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IN-HOSPITAL MORTALITY OF HIV-ASSOCIATED CRYPTOCOCCAL DISEASE IN PATIENTS TREATED WITH AMPHOTERICIN B VERSUS FLUCONAZOLE

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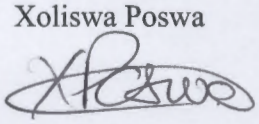
in partial fulfillment of the requirements for the degree of

Master of Science (Med) in Epidemiology & Biostatistics

DECLARATION

I declare that this research report is my own, unaided work. It is being submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Xoliswa Poswa 30 April 2012



ABSTRACT

Introduction

Cryptococcal disease (CD) is the most common cause of morbidity and mortality among patients living with HIV/AIDS in many parts of sub-Saharan Africa. Globally the highest number of HIV-associated CD cases occur in sub-Saharan Africa (720 000/957 900) and mortality rates among patients on antifungal treatment remain unacceptably high. This study aimed to estimate and compare in-hospital mortality of HIV-associated CD among patients who were treated with amphotericin B versus fluconazole versus mixed treatment with amphotericin B and fluconazole.

Materials and Methods

We performed an analytical, cross-sectional analysis of data from a national laboratory-based surveillance programme through a network of public sector laboratories in South Africa. The study period was 1 January 2005 to 31 December 2006. The analysis used a subset of data from laboratory confirmed cases with completed case report forms and available data on outcome. The exposure was measured in 3 levels defined as treatment during the induction phase of therapy for at least 7 days with either amphotericin B or fluconazole or mixed treatment (initiation of treatment with one regimen and switching on to the other within 7 days of treatment). Outcome was defined as: patients who died between 7 and 30 days in hospital. Chi-squared test was used to compare characteristics among the treatment groups and multiple logistic regression models were constructed to identify risk factors for in-hospital death.

Results

Sixty two percent of the patients (1,363/2,211) were treated for ≥7 days and 38% (848/2,211) for <7 days. In the group treated for ≥7 days, mortality was 359/1,363 (26%) and the median time to death was 12 days (IQR 9-18). There was no significant difference in case fatality among patients treated with: amphotericin B (29%), fluconazole (27%) or mixed treatment (24%), (p-value = 0.28). On multivariate analysis, factors significantly associated with in-hospital mortality were: patients between the age group of 40–59 years, province in which the patients resided and altered mental status. In the group treated for <7 days patients treated with fluconazole were 82% less likely to die than patients treated with amphotericin B.

Discussion and Conclusion

In-hospital mortality was high and similar among all treatment regimens in the ≥7 days group. However a significant reduction in mortality was noted in the <7 days group treated with fluconazole. The reason for the later findings is unclear. It may be that amphotericin B alone is not superior but equivalent to fluconazole in the first 7 days of treatment and 5-flucytocine may be the intervention required to improve outcome in the first 7 days. Or it may be that patients are reaching care too late for a significant impact in disease outcome to be observed and prevention of CD is required in the form of ART and primary prophylaxis. Selection bias in that patients in the fluconazole group are less sick than in the amphotericin B group is the most likely reason for lower mortality. The study findings call for more standardization of optimum treatment as well as advocacy for the availability of 5-flucytocine. Factors associated with high mortality require further investigations for interventions to improve patient outcomes.

DEDICATION

I dedicate this report to my parents for the gift of life and love.

Justice Ntsikelelo Mandlenkosi Poswa

and

Nombulelo Getrude Poswa

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CONTENTS

Declaration	ii
Abstract	iii
Dedication	v
Acknowledgments	vi
Contents	vii
List of figures	x
Abbreviations	xi

CHAPTER 1 1

1. GENERAL INTRODUCTION AND LITERATURE REVIEW 1

1.1 Background 1

1.2 Statement of the problem and justification of research 2

1.3 Literature review 5
1.3.1 Estimates of CD-associated mortality 5
1.3.2 Treatment of CD 10
1.3.3 Predictors of treatment failure and mortality 15

1.4 Objectives 19

CHAPTER 2 20

2 MATERIALS AND METHODS 20

2.1 Study design 20

2.2 Hypothesis 20

2.3 National surveillance programme for cryptococcal disease 21

2.4 Study population and sampling 22

2.5 Data management 23

2.6 Definitions 23
2.6.1 Exposures of interest 23
2.6.2 Outcome 25

2.7 Inclusion criteria 26

2.8 Exclusion criteria 26

2.9 Laboratory methods 27
2.9.1 Diagnosis of CD 27

2.9.2 Diagnosis of HIV 28

2.10 Statistical methods and data analysis 28

2.11 Ethical approval 30

CHAPTER 3 31

3 RESULTS 31

3.1 Overview of all patients with CD 31

3.2 Comparison of cases presenting to enhanced and non-enhanced sites 34

3.3 Description of all patients with cryptococcal disease at enhanced surveillance sites 36

3.3.1 Frequency of distribution of demographic and microbiologic characteristics of cases with cryptococcal disease at enhanced surveillance sites 36

3.4 Description of cases included in analysis of risk factors for mortality 39

3.4.1 Frequency distribution of demographic and microbiologic characteristics of patients with cryptococcal disease treated for at least 7 days 39

3.4.2 Comparison of characteristics of patients on treatment with amphotericin B, fluconazole and amphotericin B plus fluconazole in patients treated for at least 7 days 39

3.4.3 Characterization of fluconazole dosing among patients treated for at least 7 days 42

3.5 Outcome 42

3.6 Risk factors associated with mortality in patients with cryptococcal disease treated for at least 7 days 43

3.6.1 Univariate analysis 43

3.6.2 Multivariate analysis 46

3.7 Risk factors associated with mortality among patients with cryptococcal disease treated for less than 7 days 47

3.7.1 Univariate analysis 47

3.7.2 Multivariate analysis 49

CHAPTER 4 50

4 DISCUSSION 50

4.1 Introduction 50

4.2 Representativeness of the study sample 51

4.3 Systematic overview of study findings 52

4.3.1 Description of the adult population with a laboratory confirmed incident episode of HIV-associated cryptococcal disease 52
4.3.2 Description of the adult population from enhanced sites with a laboratory confirmed incident episode of HIV-associated cryptococcal disease 54
4.3.3 Risk factors of in-hospital mortality 55

