organ transplantation and potent immunosuppressive drugs, rapidly altering the immune system can put patients at risk for cryptococcal disease and *Cryptococcus*-associated immune reconstitution inflammatory

syndrome (IRIS) [43]. Between 20-60% of cryptococcosis in HIV-*negative* patients occurs in solid organ transplant patients [3,21,47,55].

Cryptococcosis disease is diagnosed at a median of 21 months after transplantation [56] and most commonly in renal transplant patients; though liver transplant patients are more likely to have disseminated disease [56]. Studies have reported a lifetime cryptococcosis prevalence of 3-4% post-transplantation [52,57,58,59]. Immunosuppressive drug administration for transplant recipients to prevent organ rejection can place them at increased risk for cryptococcal infection. Corticosteroid use is associated with an increased risk of cryptococcosis [16,43,60]. The use of calcineurin inhibitors as an immunosuppressive agent appears to be associated with lower mortality, but the interaction of this class of drug with amphotericin B deoxycholate (AmpBd) can contribute to nephrotoxicity [3,61]. Therefore, lipid formulation amphotericin B (LFAmpB) is recommended for organ transplant recipients (Table 2.2). A study by Sun et al (2009) demonstrated that LFAmpB was significantly associated with better survival in transplant patients compared to AmpBd (OR, 0.11; 95% CI, 0.02-0.57) [61].

*HIV-negative, non-transplant*

The healthy human population is rarely infected by cryptococcosis, but cases with limited frequency have been reported [3,4,21,62]. High risk patients other than HIV-positive individuals and organ transplant recipients are a heterogeneous group. Patients with malignancies, specifically those in the classical tumor risk groups (Hodgkin's disease, lymphoma, and chronic leukemia) are considered high risk [3,14,63,64,65,66,67]. Diabetes

or chronic lung diseases may contribute to susceptibility, and simultaneous infection with other fungi cannot be ruled out [3]. There are still many questions surrounding this patient group, particularly with risk factors for cryptococcosis disease given their "healthy" immune status. Therapeutic approaches are primarily based on expert opinion, out-dated explorative and retrospective cohort studies. Lacking a consensus, further study is needed.

It is difficult to consistently treat such a diverse group with mortality rates still as high as 24% [19,22]. More studies to inform the management of the HIV-negative/non-transplant group and to understand how host immunity plays a role in poorer prognosis are needed so as to reduce observed elevated mortality in this group. In an important study by Pappas et al (2001), 306 HIV-negative cryptococcosis patients from 15 different centers in the U.S. were shown to have successful treatment in 74% of cases. Relapse occurred in 4% of patients, all-cause mortality was 30% and death due to cryptococcosis was 12% [21]. Factors influencing mortality among all patients included organ failure, hematologic malignancy, age ≥60 years, unsuccessful therapy, site of infection other than pulmonary and a positive blood culture for *C. neoformans* (Table 2.1) [21]. A recent multi-center study of 86 cryptococcal meningitis patients also found the highest mortality in the non-immunosuppressed group (46%) compared to immunosuppressed (19%) and HIV-positive (15%) cryptococcal meningitis patients [62], and previous studies have reported 30 - 44% overall mortality in the HIV-negative population [21,68,69,70], and in one of these studies this was compared to a 22% mortality among HIV-positive patients [69]. However, a couple of these studies included *C.gatii* cases, which many have influenced rates of poor outcome [69,70].

**Diagnostics**

- India ink stain (direct observation)
- Latex agglutination test (antigen detection) primarily in CSF and serum
- Culture (24-72 hours up to 5-7 days)

*Cryptococcus neoformans* can be rapidly diagnosed with an India ink stain of the CSF fluid. It is not as sensitive or specific as serological tests, but serves more as an immediate diagnostic tool that can also reveal a general idea of organism burden. The detection of the cryptococcal polysaccharide antigen in body fluids (latex agglutination test) is another highly sensitive and specific (~95%) method to identify infection and indicate fungal burden. A high antigen titer level (≥1:1024) translates into a higher burden of organisms. Generally, the CSF is tested to rule out meningoencephalitis and the serum is tested for disseminated disease using the antigen test. Other body fluids can be tested, such as urine or pulmonary fluids. In geographic areas with a high burden of HIV, the serum cryptococcal antigen is now being evaluated as a screening tool for patients at-risk for cryptococcosis. Culturing *Cryptococcus neoformans* is another diagnostic tool, but takes longer than the other two tests, and CSF cultures for viable organisms may be difficult if there is a case of chronic cryptococcosis (low fungal burden in the CSF) and after treatment has started (India ink is positive but yeast is not viable).

**Treatment**

Before 1950 cryptococcosis was uniformly fatal, however its treatment has improved dramatically in the last 20 years [1,3]. There was a dramatic rise in cryptococcal infections in parallel with the AIDS epidemic in the early 1980's. The widespread use of fluconazole for antifungal prophylaxis is thought to be a contributing factor of the decline in incidence beginning in the early-mid 1990s [47]. Currently the most common antifungal therapies used to treat cryptococcal disease are: amphotericin B (a polyene antimycotic) and flucytosine, fluconazole, and lipid formulations of amphotericin B. These drugs are used alone or in combination therapy dependent on the underlying disease state [1]. Success of treatment varies due to a lack of randomized clinical trials; selection of a treatment regimen is largely arbitrarily decided by the prescribing physician [1].

Treatments have been more widely studied among HIV-related cryptococcal disease, as many of these patients must receive lifetime antifungal therapy. Side effects of combination therapy are rather common in this immunocompromised group and despite advances, mortality associated with cryptococcal meningitis can be up to 25% among persons with AIDS [15]. For HIV-positive patients with a cryptococcal infection that has no CNS involvement, the goal of treatment is preventing dissemination through drug regimens that vary based on symptom presentation and culture results [15]. Therapy for cryptococcal meningitis aims to lower elevated intracranial pressure and eradicate the infection (though eradication is uncommon in those with HIV) [15]. More often, the goal is long-term suppression of infection and resolution of clinical signs and symptoms of disease [15]. Flucytosine is commonly used in combination with fluconazole or amphotericin B to treat

cryptococcal meningitis in patients with AIDS, despite the substantial danger of drug-related toxicities and fluconazole resistance [15].

*Amphotericin B*

Amphotericin B (AmpB) began as cryptococcal therapy in 1956 [71] and to date, is the most potent and effective drug for treating cryptococcal meningitis with success rates ranging between 60-70% [3]. This drug is limited by its poor penetration of the subarachnoid space accompanied risk of nephrotoxicity; therefore it is commonly administered in combination with flucytosine or fluconazole [3,72]. Amphotericin B is also available in more expensive lipid formulations (LFAmpB) that reduce the risk of renal toxicity and thus allow higher doses to be administered (3-6mg/kg/day for liposomal AmpB [L-AmpB] or amphotericin lipid complex [ABLC] 5mg/kg/day). Lipid formulations are also beneficial in patients with baseline renal concerns [1,3]. Optimal dosing of LFAmpB and effectiveness in combination therapy are still unclear. There is some evidence that LFAmpB at 4mg/kg/day is equal or more fungicidal than AmpBd [1,73,74]. Human studies are still undersized, making clear recommendations for treatment difficult.

*Flucytosine (5FC)*

5-Fluorocytosine (flucytosine) was initially used to treat cryptococcal meningitis as a single drug therapy, but resistance and consequent patient relapse were quickly identified [75,76,77]. Combined with amphotericin B, it has synergistic or additive effects [13,78]. This has the benefit of lowering the dose of AmpBd to address toxicity issues [18,22]. In

AIDS patients it has been demonstrated that antifungal regimens containing flucytosine is an independent predictor of treatment success [13,78]. This drug is not without some serious drawbacks that generally arise between days 4-14 of treatment [79]. Side effects include bone marrow suppression, leucopenia and gastrointestinal disturbances [80,81]. Eliminated primarily by the kidneys, changes in renal function should also be checked [3]. Drug levels should be monitored to avoid bone marrow toxicities, with therapeutic ranges between 30-80 µg/ml (and not exceed 100µg/ml) two hours after first recommended dose of 100 mg/kg/day. This dose was found effective by van der Horst et al. (1997) with only a 3% withdrawal rate [13]. It is still uncertain what drug levels correspond with gastrointestinal side effects and pancytopenia.

*Fluconazole*

Fluconazole is the backbone of many cryptococcosis treatment regimens. It is recommended as primary therapy for mild or moderate cryptococcosis and consolidation therapy/maintenance therapy for severe cryptococcosis [1]. Fungistatic (not fungicidal) against *C. neoformans*, it remains an attractive treatment choice because of its availability, affordability and tolerability. Long-term use of fluconazole for prophylaxis or suppression has led to higher minimum inhibitory concentrations (MICs) and increasing resistance in parallel with the AIDS pandemic [82,83,84,85]. Factors also contributing to relapse among patients on fluconazole are non-compliance to therapy as well as severe immunosuppression resulting in uncontrolled fungal burden.

*Treatment Recommendations*

Treatment recommendations based on the most recent 2010 IDSA Guidelines for effective treatment of cryptococcosis according to each category of presentation of cryptococcosis are in Tables 2.1 and 2.2 [1]. Proper induction therapy for cryptococcal meningoencephalitis was defined using the categories in Table 2.1. For pulmonary or other types of cryptococcosis, appropriate therapy was defined by IDSA recommendations in Table 2.2 [1].

Fluconazole, flucytosine and amphotericin (deoxycholate or lipid preparation) are the antifungal medications that comprise the 2010 IDSA Guidelines for effective treatment of cryptococcosis and were also primary treatments recommended for use in the previously published 2000 guidelines [1,15]. These four drugs are sometimes used in combination or alone (except for flucytosine, which is not given alone), and choice of treatment relies on the severity of disease, immune and HIV status, and other possible underlying conditions. They are the same four treatments used and available to patients dating back to the beginning of our cohort study in 1996; part of the reasoning for our study period range along with the availability of HAART among HIV infected patients. Interestingly and considering their long history of use, there have been very few reports of drug-resistant strains (either azole or polyene) of *C. neoformans* [86].

**Clinical management**

Aside from effective antifungal treatment, the prognosis for cryptococcal meningitis relies on the underlying disease, burden of organisms, symptoms at presentation, and host

inflammatory reactions [34]. Management of patients with severe disease that have elevated intracranial pressures (≥ 20 – 25 cm water) is important in preventing poor outcome, but its requirement in conjunction with symptom development (e.g., increasing headache, mental status changes, new neurologic findings) and a precise opening pressure for treatment has not yet been established [34]. One study of HIV patients with cryptococcal meningitis showed elevated pressures two weeks after starting treatment predicted poor clinical response [15], however another study did not find a significant association between opening pressure at day 14 and mortality at 10 weeks [87]. A recent study found increased intracranial pressure was more common among HIV-infected (49%) and non-immunosuppressed (67%) cryptococcosis patients compared to immunosuppressed patients. However, HIV-patients were more likely to receive repeat lumbar punctures that other patient groups [62]. Options for managing acute elevated intracranial pressure include: repeated lumbar punctures, lumbar drain insertion, ventriculostomy, or ventriculoperitoneal shunt. In patients who have IRIS, corticosteroids are needed to control symptoms [34].

Resolution of signs and symptoms of patients who have cryptococcal meningitis should resolve within two weeks after starting antifungal therapy [34]. A study found that patients who do not have negative CSF cultures by day 14 have a five times higher risk for treatment failure at 10 weeks of follow-up than those with negative cultures [88]. The baseline organism load and rate of clearance of organisms in the CSF is an important factor in patient outcome. A recent study demonstrated that CSF cryptococcal colony-forming unit (CFU) counts and CSF cryptococcal antigen titers are highly correlated at baseline [89]. CSF cryptococcal CFU counts decreased readily during the first 2 weeks of treatment, although no correlation occurred between the rate of decline in CSF cryptococcal CFU counts and drop in

CSF cryptococcal antigen titers [89]. Both of these measures can serve as a measure of organism load and can be used in follow-up evaluation after cryptococcal treatment. A larger combined cohort study of 262 HIV-infected cryptococcal meningitis cases found an association between the rate of clearance of infection and survival [90]. The strength of the association in multivariate analysis was stronger with survival at 2 weeks (HR 1.34; 95% CI, 1.06–1.68) than at 10 weeks (HR 1.18; 95% CI, 1.04–1.33) [90].

**Immune reconstitution inflammatory syndrome (IRIS)**

Rapid changes in immunity, such as with the introduction of HAART among HIV-infected patients, can result in a clinical worsening or radiographic manifestations consistent with an inflammatory process but produces negative results for biomarkers or cultures [34]. Referred to as IRIS, this condition has been reported in up to one-third of HIV patients with cryptococcosis upon initiation of HAART [91]. Timing of onset varies, generally 4 – 6 weeks after starting HAART and is associated with increasing CD4 counts and diminishing viral load [91,92]. There is evidence that IRIS is more common in patients with a higher fungal burden and disseminated infection or fungemia [91,93]. The recommendations on when to begin HAART following cryptococcosis infection vary widely between 2 – 10 weeks [1].

Not limited to HIV-infected cryptococcosis patients, IRIS has been observed in 5% of solid organ transplant recipients nearly 6 weeks (range, 4 – 12 weeks) from the start of antifungal therapy [93]. More potent immunosuppressive therapy has been shown to be a risk factor for IRIS in this population [94]. Similar risk factors to HIV patients, such as high

fungal burden, influence the development of IRIS, and graft survival is also reduced in this patient group [95]. Management of IRIS in transplant recipients and other immunosuppressed groups, as well as apparently "normal" hosts can be complex as it is difficult to diagnose and treat as clinicians want to cure fungal disease without a strong host immune response [34].

**Tables**

Table 2.1. Primary therapy recommendations for cryptococcal meningoencephalitis based on the 2010 IDSA Guidelines [1].

| Underlying condition | Primary induction therapy | Maintenance therapy |
|---|---|---|
| HIV-positive | | |
| | AmpBd (0.7 – 1.0 mg/kg/d IV)[a] plus 5FC (100mg/kg/d po) for at least two weeks | Fluconazole 400mg (6mg/kg) po daily for a minimum of 8 weeks, then fluconazole 200mg po daily ≥1yr[b] |
| Organ transplant recipients | | |
| | Liposomal AmpB 3-6mg/kg/d IV or ABLC 5mg/kg/d IV plus 5FC 100mg/kg/d for at least 2 weeks | Fluconazole 400-800mg po daily for 8 weeks; followed by fluconazole 200mg po daily for 6-12 months |
| HIV-uninfected, non-transplant | | |
| | AmpB deoxycholate ≥0.7 – 1.0mg/kg/d + 5FC (if tolerant) 100mg/kg/d for ≥4 weeks, ≥6 weeks if intolerant to flucytosine | Fluconazole 400mg po daily for 8 weeks; followed by fluconazole 200mg po daily for 6-12 months |

[a] Can substitute amphotericin B deoxycholate for lipid formulations AmpB (3-6 mg/kg/d) for at least 2 weeks if patients have or are predisposed to renal dysfunction

[b] Dependent upon the following: successful introduction of HAART, CD4 ≥ 100cells/μl and negative HIV viral load for ≥3 months with minimum of 1 year of antifungal therapy

Table 2.2. Primary therapy recommendations for non-meningeal cryptococcosis (pulmonary or other) adapted from the 2010 IDSA Guidelines [1].

| Underlying condition | Characteristics of disease | Treatment |
|---|---|---|
| Immunosuppressed patients | | |
| | Pneumonia associated with CNS or evidence of dissemination and/or ARDS[a] | Treat like CNS disease (Table 2.1) including maintenance therapy; due to risk of nephrotoxicity, AmpBd is not recommended for first line therapy (LFAmpB should be used)[b] |
| Immunocompetent | | |
| | No evidence of dissemination, mild-to-moderate symptoms, absence of diffuse pulmonary infiltrates, absence of severe immunosuppression | Fluconazole 400mg/d for at least 6-12 months |
| | Severe disease | Treat like CNS disease (Table 2.1) including maintenance treatment |
| | Mild to moderate symptoms | Fluconazole 400mg daily for 6-12 months |

[a] ARDS = Acute Respiratory Distress Syndrome

[b] Recommendation for organ transplant recipients. Immunosuppressive management should include: sequential or step-wise reduction of immunosuppressants – lowering corticosteroids dose first (recommendation based on expert opinion with moderate evidence).

# CHAPTER III. RESEARCH DESIGN AND METHODS

## Study Population

The clinical cohort used for this study originates from the Duke University Medical Center (DUMC) in Durham, North Carolina in the Southeastern United States. During the study period from January 1, 1996 through October 31st, 2009 we retrospectively collected demographic and clinical information on all adult patients discharged from DUMC with ICD-9 diagnosis codes of cryptococcosis, cryptococcal meningitis, pulmonary *Cryptococcus*, and disseminated *Cryptococcus*. Subjects were considered eligible if they had confirmed cryptococcal disease and received treatment for their cryptococcal infection at DUMC with a sufficient electronic medical record or paper chart available for review. The complete medical record for each patient was reviewed and data regarding different interventions and outcomes were extracted. Investigators recorded all data on a standardized abstraction form that was reviewed by an epidemiologist and clinician prior to and during data entry. Abstraction forms were entered in to Microsoft Office Access (2007) and data analysis was performed using SAS v9.2 (SAS Institute, Cary, NC). This study including primary data collection was approved by the Duke University Medical Center IRB. Secondary data analyses required for Chapters Four and Five were approved by the UNC-Chapel Hill IRB and the Duke Medical IRB.

**Definitions and Data Collection**

A cryptococcosis case was confirmed by having at least one of the following: positive cerebral spinal fluid (CSF) antigen or fungal culture, direct histological examination of *Cryptococcus*, positive serum cryptococcal antigen (CRAG) test, or positive culture from blood or pulmonary sites. Positive CSF India ink staining alone was not an acceptable diagnostic tool, but was used for supportive evidence for infection.

Basic demographics, such as age, gender, state of residence, and race/ethnicity were collected. Although race and gender are possibly associated with cryptococcosis disease, HIV status, or organ transplant receipt, there is no strength of evidence that these demographics are associated with which antifungal induction treatment a patient is given, but other factors such as immunosuppression and severity of cryptococcosis, are more important predictors of what antifungal regimen is chosen.

Upon the first admission for cryptococcosis, patient information regarding presenting symptoms, self-reported duration of symptoms and radiological findings (brain CT, brain MRI, chest CT and chest X-ray) were recorded closest to the date of diagnosis. Abnormal radiology was defined as evidence of hydrocephalus, gyral enhancement, and/or multiple nodules that may be enhancing or non-enhancing [96]. Nodules can be either single or multiple [97]. Demographic information included birth date, race/ethnicity, gender, and country of origin if not the United States. Patient weight (kg) closest to first cryptococcosis diagnosis was recorded to assess accurate antifungal dosing.

For HIV-positive patients, last CD4 count prior to cryptococcosis diagnosis was recorded along with all subsequent CD4 counts until "lost to follow-up" or death. Also noted

were: the date of HIV diagnosis, any documented evidence of non-compliance with antiretroviral (ARV) therapy and/or antifungals, whether cryptococcosis was an AIDS-defining illness, and the use of ARV therapy before, during and after cryptococcosis diagnosis until lost to follow-up or death.

Information on solid organ transplant recipients included: date and type of transplant, if they experienced graft-versus-host disease (GVHD) status ≤6 months after transplant, and type and dose of immune suppression medications at the time of cryptococcosis diagnosis (corticosteroid, calcineurin inhibitor, glucocorticoid, monoclonal antibodies, methotrexate, sirolimus, mycophenolate mofetil, azathioprine, or other). It was also noted if these drugs were changed or stopped due to cryptococcosis disease. There was one patient with a stem cell transplant (not solid organ) that was included in this immunosuppressed transplant group.

Regardless of whether patients had HIV or received and organ donation, other possible causes of immune suppression (hematological or other malignancy, rheumatologic disorder, chronic organ failure, or steroid therapy) were noted. If there was no apparent immunosuppression, patients were classified as "immunocompetent."

Clinical data in existence as of October 31$^{st}$, 2009 was collected. The study period spanned the time patient was followed up at DUMC following the diagnosis of cryptococcal infection. Starting at the first admission for cryptococcosis and continuing through all subsequent available follow-up time, investigators recorded lumbar puncture data (opening pressure, RBC, number of nucleated cells, glucose, CSF antigen titer, CSF culture positivity, India ink stain, and antifungal MIC), serum cryptococcal antigen titers, blood and pulmonary fungal cultures, and other sites positive for *Cryptococcus*. For every LP performed, values

for collection tube #4 were recorded, and if not available, the highest tube number was used for LP data. Co-morbid infections, positive cultures for other organisms and new cancer diagnoses were noted during the time of hospitalization or while on treatment for cryptococcosis. Creatinine levels and positive culture results were recorded at each admission, after induction therapy, and at 2 weeks, 10 weeks, 1 year and >1 years of follow-up. Peak creatinine was recorded while the patient was on induction therapy. All available flucytosine serum drug levels were obtained. Hematologic parameters (hemoglobin, hematocrit, WBC count and platelets) at the start date of flucytosine treatment and at the nearest available date with valid measurements 14 days after starting flucytosine were collected. Treatment information (type, dose and date of start and date of stopping) was abstracted from the first admission for cryptococcosis until lost to follow-up, death or until the end of the study period.

Organ failure before, during and after antifungal therapy was an important variable collected in this study, particularly transplanted organ failure during or after antifungal therapy among transplant recipients. Poor clinical response either from treatment failure (persistence of clinical findings or positive microbiology) or treatment toxicity (notably renal failure) was noted along with modifications made to treatment due to these adverse events. Date of death and death due to cryptococcosis were also recorded.

In a paper by Seagal et al (2008), authors proposed guidelines for defining treatment responses and study outcomes to invasive fungal diseases in clinical trials (Table 3.1) [98]. These recommendations informed our study definitions used for persistence of cryptococcosis. Although the guidelines only address treatment response in cryptococcal meningitis cases (CNS disease), we have extrapolated these definitions for this study to

include success and failure in non-CNS cases. "Relapse" was defined as clinical, mycological or radiographic evidence of recrudescence after stopping antifungal therapy, if the patient had initially experienced "success" [21].

Figure 3.1 is a conceptual diagram of cohort patient flow from first diagnosis (1) and admission for cryptococcosis through death or lost to follow-up. After diagnosis, patients are typically started on treatment immediately—sometimes even prior to positive culture results if clinical signs indicate cryptococcosis disease (2). Treatment exposure depends on multiple factors and recommendations for treatment are listed in Tables 2.1 and 2.2. Two major considerations for treatment shown in these tables are HIV status or alternate possible immunosuppression and CNS involvement. Patients will then either: die while on treatment (2a), fail therapy due to toxicity or persistence of disease (2b), or experience mycological cure or suppression (3). Patients cured or suppressed on maintenance therapy (3) are followed until they die or are lost to follow-up. Some patients will experience relapse (3a) and have to restart antifungal maintenance therapy or have the dose increased. In some cases, patients will undergo re-induction therapy. Patients experiencing immune reconstitution inflammatory syndrome (IRIS) can also have antifungal or HIV medications changed or stopped. Sometimes IRIS is treated with corticosteroids. Cases of relapse can be due to factors such as antifungal drug resistance, declining immune status that results in increasing fungal burden, or inconsistent use of antifungal therapy or HIV HAART therapy. Patients who fail initial therapy indicated by persistence of disease or drug-related toxicity (2b) will likely undergo a change in antifungal treatment; either in dose, the drug itself, or with regard to other contraindicated drugs such as corticosteroids. The patients are at risk for death at any point during follow-up (Figure 3.1).