4.4 Potential study biases 62
4.4.1 Collection of exposure and outcome data 62
4.4.2 Underestimation of incidence 62
4.4.3 Bias in risk factor analysis 63

4.5 Residual confounding 63

4.6 Generalizability 64

4.7 Study strengths 64

4.8 Study limitations 64

4.9 Suggestions for further studies 65

5 CHAPTER 5 67

5.1 CONCLUSION 67

5.2 SUMMARY AND RECOMMENDATIONS 67

APPENDIX A 69

APPENDIX B 72

REFERENCES 73

LIST OF FIGURES

Figure 1: Flow chart indicating numbers of patients with cryptococcal disease reported to NICD and patients included in the current study for the period 2005 through 2006 33

LIST OF TABLES

Table 3.1: Demographic and microbiologic characteristics of patients with cryptococcal disease from GERMS-SA enhanced and non-enhanced surveillance sites, South Africa, 2005-2006 35

Table 3.2: Distribution of demographic and microbiologic characteristics of patients with cryptococcal disease from GERMS-SA enhanced surveillance sites by duration of treatment, 2005-2006 38

Table 3.3: Comparison of the characteristics of patients receiving treatment with amphotericin B, fluconazole and amphotericin B plus fluconazole in patients treated for at least 7 days 41

Table 3.4: Comparison of case fatality ratio among patients with cryptococcal disease patients treated with different fluconazole dose regimens 42

Table 3.5: Univariate analysis of demographic factors associated with in-hospital mortality among patients with cryptococcal disease treated for at least 7 days 44

Table 3.6: Univariate analysis of clinical factors associated with in-hospital mortality in patients with cryptococcal disease treated for at least 7 days 45

Table 3.7: Multivariate analysis of factors associated with in-hospital mortality in patients with cryptococcal disease treated for at least 7 days 46

Table 3.8: Univariate analysis of demographic and clinical factors associated with in-hospital mortality among patients with cryptococcal disease treated for less than 7 days 48

Table 3.9: Multivariate analysis of factors associated with in-hospital mortality among patients with cryptococcal disease treated for less than 7 days 49

ABBREVIATIONS

AIDS – Acquired immune deficiency syndrome

ART – Antiretroviral therapy

HAART – Highly active antiretroviral therapy

CD – Cryptococcal disease

CFR – Case fatality ratio

CFU – Colony forming units

CNS – Central nervous system

CMI – Cell mediated immunity

CSF – Cerebrospinal fluid

CRF – Case report form

EFA – Early fungicidal activity

ELISA - enzyme-linked immunosorbent assay

GERMS-SA - Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa

HIV – Human immunodeficiency virus

IDSA – Infectious Disease Society of America

IQR – Interquartile range

KCL – Potassium chloride

mg/kg/d – milligram per kilogram per day

mg/d – milligram per day

MRU - Mycology Reference Unit

NHLS – National Institute of Laboratory Health Services

NICD - National Institute for Communicable Diseases

OI – Opportunistic infections

TB - Tuberculosis

CHAPTER 1

1 General introduction

This chapter gives an overview of the burden of human immunodeficiency virus (HIV)-associated cryptococcal disease and why it is an important public health problem. The chapter also contains a statement of the problem, justification for the study, objectives of the study and a literature review from relevant publications.

1.1 Background

Cryptococcal disease (CD) is caused by encapsulated fungi belonging to the genus *Cryptococcus* [1]. The genus comprises of approximately 39 species, with *Cryptococcus neoformans* and *Cryptococcus gattii* being the most common cause of human infection [1]. *C. neoformans* is subdivided into two varieties: *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *neoformans* [2]. The majority of patients are infected with *C. neoformans* var. *grubii* [1]. It is a ubiquitous organism found in the soil contaminated by pigeon/bird droppings. It is thought that infection is acquired by inhalation of infectious particles from the environment. No transmission among humans has been reported [1]. Infection is initiated in the lung after inhalation of basidiospores of *C. neoformans* [3]. Earlier studies using restriction fragment length polymorphism analysis suggest that initial infection often occurs early in childhood. This could be followed by a long latent phase [3]. The primary interaction between the local alveolar macrophages and the basidiospores is a crucial determinant of whether or

not disease ensues [1]. In a normal host this interaction contains the fungus and stimulates effective cell-mediated immunity (CMI). An intact CMI is crucial to prevent the development of CD [4]. The vast majority of patients with CD have an underlying immune deficiency. Individuals with HIV infection, extensive corticosteroid use, organ transplant, leukemia, lymphoma and sarcoidosis are at high risk for CD [1]. The number of CD cases increased drastically with the onset of the AIDS pandemic [5].

The main target organs for cryptococcal CD are the central nervous system (CNS) and the lung, although any organ can be involved including bone, urinary tract, prostate gland, skin, and liver [1]. Cryptococcal meningitis is a major opportunistic infection among the HIV-infected population and it predominantly occurs in patients with a CD4+ T cell count of less than 100 cells/μL [6]. The incidence of cryptococcal disease is generally low among immunocompetent people. It has been estimated that approximately 20% of patients who have CD without HIV infection have no apparent underlying disease or risk factor [7].

1.2 Statement of the problem and justification of research

Cryptococcal disease is the most common cause of morbidity and mortality among patients living with AIDS in many parts of sub-Saharan Africa [5]. Of the estimated 957 900 cases of CD that occurred globally in 2006, sub-Saharan Africa had the highest number of estimated cases (~720 000 cases) and North America the lowest (7800 cases). Currently, South Africa has the largest number of people living with HIV in a single country, and is consequently experiencing a high

burden of CD [8-9]. Due to this high population HIV prevalence, CD is currently the most common cause of community-acquired meningitis in South Africa [5, 10]. A total of 7149 incident cases of laboratory-confirmed CD were reported in South Africa in 2006. The overall incidence was estimated to be 15 cases per 100000 persons in the general population. The incidence rate amongst HIV-infected individuals was estimated to be 133/100000 HIV cases and amongst people sick with AIDS was 12/1000 AIDS cases [8]. These figures represent the minimum estimate of the true burden of disease in South Africa given that only laboratory confirmed cases are counted.