## Data Analysis

**Specific Aim 1.** How do the following three groups with cryptococcosis differ with respect to clinical presentation and management: HIV-positive, transplant recipients, and HIV-negative/non-transplant patients?

Exposure definitions

Clinical presentation variables used for comparison included demographics, symptoms and duration of symptoms of cryptococcosis, diagnostic tools used for diagnosis, and underlying conditions and possible risk factors of disease (Table 3.2).

- *Demographics* – Gender, race/ethnicity, and age at time of first diagnosis of cryptococcosis (at DUMC) were collected. As this is the first time this study cohort will be presented in the literature, demographics showing patient composition are valuable to readers.
- *Immune status* – HIV, transplant recipients, and HIV-negative/non-transplant patients (3 general categories); we know from prior research that baseline immune status is associated with treatment exposure, treatment failure, and mortality.
- *Disease severity and disease-related morbidity* – Cryptococcosis can exist in mild-to-moderate or severe forms. For this study, severe and non-severe categories were created to simplify baseline cryptococcosis severity. Based on the IDSA Guidelines [1], all patients that would receive induction therapy with amphotericin B (CNS disease, or treat as CNS disease) were considered severe cases; non-severe cases would be non-CNS cryptococcosis where fluconazole therapy for primary treatment

is recommended as sufficient. Clinical diagnostics, such as serum and CSF antigen, CSF culture, and radiographic tests will likely contribute to what treatment patients will receive. These clinical and microbiological results can also be indicators of future treatment failure or mortality. Because they are associated with treatment exposure and patient treatment success and mortality, the following are possible confounders and may need to be adjusted for in subsequent analyses.

Outcome definitions

Clinical management and patient outcomes during follow-up were abstracted from patient charts. HAART during and after treatment for cryptococcosis, including regimen and start and stop dates were recorded when available. It was noted whether HAART was stopped during antifungal induction treatment and if so, when it was re-started (if at all). Similarly, trends of immunosuppressive medications for HIV-negative patients were examined, including dose at the time of cryptococcosis diagnosis and any changes to dose during antifungal treatment.

Management of elevated intracranial pressures using serial LPs, ventricular shunting, lumbar drain, and/or pharmacological therapy was recorded.

The diagnosis of immune reconstitution syndrome (IRIS) can be complex. We defined it as new or worsening clinical or radiographic manifestations consistent with an inflammatory process such as contrast enhancing lesions on imaging studies (CT/MRI), CSF pleocytosis >5WBC, increased ICP, histopathology showing granulomatous lesions, and/or unexplained hypocalcaemia. These symptoms must have occurred during the receipt of appropriate therapy and could not be explained by newly acquired infection. Also, there had

to be negative results for cultures or stable/reduced biomarkers for the initial fungal pathogen during diagnostic work-up for the inflammatory process.

Analysis Plan

**Objective 1:** Examine the changing populations for acquiring Cryptococcus (HIV positive, transplant recipients, HIV-negative/non-transplant)

Variables were examined using descriptive statistics and stratified based on the three groups and/or severity of cryptococcosis as needed. Where appropriate, the Student's t-test was used to test the difference of two means and the Kruskal-Wallis test was used for the difference between medians for non-parametric continuous data. Chi-square ($X^2$) tests were used to examine differences between categorical frequency distributions. The statistical significance level of alpha ($\alpha$) equal to 0.05 was used for each two-tailed test performed, thus a "significant" result refers to a p-value $<0.05$.

**Objective 2:** Summarize temporal trends in diagnosis and amphotericin B formulations as initial therapy

Graph the percentage of patients who received amphotericin B deoxycholate and lipid formulations for initial therapy each year. Examine any notable trends in use between these two regimens over time.

**Objective 3:** Describe and assess differences and similarities of underlying three groups with respect to initial presentation, diagnostics and approaches to clinical management

We will report prevalence and type of immunosuppression in the three groups—HAART exposure in HIV-infected patients, corticosteroid use and immunosuppressive

agents among transplant and HIV-negative/non-transplant, as well as prevalence of patients appearing "immunocompetent."

**Specific Aim 2.** How does initial antifungal therapy for cryptococcosis influence patient outcomes of persistence of infection, attributable mortality and overall mortality?

Exposure definitions

***Initial therapy*** - antifungal therapy regimen based on criteria provided in Tables 2.1 and 2.2.

Each patient was checked to see if they follow the 2010 IDSA guidelines for the treatment of cryptococcosis. Appropriate initial treatment would be amphotericin B for severe disease and fluconazole for non-severe disease. Induction therapy refers to the entire period of initial therapy, not the initial antifungal drug that was used.

***Treatment dose*** – the patient was given the appropriate dose of initial antifungal therapy.

This study exposure is based on mg/kg units calculated using the daily dose of antifungal therapy given to the patient divided by the patient's measured weight in kg at the closest date to cryptococcosis diagnosis. For example, if 0.7-1.0 mg/kg/day is the recommended dose of amphotericin B for a patient and the patient weighs 50kg, then 35 - 50mg, is an acceptable range of antifungal doses. To account for initial dosing adjustments that can occur in the first few days of induction, the averaged dose of continuous antifungal therapy (no change of drug) was used to define acceptable dosing. Acceptable dosing was defined as follows: 0.7-1.0mg/kg/day amphotericin B deoxycholate (AmpBd), 3-6mg/kg/day liposomal AmpB (L-AmpB), 4-6mg/kg/day AmpB lipid complex (ABLC), and ≥400mg/day

fluconazole. Rounding to the nearest tenth for AmpBd and the nearest integer for AmpB lipid products were used to categorize appropriate dosing.

***Flucytosine-containing regimens*** – flucytosine was given as a combination regimen with primary initial amphotericin B therapy among patients with severe disease (1=yes, 0=no). Secondary data analysis will examine relative differences between 0 – 7 days and >7 days of flucytosine in combination with initial therapy. Unique to this exposure variable, we hypothesized that flucytosine is more frequently paired with amphotericin B deoxycholate than other polyene formulations, so it was listed as a possible confounding factor (Figure 3.3).

Outcome Definitions:

***Persistent cryptococcosis*, two weeks** (severe disease only): Positive cultures (CSF, blood, pulmonary sites, other) two weeks (14 days) after starting therapy. The patient had to have survived two weeks to be eligible for this outcome because at least two weeks of induction therapy is recommended in this group. We acknowledge that the IDSA Guidelines recommend that HIV-negative, non-transplant patients receive a minimum of four weeks of treatment (Table 2.2), but due to limited sample size and interest in whether or not the presence of disease is evident after two weeks of antifungal therapy in this group, we included this subgroup in this outcome. Deaths among this patient group within two weeks were considered persistent infection as a sensitivity analysis.

***Persistent cryptococcosis*, four weeks** (severe and non-severe disease)**:** Positive cultures (CSF, blood, pulmonary sites, other) four weeks after starting therapy and/or the positive indication of the presence of symptoms (headache, photophobia, fever, etc.) four weeks (30 days) since starting primary therapy. The patient had to have survived four weeks to be eligible for this outcome. Deaths among this patient group in this time period were considered persistent infection as a sensitivity analysis.

Because data were observational, measures for indicating persistent infection were not taken at exactly two weeks and four weeks to test for positive culture, antigen testing, and/or infection-related symptoms. Acceptable values were used if they did not overlap with the preceding measurement (e.g. a baseline culture could not be used for a two week test result), and did not extend beyond the designated time point (e.g. a measure at 3 weeks would not be counted as a week 2 measure, but instead a 4 week measure if there was not an observation at 4 weeks). Persistence measures for two weeks had to have occurred ≥1 week of therapy. Measures beyond the final time point (4 weeks, 30 days) were accepted for that final time point if within 90 days since starting therapy.

***Cryptococcal-attributable mortality:*** A determination made by a panel of experts at DUMC. Death was attributable to disease if at the time of expiration, patients experienced conditions directly related to cryptococcal disease, such as: increased central nervous system (CNS) pressure, persistence or relapse of infection, while receiving initial induction treatment or due to organ failure during treatment for cryptococcosis.

***All-cause mortality through one year*:** In order to assess one-year mortality risk for all patients, we obtained data on survival and mortality up to one year after their date of cryptococcosis diagnosis from the Duke Data Support Repository (DSR), which uses the Social Security Administration death index, the Tumor Registry and The Duke Information System for Cardiovascular Care death data to report mortality status.

***Secondary outcomes*:** frequency of re-induction(s) with amphotericin B, IRIS and renal toxicity during initial therapy, receipt of ≥7 days of flucytosine compared to receipt of 0 – 7 days of flucytosine (severe disease), and the changing initial therapy (interrupted therapy).

*Re-induction*: Patient had to have finished initial induction therapy for at least three days or have been placed on consolidation or maintenance therapy, then placed back on amphotericin B as part of re-induction status.

*IRIS*: The definition for IRIS for this study, adapted from Singh and Perfect [94] was the following: A clinical or radiographic manifestations consistent with an inflammatory process such as contrast enhancing lesions on imaging studies (CT/MRI) along with (a) and (b) and at least one of (c) through (f):

a) Symptoms occurred during receipt of appropriate therapy and could not be explained by newly acquired infection
b) Negative results for cultures or stable/reduced biomarkers for the initial fungal pathogen during diagnostic work-up for the inflammatory process
c) CSF pleocytosis >5 WBC/mm3
d) Increased intracranial pressure (ICP)

e) Histopathology showing granulomatous lesions

f) Unexplained hypercalcemia

*Renal toxicity:* Creatinine values measured closest to day 0 and day 14 of treatment for patients with severe disease who received induction therapy with amphotericin B were used to determine renal toxicity. Defined as a >50% decrease in Glomeruler Filtration Rate (GFR), also known as estimated creatinine clearance, during initial induction treatment. GFR was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula [99].

Analysis Plan

**Objective 1:** Determine the association of aspects of initial treatment on poor patient outcomes that include the risk of persistent infection and rates mortality due to cryptococcosis and overall 1-year mortality.

1. Estimate the effect of *initial antifungal treatment type* on persistence and mortality
2. Estimate the effect of *initial treatment dosing* on persistence and mortality
3. Estimate the effect of *flucytosine exposure* among patients with severe cryptococcosis disease on persistence and mortality
    a. Estimate the effect of ≤ 7 days or >7 days of flucytosine on the rate patient mortality and the risk of persistence among patients surviving at least 14 days from the time starting antifungal therapy
4. Assess other strong predictors for the above outcomes in (1) (Table 3.2, Figures3. 2 and 3.3)

Follow-up begins on the first day of starting treatment. Because the follow-up periods were short (two weeks and four weeks) for persistence outcomes, thus minimizing competing risks, binomial regression was used to estimate the risk ratios (relative risk [RR]) for the separate (main) effects of receipt of recommended initial treatment type, recommended initial treatment dose and flucytosine combination treatment exposure (severe disease only) on the dichotomized outcomes of persistence at two weeks (severe disease only) and persistence at four weeks, adjusting for important covariates (Figures 3.2 and 3.3). Patients who were untreated with anti-cryptococcal therapy (n=3) were excluded from this analysis.

To evaluate confounding we will assess the bivariate associations between all covariates and main exposures and outcomes. Minimum adjustment sets will be determined using the Directed Acyclic Graph (DAG) program (v0.21) [100]. Should minimum adjustment sets exceed what our limited study size could operably model, the minimum adjustment sets for each of our three chosen exposures (with slight variation), based on previous studies that predicted poor outcomes [13,15,16,17,18,19,20,21], variables associated with severe underlying condition and high fungal burden from our minimal adjustment sets will be prioritized and we will proceed with multivariate adjustment using a change-in-estimate approach with a 10% cut-off criterion [101]; eliminating variables chosen by the DAG program that did not confound the association of effect estimates between the main exposures and outcomes. Effect measure modification by confounding variables will be examined through the inclusion of interaction terms in the models and using the spreadsheet by Andersson et al. (2005) to determine the relative excess risk due to interaction (RERI)[102]. Corresponding 95% CIs will be estimated to measure the precision for each

estimate of exposure and outcome association. Changes in the precision of estimates will be examined with the confidence limit ratio (CLR).

Binomial regression will be used to estimate the risk ratio (RR) of the association between treatment exposures and these outcomes. Cox proportional hazards models will be used to estimate hazard ratios (HR) for the association between treatment exposures and mortality outcomes. Assessment of the proportional hazards assumption (PHA) will be performed using graphical methods (ln – ln survival plot) and by adding an interaction between exposure and (log) time.

Abstraction forms were entered into Microsoft Office Access (2007) and data analyses were performed using SAS v9.2 (SAS Institute, Cary, North Carolina). Investigators recorded all information on a standardized abstraction form developed in collaboration with epidemiologists and clinicians.

**Objective 2**. Report the frequency of secondary outcomes of Immune Reconstitution Inflammatory Syndrome (IRIS), renal toxicity measured by at least a 50% decline in Glomerular Filtration Rate (GFR), re-induction of antifungal therapy, and changing antifungal therapy during initial induction.

Understanding the distribution frequencies of these secondary outcomes is important in understanding clinical management strategies and making future recommendations. Prevalence of these events will be assessed and patterns of changing therapy described in the context of the three groups.

**Strengths and limitations of methods**

Despite its limitations, this study represents an important insight into how the cryptococcosis patient is being managed and what the outcomes have been. To our knowledge it is the largest single-center cryptococcosis cohort and provides in-depth information on a heterogeneous group of patients experiencing disease (HIV-infected, transplant recipients and other HIV-negative patients). The importance of trends within and between these three groups will help to inform clinicians regarding at-risk populations, and how these groups may be shifting as HAART among HIV-infected patients expands and immunosuppression places other groups at a higher potential risk for cryptococcosis. With careful retrospective chart review we were able to capture the intricacies of patient treatment, which included: halting corticosteroid use, dose changes, switching of initial antifungal therapy, and duration of therapy. Future studies combining our cohort with additional patient groups from other institutions would provide beneficial robustness for treatment effectiveness analyses and encourage this group cooperation.

This review was limited to a single tertiary care center and teaching hospital. Our medical center averaged nearly 15 cases of cryptococcosis per year and this likely reflects both an endemic exposure to this yeast in the environment within the Southeastern USA and an enriched population of immunosuppressed individuals due to our hospital's care patterns. The actual number of cases seen in a particular medical center certainly varies within the U.S. Furthermore, using hospital records favors cases with severe disease and could result in selection bias against asymptomatic disease. Determining the total population-at-risk was

not estimable in this study and the underlying source population and referral patterns could shift over time.

Retrospective chart review has the potential for incomplete or incorrect information capture due to loss of paper documentation or lack of entry in electronic medical record. We used both sources to ensure data was as complete as possible and discrepancies were minimized. Erroneous self-report of symptoms or symptom duration was a possibility, but this is a limitation of many observational clinical studies. Despite our careful abstraction process, missing or incomplete data could lead to bias in categorization of symptoms or derived outcome definitions, such as IRIS. Lumbar puncture opening pressure data was inconsistent and missing in about 40% of initial procedures. However, knowing how infrequent lumbar punctures are performed is an informative fact of real-world clinical practice. Other measures may be needed to identify elevated intracranial pressures and clinically manage patients with severe cryptococcosis.

Being a rare disease, limited numbers of cases prevented robust statistical analyses. Importantly, much of the clinical attention over the last two decades has centered around two groups (HIV-infected and transplant recipients). There has been less focus on treatment of HIV-negative/non-transplant patients and yet this group accounts for over one-third of the total cases.

In order to obtain a reportable picture of various outcomes, we created definitions of severe versus non-severe, persistence of infection (two and four weeks), attributable mortality, and we used the IDSA guidelines to define "appropriate" therapy. Others could definitely choose these definitions, but one fact clearly remains—the overall mortality rate in

severe cryptococcosis is almost 25% within one year and in our opinion that rate remains too high.

## Tables

Table 3.1. Antifungal treatment response definitions in patients with cryptococcal disease. Adapted from Seagal et al, 2008 [98].

| | |
|---|---|
| **Success** | |
| Complete (or partial) | Survival and resolution (or improvement) of attributable signs and symptoms of disease;<br>AND documented clearance of pathogen from CSF, blood, other sites;<br>AND improvement or stabilization of positive radiologic findings |
| **Failure** | |
| Stable | Survival and minor or no improvement in attributable signs and symptoms of disease;<br>AND persistently positive CSF or other cultured specimens |
| Progression | Worsening of clinical signs or symptoms;<br>AND persistently positive CSF or other cultured specimens;<br>OR new sites of disease or worsening of preexisting radiologic lesions; |
| Death | Death during the period of evaluation[a] |

[a] Period of evaluation subjective to the research study and will be death ≤1 year from diagnosis for our investigation.

Table 3.2. Binary variable specification used for cohort description and analysis. Unless specified, Yes = 1 and No = 0. Table is continued on following two pages.

| Category | Variable list[a] | Brief Description |
|---|---|---|
| Exposures | | |
| | Initial therapy | Patient received appropriate initial therapy disease given their diagnosis of severe or non-severe cryptococcosis (1=No, 0=Yes) |
| | Dose of initial therapy | Patient received a recommended dose of initial therapy (1=No, 0=Yes) |
| | Flucytosine | Patient received flucytosine in combination with amphotericin B for their initial treatment regimen (severe disease only). Categories used for secondary analysis were: 0 – 7 and >7 days of flucytosine among those who survived ≥14 days from the start of initial therapy. |
| Outcomes | | |
| | Persistent cryptococcosis | (1) Positive cultures 2 weeks after starting initial therapy (severe disease only); (2) Positive cultures 4 weeks after starting initial therapy, *OR* positive indication of the presence of crypto-related symptoms 4 weeks since starting primary therapy. Both outcomes were conditional on survival ≥14 days from the start of initial therapy. |
| | Attributable mortality | Determined by a panel of experts that death was attributable to conditions that were directly related to cryptococcal disease |
| | Overall mortality | Timing of death within one year of follow-up with patients alive or lost to follow-up by the end of one year as censored subjects |
| Demographics | | |
| | Male gender | Patient was male |
| | Black Race | Patient was African American |
| | Age | <44 years (0) or ≥44 years (1); median age as a cut point |
| Underlying Condition[b] | | |
| | HIV positive | Patient had HIV at the time of cryptococcosis diagnosis |
| | Organ transplant recipient | Patient received organ transplant before cryptococcosis diagnosis |
| | HIV-negative, non-transplant | Patient was negative for HIV and had not received a solid organ transplant |
| | Renal insufficiency | Evidence of renal failure on admission |
| | Liver insufficiency | Evidence of liver failure on admission |
| | Hematologic malignancy | Underlying hematologic malignancy at the time of cryptococcosis diagnosis |

| Category | Variable list[a] | Brief Description |
|---|---|---|
| Baseline Disease | | |
| | Severe disease | Patients with disease where induction therapy with amphotericin B is recommended by IDSA Guidelines (CNS disease, or treat as CNS disease) [1]; non-severe cases were non-CNS disease where fluconazole therapy for primary treatment is recommended |
| | Positive CNS culture | Patient had positive fungal culture from CSF |
| | Positive blood culture | Patient had positive fungal culture from blood |
| | Positive pulmonary culture | Patient had positive fungal culture from lung biopsy or bronchoalveolar lavage |
| | Other histological evidence | Other histological test positive for *Cryptococcus* |
| Symptoms | | |
| | No symptoms | Patient reported no disease-attributable symptoms |
| | Altered mental status | Yes/No |
| | Headache | Yes/No |
| | Cough | Yes/No |
| | Shortness of breath | Yes/No |
| | Night sweats | Yes/No |
| | Fever | Yes/No |
| | Nausea | Yes/No |
| | Vomiting | Yes/No |
| | Seizures | Yes/No |
| Initial LP[c] | | |
| | CSF CRAG[d] titer ≥1:1024 | High was defined as ≥1:1024 |
| | Low CSF:serum glucose ratio | CSF:serum glucose ratio was <0.6 |
| | Low CSF glucose | CSF glucose ≤40mg/dL |
| | High CSF protein | CSF protein ≥45mg/dL |
| | High nucleated cells | Nucleated cells >20cells/$mm^3$ |
| | High LP OP[e] | OP ≥20cm $H_2O$ |
| | Positive India Ink | Patient had a positive India ink stain |

| Category | Variable list[a] | Brief Description |
|---|---|---|
| Other | | |
| | High serum CRAG titer | Serum CRAG titer ≥1:1024 |
| | Corticosteroid exposure | Patient on corticosteroid therapy at time of cryptococcosis diagnosis |
| | Calcineurin inhibitor | Patient taking calcineurin inhibitor |
| | Mycophenolate mofetil | Patient taking mycophenolate mofetil |
| | Azathioprine | Patient taking azathioprine |
| | Methotrexate | Patient taking methotrexate |
| | Monoclonal antibodies | Patient taking monoclonal antibodies |
| | Sirolimus | Patient taking sirolimus |
| | HAART | HIV-infected patient reported prior or current exposure to HAART |

[a] Unless unavailable and where applicable, all information was gathered from the procedure or chart entry closest to cryptococcosis diagnosis date.
[b] Since HIV positive, transplant recipients and HIV-negative/non-transplant groups were exclusive categories, dummy variables indicating the three groups were used for multivariate models.
[c] LP = Lumbar puncture; first measured value for each patient
[d] CRAG = Cryptococcal antigen
[e] OP = Opening pressure

**Figures**

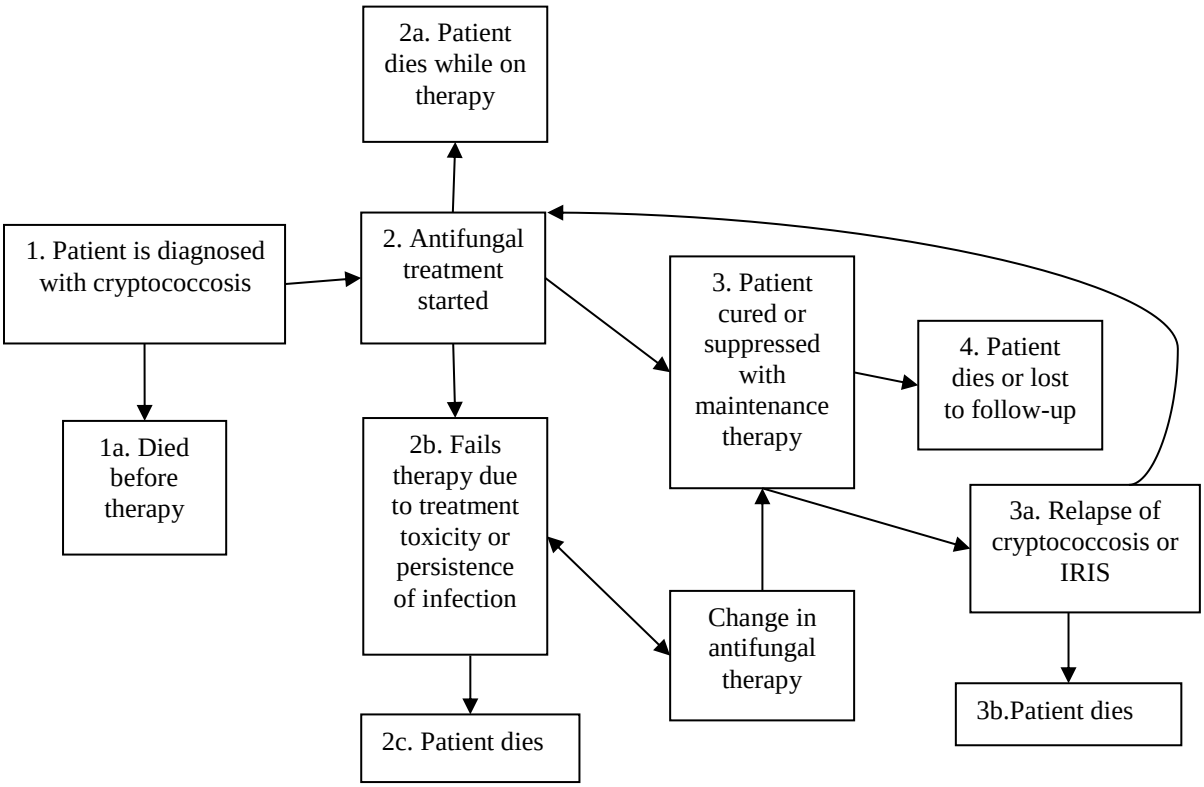


Figure 3.1. Conceptual model showing timing of patient cohort conditions and events. All patients enter the study at the time of diagnosis and are followed until death, lost to follow-up or until the end of the study period (October 31st, 2009).