The incidence of CD in the developed countries seems to have declined following the introduction of antiretroviral therapy (ART). The current estimation of incident cases is 500 and 780 cases per year in Western and Central Europe and North America respectively [5, 11-12]. A population based surveillance for CD conducted in the USA between 1992 and 2000 showed a declining incidence of this infection among persons with AIDS attributed to use of effective antiretroviral therapy [11]. The overall annual incidence of CD in the general population of Atlanta Georgia declined from 5 cases per 100 000 in 1992 to 1.3 cases per 100 000 population in 2000. Almost 90% of cases occurred among persons infected with HIV. Similarly the French national surveillance program demonstrated a decrease of 46% in CD among HIV positive individuals during the post ART period [12].

ART only became widely available to public hospitals in South Africa in 2004 [13-14]. Despite the rapid scale up of ART coverage and the stabilizing incidence of CD in South Africa, the overall incidence remains high [15-16]. It has been shown

that successful treatment of the acute infection of CD followed by initiation of ART regimen improves outcome of patients with HIV-associated CD [17-18]. It has thus become essential to improve the initial acute management of CD, so as to optimize the patient's chances of initial survival and subsequent entry into the ART program. Infectious Disease Society of America (IDSA) guidelines for the treatment of CD recommend amphotericin B combined with 5-flucytosine as optimal treatment for CD in patients with HIV [19-20]. 5-Flucytosine is however not available in sub-Saharan Africa including South Africa. South African guidelines therefore recommend use of high-dose amphotericin B as the primary drug of choice in these patients, to be administered for 7-14 days [21]. Quite often in sub-Saharan African countries, the administration of amphotericin B is not possible. This is because it is either not available, or it cannot be administered safely, due to the requirement for intravenous administration and close monitoring of toxic effects [22].

A population-based surveillance in one of the provinces in South Africa during the pre-ART era demonstrated that, contrary to the guidelines, fluconazole is widely used as primary therapy for HIV-associated CD (fluconazole alone 72%, amphotericin B alone 18% and amphotericin and fluconazole 9%) [23]. This is because fluconazole is more readily available, can be given orally and is well tolerated even though it is considered to be sub-optimal therapy during the initial phase of treatment [21-22].

Although earlier studies had shown amphotericin B to be superior to fluconazole, more recent studies have shown benefit to high-dose fluconazole (800mg/d) compared with standard-dose fluconazole (400mg/d) [24-25]. In this setting, where

use of fluconazole as induction therapy appears to be common practice, it is imperative to determine if this regimen compares to the current recommendations for treatment of HIV-associated CD. There are currently no satisfactory data to this effect. Analysis of South African surveillance data provides the opportunity to begin to explore this question which may motivate for large clinical trials in the quest for optimal therapy that is readily accessible in the setting of a developing country.

1.3 Literature review

1.3.1 Estimates of CD-associated mortality

Developed world: Earlier studies in the developed world reported that HIV-associated CD was associated with considerable mortality of about 30% [11, 26]. In most of these studies however, monotherapy or lower doses of antifungal drugs that have now been proven to be suboptimal were used. A retrospective review of patients with HIV-associated cryptococcal meningitis, treated with amphotericin B plus 5-flucytosine in a university hospital reported high rates of treatment failure [27]. Mortality was 14% at 2 weeks and 26% at 10 weeks. In the earlier stages of this study (1986-1990) patients were treated with high dose amphotericin B 0.7 mg/kg/d while in the later stages (1990-1993) of the study the institution had changed to a lower dose, 0.3 mg/kg/d. Factors found to have the strongest associations with failure to have negative cultures by day 14 were; a CSF cryptococcal antigen titer $\geq$1:1,024, low serum albumin, and low dose of

amphotericin B. However analysis to determine if mortality was different between the treatment groups was not done.

In a randomized multicentre trial by Saag et al comparing amphotericin B and fluconazole, treatment was successful in 40% of the amphotericin B group vs. 34% of the fluconazole group [28].This was however not statistically significant (p=0.40) and the overall mortality was 14% and 18% respectively with no significant difference between the treatment groups (p=0.48) [27]. Abnormal mental status was found to be the most important predictive factor of a high risk of death during therapy. It is important to note that in this study, treatment was with lower doses for both amphotericin B and fluconazole and death was not the end point. The study was also not powered to determine the difference in mortality rates.

In another study however van der Horst.et.al. demonstrated much lower mortality compared to other studies in a randomized control study using a higher dose amphotericin B (0.7mg/kg/d) combined with flucytosine [20]. The overall mortality in this study was 5.5% at 2 weeks and 3.9% at 10 weeks. This was one of the landmark studies that informed the current guidelines for management of HIV-associated cryptococcal meningitis.

The use of antiretroviral therapy has been shown to be associated with a reduction in the incidence of opportunistic infections (OI) including CD, as well as an increase in the survival of persons with AIDS [29].The recent estimates of annual case fatality for HIV-associated CD are approximately 45 deaths in Western and Central Europe and 700 in North America compared to 504 000

reported in sub-Saharan Africa [5].The USA saw a decrease in the estimated incidence and deaths of AIDS defining OI declining by 6% and 23% respectively during 1996 compared to 1995 [30-32]. This was attributed largely to successful prophylactic regimens to prevent OIs and the use of ART. ART decreases plasma HIV RNA levels and increases CD4+ T-lymphocyte counts (a crucial component of the immune system for resistance to CD) among HIV-infected persons [33].

Africa, including South Africa: Reported mortality rates among patients with HIV-associated CD on antifungal treatment remain unacceptably high in sub-Saharan Africa [18, 22, 34]. Here, patients present with advanced HIV disease and cryptococcal meningitis is often the initial AIDS-defining illness. Annually, more than 600 000 deaths associated with CD occur globally and the majority of these deaths (504 000) occur in sub-Saharan Africa. Deaths due to CD in Sub-Saharan Africa are estimated to be more frequent than tuberculosis (504 000 vs. 350 000) [5].