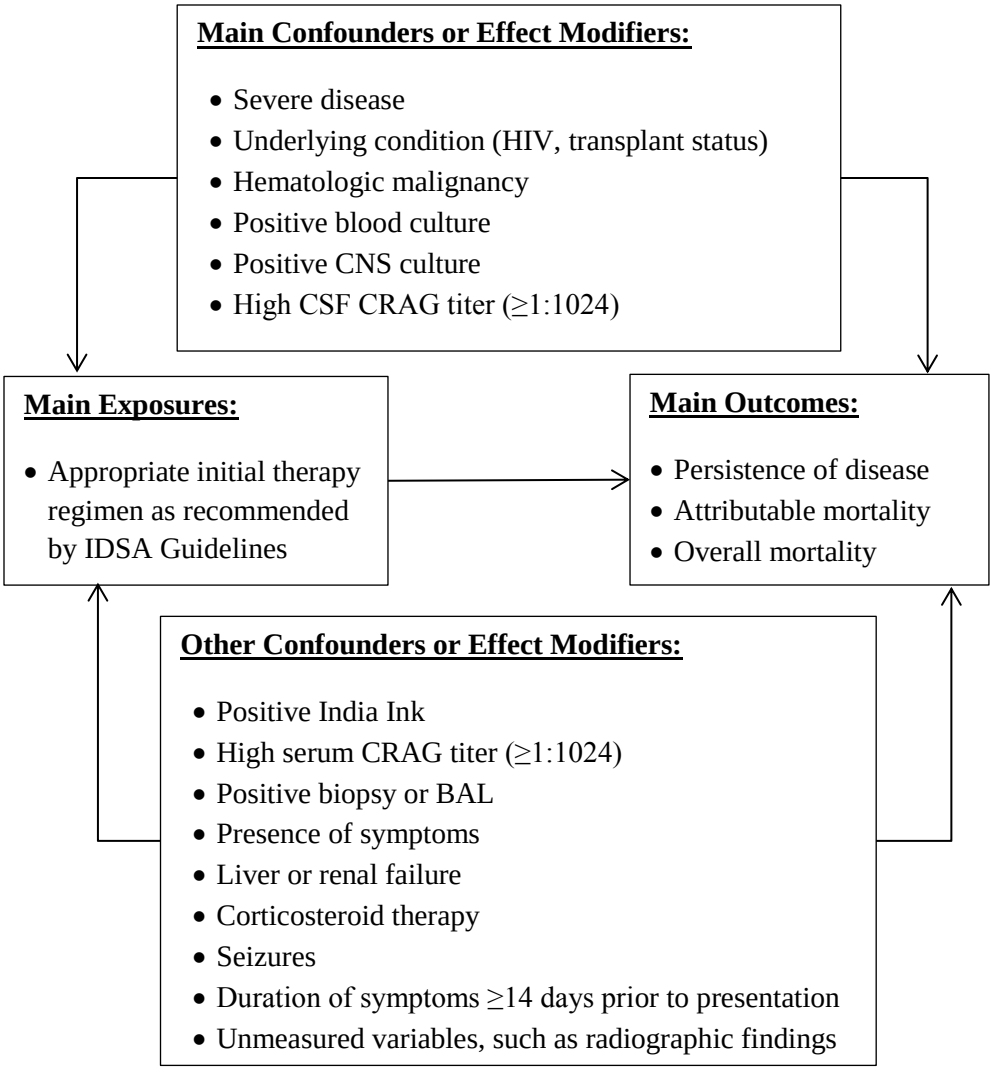


Figure 3.2. Simplified causal diagram for specific aim 2: initial treatment and dose of initial treatment. Covariates listed were determined as the minimum adjustment set for models by the DAG program by Knüppel [100].

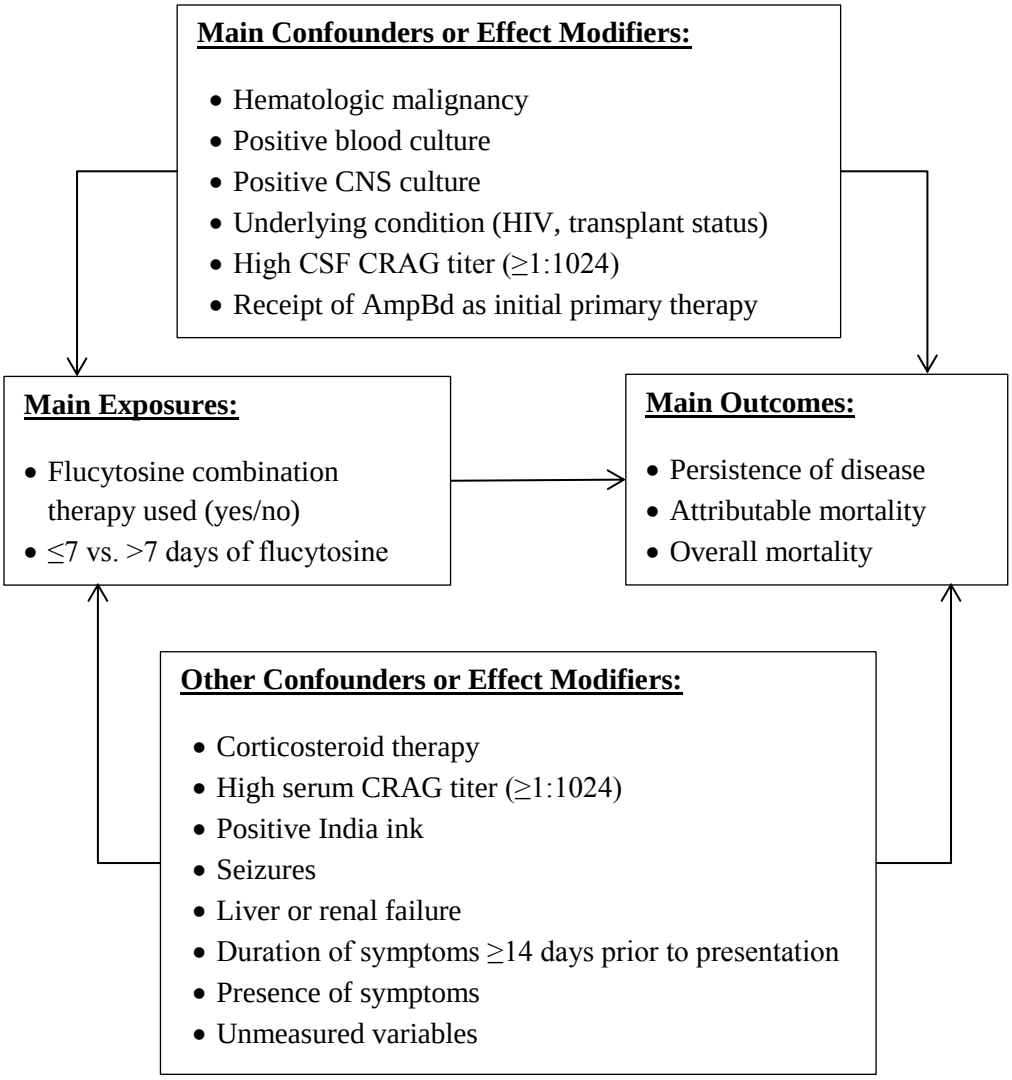


Figure 3.3. Simplified causal diagram for specific aim 2: flucytosine exposure (severe disease only). Covariates listed were determined as the minimum adjustment set for models by the DAG program by Knüppel [100].

# CHAPTER IV. COMPARISON AND TEMPORAL TRENDS OF THREE GROUPS WITH CRYPTOCOCCOSIS: HIV-INFECTED, SOLID ORGAN TRANSPLANT AND HIV-NEGATIVE/NON-TRANSPLANT

## Overview

The Infectious Disease Society of America (IDSA) 2010 Clinical Practice Guidelines for the management of cryptococcosis outlined three key populations at risk of disease: (1) HIV-infected, (2) transplant recipient, and (3) HIV-negative/non-transplant. However, direct comparisons of management, severity and outcomes of these groups have not been conducted. Annual changes in frequency of cryptococcosis diagnoses, cryptococcosis-attributable mortality and mortality were captured. Differences examined between severe and non-severe disease within the context of the three groups included: demographics, symptoms, microbiology, clinical management and treatment. An average of nearly 15 patients per year presented at Duke University Medical Center (DUMC) with cryptococcosis. Out of 207 study patients, 86 (42%) were HIV-positive, 42 (20%) were transplant recipients, and 79 (38%) were HIV-negative/non-transplant. HIV-infected individuals had profound CD4 lymphocytopenia and a majority had elevated intracranial pressure. Transplant recipients commonly (38%) had renal dysfunction. Nearly one-quarter (24%) had their immunosuppressive regimens stopped or changed. The HIV-negative/non-transplant population reported longer duration of symptoms than HIV-positive or transplant recipients and 28% (22/79) had liver insufficiency or underlying hematological malignancies. HIV-

positive and HIV-negative/non-transplant patients accounted for 89% of severe disease cryptococcosis-attributable deaths and 86% of all-cause mortality. In this single-center study, the frequency of cryptococcosis did not change in the last two decades, although the underlying case mix shifted (fewer HIV-positive cases, stable transplant cases, more cases with neither). Cryptococcosis had a relatively uniform and informed treatment strategy, but disease-attributable mortality was still common.

## Introduction

*Cryptococcus neoformans* is an invasive mycoses that can cause meningoencephalitis, particularly among those who are immunocompromised, but in some cases immunocompetent individuals [3]. The 2010 IDSA Cryptococcal Guidelines defined three distinct risk groups for induction treatment of cryptococcosis [1,3]: (1) HIV-positive; (2) transplant recipients; and (3) a heterogeneous group with neither of these conditions (i.e., HIV-negative/non-transplant). A major component of this review was to describe outcomes of recent management of these three groups. During this study, four important factors were in play that justified our decision to use the broad 14-year study period in order to maximize cohort size. First, HAART became readily prescribed in 1996 with supportive evidence of the superiority of combination antiretroviral therapy over monotherapy in reducing AIDS morbidity and mortality [103] . Second, lipid products of amphotericin B, for patients with renal impairment or unacceptable toxicity that prevents the use of conventional amphotericin B, were in use since their initial FDA approval in November, 1995 [104]. Third, in 2000 the original IDSA Guidelines were published as a standard of treatment [15]. Fourth, there was

an active Infectious Disease group at our institution with a particular interest in the pathogenesis and treatment of cryptococcosis.

HIV-positive populations with cryptococcosis have been the most widely studied group over the last two decades [5,13,41,42,47,78,105,106,107,108,109,110,111] and have received greater attention recently due to the recognition that cryptococcosis incidence in this group remains high and paralleled with the AIDS epidemic in sub-Saharan Africa [23]. Starting in the 1960 – 1980's, use of immunosuppressive medications to treat severe diseases or for solid organ transplantation has increased the pool of patients susceptible to *Cryptococcus* and, in the late 1990's *Cryptococcus gattii* emerged in Vancouver Island, British Columbia, Canada, resulting in an outbreak of infections in both immunosuppressed and immunocompetent hosts [38,39]. Though clinical isolates were not typed in this study, serotype A (*C.neoformans* var. *grubii*) predominates this region [112]; *C.gattii* is rare in the Southeastern U.S. with only one identified clinical case in an immunocompromised adult [40]. The HIV-negative cryptococcosis patient group had been excluded from clinical review for several decades but has gained more attention recently [21,45,62,68,69,70]. Cryptococcal patients who are HIV-negative, particularly those who have few or no underlying risk factors (i.e., "apparently immunocompetent"), may experience more of a delay in time to presentation and diagnosis than HIV-positive or transplant recipient patients [21]. In particular, recent evidence from a study showed that HIV-negative, non-immunosuppressed cryptococcal meningitis patients suffered higher mortality rates than HIV-positive patients [62].

Due to its rare occurrence, prospective observational studies of this disease are logistically problematic. Interventional approaches have historically been based on expert

opinion and outdated and retrospective cohort studies, with few representing HIV-negative populations and comparatively developed countries [19,21,22,48]. In this relatively large, retrospective single-center study, our goal was to provide an in-depth look at how cryptococcosis was managed clinically in the HIV-positive, transplant recipient and HIV-negative/non-transplant patient groups in order to improve our understanding of this disease.

## Methods

### *Objectives*

The goals of this study were to describe trends in cryptococcosis symptoms, diagnosis, treatment and mortality through a 14-year study period (1996–2009) within the context of the three groups defined by the IDSA Guidelines.

### *Participants*

We identified all consecutive adult patients (≥18 years old) discharged from DUMC with International Classification of Diseases, 9th Revision (ICD-9) diagnosis codes of cryptococcosis (117.5), and cryptococcal meningitis (321.0) between January 1, 1996 and October 31, 2009 through electronic medical records. Eligible subjects had confirmed cryptococcal disease and a sufficient medical record (electronic and/or paper chart) available for review. A cryptococcosis case was confirmed by having ≥1 of the following: positive cerebral-spinal fluid (CSF) cryptococcal antigen (CRAG) or fungal culture, direct histological examination of tissue or fluid with characteristic yeast forms of *Cryptococcus*,

positive serum cryptococcal antigen test with a consistent disease process or positive culture from blood or pulmonary sites.

*Description of Investigations*

Demographics, presenting symptoms (including duration), and underlying conditions at the time of diagnosis were collected. Clinical differences examined included: presentation and duration of symptoms, microbiological evidence of cryptococcal disease, and initial antifungal treatment. Follow-up visit information relevant to cryptococcosis (laboratory testing, clinic visits, and readmission) was also captured. Patients were followed from the date of diagnosis and/or admission until loss-to-follow-up, death, or until the end of the study period. In order to assess one-year mortality prevalence for all patients, we obtained data on survival and mortality up to one year after their date of cryptococcosis diagnosis from the Duke Data Support Repository (DSR), which uses the Social Security Administration death index, the Tumor Registry and The Duke Information System for Cardiovascular Care death data to report mortality status. Investigators recorded all information on a standardized abstraction form developed in collaboration with epidemiologists and clinicians.

We report on annual changes in frequency of cryptococcosis diagnoses, treatment, and outcomes including: overall mortality through one year, deaths attributable to cryptococcosis, and occurrence of immune reconstitution inflammatory syndrome (IRIS). Defined and determined by a panel of experts at DUMC, death was attributable if patients experienced conditions directly related to cryptococcal disease, such as: increased central nervous system (CNS) pressure, persistence or relapse of infection, while receiving initial induction treatment or due to organ failure during treatment for cryptococcosis. The criteria

used to identify IRIS, adapted from Singh and Perfect (2007), included clinical or radiographic manifestations consistent with an inflammatory process, such as contrast enhancing lesions on imaging studies (CT/MRI), combined with symptoms that occurred during receipt of appropriate therapy and could not be explained by newly acquired infection, and at least one of the following: (1) negative results for cultures or stable/reduced biomarkers for the initial fungal pathogen during diagnostic work-up for the inflammatory process, (2) CSF pleocytosis >5 WBC/mm3, (3) Increased ICP, (4) histopathology showing granulomatous lesions, or (5) unexplained hypercalcemia [94].

Central nervous system (CNS), pulmonary and 'other' cryptococcosis patients were collapsed into two categories based on specific indicators described in the 2010 IDSA Treatment Guidelines [1]: severe disease (all patients with evidence of central nervous system (CNS) involvement, or those with cryptococcemia or dissemination with evidence of high fungal burden based on CRAG ≥1:512) or non-severe disease.

*Ethics*

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

*Statistical Methods*

All data was entered into Microsoft Office Access (2007) and analyses were performed using SAS v9.2 (SAS Institute, Cary, NC). Variables were examined using descriptive statistics and stratified based on the three groups and/or severity of cryptococcosis as needed. Where appropriate, the Student's t-test was used to test the difference of two means and the Kruskal-Wallis test was used for the difference between medians for non-parametric continuous data. Chi-square ($X^2$) tests were used to examine differences between categorical frequency distributions. The statistical significance level of alpha ($\alpha$) equal to 0.05 was used for each two-tailed test performed, thus a "significant" result refers to a p-value $<0.05$.

## Results

There were 223 study patients identified; 16 were excluded due to the following reasons: unable to locate chart (n=1), transferred out of care prior to diagnostic test results and did not receive treatment at DUMC (n=4), patient <18 years (n=1), and the patient did not have verified cryptococcal disease (n=10). A total of 207 patients were used for analysis. The majority of cases were CNS disease (61%), followed by pulmonary (34%) and other sites (Table 4.1). Consistent across the three clinical groups, nearly two-thirds of the cohort were male (65%) and African Americans were more prevalent among HIV-positive patients than the other two groups (Table 4.1). HIV-positive patients were significantly younger than the other two groups.

Overall annual case frequencies of cryptococcosis did not significantly change over time (Figure 4.1). During the study period, the number of transplant patients per year averaged three (range, 0–5 cases/yr.).The frequency of HIV-infected cases averaged six annually (range, 2–12 cases/yr.). Among HIV-negative, non-transplant cases there was a slight increasing trend with time; the annual average number of cases was six (range, 3–9 cases/yr.). Although the total cases have remained relatively steady (~15/yr.), there appeared to be a shift to a decreasing proportion of HIV-positive patients with a concomitant increase in HIV-negative cases. HIV-positive patients accounted for half of all cases in the first seven years of this study then fell to less than one-third in the latter seven years.

*Within-group observations*

Within the **HIV-positive** patient group (n=86), 74 (86%) had CNS disease, 9 (11%) had pulmonary cryptococcosis and three patients had another form of cryptococcosis (Table 4.1). Twenty-seven (31%) patients were newly identified as positive for HIV infection during their hospital admission for cryptococcosis. CD4 counts were available for 62 (72%) patients during hospitalization (median, 22 cells/μL; range: 1-300 cells/μL). Forty-two percent of HIV-positive patients with severe (n=31) and non-severe (n=5) cryptococcosis reported current or previous exposure to HAART therapy, but management compliance was heterogeneous. Prevalence of reported HAART exposure did not differ significantly comparing earlier (1996 – 2002) and later cases (2003 – 2009). During admission 18 patients (21%) continued their known HAART therapy, four patients (5%) changed to another regimen, six patients (7%) continued their current therapy but changed their regimen at the time of discharge and six patients had their therapy held on admission. Seventeen patients

(20%) had no confirmed previous exposure to HAART; eight died with cryptococcal disease-related deaths. Thirty-five patients (41%) were started newly on HAART therapy at the time of or after discharge. The median time-to-start of HAART therapy was 67 days after the start of initia therapy.

Among **transplant recipients** (n=42), 18 (43%) had CNS disease (severe) and 24 (57%) had pulmonary disease (non-severe; Table 4.1). The majority of transplants were renal (n=17; 4 included pancreas) followed by cardiac (n=11) and pulmonary (n=9). The median time from transplant to diagnosis of cryptococcosis (n=41) was 26 months (Inter-Quartile Range [IQR]: 10–56 months). Over one-third (n=16) of transplant recipients had renal insufficiency at the time of diagnosis (Table 4.2), but only two patients (11%) with severe disease experienced a >50% decrease in Glomerular Filtration Rate (GFR) during induction treatment. Current steroid exposure at the time of cryptococcosis was high (93%), and 19 patients (24%) had their immunosuppressive therapy stopped or changed at the time of their cryptococcosis diagnosis. Of the 39 patients taking steroids prior to cryptococcosis diagnosis, 37 (95%) had dose information. The median daily dose was 10mg of prednisone or prednisone equivalent (range: 4 – 30mg/day). Most of these patients had been on extended immunosuppressive therapy. The median duration of immunosuppression (n=39) was 19 months (IQR: 8 – 56 months).

In the **HIV-negative/non-transplant group** (n=79), 34 (43%) had CNS disease, 38 (48%) had pulmonary cryptococcosis and 7 (9%) had another form of cryptococcosis (Table 4.1). There were 37 (47%) patients with no underlying malignancy or immunosuppressive therapy at the time of diagnosis. Ninety percent of all cancers in the cohort were among HIV-negative, non-transplant patients (Table 4.2). Of the 31 patients (39%) taking steroids

prior to cryptococcosis diagnosis, 24 (77%) had dose information. The median daily dose was 20mg prednisone or prednisone equivalent (range: 5–267mg/day). The median duration of any type of immunosuppressive therapy (N=21) was 7 months (IQR: 1 – 36 months). Eight patients (21%) with severe disease experienced a >50% decrease in GFR during induction treatment.

*Clinical symptoms*

There were 21 patients (10%) who were asymptomatic at the time of diagnosis (Table 4.2). Of the 19 with asymptomatic pulmonary disease, 10 were transplant recipients and nine were HIV-negative/non-transplant patients.