Prior to the wide availability of ART in the public sector, hospital-based studies reported a high early mortality ranging from 25%-64% while receiving antifungal treatment [18, 22, 25, 34-35]. A retrospective review of patients treated in a tertiary hospital in Durban, South Africa, reported extremely high rates of in-hospital mortality at 64% [34]. Although most patients would have been treated with amphotericin B or fluconazole, due to poor record keeping, analysis to determine if patients received optimal treatment and if mortality was different between the treatment groups was not done. Cryptococcal meningitis

was the initial AIDS-defining illness in 84% of their patients. Patients presented late with high rates of neurological complications and this is likely to have contributed to the high mortality [34].

A hospital-based Zambian study also reported poor outcomes with a median survival of 19 days and a 100% mortality ≤6 months after presentation even when antifungal treatment was given [35]. Cryptococcal meningitis was the first AIDS-defining illness in 91% of patients, a standard dose of fluconazole (400mg/d) was used and there was no survival advantage between patients receiving fluconazole and those who did not [35]. Schaars et al in a retrospective review to assess the outcome of AIDS associated cryptococcal meningitis in patients treated with fluconazole at 200mg/d or 400mg/d reported a mortality of 25% at 2 weeks. There was no significant difference between the treatment groups [22]. Only 10% were known to be alive after 6 months and 3% after 1 year. In this study once again low doses of fluconazole were used.

Longley et al in a Ugandan prospective, non-randomized study to determine the effect of increasing fluconazole dosage on the rate of cryptococcal clearance of infection also reported high mortality rates despite a statistically significant increase in early fungicidal activity when fluconazole dosage was increased [25]. Overall 2 week and 10-week mortality in this study were still high, 30% and 54% respectively. A population-based surveillance in South Africa during the period of 2006 to 2007, reported 31% mortality during the first hospital admission [23].

A study comparing the outcomes of cryptococcal meningitis among antiretroviral naïve and experienced patients who were on amphotericin B, reported in-hospital mortality of 29% in ART experienced and 31% in ART naïve (p=0.77). The median time to death was 13 and 14 days respectively [36]. The relationship between ART status and in-hospital mortality remained non-significant in multivariate analysis. Kambugu et al examined the clinical presentation and mortality of cryptococcal meningitis among HIV-infected adults receiving amphotericin B in a prospective cohort study pre- and post-ART [18]. They reported a 2-week survival of 49% in the pre-ART era and 80% in the post-ART era. However the 6-month survival after ART was still low, 41%. The observation of a high early mortality by Jarvis and Kambugu in the era of ART underscores the need for finding optimal strategies for management of acute CD.

Long-term maintenance antifungal therapy and use of ART minimize the development of CD including other opportunistic infections [21, 37]. Bicanic et al demonstrated in a smaller series a significantly lower mortality at 1 year in the ART experienced group [17].This suggests that while ART may not affect outcomes in acute setting, it leads to substantial reduction in mortality by reducing susceptibility to other opportunistic infections. This is supported by data from a study comparing outcomes of cryptococcal meningitis in pre-and post-ART era in France [38]. Although acute mortality remained unchanged following introduction of ART (18% vs. 21%), long-term outcomes were markedly improved.

1.3.2 Treatment of CD

Antifungal agents with activity against *C. neoformans* and commonly used for treatment of HIV-associated CD include amphotericin B, 5-flucytocine and fluconazole [19]. Amphotericin B is a polyene antifungal agent, prescribed as an intravenous once daily dose according to the patient's weight and it is infused over 4 hours[39]. Amphotericin B penetrates poorly into the cerebrospinal fluid (CSF) irrespective of inflamed or normal meninges. It is however fungicidal and has been associated with early fungicidal activity and rapid clearance of fungi from the CSF [40-41]. It is uniformly active against *C. neoformans* with low MICs and resistance has infrequently been encountered [39].

The side effects include renal toxicity and electrolyte abnormalities and it is thus mandatory to pre-hydrate the patient with 1 liter of normal saline containing 20mmol of KCl, which is infused over 2 hours prior to the administration of amphotericin B [39]. Baseline and twice weekly monitoring of creatinine, magnesium and potassium is also recommended. Other frequently encountered side effects include phlebitis, febrile reactions and anemia. In recent studies where the recommended dose of 1mg/kg/d of amphotericin B has been used, it is generally well tolerated with no significant nephrotoxicity [17, 42].

Fluconazole is an azole compound available in both oral and intravenous formulation. It has excellent bioavailability with more than 80% of the drug available in serum after ingestion thus obviating the need for intravenous administration [43]. Concentrations of fluconazole in CSF are approximately 70% of serum levels and it penetrates the blood brain barrier well irrespective of

whether meninges are inflamed or not but it is fungistatic [39]. Although resistance is very rare, a few relapses have been associated with an increase in MIC of relapse isolate than original isolate [39]. It is generally well tolerated and side effects include nausea, vomiting, abdominal pain, rash and drug induced hepatotoxicity. It requires clinical monitoring of these gastrointestinal symptoms for hepatitis and laboratory tests should be performed for confirmation [39].

5-Flucytosine is a pyramidine antagonist, prescribed as an oral formulation [39]. It also has an excellent bioavailability following ingestion and excellent CSF penetration with concentrations approximating 74% of serum concentrations. Resistance develops quickly with the high organism load experienced in cryptococcal meningitis when the drug is used as monotherapy. Side effects include diarrhea and leucopenia. Development of life-threatening leucopenia is minimized by using lower dose (100mg/kg/d) than previously used and by monitoring to maintain serum levels at <100μg/ml [44].

These agents may be used alone or in combination with other agents, with varying degrees of success [20, 25, 40, 45]. The Infectious Diseases Society of America (IDSA) recommend induction treatment with amphotericin B 0.7-1 mg/kg/d and 5-flucytosine 100mg/kg/d in the first 2 weeks [19]. The combination has been demonstrated to have synergistic activity both in vitro and in vivo against *C. neoformans* [40]. Evidence demonstrating that this regimen was superior to amphotericin B or fluconazole monotherapy in clinical trials forms the basis of this recommendation [20, 40-41]. Bennett et al in a

randomized double blinded multi-center trial showed that combination regimen of low dose amphotericin B and 5-flucytosine cured or improved more patients, produced fewer failures or relapses and more rapid sterilization of CSF and less nephrotoxicity than amphotericin B alone [40]. In all the mortality rate was 47% for amphotericin B alone and 24% for the combination of amphotericin B and 5-flucytosine. This study was stopped earlier than initially planned because the goal of the study had been achieved while they also felt that monotherapy was inadequate.