Among the patients reporting symptoms (n=186), the duration of symptoms was unknown for 30 patients; 10 (12%) patients among those with HIV, seven (22%) of transplant recipients and 13 (18%) of the patients in the third group. Excluding these patients, the mean length of symptoms prior to presentation was not significantly different between severe and non-severe disease. The mean symptom duration was longer for the HIV-negative/non-transplant patients than either of the other two groups (Table 4.3). When compared to this group, the difference in means was -25 days (95% Confidence Limits [CL] -50, 1) for the HIV-positive patients and -20 days (95% CL -48, 8) for transplant recipients with severe disease. The differences in means were even greater when comparing the groups with non-severe disease (Table 4.3).

Patients with severe disease frequently experienced headaches, altered mental status, fevers, nausea and vomiting across all three groups at initial presentation (Table 4.2). The prevalence of nausea and vomiting was significant between groups with them being more

common in HIV and transplant recipients. Furthermore, headache was significantly more prevalent among HIV-positive patients (73%) and similar between the other two groups. Symptoms among non-severe cryptococcosis patients were similar between transplant recipients and HIV-negative/non-transplant groups. However, the prevalence of corticosteroid exposure was significantly higher in transplant recipients (Table 4.2).

*Patient diagnostics*

Among patients with **severe disease**, patients had similar lumbar puncture (LP) results among all three groups (Table 4.4). At least one opening pressure (OP) measurement was available for 79 (63%) patients. Peak OP distributions were very similar between all three groups with a mean of 33cm $H_2O$ (SD±16cm $H_2O$). The proportion of HIV-positive patients (48%) with elevated CSF host cells (≥20 cells/$mm^3$) was significantly less than the other groups, which had frequencies of 78% and 61% (Table 4.4). The difference in frequencies across all three patient groups having an elevated CSF CRAG titer (≥1:1024) and positive India ink neared significance. With regard to the frequency of these two CSF diagnostic measures, further comparison of non-HIV/non-transplant with the other two groups combined (differences in frequencies between HIV-positive and transplant patients were not significant) indeed reached the level of significance. CSF glucose and protein levels were similar in all groups (~40% of all patients had hypoglycorrhachia). There was no significant difference between groups in regards to cryptococcemia (Table 4.4).

Among **non-severe** disease patients, histological evidence of *Cryptococcus* was identified in over 60% of the two HIV-negative groups (Table 4.4). Positive pulmonary cultures were identified by at least one positive culture, biopsy or broncho-alveolar lavage in

>50% of HIV-negative patients, whereas only 17% (n=2) of HIV-positive patients had documentation of pulmonary disease. Of the 40 HIV-negative/non-transplant patients, 22 (55%) received an LP to rule out CNS disease, which was not significant compared to the 18 (75%) transplant recipients but was significant compared to the 11 (92%) HIV-infected patients who received an LP when non-severe disease was identified.

*Initial Treatment*

During the study period, 132 patients (64%) received amphotericin B (either formulation) for initial therapy. Utilization of amphotericin B deoxycholate (AmpBd) decreased over time, indicating that lipid formulation amphotericin B (LFAmpB) was used more frequently as initial therapy in recent years (Figure 4.2). Despite this observed trend, AmpBd was used as initial therapy for 80% of patients across the entire study period.

Initial antifungal regimens are summarized in Table 4.5. Eighty percent of patients with non-severe disease were given fluconazole for initial treatment; this was not significantly different across the three risk groups. In the severe disease group, the frequency of polyene use as initial therapy was high for all three patient groups (89% of HIV-positive, 100% of transplant, and 87% of HIV-negative/non-transplant), as was the use of flucytosine in combination with the polyene (78% of HIV-positive, 83% of transplant, and 72% of HIV-negative/non-transplant).

*Mortality and IRIS*

Mortality attributable to cryptococcosis was 15% (n=31) and there was a total of 52 deaths (25%) through one year of follow-up (Table 4.5). HIV-positive and HIV-

negative/non-transplant patients accounted for 89% of severe disease cryptococcosis-attributable deaths and these two groups accounted for 86% of all-cause mortality. IRIS was identified in seven (3%) cases and most of these cases had severe cryptococcosis. Four out of the seven patients were HIV-positive but IRIS was observed in the other two groups.

The HIV-negative/non-transplant group experienced both greater mortality attributable to cryptococcosis and overall mortality, since they accounted for nearly half of all cryptococcosis-attributable deaths (15/31) and more than half of all-cause mortality (29/52). Within this group, patients who died were older at diagnosis (mean, 63 years) than those who did not (mean, 51 years). However, the average age at diagnosis was not significantly different between survivors and those who died within either the HIV-positive or transplant groups.

**Discussion**

The 2010 IDSA Guidelines divided cryptococcal disease into three risk groups because of their different management issues in an attempt to better describe the issues around treatment and outcome [1]. The results from our study found notable trends and important clinical differences between and within these groups and uniquely describes the realities in the management of this disease at one institution.

In the early parts of the 14-year study period, the highest number of cases occurred in the HIV-infected population and appeared to experience fewer of these infections recently, coinciding with the widespread use of HAART. However, 42% of HIV-positive patients in this cohort had been exposed to HAART, emphasizing that despite therapies to control HIV

infection, cryptococcosis will continue to be an opportunistic infection in HIV-infected persons. The HIV-negative/non-transplant patients appeared to offset the reduction of cryptococcosis seen in HIV-infected patients in more recent years. As we continue to aggressively treat serious underlying diseases with immunosuppressants and the denominator of persons-at-risk enlarges, this group will likely increase since there is no strategy for prophylaxis. There were a consistent number of cases of cryptococcosis in the transplant recipients over time despite the widespread use of the potentially anti-cryptococcal agents, the calcineurin inhibitors [56,113,114,115,116]. The steady annual prevalence of cryptococcosis in this group supports the continued routine use of immunosuppressants and thus a persistent need for careful diagnostic surveillance for detection of early cryptococcosis [117].

The differences within the groups were several. For HIV-infected individuals, there was a variety of antiretroviral strategies employed during anti-cryptococcal therapy that reflects the lack of precise guidelines on when to initiate HAART [118]. Similar to previous studies, most of these patients had profound CD4 lymphocytopenia and a majority of these patients (76%) had elevated intracranial pressure [2,4,42,111]. The important underlying issues surrounding transplant recipients were immunosuppressive drugs and frequent renal dysfunction. All had some form of immunosuppression but only one-quarter had their immunosuppressive regimens stopped or changed and the prevalence of IRIS was low. Also, one-third of patients started treatment with evidence of renal dysfunction, emphasizing that lipid products of amphotericin B may be essential therapeutic choices in this group and that monitoring flucytosine levels and/or complete blood counts may be necessary to prevent treatment toxicity during worsening of renal dysfunction caused by polyene treatment [43].

Moreover, the average time from transplant to cryptococcal infection was within range of the 17 – 28 months reported in previous studies [43,61,115,119,120]. There were two important findings in the HIV-negative/non-transplant group. First, the duration of symptoms in this group with severe disease averaged 44 days prior to diagnosis and although not reaching significance from the other groups (it was significant among those with non-severe disease), this notable delay deserves greater attention and has been observed in a previous case series [68]. This delay may have contributed to the observed poorer outcome of the group. Another study also found a lack of significance between HIV-infected, immunocompromised, and immunocompetent groups, where the symptom duration averaged approximately 15 days [62]. It is possible that the other two groups have specialists aware of the risk of cryptococcosis, while in this group diagnosis gets delayed because it is not considered in the initial differential diagnosis. Second, 33% had liver insufficiency or hematological malignancies. These are important findings as this subgroup had the highest mortality and both factors have been shown to be predictors of mortality in HIV-negative cryptococcosis patients [69,121] Previous results emphasize that disseminated cryptococcosis among HIV-negative patients experienced the worst prognosis secondary to the stage of underlying disease and the immunosuppressive medication used [21,56]. It has been clearly shown in the HIV-positive population with cryptococcal disease that stage of HIV is strongly associated with poor outcome [122]. The underlying disease and its stage are a major factor in cryptococcosis outcome.

Since delay in diagnosis may be a prognostic factor, we investigated whether there were additional differences in symptoms and laboratory findings between the three groups. There were little differences between HIV-infected patients and transplant recipients who

generally had similar symptoms and CSF laboratory parameters. However, headaches (known to be a prognostic factor) [19], were similar between both HIV-negative groups and significantly more prevalent in the HIV-positive group. While all three groups had a high prevalence of poor prognostic signs such as altered mental status (~1/3), the presence of nausea and vomiting were less common in the HIV-negative/non-transplant group with severe disease. These findings differ from another study that reported significantly more mental status changes in non-immunosuppressed patients compared to HIV-positive patients [62]. Additionally, our study supports previous evidence that suggests non-immunosuppressed patients have less fungemia [69]. The HIV-negative/non-transplant group also appeared to present with a smaller burden of yeasts by India ink and CRAG test results than the other two groups. Although appreciation for burden organisms and outcome could not be precisely understood in this retrospective review, prospective studies of cryptococcal meningitis may benefit from quantification of viable yeasts in the CSF (Colony-forming unit [CFU]/mL measurements) and understanding its rate of change during therapy in relationship to treatment strategy and outcome [78,90,123].

Importantly, compared to HIV-positive and transplant recipients, the attributable mortality in the HIV-negative/non-transplant population with severe disease was nearly two-times higher despite the fact that the majority of patients received induction therapy with a polyene and flucytosine. A recent multi-center study of 86 cryptococcal meningitis patients also found the highest mortality in the non-immunosuppressed group (46%) compared to immunosuppressed (19%) and HIV-positive (15%) cryptococcal meningitis patients [62], and previous studies have reported 30 - 44% overall mortality in the HIV-negative population [21,68,69,70]. In one of these studies this rate was compared to a 22% mortality among

HIV-positive patients [69]. However, a couple of these studies included some *C.gatii* cases so that species' factors may have influenced outcome [69,70]. Clearly more studies to inform the management of the HIV-negative/non-transplant group and to understand how host immunity and yeast strain may play a role in poorer prognosis are needed so as to reduce this elevated mortality.

One of the major complications of cryptococcosis management has been the identification and management of IRIS. We identified the occurrence of IRIS in all three groups but the prevalence (3%) was relatively low compared to other studies which primarily included AIDS patients (range, 8–19%) [92,93,124,125]. This frequency could be influenced by our definition of IRIS, the patient mix, and/or clinical management. In general, our HAART management was delayed (>60 days after start of induction therapy) and only approximately a quarter of the transplant recipients had their immunosuppressants adjusted. This lack of immune manipulation during early induction therapy may influence our lower rate of IRIS. However, it is identified in a measureable amount of all patient groups and needs to be appreciated by clinicians.

Lipid formulations of amphotericin B outside of HIV-infected patients [73,126] have limited critical appraisal of proper dosing and efficacy. However, as Figure 2 demonstrated, there is a general increase in the use of lipid formulations for induction therapy at our institution. We expect this was in relationship to the approximately third of patients who develop nephrotoxicity during management. Therefore, further investigation into lipid products of amphotericin B is still warranted to ensure their optimal use.

*Limitations*

This review was limited to a single tertiary care center and teaching hospital. Our medical center averaged nearly 15 cases of cryptococcosis per year and this likely reflects both an endemic exposure to this yeast in the environment within the Southeastern USA and an enriched population of immunosuppressed individuals due to our hospital's care patterns. The actual number of cases seen in a particular medical center certainly varies within the U.S. Furthermore, using hospital records favors cases with severe disease and could result in selection bias against asymptomatic disease. Determining the total population-at-risk was not estimable in this study and the underlying source population and referral patterns could shift over time. Retrospective chart review has the potential for incomplete or incorrect information capture due to loss of paper documentation or lack of entry in electronic medical record. We used both sources to ensure data was as complete as possible and discrepancies were minimized. Erroneous self-report of symptoms or symptom duration was a possibility, but this is a limitation of many observational clinical studies. Despite our careful abstraction process, missing or incomplete data could lead to bias in categorization of symptoms or derived outcome definitions, such as IRIS. Being a rare disease, limited numbers of cases prevented robust statistical analyses, although this study is one of the largest to date. Importantly, much of the clinical attention over the last two decades has centered around two groups (HIV-infected and transplant recipients). There has been less focus on treatment of HIV-negative/non-transplant patients and yet this group suffered the highest mortality.

*Conclusion*

In summary, dividing patients with cryptococcosis into three risk groups showed both differences and similarities within the groups. In a single medical center the overall frequency of cryptococcosis has not changed though the composition of the three groups has changed in the last two decades. Despite three major classes of drugs to treat severe disease and a relatively uniform and informed treatment strategy framed by the IDSA Guidelines, attributable mortality was common. Prospective multi-center studies and comparison of strategies in advanced medical centers are still needed to determine the extent high mortality revolved around underlying disease, high burden of organisms and delayed diagnosis.

## Tables

Table 4.1. Patient characteristics at baseline. Primary diagnosis, disease severity, basic patient characteristics and underlying condition of cryptococcosis patients at DUMC (N=207).

| | | | | Underlying Condition[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | | HIV | | Transplant | | HIV-/Trans-[b] | | |
| Category | Subcategory | N | (%) | n | (%) | n | (%) | n | (%) | p-value[c] |
| Primary Diagnosis | | | | | | | | | | |
| | CNS | 126 | (61) | 74 | (86) | 18 | (43) | 34 | (43) | >0.05 |
| | Pulmonary | 71 | (34) | 9 | (10) | 24 | (57) | 38 | (48) | |
| | Other | 10 | (5) | 3 | (3) | 0 | (-) | 7 | (9) | |
| Disease severity | | | | | | | | | | |
| | Severe | 131 | (63) | 74 | (86) | 18 | (43) | 39 | (49) | >0.05 |
| | Non-severe | 76 | (37) | 12 | (14) | 24 | (57) | 40 | (51) | |
| Demographics | | | | | | | | | | |
| | Male Gender | 135 | (65) | 55 | (64) | 28 | (67) | 52 | (66) | >0.05 |
| | Black Race | 106 | (51) | 69 | (80) | 13 | (31) | 24 | (30) | >0.05 |
| | Age (yrs)[d] | 47 | (15) | 40 | (9) | 50 | (14) | 54 | (18) | <0.05 |

[a] There were 86 patients in the HIV group, 42 in transplant and 79 in HIV-negative, non-transplant.
[b] HIV-negative and non-transplant
[c] Cochran Mantel-Haenszel Chi-square test for a general association between the three groups; Kruskal-Wallis test for difference between median age was used.
[d] Instead of n (%), mean (STD) are shown for age.

Table 4.2. Presenting symptoms and risk factors of cryptococcosis disease (N=207) stratified by disease severity at initial presentation and underlying condition.

| | | Severe disease (n=131)[a] | | | | | | | Non-severe disease (n=76)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HIV | | Transplant | | HIV-/Trans- | | | HIV | | Transplant | | HIV-/Trans- | | |
| Presentation | Characteristic | n | (%) | n | (%) | n | (%) | *p<.05[b] | n | (%) | n | (%) | n | (%) | *p<.05[b] |
| Symptoms | | | | | | | | | | | | | | | |
| | No symptoms | 1 | (1) | 0 | (-) | 0 | (-) | | 1 | (8) | 10 | (42) | 9 | (23) | |
| | Altered mental status | 22 | (30) | 7 | (39) | 17 | (44) | | 0 | (-) | 1 | (4) | 0 | (-) | |
| | Headache | 54 | (73) | 9 | (50) | 17 | (44) | * | 2 | (17) | 3 | (13) | 2 | (5) | |
| | Cough | 15 | (20) | 1 | (6) | 3 | (8) | | 7 | (58) | 3 | (13) | 11 | (28) | * |
| | Shortness of breath | 10 | (14) | 1 | (6) | 5 | (13) | | 6 | (50) | 5 | (21) | 14 | (35) | |
| | Night sweats | 5 | (7) | 2 | (11) | 1 | (3) | | 4 | (33) | 2 | (8) | 4 | (10) | |
| | Fever | 40 | (54) | 7 | (39) | 12 | (31) | | 6 | (50) | 4 | (17) | 15 | (38) | |
| | Nausea | 33 | (45) | 11 | (61) | 6 | (15) | * | 2 | (17) | 5 | (21) | 5 | (13) | |
| | Vomiting | 28 | (38) | 8 | (44) | 5 | (13) | * | 3 | (25) | 3 | (13) | 3 | (8) | |
| | Seizures | 8 | (11) | 0 | (-) | 3 | (8) | | 0 | (-) | 0 | (-) | 1 | (3) | |
| Condition/risk factor | | | | | | | | | | | | | | | |
| | Renal insufficiency | 3 | (4) | 10 | (56) | 5 | (13) | * | 3 | (25) | 6 | (25) | 6 | (15) | |
| | Liver insufficiency | 1 | (1) | 0 | (-) | 6 | (15) | * | 1 | (8) | 0 | (-) | 2 | (5) | |
| | Hematologic malignancy | 0 | (-) | 1 | (6) | 7 | (18) | * | 0 | (-) | 0 | (-) | 7 | (18) | * |
| | Non-hematologic Malignancy | 0 | (-) | 0 | (-) | 2 | (5) | | 1 | (8) | 0 | (-) | 3 | (8) | |
| Immunosuppressants | | | | | | | | | | | | | | | |
| | Corticosteroid | 4 | (5) | 17 | (94) | 19 | (49) | * | 1 | (8) | 22 | (92) | 12 | (30) | * |
| | Calcineurin inhibitor | 0 | (-) | 15 | (83) | 0 | (-) | * | 0 | (-) | 21 | (88) | 2 | (5) | * |
| | Mycophenolate mofetil | 0 | (-) | 13 | (72) | 2 | (5) | * | 0 | (-) | 10 | (25) | 2 | (5) | * |
| | Azathioprine | 0 | (-) | 2 | (11) | 1 | (3) | * | 0 | (-) | 8 | (33) | 1 | (3) | * |
| | Methotrexate | 0 | (-) | 1 | (6) | 1 | (3) | | 0 | (-) | 3 | (13) | 3 | (8) | |
| | Monoclonal antibodies | 1 | (1) | 2 | (11) | 3 | (8) | | 0 | (-) | 0 | (-) | 2 | (5) | |
| | Sirolimus | 0 | (-) | 2 | (11) | 0 | (-) | * | 0 | (-) | 2 | (8) | 0 | (-) | |

[a] Severe disease: HIV group had 74 patients, transplant group had 18 patients and 39 patients were in the HIV-negative/non-transplant group; Non-severe disease: HIV group had 12 patients, transplant group had 24 patients and 40 patients were in the HIV-negative/non-transplant group.

[b] Cochran Mantel-Haenszel Chi-square test for a general association between the three groups.

Table 4.3. Differences in mean duration of symptoms (days) reported prior to presentation among those reporting any symptom(s) of cryptococcosis (N=156).

| | Severe disease | | | | Non-severe disease | | | |
|---|---|---|---|---|---|---|---|---|
| Underlying condition | n | Mean | Difference | 95% CL[a] | n | Mean | Difference | 95% CL[a] |
| HIV-negative, non-transplant | 36 | 44 | 0 (ref.) | 20, 68 | 21 | 66 | 0 (ref.) | 29, 103 |
| HIV positive | 65 | 19 | -25 | -50, 1 | 10 | 26 | -40 | -80, 0 |
| Transplant recipient | 14 | 24 | -20 | -48, 8 | 11 | 20 | -46 | *-84, -8* |
| Total | 114 | 28 | | 19, 37 | 42 | 44 | | 24, 64 |

[a] Unadjusted 95% Confidence Limits (CL) of the difference in means. The 95% CL for the referent group and the total are surrounding their corresponding mean (days).

Table 4.4. Diagnostic findings of cryptococcosis disease (N=207) stratified by disease severity at initial presentation and underlying condition.

| | | Severe disease (n=131)[a] | | | | | | | Non-severe disease (n=76)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HIV | | Transplant | | HIV-/Trans- | | | HIV | | Transplant | | HIV-/Trans- | | |
| Diagnostic | Result description | n | (%) | n | (%) | n | (%) | *p<0.05 | n | (%) | n | (%) | n | (%) | *p<0.05 |
| Positive culture | | | | | | | | | | | | | | | |
| | CNS, first LP[b] | 61 | (82) | 18 | (100) | 22 | (56) | | 0 | - | 0 | - | 0 | - | |
| | Blood[c] | 35 | (47) | 8 | (44) | 11 | (28) | | 6 | (50) | 2 | (8) | 5 | (13) | * |
| | Pulmonary | 6 | (8) | 2 | (11) | 3 | (8) | | 2 | (17) | 13 | (54) | 21 | (53) | |
| Initial LP[b] | | | | | | | | | | | | | | | |
| | CSF CRAG titer ≥1:1024 | 35 | (47) | 6 | (33) | 8 | (24) | | 0 | - | 0 | - | 0 | - | |
| | CSF:serum glucose ratio <0.6 | 66 | (89) | 16 | (89) | 28 | (85) | | 7 | (64) | 7 | (39) | 6 | (27) | |
| | CSF glucose ≤40mg/dL | 43 | (58) | 11 | (61) | 20 | (61) | | 1 | (0) | 1 | (6) | 0 | - | |
| | CSF protein ≥45mg/dL | 66 | (89) | 16 | (89) | 32 | (97) | | 6 | (55) | 14 | (78) | 9 | (41) | * |
| | Nucleated cells >20cells/mm$^3$[, d] | 30 | (48) | 14 | (78) | 20 | (71) | * | 0 | - | 0 | - | 0 | - | |
| | OP ≥20cm $H_2O$ | 47 | (64) | 11 | (61) | 17 | (52) | | 6 | (55) | 3 | (17) | 4 | (18) | |
| | Positive India Ink | 41 | (55) | 9 | (50) | 11 | (33) | | 0 | - | 0 | - | 0 | - | |
| Other | | | | | | | | | | | | | | | |
| | Serum CRAG titer ≥1:1024 | 47 | (64) | 11 | (61) | 17 | (44) | | 5 | (42) | 3 | (13) | 4 | (10) | * |
| | Other histological evidence | 9 | (12) | 3 | (17) | 6 | (15) | | 0 | - | 15 | (63) | 27 | (68) | * |

[a] Severe disease: HIV group had 74 patients, transplant group had 18 patients and 39 patients were in the HIV-negative/non-transplant group; Non-severe disease: HIV group had 12 patients, transplant group had 24 patients and 40 patients were in the HIV-negative/non-transplant group.

[b] Lumbar puncture; 176 total patients had an LP; percentages relevant to the LP procedure reflect missing observations. Six HIV-negative, non-transplant patients with severe disease did not receive an LP (n=33). 25 patients without an LP (to rule out CNS disease) were non-severe cases: one HIV-positive, six transplant recipients, and 18 HIV-negative/non-transplant patients.

[c] OP = Opening pressure

[d] Eleven (15%) HIV-infected patients had missing documentation of CSF host (nucleated) cells (overall N=63); excluding the six with no initial LP, five HIV-negative, non-transplant were missing host cell counts (overall N=28).

[e] Opening pressure; maximum LP OP was used for this variable, as initial LP OP was infrequently captured. Denominators for each group with severe disease were 74, 8, and 33, respectively.

Table 4.5. Patient treatment and outcomes**.** Initial antifungal regimen used for induction therapy, patient mortality through one year, and immune reconstitution inflammatory syndrome (IRIS) by primary disease diagnosis (severe or non-severe) and major underlying condition (HIV, transplant, or HIV-negative and non-transplant).

| | | | | Severe disease (n=131)[a] | | | | | | Non-severe disease (n=76)[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | | HIV | | Transplant | | HIV-/Trans-[b] | | HIV | | Transplant | | HIV-/Trans-[b] | |
| Outcomes | Description | N | (%) | n | (%) | n | (%) | n | (%) | n | (%) | n | (%) | n | (%) |
| Initial therapy | | | | | | | | | | | | | | | |
| | AmpBd alone | 18 | (9) | 7 | (9) | 1 | (6) | 5 | (13) | 1 | (8) | 2 | (8) | 2 | (5) |
| | AmpBd+5FC | 88 | (43) | 55 | (74) | 5 | (28) | 22 | (56) | 1 | (8) | 0 | (-) | 5 | (13) |
| | LFAmpB alone | 7 | (3) | 1 | (1) | 2 | (11) | 1 | (3) | 0 | (-) | 1 | (4) | 2 | (5) |
| | LFAmpB+5FC | 19 | (9) | 3 | (4) | 10 | (56) | 6 | (15) | 0 | (-) | 0 | (-) | 0 | (-) |
| | Fluconazole | 71 | (34) | 8 | (11) | 0 | (-) | 2 | (5) | 10 | (83) | 21 | (88) | 30 | (75) |
| | Voriconazole | 1 | (<1) | 0 | (-) | 0 | (-) | 1 | (3) | 0 | (-) | 0 | (-) | 0 | (-) |
| | None | 3 | (1) | 0 | (-) | 0 | (-) | 2 | (5) | 0 | (-) | 0 | (-) | 1 | (3) |
| Result[c] | | | | | | | | | | | | | | | |
| | Attributable death | 31 | (15) | 12 | (16) | 3 | (17) | 12 | (31) | 1 | (8) | 0 | (-) | 3 | (8) |
| | Overall mortality | 52 | (25) | 15 | (20) | 5 | (28) | 16 | (41) | 2 | (17) | 1 | (4) | 13 | (33) |
| | IRIS | 7 | (3) | 3 | (4) | 2 | (11) | 1 | (3) | 1 | (8) | 0 | (-) | 0 | (-) |

[a] Severe disease: HIV group had 74 patients, transplant group had 18 patients and 39 patients were in the HIV-negative/non-transplant group; Non-severe disease: HIV group had 12 patients, transplant group had 24 patients and 40 patients were in the HIV-negative/non-transplant group.

[b] HIV-negative, non-transplant

[c] Cochran Mantel-Haenszel Chi-square test for a general association between the three groups was significant for overall mortality among non-severe disease only.

**Figures**

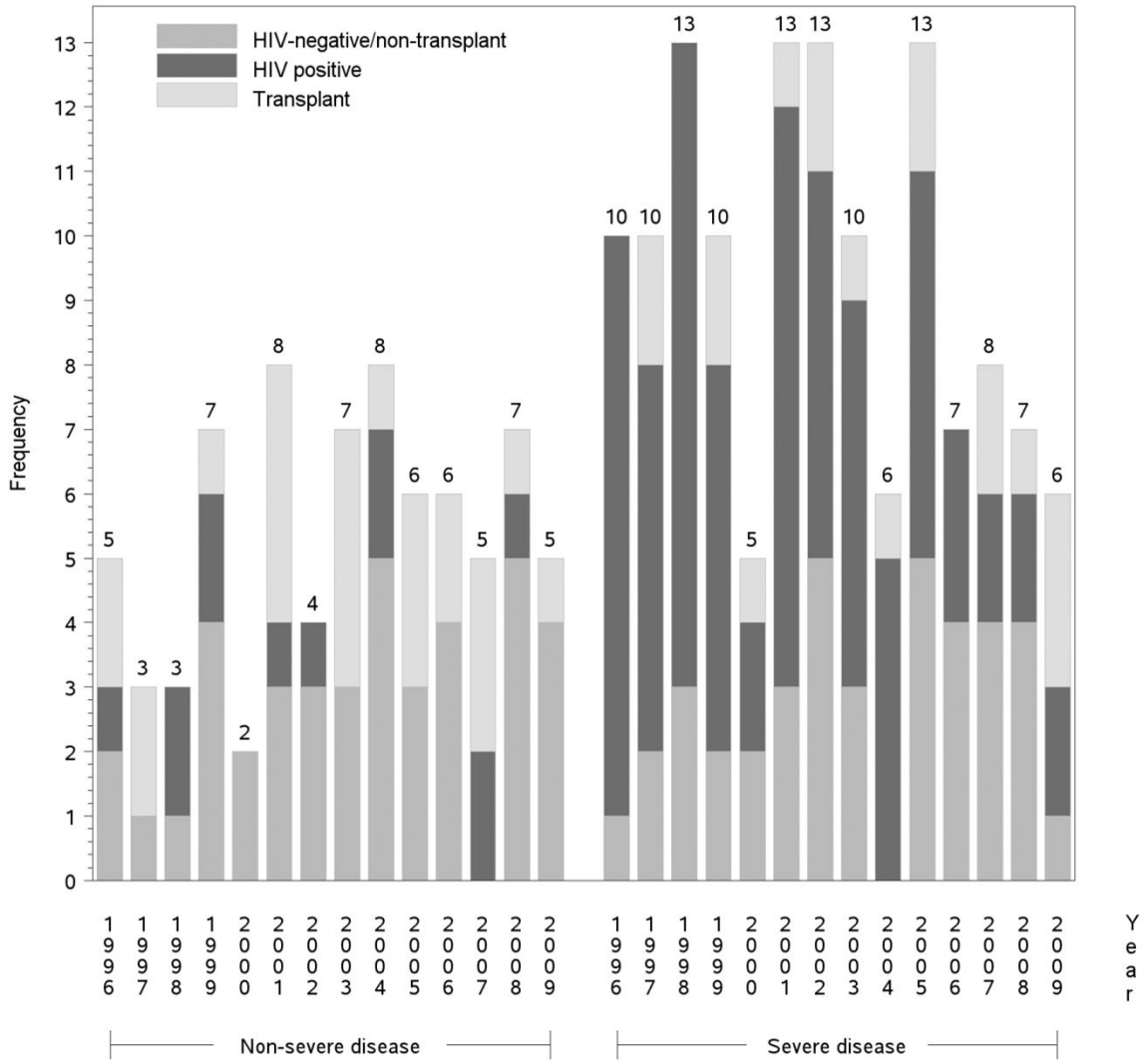


Figure 4.1. Annual cases. Annual frequency of severe and non-severe cryptococcosis cases according to underlying condition (N=207).

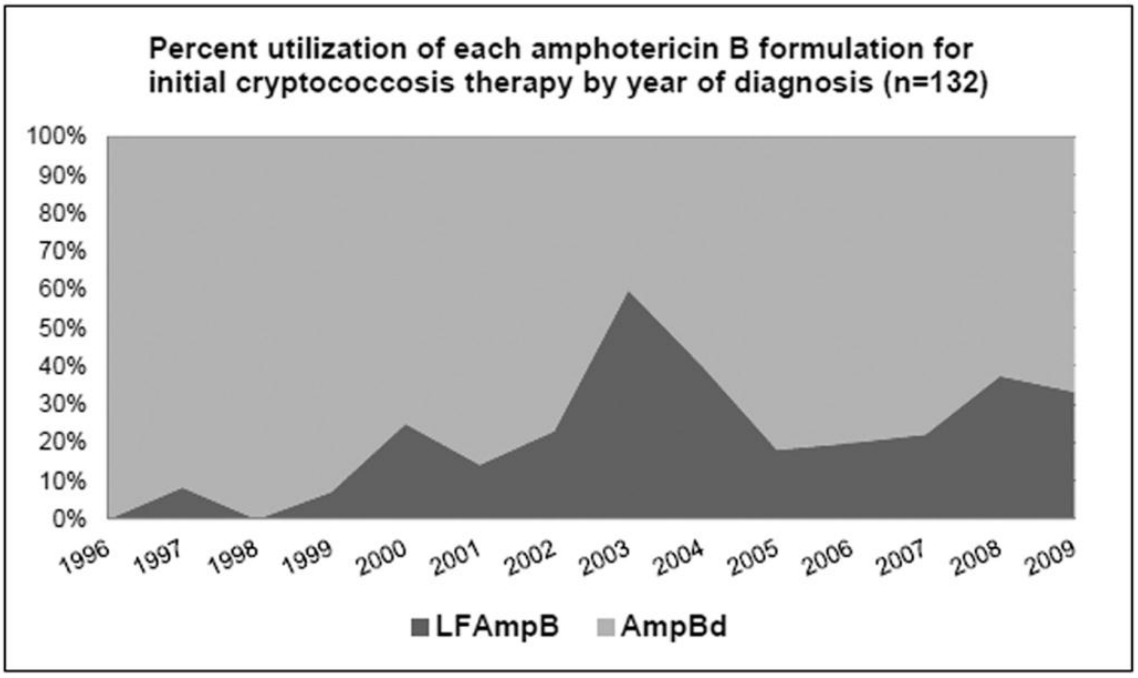


Figure 4.2. Use of amphotericin B. Amphotericin B formulation trends over time. Annual percentage of patients receiving lipid formulation amphotericin B (LF AmpB) or deoxycholate amphotericin B (AmpBd) for initial therapy (N=132).

# CHAPTER V. APPROACHES TO ANTIFUNGAL THERAPY AND THEIR EFFECTIVENESS AMONG PATIENTS WITH CRYPTOCOCCOSIS

## Overview

The goal of this study was to determine the degree to which the persistence of cryptococcosis, overall 1-year mortality, and 1-year mortality due to cryptococcosis were influenced by initial antifungal treatment regimen in a cohort of adults with cryptococcosis treated at a tertiary care medical center. Risk factors, underlying conditions, treatment and mortality information were obtained for 204 adults with cryptococcosis from Duke University Medical Center (DUMC) from 1996-2009. Adjusted risk ratios (RR) for persistence and hazard ratios (HR) for mortality were estimated for each exposure.

All-cause mortality among patients with non-severe disease (20%) was similar to the group with disease (26%). However, cryptococcosis-attributable mortality with non-severe disease (5%) was much lower than with severe disease (20%). Flucytosine exposure was associated with a lower overall mortality rate (HR 0.4, 95%CI 0.2 – 0.9) and attributable mortality (HR 0.5, 95%CI 0.2 – 1.2). Receiving a non-recommended antifungal regimen was associated with a higher relative risk of persistent infection at four weeks (RR1.9, 95%CI 0.9 – 4.3) and the rate of attributable mortality among those not receiving the recommended dose of initial therapy was higher relative to those receiving recommended dosing (HR 2.3, 95%CI 1.0 – 5.0).

Thus, the 2010 IDSA Guidelines are supported by this retrospective review as a best-practice protocol for cryptococcal management. Future investigations should consider highlighting the distinction between all-cause and attributable mortality as not to overestimate the true effect of cryptococcosis on patient death.

## Introduction

The optimal antifungal treatment strategy for patients with cryptococcosis remains in question despite the 2010 Infectious Disease Society of America Guidelines [1]. Treatment of cryptococcal meningoencephalitis is based on a small number of clinical trials, but most of the recent studies have been in resource-limited areas and may not reflect the situation with newer antifungals in advanced medical settings [106,127,128,129]. Previous studies have suggested that treatment is generally 50-80% effective [3,4,18,22], that antifungal drugs cause toxicities in roughly one-third of cases, that mortality while on antifungal therapy remains high (approximately 20%) and that mortality varies considerably by the host underlying immune status [13,21,44,48,62].

There have been few comprehensive, comparative studies of cryptococcosis that encompass all three risk groups (HIV positive, solid organ transplantation and HIV-negative/non-transplant) with and without meningeal involvement, identified by the 2010 IDSA Guidelines in the era of lipid products of amphotericin B. Therefore, we examined the effectiveness of initial antifungal treatment among these three clinical groups within a single study-center. The primary aim of this study was to determine the degree to which the risk for

persistence of cryptococcosis, rates of 1-year mortality and mortality due to cryptococcosis were influenced by initial antifungal treatment regimen in a cohort of patients with cryptococcosis treated at a tertiary care medical center. The advantage of this approach was to observe real world treatment strategies and compare risk groups where anti-cryptococcal drugs are available and the general care and management of this infection has been relatively consistent over the 14-year study period. Other treatment-related outcomes examined were: (1) changes from initial therapy to definitive therapy (2) development of renal toxicity, (3) development of Immune Reconstitution Inflammatory Syndrome (IRIS), (4) and number of cases requiring multiple courses of induction therapy using amphotericin B.

## Methods

### *Study population*

All consecutive 204 adults hospitalized with cryptococcosis and who were treated at DUMC were enrolled in the cohort using ICD-9 discharge codes of cryptococcosis (117.5), and cryptococcal meningitis (321.0) from 1996-2009. Three identified patients were excluded from this cohort because they had died prior to receiving any anti-cryptococcal therapy or refused treatment at DUMC. Risk factors, underlying conditions, treatment and mortality information were obtained by chart review. Patients presented to DUMC, were diagnosed with cryptococcosis and were assessed for severity of disease prior to starting treatment. "Severe" and "non-severe" cryptococcosis disease categories divided patients who required induction therapy with amphotericin B (severe disease) and those where fluconazole was indicated as primary therapy (non-severe disease) based on the IDSA

Guidelines [1], whether or not the patients actually received the indicated treatment. Follow-up started when initial treatment was given, preceded by the occurrence of disease, patient admission, followed by the assessment of cryptococcosis severity.

*Exposures*

There were three main treatment exposures of interest: (1) appropriate initial treatment, (2) appropriate initial treatment dose, (3) and appropriate flucytosine (5FC) use based on IDSA recommendations. Secondary treatment-related exposures of interest included the completion of at least 7 days of flucytosine combination antifungal therapy versus not receiving it or completing <7 days, confined to patients with severe disease, completion of at least 30 days of fluconazole therapy among surviving patients (non-severe disease), and completion of at least 90 days of fluconazole therapy among surviving patients (non-severe disease).

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

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

*Outcomes*

Follow-up time started when anti-cryptococcal therapy was initiated after patient admission, diagnosis and severity evaluation were complete. We assessed persistent infection at two (severe disease only) and four weeks, cryptococcal-attributable mortality and all-cause mortality through one year of follow-up. Two and four weeks were chosen because severe disease patients are recommended to receive at least two weeks of induction therapy and reliable follow-up and mortality information was available for the majority of surviving cases. Figure 5.1 illustrates overall patient flow. Follow-up for this study began at the start of anti-cryptococcal therapy up to four weeks to evaluate persistence and up to one year for mortality outcomes.

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

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

persistent infection, relapse of infection, while on treatment for cryptococcosis and an underlying disease, or organ failure while on antifungal treatment.

Additional outcomes for both severe and non-severe cryptococcosis patients included frequency of re-induction(s) with amphotericin B, IRIS and renal toxicity during initial therapy. Renal toxicity was defined as a >50% decrease in Glomerular Filtration Rate (GFR), also known as estimated creatinine clearance, during initial induction treatment. GFR was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula [99]. For patients with severe disease who received induction therapy with amphotericin B, creatinine values closest to day 0 and day 14 of treatment were used to determine renal toxicity. The definition for IRIS (adapted from Perfect and Singh, 2007) has been previously described (Bratton et al, 2012, in production; see Chapter Four).

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

*Data analysis*

To evaluate issues of confounding, we assessed the bivariate associations between all covariates and main exposures and outcomes. Minimum adjustment sets were determined using Directed Acyclic Graph (DAG) program (v0.21) [100]. However, the program resulted in 14 confounders in the minimum adjustment sets for each of our three chosen exposures (with slight variation; Figures 3.2 and 3.3), of which our limited study size could not

operably model. Based on previous studies that predicted poor outcomes [13,15,16,17,18,19,20,21], we prioritized variables associated with severe underlying condition and high fungal burden from our minimal adjustment sets and proceeded with multivariate adjustment using a change-in-estimate approach with a 10% cut-off criterion [101]; eliminating variables chosen by the DAG program that did not confound the association of effect estimates between the main exposures and outcomes. Changes in the precision of estimates were examined with the confidence limit interval (CLR); covariates that improved precision were maintained in the final model. Effect measure modification by confounding variables was examined through the inclusion of interaction terms in the models and using the spreadsheet by Andersson et al. (2005) to determine the relative excess risk due to interaction (RERI)[102].

Binomial regression was used to estimate the risk ratio (RR) of the association between treatment exposures and these outcomes. If the binomial model failed to converge, a scaled Poisson distribution was used for robust estimation. Cox proportional hazards models were used to estimate hazard ratios (HR) for the association between treatment exposures and mortality outcomes. Assessment of the proportional hazards assumption (PHA) was performed using graphical methods (ln – ln survival plot) and by adding an interaction between each of the model predictors and (log) time. Corresponding 95% CIs were estimated to measure the precision for each estimate of exposure and outcome association.

Abstraction forms were entered into Microsoft Office Access (2007) and data analyses were performed using SAS v9.2 (SAS Institute, Cary, North Carolina). Investigators

recorded all information on a standardized abstraction form developed in collaboration with epidemiologists and clinicians.

*Research Ethics*

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

## Results

*Baseline Characteristics*

There were 204 patients with records describing their antifungal treatment; 129 (63%) patients had severe disease defined as requiring amphotericin B induction treatment and there were 75 (37%) patients treated with fluconazole as recommended for non-severe disease. Transplant recipients were the smallest group (n=42, 21%) and HIV-positive (n=85, 42%) and HIV-negative, non-transplant patients (n=77, 38%) composed the remainder of the cohort. The majority of patients presenting at DUMC had CNS disease (n=126, 62%) and pulmonary disease was seen in about a third of all cases (n=69, 34%). In addition to CNS disease patients, three patients from the 'other' disease category (n=9, 4%) also fit the definition for severe disease requiring amphotericin B induction treatment. There were 32,801 total days of follow-up accrued among patients with severe cryptococcosis and 20,439 days of follow-up among patients with non-severe cryptococcosis. Average length of follow up did not differ substantially between groups (mean: 254 days for severe disease and

273 days for non-severe disease) and averages were also similar across treatment exposure strata.

Patients differed considerably with regard to presenting symptoms and conditions based on severity of cryptococcosis, although the duration of symptoms prior to presentation was similar in range. As expected, patients with non-severe cryptococcosis (n=75) had fewer neurological symptoms than CNS cases. Diagnostically, the proportion of patients with a maximum opening pressure of 20cm $H_2O$ was high for both severe (42%) and non-severe (65%) cryptococcosis patients, although one-third of non-severe patients did not undergo a Lumbar puncture (LP). If we assumed that all non-severe patients without a LP were negative for elevated opening pressure, the proportion of patients with an elevated opening pressure was 44%.

Baseline covariate measures prior to starting therapy according to patient status regarding our four main treatment exposures are shown in Tables 5.1 and 5.2. Patients who received their recommended therapy and severe disease patients who received flucytosine combination therapy had higher frequencies of symptoms and diagnostics indicating CNS disease (headache, vomiting and nausea, altered mental status, high CRAG titers), compared to patients who did not receive the recommended initial therapy. This trend was not seen when looking at initial treatment dose, which showed very few variations in covariate frequencies between exposure groups, except for altered mental status and high serum CRAG titer (Table 5.1, Table 5.2).

Severe disease patients who received flucytosine combination initial therapy had lower frequencies of pulmonary symptoms (shortness of breath and cough), but a higher proportion of patients had a longer duration of symptoms (≥14 days) and diagnostic evidence

of CNS disease (India ink, high CSF CRAG titer), compared to those who did not receive flucytosine (Table 5.1). Patients given a shorter length of flucytosine exposure (0 – 7 days) had a higher proportion of cases taking corticosteroids at the time of diagnosis, reported weight loss, and HAART exposure (among HIV-positive patients) compared to patients given ≥ 7 days of flucytosine, who had a higher proportion of patients with positive blood and CNS cultures, India ink and organ transplant recipients (Table 5.1, Table 5.2).

*Antifungal Treatment*

Initial antifungal therapy type was considered "appropriate" given disease severity for the majority of patients (88%; Table 5.3). Only 11 patients (9%) with severe disease (n=129) were not given amphotericin B therapy within 5 days of starting therapy—seven of these patients were eventually given polyene treatment (Table 5.3). Fourteen patients (19%) that fulfilled the definition of non-severe cryptococcosis did not receive fluconazole for initial antifungal therapy. Rather, amphotericin B was used. Given the severity of disease, appropriate therapy was similar in prevalence between underlying risk groups: 88% (n=75) of HIV-positive patients received appropriate therapy, 84% (n=39) of transplant recipients and 93% (n=65) of HIV-negative/non-transplant patients received appropriate antifungal therapy.

There were 192 patients (94%) with available initial treatment dose information (Table 5.4). The mean dose of AmpBd was 0.66 mg/kg/day (interquartile range [IQR]: 0.56 – 0.73 mg/kg/day). Mean doses for lipid products of amphotericin B were similar and generally in the recommended range, but ABLC was used more often and slightly higher in doses than L-AmpB (Table 5.4). The fluconazole dose averaged 350mg/day (IQR: 208-400

mg/day; Table 5.4), which was close to the recommended ≥400mg/day for primary therapy and was lowest for HIV-positive patients (data not shown).

Overall, 66% of patients received appropriate dosing of their initial therapy (Table 5.4). Dosing for severe patients was outside the recommended range for 42 (36%) patients and this was similar for non-severe patients (n=24, 32%). Furthermore, 43% of patients who received AmpBd and 27% of patients who received fluconazole did not receive appropriate dosing of therapy, while approximately 13% of patients who received LFAmpB did not receiving appropriate dosing. Among patients with severe disease, AmpBd formulation was within the recommended range of 0.7 – 1.0 mg/kg/day for 50 patients (57%; Table 5.4), and tended to be outside the recommended range for HIV-negative patients in particular (data not shown). Median cumulative dose of initial therapy for patients who did not switch therapy is shown in supplemental information (Appendix B).

*Treatment for Severe Disease*

Flucytosine (5FC) was incorporated into initial therapy in 77% of patients with severe cryptococcosis (Figure 5.2). In this group, 79% of HIV-positive patients received flucytosine with initial therapy; 83% of transplant patients and 70% of HIV-negative/non-transplant also received flucytosine in combination with amphotericin B. The mean number of days of initial flucytosine was 11.8 days (standard deviation [SD] ± 6.3 days). Only 37% of patients who received flucytosine continued the drug for at least 14 days as recommended (Figure 5.2).

Among patients who received any formulation of amphotericin B as initial therapy (n=118) and survived at least 14 days since the start of therapy (n=106), 56 patients (53%)

completed ≥14 days of amphotericin B treatment. Seventy-five patients (58%) did not switch from their initial amphotericin B formulation (Table 5.3). Among the 14-day survivors (n=106), patients who switched from their initial amphotericin B treatment experienced significantly longer treatment exposure than those who did not (difference in means=6 days, 95% CI 3 – 9 days).