Subsequent studies with bigger series and using higher doses of amphotericin B showed significant improvement in outcomes with shortened duration of treatment with amphotericin B and 5-flucytosine [20, 37]. A randomized double blind multi-center study showed that the use of a higher dose of amphotericin B (0.7 mg/kg/d) plus 5-flucytosine is associated with increased rate of CSF clearance and decreased mortality at 2 weeks compared to regimens in previous studies that used lower doses of amphotericin B (0.3–0.5mg /kg/d) [20]. Adding 5-flucytocine was an independent factor associated with sterilization at 2 weeks. Bicanic et al in a smaller study also found 1mg/kg/d of amphotericin B plus 5-flucytocine to be more rapidly fungicidal than 0.7mg/kg/d [46]. Dromer et al also demonstrated these findings in a large observational cohort [47].

5-Flucytosine is, however, not available in southern Africa, and local guidelines recommend the use of a higher dose of amphotericin B 1mg/kg/d as monotherapy as it is considered an acceptable alternative [21]. They recommend a 2-week regimen or a minimum of 1 week, if 2 weeks of treatment is not

feasible. The recommendation for a minimum duration of 1 week is in recognition of the challenges associated with administration of amphotericin B, such that most of the patients in our setting are unlikely to receive the 2-week regimen. Surveillance data showed the median duration of amphotericin B prescription during hospitalization was 6 days (range 1–14 days) [15]. Data are sparse on the efficacy of shortening the initial phase from 2 weeks to 1 week but a Thailand study concluded that the 1-week regimen may be comparable to the 2-week regimen [42]. This study was however small and not powered to determine the difference between the two regimens. It is recommended that the induction phase of treatment is followed by fluconazole (400mg/d) in the continuation phase for a period of 8 weeks, or until CSF cultures are sterile [20, 26, 28].

The objective of treatment of cryptococcal meningitis is eradication of the infection (CSF sterilization), and control of elevated intracranial pressure [20]. Failure to eradicate infection is however common in HIV disease, as evidenced by a high relapse rate, even among patients successfully treated [20]. Long-term control, aimed at preventing relapse of CD, is achieved by use of fluconazole (200mg/d after the 10-week continuation phase) for life, or until CD4+ T-cell count >200 cells/μl for more than 6 months on highly active antiretroviral therapy (HAART) [20, 26].

High-dose fluconazole has been proposed as the alternative for induction phase in the treatment for CD (fluconazole 800mg/d orally for four weeks followed by 400mg/d for 8 weeks in the continuation phase) [24]. Haubrich et al assessed the

safety and efficacy of high dose fluconazole (800mg/d) as primary therapy for CD among patients with HIV [24]. The median time to sterilization of CSF was faster (21 days) compared to the lower doses (200 and 400mg/d) used in the past. There was clinical resolution in all but one patient who was changed to amphotericin B on day 18 but this patient's CSF culture was negative on day 15. Although they concluded that high dose fluconazole was an effective and well tolerated therapeutic option for patients with HIV-associated cryptococcal meningitis, the study was small.

In slightly larger series in Uganda, Longley et al in a prospective non-randomized study reported a significant increase in early fungicidal activity when fluconazole dosage was increased from 800mg/d to 1,200mg/d [25]. Comparing these results with those of Bicancic et al in Cape Town where 400mg daily fluconazole dosage was used, Longley et al seem to suggest that early fungicidal activity of fluconazole increases with an increase in fluconazole dosage above 400-1200mg (EFA, -0.02 log CFU/mL, -0.07 log CFU/mL and 0.18 CFU/mL) [17]. Overall 2 week and 10-week mortality in this study was still high (30% 17/57 and 54% 31/57) and the study was not powered to determine differences in mortality between treatment groups. At 6 and 9 months 30% of patients who had 800mg and 43% who had 1200 mg were known to be alive. The study was once again small and had a severely ill group of patients. Previous studies, comparing amphotericin B and fluconazole, showed that recipients of fluconazole required twice as long to become negative as those of amphotericin B recipients [28]. Early deaths within the first two weeks of

therapy were more frequent in the fluconazole group, compared to the amphotericin B group, where deaths were evenly distributed. The doses of amphotericin B and fluconazole used in the earlier studies were, however, suboptimal for HIV-associated CD [20].

High-dose fluconazole and high-dose amphotericin B have not been compared head-to-head in clinical trials. This lack of comparison, together with the fact that fluconazole is fungistatic as compared to amphotericin B which is fungicidal, makes amphotericin B the primary drug of choice for treatment of CD [20]. Despite this, approximately 72% of patients in SA are still treated with fluconazole alone as induction therapy [23]. For this reason it is important to describe the clinical outcomes of patients treated with amphotericin B as compared to fluconazole or a mixture of these agents.

1.3.3 Predictors of treatment failure and mortality

Various studies have defined treatment failure at various points of patient evaluation, e.g. at 2 weeks, 10 weeks, 3 months or 6 months [20, 28, 40, 47]. Treatment failure is defined as clinical or mycological failure. Clinical failure is usually considered to be exacerbation of symptoms or death and clinical cure is considered as resolution of all initial clinical abnormalities at the specified point of evaluation [20]. Mycological failure refers to the presence of viable *C. neoformans* from at least one of the cultured sites, and mycological cure refers to negative cultures for initially infected sites at the specified point of patient evaluation [47]. Evaluation at week 2 is now considered to be a major milestone

in the management of CD [20]. Previous studies in HIV-associated cryptococcal meningitis have shown that early mycological failure is associated with death [28, 48]. A therapeutic trial comparing the efficacy of orally administered fluconazole with that of intravenously administered amphotericin B, as primary therapy for crytococcal meningitis in patients with AIDS, identified the following pre-treatment factors predictive of early death during treatment: an abnormal mental status (RR = 5.99 95% CI 2.44-14.73, p=0.00002), CSF cryptococcal antigen >1:1024 (RR = 4.31; 95% CI 1.17-15.80, p=0.02) and a positive blood culture for *C. neoformans* (p=0.03, RR=3.76, 95% CI 1.05-13.33, p=0.03) [28]. In their multivariate analysis, abnormal mental status (p <0.001), CSF cryptococcal antigen >1:1024 (p=0.01) and CSF WCC less than $0.02x10^9$ per liter (20 cells per cubic millimeter) (p=0.04) were found to be independently associated with early death.

In this study mortality amongst patient at high risk for death was 33% in the amphotericin B group (8/24) and 40% (20/50) in the fluconazole recipients compared to the low mortality in the low risk group 2.9% and 4.9% in amphotericin B and fluconazole groups respectively [28]. These findings suggest that other factors may be more important in predicting death among patients with HIV-associated CD.

In a retrospective review of AIDS patients who were treated for cryptococcal disease, Chuck et al found hypernatremia (p <0.001) and growth of organism outside the CSF in addition to meningitis (p <0.001) to be significantly associated with a shorter survival time [26]. Patients were treated with

amphotericin B alone or a combination of amphotericin B and 5-flucytosine followed by suppressive therapy after initial treatment. Suppressive therapy was associated with a significantly better survival than that of patients not given continued antifungal treatment [26].

Initial antifungal treatment regimen is a critical determinant of outcome as seen in earlier studies supporting the use of 5-flucytosine in combination with amphotericin B where the addition of 5-flucytosine in the induction phase was an independent factor for sterilization of CSF [20]. Dromer et al using data from a prospective observational cohort of HIV positive and negative patients treated for CD with or without CNS involvement, analyzed the impact of antifungal treatment strategies prescribed in routine clinical practice [47]. The main outcome measured was treatment failure (death or mycological failure) at week 2 and month 3. The treatment strategies compared were amphotericin B and 5-flucytosine, amphotericin B only, fluconazole only and other combinations. Lack of induction therapy with amphotericin B and 5-flucytosine versus any other therapy (OR=5.16, 95% CI 2.44-10.91, p <0.001) was predictive of treatment failure at week 2. Other factors predictive of treatment failure at week 2 in this study were presence of meningoencephalitis (OR=4.45, 95%CI 1.34-14.79, p=0.015) and a high serum antigen titer (OR=2.99 95% CI 1.43–6.24, p=0.003). Within the group with meningoencephalitis, lack of induction therapy with amphotericin B and 5-flucytosine versus any other therapy (OR 51.25 95% CI 9.67–271.52, p <0.0001), an abnormal brain imaging (OR=4.18 95% CI 1.33–123.17 p=0.014), presence of fungemia (OR=3.07 95% CI 1.13–8.31

p=0.027) and a high CSF antigen titer (OR=17.80 CI 3.79–83.54 p <0.0001) were also predictors of early outcome.

A high serum antigen titer (OR=4.43 CI 1.21–16.23, p=0.025) and abnormal brain imaging (OR=3.89 CI 1.23–12.31, p=0.021) remained significant determinants of early outcome (week 2) after induction therapy with amphotericin B and 5-flucytosine. Combination therapy of amphotericin B and 5-flucytosine has also been shown to be associated with a faster clearance of cryptococci from CSF compared to amphotericin B alone amongst HIV-positive patients [41].

Some of the factors that have been considered as possible contributors to the high mortality in Africa include limited access to antifungal therapy (specifically, limited access to amphotericin B and 5-flucytosine), late presentation, inability to adequately monitor and manage raised intracranial pressure, limited use of cotrimoxazole prophylaxis and ART, and co-morbid conditions (like tuberculosis) in severely immunocompromised patients [18, 23, 34].

1.4 Objectives

1. To estimate the in-hospital mortality of HIV-associated CD (overall mortality, mortality by type of antifungal treatment, mortality by age-group and mortality by duration of hospitalization)
2. To compare in-hospital mortality among patients who received treatment for at least 7 days with:
    - amphotericin B
    - fluconazole
    - mixed treatment with amphotericin B and fluconazole

CHAPTER 2

2 Materials and Methods

In this chapter study population, data sources, data management are discussed. In addition, definition of variables, study hypothesis as well as statistical methods and data analyses are reported.

2.1 Study design

This was an analytical, cross-sectional study, using data collected for laboratory-based surveillance of laboratory-confirmed CD at the National Institute for Communicable Diseases (NICD) in South Africa. The study period was 1 January 2005 to 31 December 2006 and the data were collected from all nine provinces of South Africa. The analysis used a subset of data from the enhanced surveillance sites with completed case report forms and available data on in-hospital outcome.

2.2 Hypothesis

We hypothesized that treatment of patients with CD with amphotericin B for a minimum of 7 days of the induction period is associated with lower in-hospital mortality than in patients with CD treated with fluconazole or a combination of amphotericin B and fluconazole.

2.3 National surveillance programme for cryptococcal disease

The national laboratory-based surveillance for CD is performed by the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA), through a nationwide network of public and private sector laboratories, and is coordinated by the NICD. The incidence rate of CD was based on an estimated South African population of 47 million in 2005 and 2006 obtained from statistics South Africa [49-50]. Using standard case definitions, when a participating laboratory has identified a case of CD, the corresponding isolate or specimen is submitted along with the standard GERMS-SA laboratory case report form (CRF) (including limited data on patient age and date of specimen collection) to the Mycology Reference Unit (MRU), NICD (Appendix A).

To enhance the quality of the surveillance data, the laboratory-based surveillance system is supplemented with clinical data obtained at 16 enhanced surveillance sites at public sector hospitals in all 9 provinces of South Africa. Surveillance officers at these sites complete clinical case report forms for all patients with laboratory-confirmed CD by patient interview or hospital medical record review. Clinical data obtained includes use of antimicrobials, HIV status, patient outcome and previous hospital admissions.

Laboratory audits, which are done by the National Microbiology Surveillance Unit (NMSU) at intervals of 3, 6, or 12 months, compare the number of cases detected at a participating laboratory with the number of cases reported to NICD. Additional cases detected through audit are added to surveillance databases. One hundred and

fifteen clinical microbiology laboratories nationwide participated in the surveillance programme in 2005-2006.