*Treatment for Non-severe Disease*

Most non-severe disease patients (81%) received fluconazole as initial antifungal therapy (Table 5.3). There were 10 (13%) patients with non-severe disease who later changed initial treatment compared to 50 (39%) severe disease patients (Table 5.3). This continuity of initial therapy was significantly higher than that among patients with severe disease ($RR_{crude}$ 1.4, 95% CI: 1.2 – 1.7). Of the patients given fluconazole as initial therapy, 33 patients (54%) completed 90 days of treatment, while prior to 90 days two patients were lost to follow-up and six died (one attributable to cryptococcosis). Fifty-two patients (72%) completed 30 days of fluconazole treatment among those surviving at least that long (N=72).

*Persistence of Cryptococcosis*

Persistence of infection was common two weeks after starting therapy (47%, Table 5.5) among patients with severe disease. Overall persistence at four weeks after starting therapy was 25%; 33% among severe and 11% among non-severe patients.

*Patient Mortality*

Mortality in the first year after starting cryptococcosis treatment was high (Table 5.5) with attributable mortality through one year of 15% (n=30). All-cause mortality through one year of follow-up was 25% (n=52). Notably, half of these deaths (n=15) were among HIV-negative/non-transplant cases. Acute mortality was high among patients with severe disease. Twenty-six (20%) patients died due to cryptococcosis and half of these were during the first two weeks while receiving induction treatment; three additional patients died through four weeks of follow-up. Among patients with severe disease, 10 (10%) who did not complete full induction therapy died. Of those who did not switch from their initial amphotericin B therapy (n=75), there were 12 deaths attributable to cryptococcosis (16%), which was similar to the 9 deaths among the 43 patients (21%) who changed their amphotericin B induction regimen. Non-severe disease patients had lower mortality; three deaths (5%) occurred within the first month of follow-up from the start of initial therapy.

*Appropriate Initial Treatment*

The risk of persistence two weeks from starting therapy among surviving severe disease patients who did not receive recommended treatment was 1.4 (95% CI 0.6 – 3.0) relative to those who received appropriate initial therapy (Table 5.6). The risk of persistence at four weeks out from treatment among all surviving patients who did not initially receive the recommended antifungal treatment was higher (RR 1.9, 95% CI 0.9 – 4.3; Table 5.6). If patient deaths during two and four weeks were considered persistent infection, the corresponding RRs were 1.2 (95% CI 0.6 – 2.7) and 1.6 (95% CI 0.7 – 3.3).

The association between appropriate initial treatment and patient mortality was weak (Table 5.6). The adjusted HR cryptococcosis-attributable mortality through one year of follow-up for initial treatment was 0.8 (95% CI 0.3 – 1.8). The hazard of overall mortality through one year of follow-up among patients who did not receive the recommended antifungal treatment was 1.1 times the hazard of those who received the recommended initial treatment (95% CI 0.4 – 3.2), adjusted for underlying hematologic malignancy and severe disease.

*Appropriate Initial Treatment Dose*

Treatment dose had no discernible association with the outcome of persistence (Table 5.6). There was no significant association between the relative risk of persistence at four weeks out from treatment among surviving patients who did not receive recommended antifungal treatment dosing compared to those who initially received the recommended dosing (RR 1.1, 95% CI 0.6 – 1.8). Among patients with severe disease, the adjusted RR of treatment doses outside that of the recommended range and two-week persistence was also close to null (Table 5.6). If patient deaths during two and four weeks were considered persistent infection, the corresponding RRs were similar: 1.1 (95% CI 0.9 – 1.4) and 1.2 (95% CI 0.8 – 1.8).

The hazard rate of cryptococcosis-attributable mortality among patients who received treatment dosing outside what was recommended was 2.3 times the rate compared to patients who received the recommended dosing (95% CI 1.0 – 5.0) after adjusting for underlying hematologic malignancy, severe disease, and positive blood culture (Table 5.6).The adjusted

HR was 1.3 (95% CI 0.7 – 2.4), adjusting for underlying hematologic malignancy and positive blood culture.

*Flucytosine Use among Severe Cases*

The RR of two-week persistence and receiving flucytosine was similar to four weeks (Table 5.6). Among patients with severe disease (n=129) where flucytosine in combination with amphotericin B treatment is recommended, the adjusted risk of persistence at four weeks from initiation of treatment among surviving patients who received flucytosine as part of initial therapy was 0.6 times the risk of those who did not receive any flucytosine (Table 5.6; 95% CI 0.3 – 1.3). If patient deaths during two and four weeks were considered persistent infection, the corresponding estimates were: RR 0.8 (95% CI 0.5 – 1.4) and RR 0.8 (95% CI 0.4 – 1.4).

The adjusted hazard of overall mortality through one year of follow-up among patients who received flucytosine with their initial antifungal therapy was 0.4 times the hazard of those who did not receive flucytosine (95% CI 0.2 – 0.9) (Table 5.6). The HR of attributable mortality through one year of follow-up for flucytosine exposure was similar, though less precise (HR 0.5, 95% CI 0.2 – 1.2).

There was no clear association with risk of persistence (two or four weeks) between patients who received >7 days of flucytosine in combination with their primary antifungal therapy and those who received ≤7 days (Table 5.6). Receiving >7 days of flucytosine was protective of cryptococcosis-attributable mortality hazard compared to those who received ≤7 days of flucytosine, though the association was not significant (Table 5.6). Notably, full

flucytosine exposure was not possible for those that succumbed to acute mortality within 14 days of starting treatment (n=13, 50% of all attributable deaths).

*Additional Outcomes*

Re-induction with amphotericin B treatment was performed in 25% (n=29) of severe disease patients and 7% (n=5) of non-severe disease patients (Table 5.6). IRIS was diagnosed rarely (n=7; 3%) among all patients surviving through the end of initial therapy, and most of these were among patients with severe disease (n=6).

Pertaining to severe disease patients, renal toxicity during initial treatment occurred in approximately one-third of cases (n=33) and 26 of these patients did not complete 14 days of induction treatment. Out of the 33 cases with renal toxicity, 19 (58%) patients switched from their initial therapy. Fifteen of these 19 patients who experienced renal toxicity and switched initial therapy did not complete 14 days of induction treatment. Of the remaining 14 patients who experienced renal toxicity but did not switch from their initial therapy, 11 did not complete 14 days of induction treatment. Among the patients surviving at least 14 days (eligible to switch therapy within recommended treatment length), evidence of renal toxicity was associated with a higher relative risk of switching initial treatment ($RR_{crude}$ 1.9, 95% CI: 1.0 – 3.3).

**Discussion**

This study of cryptococcal management provides important insights into the disease in an advanced treatment center and presents additional data and support for the current

IDSA Guidelines. Retrospective reviews have limitations. However, they can provide crucial knowledge into "real world" effectiveness of therapy that is not captured in randomized controlled studies. The outcomes of individual cases are subject to their underlying disease, toxicity of medications and difficulties in understanding complications, such as IRIS and increased intracranial pressure. By studying the effectiveness of therapy we can evaluate the current standards of care, their real world outcomes, and identify obstacles for improving that care.

There were important differences in symptoms, underlying conditions and diagnostics based on cryptococcosis disease severity. Of note, cerebrospinal fluid opening pressure was similar between two divergent groups (one with and one without indicators of central nervous system disease) — even when the absence of a lumbar puncture for non-severe disease patients was considered negative results. This result was interesting knowing that the non-severe group was negative for central nervous system cryptococcosis. It demonstrates that an elevated cerebrospinal fluid opening pressure in patients with neurological symptoms can be an indicator for intracranial management [130], but the potentially low specificity observed in our study suggests that it may not serve as a precise diagnostic tool as it is presently measured at the bedside with a manometer. However, in clinical practice intracranial pressure measurements are importantly linked to other neurologic symptoms of disease such as headache or altered mental status. All of these factors remain essential for treatment and resolution of cryptococcal meningitis [34], but better and more precise measurement technology may be warranted.

Overall, appropriate initial treatment of cryptococcosis was high over the 14-year study period, with a recommended therapeutic regimen being used for 88% of patients and

the recommended dosing being followed in 66% of patients. Despite small numbers and retrospective nature of this study, there was favorable evidence for compliance and clinical results supporting the IDSA Guidelines. For instance, patients not receiving initial recommended treatment regimens had a higher risk of persistent infection at four weeks (RR 1.9) and a higher attributable mortality (RR 2.3) in those not receiving the recommended dose. However, over one-third of patients did change from their initial therapy; 28% of these patients were switched from a non-recommended to a recommended therapy given their disease severity. The consequences of this change in therapy resulted in patients who experienced an overall longer duration of induction compared to those who did not.

Flucytosine was used in 78% of severe disease patients for initial therapy, but only 37% of these recipients continued combination treatment for at least 14 days. High acute mortality (prior to completion of induction) and renal toxicity among severe disease patients likely contributed to this high incompletion rate. Nonetheless, patients receiving flucytosine combination therapy experienced lower rates of overall and attributable mortality than those patients who did not receive any flucytosine. However, receiving more than seven days of flucytosine was not significantly associated with lower mortality rates compared to those who received at most seven days, suggesting that early acute mortality may not be preventable with flucytosine use, but is a consequence of other underlying factors, such as malignancy or AIDS, contributing to poor patient outcome. On the other hand, the observed protective effects of flucytosine supports a growing body of evidence that combination therapy is important to a positive outcome [4,13,78]. The polyene and flucytosine combination has consistently demonstrated its superior success with retrospective data identifying better outcomes at two weeks[4], prospective randomized trial data on fungicidal

activity in the CSF [78], and correlation with this fungicidal activity and outcome [87,131]. Thus, our study highlights the importance for increased use of flucytosine. This may be improved with rapid access flucytosine levels and/or close follow-up of complete blood counts— elements needed to increase the likelihood patients will receive at least two weeks of flucytosine.

By our definition persistence at two weeks was a common outcome, but 75% of patients through four weeks did have documented resolution of symptoms and microbiological signs of disease. The percent of patients with disease resolution through four weeks falls into the same range of outcomes as previously published cohorts [2-5]. This persistence of infection rate likely reflects the high burden of *Cryptococcus* in the CSF of these patients and challenges us to be more aggressive about measuring how our treatment regimens are impacting the killing of yeasts during induction therapy.

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

This difference between attributable and overall mortality emphasizes how the specific underlying disease was a risk factor for poor outcomes. For example, a hematologic malignancy was a strong independent predictor of patient mortality in our cohort and likely reflects the end-stage of the underlying disease when cryptococcosis appears [111,115].

Fourteen out of 15 patients with hematologic malignancy were HIV-negative and had not received a solid organ transplant. In fact, 50% of cryptococcosis-attributable deaths were in HIV-negative, non-transplant patients with a variety of underlying diseases. Thus, identifying background rates of cryptococcosis and baseline risk factors for earlier diagnosis and assessing present treatment strategies in relationship to how they are efficiently handling the fungal burden could be very useful in reducing morbidity and mortality for this group.

Four major randomized treatment trials served as important comparators to this study and informed the treatment recommendations in the current IDSA Guidelines [13,18,19,22]. The key differences between these studies and this cohort was that this study was observational (non-randomized) and used a single-center instead of multiple sites for patient recruitment. Only one study [13] used a larger patient group for analysis and all but one study [19] recruited only cryptococcal meningitis cases. Our study spanned a much longer period of time (14 years compared to ≤5 years for the randomized trials). The continuity of care at a single-center allowed for such an extended period of in-depth retrospective observation. However, there were striking similarities between results in our study to these randomized trials. First, we observed a prevalence of cure or improvement through four weeks of 75% and Dismukes et al (1987) observed 80% cure through four weeks [19]. Second, through two weeks of combination induction therapy van der Horst et al (1997) reported 60% patients were culture negative [13]. In our study, 42% patients with severe disease had signs of persistent infection through two weeks. Third, attributable mortality and overall mortality were consistent with those found by three other randomized trials [18,22,108]. Among patients with severe disease in our study, 10% died in the first two weeks. This sobering figure demonstrates that acute mortality continues to be a serious

problem despite over three decades of clinical study and experience. Fourth, since timing of relapse was difficult to categorize in this observational study, re-induction might be considered a proxy for relapse—19% of our patients were re-induced. This finding was similar to the prevalence of relapse (16% in six week arm and 27% in four week arm) reported by Dismukes et al [19]**.** This relapse rate may be artificially high secondary to lack of IRIS appreciation, although in retrospective in our review, we found a relatively low incidence of this condition in our cohort.

Although an observational study design was used, we observed similar results as previous randomized trials. Taking into consideration our single-center attribute, the observed consistency in treatment may have had the unintended consequence of revealing underlying factors contributing to patient failure rather than determining whether variations in initial treatment were contributing to persistence of cryptococcosis or its attributable-mortality. In fact, positive CSF cultures, high CSF antigenemia, absence of headache and long duration of symptoms prior to admission continue to emerge in our analyses as strong predictors of patient failure rather than treatment regimens. Within these risk factors are likely buried important features of outcome regarding high burden of yeasts and poor host inflammatory responses within the CNS which must be carefully defined and monitored in relationship to therapy [132].

*Strengths and Limitations*

Our results may not be applicable to all centers in the USA as approaches for ancillary care may differ. Furthermore, using hospital records favors cases with severe disease and result in selection bias against asymptomatic or mild disease. Determining the

total population-at-risk was not estimable in this study and the underlying source population and referral patterns could shift over time.

In order to obtain a reportable picture of various outcomes, we created definitions of severe versus non-severe, persistence of infection (two and four weeks), attributable mortality, and we used the IDSA guidelines to define "appropriate" therapy. Regardless, the overall mortality rate in severe cryptococcosis remains high at almost 25% within one year.

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

Table 5.1. Baseline covariates prior to starting antifungal therapy by exposure status (recommended therapy according to IDSA Guidelines and flucytosine [5FC] exposure among patients with severe disease).

| | **Recommended initial treatment** | | | | **Recommended initial treatment dose** | | | | **5FC combination therapy, severe only** | | | | **5FC (days), severe 2-week survivors** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | | No | | Yes | | No | | Yes | | No | | ≤7 days | | >7days | |
| | n=179 | (%) | n=25 | (%) | n=126 | (%) | n=66 | (%) | n=101 | (%) | n=28 | (%) | n=35 | (%) | n=81 | (%) |
| Age >44 yrs.[a] | 97 | (54) | 9 | (36) | 69 | (55) | 30 | (45) | 46 | (46) | 16 | (57) | 14 | (40) | 41 | (51) |
| Symptoms, ≥14 d[a] | 70 | (39) | 12 | (48) | 53 | (42) | 27 | (41) | 48 | (48) | 7 | (25) | 11 | (31) | 40 | (49) |
| Severe disease | 118 | (66) | 11 | (44) | 75 | (60) | 42 | (64) | 100 | (100) | 0 | - | 35 | (100) | N/A | - |
| No symptoms | 18 | (10) | 3 | (12) | 15 | (12) | 6 | (9) | 1 | (<1) | 0 | - | 1 | (<1) | 0 | - |
| Altered mental status | 46 | (26) | 1 | (4) | 27 | (21) | 1 | (2) | 36 | (36) | 10 | (36) | 12 | (34) | 26 | (32) |
| Headache | 80 | (45) | 7 | (28) | 51 | (40) | 31 | (47) | 69 | (68) | 11 | (39) | 23 | (66) | 54 | (67) |
| Cough | 33 | (18) | 7 | (28) | 25 | (20) | 13 | (20) | 13 | (13) | 6 | (21) | 6 | (17) | 10 | (12) |
| Shortness of breath | 29 | (16) | 11 | (44) | 26 | (21) | 12 | (18) | 8 | (8) | 7 | (25) | 5 | (14) | 8 | (10) |
| Night sweats | 16 | (9) | 4 | (16) | 13 | (10) | 6 | (9) | 8 | (8) | 2 | (7) | 2 | (6) | 7 | (9) |
| Fever | 72 | (40) | 12 | (48) | 52 | (41) | 24 | (36) | 45 | (45) | 14 | (50) | 14 | (40) | 36 | (44) |
| Nausea | 57 | (32) | 5 | (20) | 38 | (30) | 19 | (29) | 39 | (39) | 11 | (39) | 15 | (43) | 33 | (41) |
| Vomiting | 47 | (26) | 3 | (12) | 29 | (23) | 16 | (24) | 32 | (32) | 9 | (32) | 12 | (34) | 27 | (33) |
| Seizures | 10 | (6) | 2 | (8) | 5 | (4) | 7 | (11) | 9 | (9) | 2 | (7) | 3 | (9) | 5 | (6) |
| Weight loss | 27 | (15) | 4 | (16) | 17 | (13) | 12 | (18) | 20 | (20) | 5 | (18) | 11 | (31) | 13 | (16) |
| Renal insufficiency | 28 | (16) | 4 | (16) | 21 | (17) | 8 | (12) | 14 | (14) | 3 | (11) | 4 | (11) | 13 | (16) |
| Liver insufficiency | 10 | (6) | 0 | (-) | 6 | (5) | 3 | (5) | 6 | (6) | 1 | (4) | 1 | (<1) | 5 | (6) |
| Corticosteroid | 65 | (36) | 10 | (40) | 47 | (37) | 23 | (35) | 33 | (33) | 7 | (25) | 6 | (17) | 28 | (35) |
| Hematologic malignancy | 10 | (6) | 5 | (20) | 11 | (9) | 4 | (6) | 5 | (5) | 3 | (11) | 4 | (11) | 1 | (1) |
| Non-hematologic malignancy | 5 | (3) | 1 | (4) | 4 | (3) | 2 | (3) | 1 | (1) | 1 | (4) | 1 | (<1) | 1 | (1) |
| Transplant recipient | 39 | (22) | 3 | (12) | 30 | (24) | 9 | (14) | 15 | (15) | 3 | (11) | 3 | (9) | 15 | (19) |
| HIV positive | 76 | (42) | 10 | (40) | 53 | (42) | 29 | (44) | 59 | (58) | 15 | (54) | 23 | (66) | 45 | (56) |
| Exposure to HAART[b] | 30 | (39) | 6 | (60) | 22 | (42) | 12 | (41) | 20 | (34) | 11 | (73) | 16 | (70) | 14 | (31) |

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

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

Table 5.2. Patient diagnostics at baseline prior to starting antifungal therapy, stratified by to exposure status (recommended therapy according to IDSA Guidelines and flucytosine exposure among patients with severe disease).

| | **Recommended initial treatment** | | | | **Recommended initial treatment dose** | | | | **Flucytosine combination therapy, severe only** | | | | **Flucytosine exposure (days), severe 2-week survivors** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes n=179 | (%) | No n=25 | (%) | Yes n=126 | (%) | No n=66 | (%) | Yes n=101 | (%) | No n=28 | (%) | ≤7 days n=35 | (%) | >7days n=81 | (%) |
| Positive cultures | | | | | | | | | | | | | | | | |
| CNS– first LP[a] | 96 | (54) | 6 | (24) | 59 | (47) | 35 | (53) | 84 | (83) | 17 | (61) | 24 | (69) | 69 | (85) |
| Blood | 56 | (31) | 10 | (40) | 38 | (30) | 24 | (36) | 42 | (42) | 10 | (36) | 10 | (29) | 35 | (43) |
| Pulmonary | 39 | (22) | 7 | (28) | 29 | (23) | 15 | (23) | 7 | (7) | 3 | (11) | 3 | (9) | 8 | (10) |
| Positive histology | 52 | (29) | 8 | (32) | 38 | (30) | 20 | (30) | 15 | (15) | 3 | (11) | 7 | (20) | 10 | (12) |
| Serum antigen titer ≥1:1024 | 78 | (44) | 10 | (40) | 58 | (46) | 26 | (21) | 64 | (51) | 11 | (39) | 21 | (60) | 49 | (60) |
| First LP[b] | | | | | | | | | | | | | | | | |
| CSF antigen titer ≥1:1024 | 48 | (56) | 2 | (13) | 87 | (82) | 45 | (82) | 44 | (44) | 5 | (23) | 10 | (30) | 32 | (40) |
| CSF:Serum glucose ratio <0.6 | 117 | (82) | 14 | (70) | 78 | (78) | 45 | (83) | 90 | (93) | 20 | (87) | 27 | (82) | 74 | (95) |
| CSF glucose ≤40 | 69 | (45) | 6 | (30) | 47 | (44) | 20 | (36) | 58 | (58) | 16 | (67) | 18 | (53) | 52 | (64) |
| CSF protein ≥45 | 143 | (86) | 14 | (70) | 31 | (32) | 17 | (33) | 91 | (94) | 23 | (96) | 29 | (88) | 77 | (97) |
| Positive India ink stain | 61 | (44) | 1 | (5) | 36 | (37) | 23 | (46) | 55 | (63) | 6 | (2) | 11 | (35) | 44 | (60) |
| LP OP ≥20cm $H_2O$[b] | 76 | (73) | 10 | (63) | 54 | (72) | 29 | (73) | 39 | (66) | 14 | (78) | 18 | (55) | 28 | (61) |

[a] Lumbar puncture; 176 total patients had an LP performed. Denominators used for percentages according to exposure (across) were: 85, 15, 106, 55, 101, 23, 33 and 81 (CSF antigen titer ≥1:1024); 143, 20, 100, 54, 97, 23, 33, and 78 (CSF: Serum glucose ratio <0.6); 153, 20, 107, 55, 100, 24, 34, and 81 (CSF glucose ≤40); 166, 20, 98, 51, 97, 24, 33, and 79 (CSF protein ≥45); 138, 19, 97, 50, 88, 23, 31 and 73 (positive India ink stain).

[b] In an effort to capture more opening pressure measurements, maximum LP opening pressure was used for this variable, as first LP did not always measure opening pressure. There were 157 (60%) patients who had an LP opening pressure reading. Denominators used for percentages according to exposure (across) were: 104, 16, 75, 40, 59, 18, 33, and 46.