2.4 Study population and sampling

The study population included all adult patients (≥15 years old), with a first episode of laboratory-confirmed CD, detected at GERMS-SA enhanced surveillance sites, from 1 January 2005 through 31 December 2006, with a known outcome at the end of hospitalization and where in-hospital treatment was known. For the main analytic component of the study, only patients receiving therapy for at least 7 days were included. This is because the minimum recommended duration of amphotericin B induction therapy is 7 days and death in the first 7 days of treatment is likely to be a result of underlying severity of illness and unaffected by treatment regimen [21].

Preliminary data analysis was done to compare patients included in the in-hospital mortality risk factor analysis to the overall CD population to determine the comparability of the two groups. For completeness an additional analysis was conducted to determine the factors associated with mortality amongst patients hospitalized and treated for less than 7 days. Findings of this analysis were compared with the main analysis.

2.5 Data management

Data for CD surveillance were entered in Epi Info version 6.04 statistical software package and exported to MS Excel spreadsheet for cleaning and validation. Cleaning of data included simple descriptive methods such as frequency tables and cross-tabulations to get a better understanding of the dataset, to identify missing or duplication of data and to determine inconsistencies. A verification exercise of data entered against data on original questionnaires was performed to correct errors identified and to increase completeness of data. Once considered clean and complete the data was exported to Stata 10 software package version for analysis.

2.6 Definitions

2.6.1 Exposures of interest

The exposure variable was measured in 3 levels. It was defined as treatment during the induction phase of therapy for at least 7 days with either: (i) amphotericin B or (ii) fluconazole or (iii) mixed amphotericin B and fluconazole.

Induction-phase therapy was defined as the antifungal treatment given in the first 7 days after diagnosis of CD For the main analytic component of the study, only patients receiving therapy for a minimum of 7 days were included. Seven days was chosen a minimum time period for treatment with amphotericin B, as this is the minimum duration of amphotericin B therapy, recommended by the Southern African HIV Clinicians' Society guidelines [21]. In addition, it is likely that patients who die before receiving at least 7 days of induction phase

treatment may have died due to severity of underlying illness, the outcome of which would not have been modified greatly by antifungal treatment.

Mixed treatment with amphotericin B and fluconazole was defined as initiation of treatment with one regimen and switching on to the other within 7 days of treatment. The treatment guidelines recommend treatment with amphotericin B for a minimum of 7 days prior to switching to fluconazole [21]. However observation from our data was that 20% of patients eligible for inclusion in the study were treated for example for 1 or 2 days with amphotercin B and then changed to fluconazole.

An incident case of CD was defined by GERMS-SA as the first i) isolation by culture of any *Cryptococcus* species from any clinical specimen, or ii) positive India ink or iii) positive cryptococcal latex agglutination test on CSF or other body fluid, from any patient, diagnosed at a South African laboratory.

For the purposes of this study, a patient with CD, whose HIV status was unknown, was considered to be HIV infected, 99% of adults with CD are infected with HIV [23]. Where HIV testing was performed, HIV test results were captured by the surveillance. Patients known to be HIV negative were excluded from the study.

2.6.2 Outcome

Outcome was defined as in-hospital death i.e. patients who died between 7 and 30 days in hospital. In-hospital survival was defined as patients alive after 7 days in hospital and discharged alive before 30 days or hospitalized alive to 30 days. To minimize misclassification of deaths that may have occurred due to causes other than cryptococcal disease, all deaths occurring after 30 days of hospitalization were excluded from the analysis.

Case fatality ratio was defined as the number of patients with CD who died in hospital over the number of patients who were admitted with CD.

For the purposes of this study, patients discharged alive were assumed to have survived. No data on outcome post-discharge were available. The choice of a cut-off point of 7 days of treatment is based on the assumption that patients who die before receiving at least 7 days of induction phase treatment may have died due to advanced CD or co-morbidity, the outcome of which would not have been modified greatly by antifungal treatment. Patients who died before this period were not included in the main analysis, but were included in the descriptive and additional sub-analytic component of the study.

Although dose of therapy administered was not available on all cases, we performed a descriptive analysis amongst patients who received fluconazole for the subgroup of patients with available data on dose to describe the proportion of patients who receive therapy at the appropriate dosage. This was not possible for patients receiving amphotericin B as the dosage is dependent on patient weight and weight data were not available.

2.7 Inclusion criteria

For the descriptive component, all patients presenting to enhanced surveillance sites who met criteria for GERMS-SA case definition, age ≥15 years, HIV-infected (or unknown HIV status), no previous episode of CD recorded on the GERMS-SA database and outcome was known were included. Only patients with complete CRF were included.

For the main analytic component only patients who were discharged or died between 7 days and 30 days, or remained hospitalized were included. Patients discharged alive were assumed to have survived.

For completeness an additional analysis was conducted to determine the factors associated with mortality amongst the subgroup of patients hospitalized and treated for less than 7 days. Findings of this analysis were compared with the main analysis.

2.8 Exclusion criteria

Patients with a previous episode of cryptococcal meningitis (recurrent disease), HIV status tested negative and unknown outcome were excluded from the study. Patients who died or were discharged before 7th day of hospitalization were excluded from the main analytical component of the study. These patients were however included in a secondary analysis to assess risk factors for mortality amongst this subgroup.

2.9 Laboratory methods

2.9.1 Diagnosis of CD

Laboratory tests, for the diagnosis of CD, include India ink, latex agglutination for detection of cryptococcal polysaccharide antigen and culture of *C. neoformans* from clinical specimens. India ink has a reported sensitivity of more than 80% in the developed world and even higher; 80%-98% in a setting of high prevalence of HIV/AIDS [22-23]. Cryptococcal antigen test has a reported sensitivity of 93%-100% and specificity of 93%-98% [51].

Culture of *C. neoformans* from clinical specimens remains the gold standard for the diagnosis of CD. The India ink test is available at most National Health Laboratory Service (NHLS) laboratories. Culture and the cryptococcal antigen test are available at larger referral NHLS laboratories and private laboratories. In 2005 and 2006, all laboratories in the network were requested to submit all isolates of *Cryptococcus* species to the MRU. The isolates were submitted in Dorset transport medium in screw-top glass vials. In the MRU, the isolates were directly inoculated onto Niger seed agar, to confirm purity. Genus identification was confirmed by growth at 37^0C, brown discoloration on Niger seed agar, and urease hydrolysis. Isolates with indeterminate reactions were subjected to API20C Aux (BioMerieux Durham, North Carolina, USA) testing. All isolates of *Cryptococcus* species were subjected to biochemical speciation, using canavine-glycine-bromothymol blue (CGB) agar to differentiate *C. neoformans* from *C. gattii*.