Table 5.3. Initial antifungal regimen by baseline severity of disease

| **Antifungal treatment exposures** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Initial therapy | | Total (N=204) | | Severe (n=129) | | Non-severe (n=75) | |
| | | N | (%) | n | (%) | n | (%) |
| | AmpBd | 18 | (9) | 13 | (10) | 5 | (7) |
| | AmpBd+ 5FC | 88 | (43) | 82 | (64) | 6 | (8) |
| | ABLC[a] | 5 | (2) | 2 | (2) | 3 | (4) |
| | ABLC+5FC | 12 | (6) | 12 | (9) | 0 | - |
| | L-AmpB[a] | 2 | (<1) | 2 | (2) | 0 | - |
| | L-AmpB +5FC | 7 | (3) | 7 | (5) | 0 | - |
| | Fluconazole | 71 | (34) | 10 | (7) | 61 | (81) |
| | Other | 1 | (<1) | 1 | (<1) | 0 | - |
| Total (appropriate therapy)[b] | | 179 | (88) | 118 | (92) | 61 | (81) |
| Initial dose[c] | | Total (N=192) | | Severe (n=117)[d] | | Non-severe (n=75)[d] | |
| | | N | (%) | n | (%) | n | (%) |
| | AmpBd (n=98) | 54 | (55) | 50 | (57) | 4 | (36) |
| | ABLC (n=15) | 13 | (87) | 10 | (9) | 3 | (4) |
| | L-AmpB (n=8) | 7 | (88) | 7 | (6) | 0 | (-) |
| | Fluconazole (n=71) | 52 | (73) | 8 | (7) | 44 | (57) |
| Total (appropriate dose) | | 126 | (66) | 75 | (64) | 51 | (68) |
| Therapy changes | | Total (N=204) | | Severe (n=129) | | Non-severe (n=75) | |
| | | N | (%) | n | (%) | n | (%) |
| | Fluconazole only | 60 | (29) | 3 | (2) | 57 | (76) |
| | AmpBd only | 60 | (29) | 53 | (41) | 7 | (9) |
| | LFAmpB only | 23 | (11) | 22 | (17) | 1 | (1) |
| | Voriconazole only | 1 | (<1) | 1 | (<1) | 0 | - |
| | Fluconazole to AmpBd | 9 | (4) | 6 | (5) | 3 | (4) |
| | Fluconazole to LFAmpB | 2 | (1) | 1 | (<1) | 1 | (1) |
| | AmpBd to LFAmpB | 46 | (23) | 42 | (33) | 4 | (5) |
| | LFAmpB to AmpBd | 3 | (2) | 1 | (<1) | 2 | (3) |
| Total (therapy changed) | | 60 | (29) | 50 | (39) | 10 | (13) |

[a] ABLC = Amphotericin B Lipid Complex, L-AmpB = liposomal amphotericin B

[b] Therapy was appropriate (recommended) with respect to the 2010 IDSA Guidelines [1].

[c] Initial therapy dose was within appropriate (recommended) range for each drug defined by the 2010 IDSA Guidelines [1].

[d] Denominators for each drug by severity of disease are n=87, 12, 8, and 10 patients for severe disease; n=11, 3, 0 and 61 patients for non-severe disease, respectively.

Table 5.4. Dosing (mg/kg/day) of initial therapy and baseline disease severity among patients receiving antifungal therapy and with dosing information.

| | Total (N=192)[b] | | Severe (n=117)[c] | | Non-severe (n=75)[c] | |
|---|---|---|---|---|---|---|
| Antifungal Therapy[a] | Mean | IQR | Mean | IQR | Mean | IQR |
| Amphotericin B (n=121) | | | | | | |
| AmpBd | 0.66 | 0.56 – 0.73 | 0.66 | 0.57 – 0.73 | 0.65 | 0.48 – 0.84 |
| ABLC[d] | 4.90 | 4.62 – 5.20 | 4.86 | 4.54 – 5.14 | 5.06 | 4.80 – 5.47 |
| L-AmpB[d] | 4.28 | 3.29 – 5.05 | 4.28 | 3.29 – 5.05 | n/a | - |
| Fluconazole (n=71) | 350 | 208 – 400 | 367 | 400 – 400 | 346 | 208 – 400 |

[a] Fluconazole dosing is in mg/day. All other medications are in mg/kg/day.
[b] 12 patients were missing information on initial treatment dose (either dosing or body weight information).
[c] Denominators for each antifungal drug (top to bottom) are n=87, 12, 8, and 10 patients for severe disease; n=11, 3, 0 and 61 patients for non-severe disease, respectively.
[d] ABLC = Amphotericin B Lipid Complex, L-AmpB = liposomal amphotericin B.

Table 5.5. Patient outcomes after antifungal therapy, according to treatment exposure status.

| | **Recommended initial treatment** | | | | **Recommended initial treatment dose** | | | | **Flucytosine combination therapy, severe only** | | | | **Flucytosine exposure (days), severe, 2-week survivors** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcomes | Yes | (%) | No | (%) | Yes | (%) | No | (%) | Yes | (%) | No | (%) | ≤7days | (%) | >7days | (%) |
| Persistence, 2 weeks[a] | | | | | | | | | | | | | | | | |
| Yes | 52 | (50) | 4 | (40) | 33 | (42) | 17 | (47) | 46 | (50) | 10 | (43) | 16 | (46) | 40 | (50) |
| No | 53 | (50) | 6 | (60) | 37 | (47) | 19 | (53) | 46 | (50) | 13 | (57) | 19 | (54) | 40 | (50) |
| Persistence, 4 weeks | | | | | | | | | | | | | | | | |
| Yes | 39 | (24) | 6 | (27) | 26 | (25) | 15 | (26) | 29 | (33) | 8 | (33) | 11 | (34) | 26 | (33) |
| No | 121 | (76) | 16 | (73) | 89 | (85) | 43 | (74) | 60 | (67) | 14 | (67) | 21 | (66) | 53 | (67) |
| Cryptococcosis-attributable mortality | | | | | | | | | | | | | | | | |
| Yes | 26 | (15) | 4 | (16) | 12 | (10) | 14 | (21) | 18 | (11) | 8 | (29) | 6 | (17) | 7 | (9) |
| No | 153 | (85) | 21 | (84) | 114 | (90) | 52 | (79) | 83 | (89) | 20 | (71) | 29 | (83) | 74 | (91) |
| One-year mortality | | | | | | | | | | | | | | | | |
| Yes | 41 | (23) | 8 | (32) | 26 | (21) | 18 | (27) | 22 | (22) | 12 | (43) | 7 | (20) | 14 | (17) |
| No | 138 | (77) | 17 | (68) | 100 | (79) | 48 | (73) | 79 | (78) | 16 | (57) | 28 | (80) | 67 | (83) |
| Patient re-induced[b] | | | | | | | | | | | | | | | | |
| Yes | 29 | (18) | 5 | (23) | 20 | (17) | 11 | (19) | 22 | (19) | 7 | (30) | 8 | (23) | 21 | (26) |
| No | 132 | (82) | 17 | (77) | 95 | (83) | 48 | (81) | 71 | (81) | 16 | (70) | 27 | (77) | 60 | (74) |
| IRIS[c] | | | | | | | | | | | | | | | | |
| Yes | 6 | (4) | 1 | (5) | 4 | (3) | 3 | (5) | 6 | (6) | 0 | (0) | 1 | (3) | 5 | (6) |
| No | 155 | (96) | 21 | (95) | 111 | (97) | 57 | (95) | 87 | (94) | 23 | (100) | 34 | (97) | 76 | (94) |
| Renal toxicity[d] | | | | | | | | | | | | | | | | |
| Yes | 30 | (31) | 3 | (43) | 23 | (35) | 9 | (28) | 27 | (32) | 6 | (30) | 10 | (32) | 23 | (32) |
| No | 67 | (69) | 4 | (57) | 42 | (65) | 23 | (72) | 57 | (68) | 14 | (70) | 21 | (68) | 50 | (68) |

[a] Among severe cases only: 2-week persistence: N=115 (13 deaths; 1 missing information), for 4-week persistence; N=182 (5 additional deaths since 2 weeks and 3 missing information).

[b] Patient had to survive through 2 weeks for severe disease and 90 days for non-severe disease (end of recommended treatment duration) to be eligible for denominator of re-induction status.

[c] Immune Reconstitution Inflammatory Syndrome; patient had to survive through 2 weeks for severe disease and 90 days for non-severe disease (end of recommended treatment duration) to be eligible for IRIS diagnosis.

[d] >50% drop in Glomerular Filtration Rate (among severe disease only)—measured from the date closest to the start of therapy (week 0) to the date closest to 2 weeks after starting therapy (week 2). Patient had to survive through 2 weeks (end of recommended treatment duration) to be eligible for renal toxicity outcome.

Table 5.6 Final adjusted models of the influences of treatment type, dosing, and flucytosine use (severe disease only) on the risk of persistence of disease at two weeks (severe disease only) and four weeks (both groups) and mortality hazard. Persistence outcomes were contingent on survival through two weeks and four weeks since the date of starting antifungal therapy.

| | Initial therapy type[a] | | Initial therapy dose[b] | | Flucytosine[c] | | Flucytosine[d] | |
|---|---|---|---|---|---|---|---|---|
| Outcomes | RR | (95% CI) | RR | (95% CI) | RR | (95% CI) | RR | (95% CI) |
| Persistence at two weeks (severe only) | 1.4 | (0.5 – 3.5) | 1.1 | (0.8 – 1.4) | 0.8 | (0.4 – 1.6) | 0.9 | (0.7 – 1.2) |
| Persistence at four weeks | 1.9 | (0.9 – 4.3) | 1.1 | (0.6 – 1.8) | 0.6 | (0.3 – 1.3) | 0.8 | (0.4 – 1.3) |
| | HR | (95% CI) | HR | (95% CI) | HR | (95% CI) | HR | (95% CI) |
| Cryptococcosis-attributable mortality | 1.1 | (0.4 – 3.2) | 2.3 | (1.0 – 5.0) | 0.5 | (0.2 – 1.2) | 0.6 | (0.2 – 1.8) |
| One-year all-cause mortality | 0.8 | (0.3 – 1.8) | 1.3 | (0.7 – 2.4) | 0.4 | (0.2 – 0.9) | 1.1 | (0.4 – 2.7) |

[a] Patient not given recommended antifungal therapy regimen initially(1) or received recommended therapy (0); Persistence at two weeks adjusted for high cryptococcal CSF antigen titer (≥1:1024). Persistence at four weeks adjusted for high cryptococcal CSF antigen titer (≥1:1024) and severe disease. Cryptococcosis-attributable mortality was adjusted for underlying hematologic malignancy and positive blood culture. One-year all-cause mortality was adjusted for confounding by severe disease and underlying hematologic malignancy.

[b] Patient dose for initial antifungal therapy was outside the range of recommended (1) or dosing was within recommended range (0); Persistence at two weeks was adjusted for high cryptococcal CSF antigen titer (≥1:1024). Persistence at four weeks adjusted for severe disease. Cryptococcosis-attributable mortality was adjusted for underlying hematologic malignancy, positive blood culture and severe disease. One-year all-cause mortality was adjusted for confounding by positive blood culture and underlying hematologic malignancy.

[c] Flucytosine was used with initial therapy among severe disease only (n=129). Persistence at 2 weeks adjusted for high cryptococcal CSF antigen titer (≥1:1024); persistence at 4 weeks adjusted for high cryptococcal CSF antigen titer (≥1:1024) and ≥14 days of symptoms prior to presentation; both cryptococcosis-attributable mortality and overall mortality adjusted for underlying hematologic malignancy and receipt of amphotericin B deoxycholate.

[d] Flucytosine exposure categories were >7 days (referent) and ≤ 7 days of exposure. Two-week persistence adjusted for high CSF cryptococcal antigen titer (≥1:1024) at first LP; four-week persistence adjusted for ≥14 days of symptoms prior to presentation and high initial CSF cryptococcal antigen titer (≥1:1024) at first LP; attributable mortality and overall mortality adjusted for underlying hematologic malignancy.

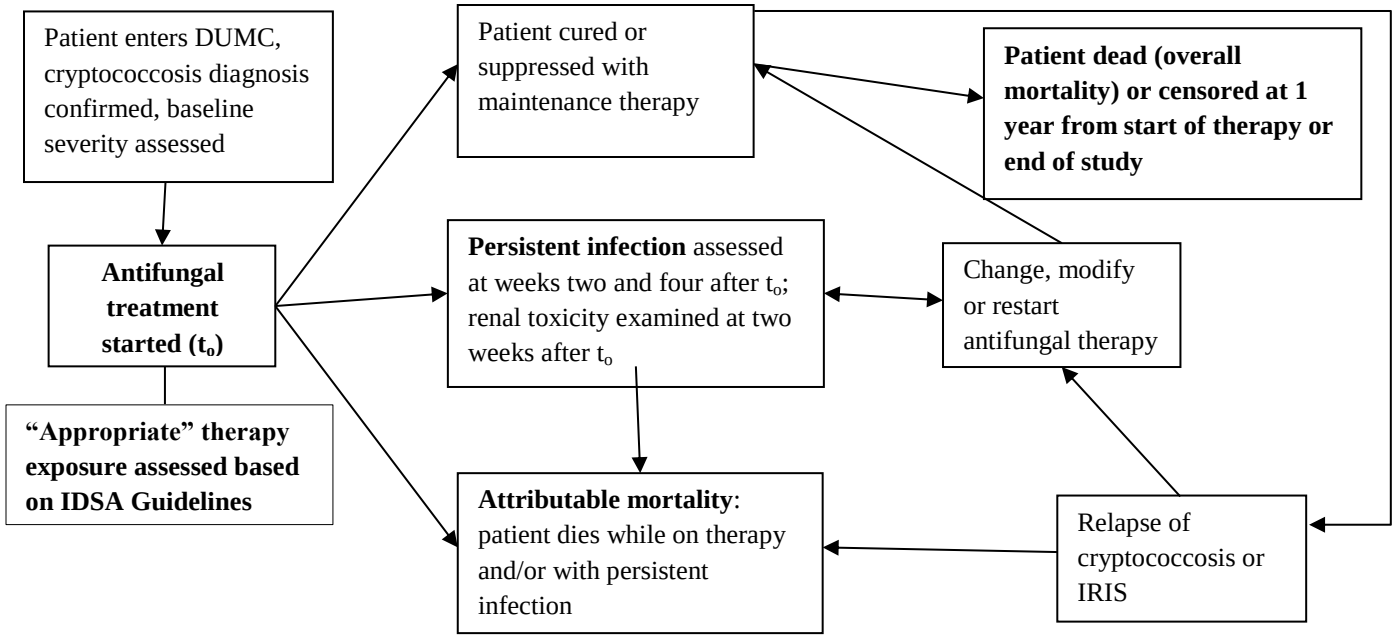


Figure 5.1. Diagram of patient flow scenarios from entry through one year of follow-up.

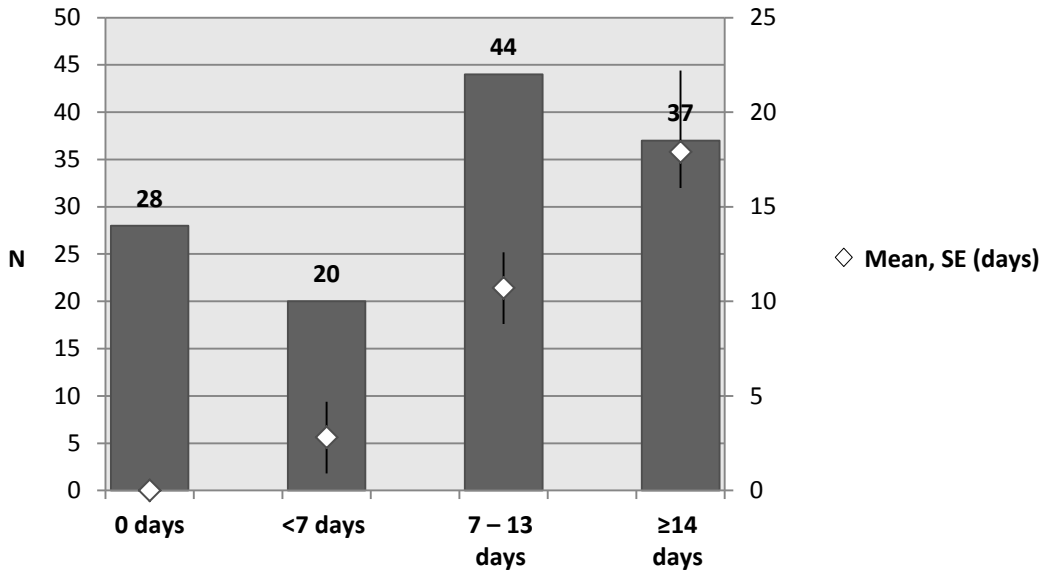


Figure 5.2. Duration of flucytosine combination treatment with initial primary therapy (severe disease, n=129)

# CHAPTER VI. DISCUSSION

## Study summary

There were two main goals for this study: to provide an in-depth description of three underlying risk groups with cryptococcosis (HIV-infected, transplant recipients and HIV-negative, non-transplant) and to investigate antifungal treatment effectiveness from 1996 through 2009 at Duke University Medical Center. Importantly, both of these aims were developed in order to assess the practical implementation of the 2010 IDSA Guidelines as a tool for clinical management of cryptococcosis patients. In the first aim the three groups were described and compared with regard to clinical presentation, immunosuppression, initial antifungal therapy and mortality. Also, global outcomes for utilization of lipid amphotericin B as initial therapy as well as trends in frequency of diagnoses between the three groups were examined through the 14-year study period. In the second aim we evaluated the effect of initial treatment on the outcomes of persistent cryptococcosis, cryptococcosis-attributable mortality and overall mortality through one year from the initiation of antifungal therapy.

## The three groups

There were notable trends observed between and within each of the three cryptococcosis groups. We found that the mean duration of symptoms reported prior to diagnosis was longest in the HIV-negative, non-transplant group. In this group, duration of symptoms was approximately three weeks longer among those with severe disease and six

weeks longer among patients with non-severe disease compared to the other two groups. Between-group means were not all significant. However among non-severe patients, transplant recipients and HIV-negative, non-transplant cases had statistically significant different means of symptom duration. Longer duration of symptoms prior to presentation in this latter group was observed in a previous study [68], while another study found a lack of significance between HIV-infected, immunocompromised, and immunocompetent groups [62]. Greater attention should be placed on barriers to earlier diagnosis, especially considering its potential influence on poorer outcome. Headache, nausea, and vomiting are symptoms commonly associated with severe disease, yet in our study HIV-negative, non-transplant patients with severe disease reported these symptoms markedly less than the other two groups. Altered mental status, another symptom of CNS disease, was similar across the three groups in our cohort, while another study reported significantly more mental status changes in non-immunosuppressed patients compared to HIV-positive patients [62]. Based on our evidence, the absence of indicators for severe disease could be a contributing factor in delayed diagnosis in the HIV-Negative, non-transplant group.

Diagnostic testing by lumbar puncture among severe disease cases revealed that the proportion of elevated CSF CRAG titer (≥1:1024) and positive India ink tests in the HIV-negative, non-transplant group were significantly lower than the other two groups. Non-severe disease patients in this group also underwent fewer lumbar puncture tests to rule out CNS disease; highlighting the possibility that this group lacks clinical and diagnostic evidence of disease that results in delayed diagnosis. This may in turn influence downstream outcomes such as attributable mortality. These diagnostic findings, despite lacking a measurable appreciation for burden of organisms, underscore the potential value in the

quantification of viable yeasts in the CSF (Colony-forming unit [CFU]/mL measurements). Given the relationship of fungal burden to treatment strategy and outcome, understanding its rate of change during therapy is important [78,90,123].

**Temporal trends**

Case frequencies at DUMC hovered around 15 cases annually, with an apparent shift to a decreasing proportion of HIV-positive patients with a concomitant increase in HIV-negative cases. HIV-positive patients accounted for half of all cases in the first seven years of this study then fell to less than one-third in the latter seven years. As we continue to aggressively treat serious underlying diseases with immunosuppressants and the denominator of persons-at-risk enlarges, we hypothesize that the HIV-negative, non-transplant group will continue to increase given there is currently no strategy for antifungal prophylaxis for this population. There were a consistent number of cases of cryptococcosis in the transplant recipients over time despite the widespread use of calcineurin inhibitors, which potentially have anti-cryptococcal properties [56,113,114,115,116]. The steady annual prevalence of cryptococcosis in this group supports a continued need of careful diagnostic surveillance for the early detection of cryptococcosis while patients are on sustained immunosuppressive therapy [117].

During the 14-year study period, 132 patients (64%) received amphotericin B (either formulation) for initial therapy. Of these 132 patients, AmpBd was used as initial therapy for the majority of patients (80%). Utilization of AmpBd for initial therapy decreased over time, indicating that LFAmpB was used more frequently as initial therapy in recent years. There is a lack of studies reporting site-specific annual patterns of initial therapy for the basis of

comparison. Given the approximately one-third of patients who experienced nephrotoxicity during management in our study we expect LFAmpB to continue to play an important role in anti-cryptococcal therapy. Further investigation into lipid products of amphotericin B is warranted to ensure their optimal use for patients where this formulation is recommended as initial therapy, as well as for those who may need to switch to it due to the toxic effects of AmpBd.

**Persistence, mortality and secondary outcomes**

Among patients with severe disease, persistence was 55% two weeks after starting initial therapy then decreased to 33% at four weeks. Comparatively, among non-severe disease patients, persistence was 11% at four weeks. The percent of patients with disease resolution through four weeks falls into the same range of outcomes as previously published cohorts [2-5]. Mortality attributable to cryptococcosis was 15% (n=31) and overall mortality was 25% (n=52) through one year of follow-up. Notably, all-cause mortality of severe disease patients (26%) was similar to non-severe disease patients (20%), yet when comparing attributable mortality, non-severe disease patient mortality (5%) was much lower than severe disease patient mortality (20%). This demonstrates the importance in distinguishing between attributable and overall mortality when presenting effect estimates of cryptococcosis on patient survival.

Among patients with severe disease, early attributable mortality during induction therapy was high, which limited the analysis of treatment-related effects. Half of the 26 severe disease patients who died due to cryptococcosis were within the first two weeks while receiving induction treatment; three more died by four weeks. Non-severe disease patients

had much lower mortality, with only three deaths (5%; two attributable to cryptococcosis) within the first month of follow-up from the start of initial therapy.

The HIV-negative, non-transplant group experienced both greater mortality attributable to cryptococcosis and overall mortality, accounting for nearly half of all attributable deaths and more than half of all-cause mortality. HIV-infected patients accounted for the majority of the remaining deaths. A recent multi-center study of 86 cryptococcal meningitis patients also found the highest mortality in the non-immunosuppressed group (46%), compared to immunosuppressed (19%) and HIV-positive patients (15%) [62]. Previous studies have reported 30 – 44% overall mortality in the HIV-negative population [21,68,69,70]. In one of these studies, this rate was compared to a 22% mortality among HIV-positive patients [69]. Of note, some of these studies also included *C.gatii* cases, and factors of that species may have influenced outcome [69,70].

Renal toxicity and IRIS are important additional outcomes that can be influenced by initial antifungal treatment choices and impact subsequent clinical management of the patient. IRIS was identified in all three groups, but the prevalence was low (4%) compared to other studies which primarily included only AIDS patients (range, 8–19%) [92,93,124,125]. Four out of seven IRIS cases in our study were HIV-infected patients. Renal toxicity among patients with severe disease occurred in nearly one-third of cases during initial treatment, and 58% of these patients did not complete the recommended 14 days of induction therapy. Among the patients surviving at least 14 days, therefore eligible to experience switching therapy within the recommended treatment window, there was a significant association between switching therapy and evidence of renal toxicity ($RR_{crude}$ 1.9, 95% CI: 1.0 – 3.3).

**Treatment effectiveness**

Our study modeled the effect of three elements of initial treatment exposure (recommended drug, dose, and flucytosine combination therapy) on three outcomes—persistence, attributable mortality and overall mortality. We found that the administration of recommended initial treatment in this retrospective cohort adhered consistently to the IDSA Guidelines, with an appropriate therapeutic regimen being used for 88% of patients and the recommended dosing being followed as recommend in 66% of patients.

Our results of this support the current IDSA Guideline bearing in mind the limited number of cases and retrospective nature of this study. For instance, not receiving the recommended antifungal regimen initially was associated with a higher relative risk of persistent infection at four weeks (RR1.9, 95% CI 0.9 – 4.3). Also the risk of attributable mortality among those not receiving the recommended dose of initial therapy was higher relative to those receiving appropriate dosing (RR 2.3, 95% CI 1.0 – 5.0). Over one-third of patients changed from their initial therapy; 28% of these patients were switched from a non-recommended to a recommended therapy based on their disease severity. The consequences of this change in therapy resulted in patients who experienced an overall longer duration of treatment compared to those who did not. Future studies should further examine how a patient's length of induction therapy is influenced by changing amphotericin B formulations, which could be a consequence of toxicities rather than persistence of disease.

Flucytosine was used in 78% of severe disease patients for combination therapy, but only 37% of these recipients continued combination treatment for at least 14 days as recommended. Renal toxicity likely contributed to early cessation of flucytosine therapy. Flucytosine exposure was associated with a lower overall mortality rate (HR 0.4, 95% CI 0.2

– 0.9); ≥7 days of exposure was associated with a lower rate of attributable mortality relative to those with 0 – 7 days of flucytosine with initial treatment (HR 0.6, 95% CI 0.2 – 1.8). This observation stresses the importance of exposure to combination therapy with polyene and flucytosine, and supports a growing body of evidence that this fungicidal regimen is critical for treatment success. The polyene and flucytosine combination has demonstrated its superior success based on evidence from retrospective data identifying better outcomes at two weeks [4], prospective randomized trial data on fungicidal activity in the CSF [78], and correlation with this fungicidal activity and outcome [87,131].

**Conclusions**

This study of "real world" clinical management of cryptococcosis revealed some important observations. Unfortunately, acute mortality remains a serious concern. Future clinical studies should focus on earlier identification, diagnosis and treatment, as well as strategic clinical management of this high-risk group. Additionally, sustained flucytosine combination therapy was inconsistent among severe disease patients in this study. Longer exposure to combination antifungal therapy resulted in lower rates of mortality relative to patients receiving little or no flucytosine. Our study emphasized the need for clinicians to use flucytosine more consistently. In order to assess therapeutic dosing, rapid access to serum flucytosine levels could improve adherence and potentially avoid issues of toxicity. Due to our findings regarding the importance of remaining on flucytosine combination therapy to reduce patient morbidity and mortality, further investigation into factors contributing to toxicity is warranted. Renal toxicity was common and negatively influenced completion of antifungal therapy. In these cases, lipid formulation amphotericin B may be a

better alternative for initial primary therapy to amphotericin B deoxycholate, as changing during induction treatment can lead to longer durations of therapy and greater costs.

Our description of the three groups with cryptococcosis revealed that HIV-negative, non-transplant patients had a longer duration of symptoms prior to presentation. In this group a fewer proportion of patients presented with symptoms and/or diagnostics indicating severe disease. These factors could have contributed to delayed diagnosis and poorer outcomes observed relative to the other two groups. HIV-negative, non-transplant patients experienced the highest mortality, and hematologic malignancy was strongly associated with patient mortality in our cohort. This likely reflected the end-stage of the underlying disease when cryptococcosis was diagnosed [111,115]. More studies of this group are needed in order to identify background rates of cryptococcosis, baseline risk factors, and symptoms for earlier diagnosis. Additionally, it would be valuable to assess present treatment strategies and their relationship to changes in fungal burden, so as to reduce poor outcomes in this population.

Out of the four initial treatment exposure and patient outcome categories modeled in this study, there were some notable findings. First of all, not receiving the recommended initial therapy was associated with an increased risk in persistence at four weeks relative to the risk among those who received recommended therapy. Additionally, not receiving the recommended initial therapy dose was associated with a higher mortality hazard rate relative to receiving the recommended dose. Furthermore, receiving flucytosine combination therapy for initial treatment as recommended for severe disease patients was associated with a lower mortality hazard rate relative to those only receiving amphotericin B.

Antifungal therapy approaches used in our medical center were largely concurrent with the treatment algorithms provided by the IDSA Guidelines. Most patients with severe disease received amphotericin B for initial treatment and the majority of non-severe patients received fluconazole as primary therapy. While we summarized the intricacies of stopping and changing treatment choices in our study, due to limited sample size, we did not examine the causal effects of switching therapy on patient outcome. Nonetheless, examining initial therapy exposures yielded valuable insights into the treatment approaches used for cryptococcosis across patient groups with various underlying conditions and disease presentations. The future of cryptococcosis treatment and the development of new antifungal therapies should not only be informed by randomized trials, but also by key observational trends that help to identify problematic applications of drug regimens to diverse populations.

## APPENDIX A. PATIENT ABSTRACTION FORM

**Study Number_____**

(Write this number in upper right hand corner of every page)

***Cryptoccocus* infections in the era of lipid formulation of amphotericin B**

**Database form**

(This front sheet will be torn off after data entry and stored separately to de-identify patients)

**Patient name (not to be entered in database):_____**

**History Number (not to be entered into database): _____**

**All the information from hospital admissions and follow ups will be documented if these were related directly to <u>cryptococcal infections.</u>**

**Please duplicate tables if need more space. All dates should be entered as "mm/dd/yy."**

**First Date of Hospital Admission (MM/DD/YY)*: _____/_____/_____**
**Date of Hospital Discharge (MM/DD/YY): _____/_____/_____**
**Or**
**Date of Death (MM/DD/YY): _____/_____/_____**

Subsequent Admissions (with Crypto dx on discharge):
2. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
3. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
4. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
5. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
6. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
7. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____
8. Date of Hospital Admission (MM/DD/YY): _____/_____/_____
Date of Hospital Discharge (MM/DD/YY): _____/_____/_____

**Geographical location:** Where does patient live at time of Dx?
(Example: Durham, NC) _____ **Country (if not US) _____**

**Primary Discharge diagnosis**

□□CNS cryptococcus infection □ □Pulmonary Cryptococcus infection
□□Crypto infection at other site, Specify: _____

**Demographic information**

**Age** (at time of diagnosis): _____ **Sex**: □□ M □□ F
**Birthdate**: _____/_____/_____
**Race/ethnicity**:
□ African American □ □ Caucasian□ □ Hispanic□
□ Asian □ □ American Indian □ □ Other □
□Unknown□
**Weight***:_____ **Date taken**: _____/_____/_____
*important for those who received flucytosine

**IMMUNE STATUS**

**HIV Status on admission**

□ positive□ □ negative □ □ unknown □

**Diagnosis of HIV made during this admission** □ yes □ □ no □

**Date of HIV Diagnosis: _____/_____/_____**

**Antiretroviral therapy prior to 1st admission for Crypto?**

□ Yes □ □ no□

If yes please list the name of antiretrovirals:_____

Evidence of noncompliance*

□ Yes □ □ no □

**Last CD4 count prior to *therapy* for cryptococcus _____**

**Date:_____/_____/_____**

**Please list all CD4 counts <u>after</u> Cryptococcus diagnosis was made.** Circle Yes or No when applicable.

| CD4 count | Date (mm/dd/yy) | Still on Crypto therapy (Y/N) | ARV therapy (Y/N) |
|---|---|---|---|
| | | Y N | Y N |
| | | Y N | Y N |
| | | Y N | Y N |
| | | Y N | Y N |
| | | Y N | Y N |
| | | Y N | Y N |

**Notes:**

***if the patient has a history of noncompliance with regard to HIV therapy, please briefly describe here.**

****IF NOT A TRANSPLANT PATIENT, SKIP PART C****

**C. Transplant Status:**

**1. Transplanted organ** □ Yes□ □ No □

□ Renal □ □ Cardiac □ □ Pulmonary □ □ Liver□ □ Pancreas□ □ Heart-lung□

Date of transplant _____/_____/_____

**2. Stem Cell Transplant Recipient** □ Yes□ □ No □

□ Allo □ □ Auto □ □ Cord Blood □

Date of Transplant: _____

Did the patient have GVHD within 6 months of diagnosis: □ Yes□ □ No □

**Type of immunosuppression**:

□ Calcineurin inhibitor □ □ glucocorticoid □ □ MMF □ □ AZA □ □ Methotrexate □

□ Monoclonal antibodies (please specify which e.g alemtuzumab(campath), antiTNFalpha antibodies, daclizumab, OKT3, Rituximab or other) □_____

□ Sirolimus □

Type of calcineurin inhibitor: □ cyclosporine □ □ tacrolimus □

Type of steroid: _____

Daily dose of steroids the day of Cryptococcus diagnosis: _____

Other immunosuppressants: _____

Duration of immunosuppressant up to diagnosis of crypto: _____

Date of starting immunosuppressant: _____

**D. Other causes for immunosuppression**

□ Steroid therapy □ □ Immunosppressants (other than steroids) □

Chronic organ failure: □ renal □ □ hepatic□ □ rheumatologic disorder □

□ chronic lung disease □ □ hematologic malignancies□ □ other malignancies□

□ Other:_____

**Type of immunosuppression**:

□ Calcineurin inhibitor □ □ glucocorticoid □ □ MMF □ □ AZA □

□ Sirolimus □□ Methotrexate □

□ Monoclonal antibodies (please specify which e.g alemtuzumab(campath), antiTNFalpha antibodies, daclizumab, OKT3, Rituximab or other) □_____

Type of calcineurin inhibitor: □ cyclosporine □ □ tacrolimus □

Type of steroid _____

Daily dose of steroids at time of treatment_____

□ Other immunosuppressants_____

Duration of immunosuppressant up to diagnosis of crypto:_____

Date of starting immunosuppressant:_____

**E. Changes made in immunosuppressants after diagnosis of cryptococcal infection**

Was immunosuppressive therapy weaned during cryptococcal therapy: □ Yes □□ No □

If yes, which agent was dose-reduced: _____ Date: _____

Drug concentrations while receiving anti-crypto therapy (list): _____

_____

**DIAGNOSIS**

**Physical Findings/Symptoms on admission/diagnosis**

**1. General** □Night sweat□ □ Weight loss□ □Fever □Temperature if measured: _____
□Nausea□ □vomiting □
□ Other:_____

**2. CNS/neurological** □Papilledema □ Altered mental state □cranial neuropathy □
□Hearing loss□ □Meningismus □Behavioral changes □Seizures□
□Cerebellar signs□ □Headache □memory changes □ □ Pathologic reflexes□
□ Other:_____

**3. Respiratory** □Cough□ □ Shortness of breath□ □ Pleuritic chest pain □
□ Other:_____

**4. Skin** □ papules/nodules□ □ cellulitis □
□ Other:_____

**5. Other:_____**

**Head CT findings**

**Date: _____/____/_____**

□CT with contrast □ □CT without contrast □
□Meningeal enhancement □ □ intraparenchymal lesion□ □ increased intracranial pressure
□ not tested □ □normal□
□ Other:_____

**MRI findings**

**Date: _____/____/_____**

□Meningeal enhancement □ □ intraparenchymal lesion□
□ increased intracranial pressure □ □ not tested □ □normal□
□ Other:_____

**Chest Xray** (First documented after crypto diagnosis)

**Date: _____/____/_____**

□ Effusion □ □ intraparenchymal mass □ □ atelectasis □ □ lymphadenopathy□
□ Normal □ □ Not tested □ □ Not related□
□ Other:_____

**CT chest** (First documented after crypto diagnosis)

**Date: _____/____/_____**

□ Effusion □ □ intraparenchymal mass □ □ atelectasis □ lymphadenopathy□
□ Normal □ □ not tested □ □ not related □
□ Other:_____

**Histology**

**Biopsy** □ **BAL( bronchoalveaolar lavage)** □

**Date: _____/____/_____**

**Site of biopsy:** □ lung □ □ Skin □ □Brain□ □ Other:_____

If brain biopsy specify: □open brain bx □ □Needle biopsy □

If lung biopsy specify: □open thoracotomy □ □ Needle biopsy □ □ thoracoscopy□

Describe histology findings as reported:

_____

**Lumbar Puncture Data (Please include all available LP after cryptococcus diagnosis)**

| Date (mm.dd.yy) | Opening pressure◦ (in cm of water) | Number of nucleated cells | Number of RBC | glucose | protein | Antigen titer | Culture | Days positive after culture* | India ink | Minimal inhibitory concentration ( MIC) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

*Days between when the LP was done and when CSF culture became positive (Consider a month to be 30 days)

◦ If opening pressure was not measured please write NM = "Not measured"

Serum cryptococcus antigen (Please document all available data after initial crypto diagnosis)

Date_____/____/_____ titer_____ □detected□ □ not detected□ □ not tested□
Date_____/____/_____ titer_____□ detected□ □not detected□ □ not tested□
Date_____/____/_____ titer_____ □detected□ □not detected□ □ not tested□
Date_____/____/_____ titer_____ □detected□ □not detected□ □ not tested□
Date_____/____/_____ titer_____ □detected□ □not detected□ □ not tested□
Date_____/____/_____ titer_____ □detected□ □not detected□ □ not tested□

**Fungal cultures (*other than CSF*) for cryptoccocus at the time of diagnosis, treatment induction or relapse**

**1. Blood** □ Not checked□ □ Nbr of positive cxs____ □ Nbr of negative cxs ____
Dates of positive cxs **_____/____/_____, _____/____/_____, _____/____/_____**
Dates of negative cxs**_____/____/_____, _____/____/_____, _____/____/_____**

**2. Pulmonary** □ Not checked□ □ Nbr of positive cxs____ □ Nbr of negative cxs ____
Dates of positive cxs **_____/____/_____, _____/____/_____, _____/____/_____**
Dates of negative cxs**_____/____/_____, _____/____/_____, _____/____/_____**

**3. Other sites with cultures positive for cryptococcus (please specify with corresponding date (mm/dd/yy)**:**_____**

**COMORBIDITIES**

**Infections during or after diagnosis of crypto (During diagnosis or while on treatment)**

| Organism | Date of diagnosis | Site of culture | Antigen test (Y/N) | Name of Antibiotics |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
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| | | | | |

**Were malignancies diagnosed during or after diagnosis of crypto (during diagnosis or while on treatment)?**

□ Yes □ □ No □

If yes, specify

Type of malignancy:_____

Site of malignancy:_____

Date of diagnosis:_____/_____/_____

Treated? □ Yes□ □ No □Agent for treatment:_____

**TREATMENT**

**Please fill the following table starting with the first encounter:**

| Drug | Date of Initiation | Daily Dose | Date of stopping | Cumulative Dose |
|---|---|---|---|---|
| **Flucytosine** | | | | |
| | | | | |
| | | | | |
| | | | | |
| **AmB-d** | | | | |
| | | | | |
| | | | | |
| | | | | |
| **L-AmB** | | | | |

| | | | | |
|---|---|---|---|---|
| | | | | |
| | | | | |
| **ABLC** | | | | |
| | | | | |
| | | | | |
| | | | | |
| **Fluconazole** | | | | |
| | | | | |
| | | | | |
| | | | | |
| **Other:** | | | | |

Change row with each dose switch. Data can be entered from multiple admissions or follow ups.

**Flucytosine**

| Date | Serum Concentration |
|---|---|
| | |
| | |
| | |

**<u>Short-term Outcomes:</u>**

**Renal function (fill in all that applies)**

| Date of value followed by Cr value | Creatinine on admission | Cr at the end of Amphotericin B deoxycholate | Cr at end of amphotericin B lipid product | Maximum creatinine on admission while on treatment with polyene therapy |
|---|---|---|---|---|
| Date | | | | |
| Value (of Cr at date above) | | | | |
| Date | | | | |
| Value | | | | |

* Please duplicate table if reinduction with amphotericin products

**Hematologic parameters:**

| | Hemoglobin | Hematocrit | Platelets | WBC |
|---|---|---|---|---|
| **Value at onset of induction treatment w/ flucytosine Date:_____/_____/_____** | | | | |
| **Value at 2 weeks after treatment w/ flucytosine Date: _____/_____/_____** | | | | |

**1. Did the patient receive polyene therapy?** □ Yes□ □ No□ □ Unknown □

a) What was the initial polyene therapy? □ deoxycholate □ □ lipid preparation

b) Did the patient have to switch antifungal therapy (from deoxycholate to a lipid preparation) as a result of drug toxicity or side effect?

□ Yes□ □ No□

**If so, what toxicity or side effect:** □ infusion related□ □ renal dysfunction□

□ □ Other_____

**2. Did the patient have to switch antifungal therapy secondary to poor clinical response?**

□ Yes □ □ No □

□ From deoxycholate to a lipid formula □

□ From lipid formula to deoxycholate □

□ From fluconazole to amphotericin B □

□ Other:

_____

**Reason for switch (circle):** □ Persistent symptoms/physical findings □

□ Persistent cultures□ □ Persistent antigen in CSF □ □ Persistent antigen in serum □

**Antiretrovirals and Antifungals**

Was antiretroviral therapy continued along with antifungals during admission for crypto?

□ Yes □ □ No□

Was antiretroviral therapy held during admission for crypto?

□ Yes □ □ No□

If held during admission was antiretroviral therapy restarted on discharge:

□ Yes □ □ No□

Was antiretroviral therapy started newly after diagnosis of crytpococcal infection during the admission for crypto?

□ Yes □ □ No□

Was antiretroviral therapy started newly after diagnosis of crytpococcal infection on discharge?

□ Yes □ □ No□

Was antiretroviral therapy planned to be started newly after diagnosis of crytpococcal infection after discharge?

□ Yes □ □ No□

Name of antiretroviral medications that was newly started and date on which it was started (mm/day/yy):

1._____ Date_____/_____/_____

2._____ Date_____/_____/_____

3._____ Date_____/_____/_____

**Management of increased intracranial pressure (if present)**

**A. Repeated lumbar punctures:** □ Yes □ □ No □

**B. Lumbar drain:** □ Yes □ No □

Time from diagnosis of increased ICP (OP>20cm water) to lumbar drain:_____

Date of insertion: _____/_____/_____

**C. Ventricular shunting:** □ Yes □ No □

Time from diagnosis of increased ICP (OP>20cm water) to ventricular shunting:_____

Time from starting antifungal therapy to ventricular shunting:_____

Date of procedure: _____/_____/_____

**D. Pharmacological therapy:** □ Yes □ No □

Medication used: Acetazolamide □ Corticosteroids (newly started for that reason) □

Others □ _____

**Long-term outcomes:**

**Date of last follow up: _____/_____/_____**

**Outcomes**: (Documented on last available follow up)

□ □ Cure (pt off drugs and resolved symptoms)

□ Improved

□ Stable on therapy

□ **Persistence** defined by:

□ Persistence of symptoms □ □ Positive Antigen (CSF or serum) □

□ Positive culture

□ Relapse defined by following after initial sx and cultures resolved

□ Reappearance of symptoms □

□ Positive culture □

□ Death directly related to crypto infection
□ Indeterminate (lost to follow up)

**In patients with organ transplant were they diagnosed with TRANSPLANTED organ failure before, during or after *Cryptococcus* treatment**?

□ Yes □ □No □ Indeterminate

If yes please specify: □ Before□ □ During□ □ After cryptococcus treatment□

Was the patient re-transplanted?

□ Yes □ □ No□

Date of re-transplant:____/_____/_____

Were they on antifungal treatment on time of re-transplant?

□ Yes□ □ No □

Did the patient have persistent organ failure secondary to antifungal treatment? □

□ Yes □ □ No

If yes please specify: Liver failure □ □ Kidney failure □ □ Bone marrow suppression□

**Diagnosis of immune reconstitution syndrome made during hospital stay**

□ Yes □ □ No □

**Was Immune Reconstitution Syndrome treated?**

□ Yes□ □ No□

If yes please specify what medication was used for treatment: _____

If steroids were used please specify:

Name of steroid_____

Daily Dose_____

Starting date_____/_____/_____

Stopping date_____/_____/_____

***Criteria used for diagnosis: New appeareance or worsening of any of the following:***

□ Clinical or radiographic manifestations consistent with an inflammatory process such as contrast enhancing lesions on imaging studies (CT/MRI) □

□ CSF pleocytosis>5WBC □

□ Increased ICP □

□ Histopathology showing granulomatous lesions □

□ Unexplained hypercalcemia □

**AND**

□ Symptoms occurred during receipt of appropriate therapy and could not be explained by newly acquired infection □

**AND**

□ Negative results for cultures or stable/reduced biomarkers for the initial fungal pathogen during diagnostic work-up for the inflammatory process □

**MORTALITY:** □ □ **yes** □ □ **no** □ **n/a**
**Date of death: _____/_____/_____**
**Cause of death:**
Attributable to Cryptococcus □ □ Yes □ □ No
If yes, was it identified as related to the following?
□ □ Increased CNS pressure
□ □ Persistent infection
□ □ After end of treatment
□ Organ failure secondary to Cryptococcus treatment
□ □ while on treatment for crypto of underlying disease
□ □ Indeterminate
□ □ Other_____

**Notes:**

**Summary Timeline for CLINICAL OUTCOMES:**

| | Date Of last clinical assessment* | Hospitalized (H) Home (HM) SNF | # of hospital readmissions (since last assessment) | On Tx (y/n) | Reinduced with antifungal therapy(Y/N) | Creatinine |
|---|---|---|---|---|---|---|
| 2Weeks | | | | | | |
| 10 weeks | | | | | | |
| 1 Year | | | | | | |
| > 1 Year | | | | | | |

*If still hospitalized from primary adm, please use date at specified week or last available prior date

| | Old sx still present (specify which) | New sx appeared (specify which) | Old sx resolved (specify which) | Imaging findings ( CT brain, MRI brain, X ray chest, CT chest) | Improved (I) Stable (S) Worse (W) | Provider performing the follow up (PCP, ID,neuron, pulm) |
|---|---|---|---|---|---|---|
| 2 weeks | | | | | | |
| 10 weeks | | | | | | |
| 1 year | | | | | | |
| >1 year | | | | | | |

## APPENDIX B. CUMULATIVE ANTIFUNGAL DOSING

Table B.1. Cumulative dose of uninterrupted initial therapy for patients who survived through two weeks (severe) or 90 days (non-severe).

| Initial Therapy | Median cumulative dose* (mg/kg) | Range |
|---|---|---|
| Severe cryptococcosis | | |
| AmpBd (n=17) | 9 | 1 - 35 |
| ABLC (n=5) | 64 | 45 – 92 |
| Liposomal AmpB (n=4) | 54 | 5 – 163 |
| Fluconazole (n=1) | 146000 | n/a |
| Non-severe cryptococcosis | | |
| AmpBd (n=3) | 17 | 10 – 24 |
| Fluconazole (n=16) | 30100 | 4116 – 14000 |

*Fluconazole cumulative dose is in total mg.

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