2.9.2 Diagnosis of HIV

Most laboratories performed an HIV enzyme-linked immunosorbent assay (ELISA) on blood specimens from adult patients. In the majority of cases, a second confirmatory ELISA was performed on the same specimen [52]. In cases where the laboratories were unable to perform ELISA on-site, a rapid test was performed on-site and blood was sent to a reference laboratory for a confirmatory ELISA. The commonly used systems for the ELISA were the Abbot Axysm and the Roche Elecys [52].

2.10 Statistical methods and data analysis

Preliminary analyses were conducted to compare patients from enhanced surveillance sites with the population of all patients with CD. The comparison was done to determine if the two groups were similar and thus evaluate the generalizability of results as well as the potential for bias.

Statistical analysis was done using Stata statistical software, version 10 (Stata Corp.2007 College Station, Texas). The chi-squared test was used to compare differences in characteristics between categorical variables (e.g. demographic and microbiologic characteristics of cases from enhanced and non enhanced surveillance sites).

Univariate logistic regression was done for each exposure variable to assess individual association between the outcome variable (in-hospital death between 7 and 30 days after admission) and each exposure variable separately.

The exposure variables considered were gender, age, province, antifungal treatment, mental status, CD4+ T-cell count, wasting and current ART treatment, cotrimoxazole prophylaxis and anti- tuberculosis (TB) treatment. Gender, age and province were chosen to determine if demographic factors were likely to confound the association between outcome and exposure of interest i.e. antifungal treatment. The rest of the variables were included as potential confounding clinical variables. CD4+ T-cell count level below 100 cells /µl is a risk factor for developing CD in particular cryptococcal meningitis as well as other opportunistic infections [6]. CD4+ T-cell count is not analyzed as a continuous variable in this study because the CD4+ T-cell data were captured in ranges e.g. <100, 100-200 cell/µl. It is thus presented as categorical variable in 3 levels: 0-100, 101-200 and >200. Altered mental status is a marker of severe disease and has been cited as a risk factor for early mortality [18].

Treatment with ART may modify the outcome of disease and current ART was defined as whether patient was taking ART during this admission (yes or no) [16]. Duration of ART was not captured in the surveillance data. Wasting with more than 10% of body weight is generally a finding of advanced HIV disease, thus antifungal treatment may not influence the outcome of CD [14]. Cotrimoxazole has been associated with a reduction in all cause mortality by decreasing other opportunistic infections among patients infected with HIV [53-54]. Cotrimoxazole prophylaxis was defined as whether patient was on prophylaxis during this admission.

Concurrent tuberculosis treatment was used as a marker of tuberculosis infection and it has been associated with an increased risk of mortality in CD [55].

Multivariate logistic regression was performed to assess the effect of multiple risk factors that together had the strongest association with in-hospital mortality. Factors that were significant at p-value ≤ 0.1 on univariate analysis were included in logistic regression models. Stepwise logistic regression using forward and backward elimination was used. Interactions between main effects in the model were also assessed for significance and colinearity. Potential confounding variables were adjusted for in the models. Precision of estimate for odds ratio in the logistic regression model was defined as $p \leq 0.05$ significance level. Odds ratios and 95% confidence intervals were also used to summarize the contribution of each risk factor included in the multivariate logistic regression. Where data were missing proportions were presented amongst those individuals with available data. Missing values were excluded in all statistical tests.

In addition, to better understand the factors contributing to mortality we conducted an additional supplementary analysis to determine the risk factors for mortality in the subgroup of patients that was treated for <7 days on univariate and multivariate analysis. This was done for comparison with the group that was treated for ≥ 7 days.

2.11 Ethical approval

The protocol was approved by the Post Graduate and the Research Ethics Committee of the University of the Witwatersrand (Appendix B).

CHAPTER 3

3 Results

3.1 Overview of all patients with CD

The profile of patients whose specimens were referred to NICD between 2005 and 2006 is illustrated in figure 1. The number of patients excluded at each point is shown.

A total of 10,935 patients were reported to NICD with CD from 2005 through to 2006. Thirteen percent (1,473/10,935) were not eligible for inclusion in the study for the following reasons: age was not recorded in 1032, 186 were less than 15 years old, 173 had recurrent CD and 82 had missing data on surveillance site status. The number of incident case patients was 9,702. The incidence of CD in this study was 8.6 per 100 000 population in 2005 and it increased to 11.6 per 100 000 population in 2006.

Nine thousand four hundred and sixty two patients were included in the descriptive analysis. Fifty six percent (5,273/9,333) were female and 70% (6,620/9,462) were in the age group 20-39 years. The highest number of cases was reported in Gauteng followed by KwaZulu-Natal province. The HIV prevalence was 99% amongst patients who were tested for HIV. Meningitis was the clinical diagnosis in 97% of patients and CD diagnosis was confirmed from the CSF specimen (India ink, culture or cryptococcal antigen latex) in 95% of the patients. India ink was performed on 6,745/9,462 (71%) of the specimens and 6,320/6,745 (94%) tested positive. Cryptococcal antigen detection by latex was performed on 4,032/9,462 (42%) of the specimens and 4,000/4,032 (99%) tested positive. Ninety five percent

(8564/9462) of patients with CD were reported to NICD and 10% (898/9462) were only identified through laboratory audits conducted by NICD.

The majority of patients (6,123/9,462, 65%) were reported from non-enhanced surveillance sites. Of the 3,339 patients from enhanced surveillance sites a total of 1,128 (34%) patients were excluded from the risk factor analysis for the following reasons: 632 had missing data on their case record forms regarding the final outcome, 21 were recorded to be HIV negative, 59 refused hospital treatment and 416 had missing data regarding treatment regimen. Sixty two percent of the patients (1,363/2,211) were treated for ≥7 days and 38% (848/2,211) were treated for <7 days. One thousand three hundred and sixty three patients were included in the main analysis. An additional analysis of risk factors for death was conducted amongst the 848 patients treated for <7 days.

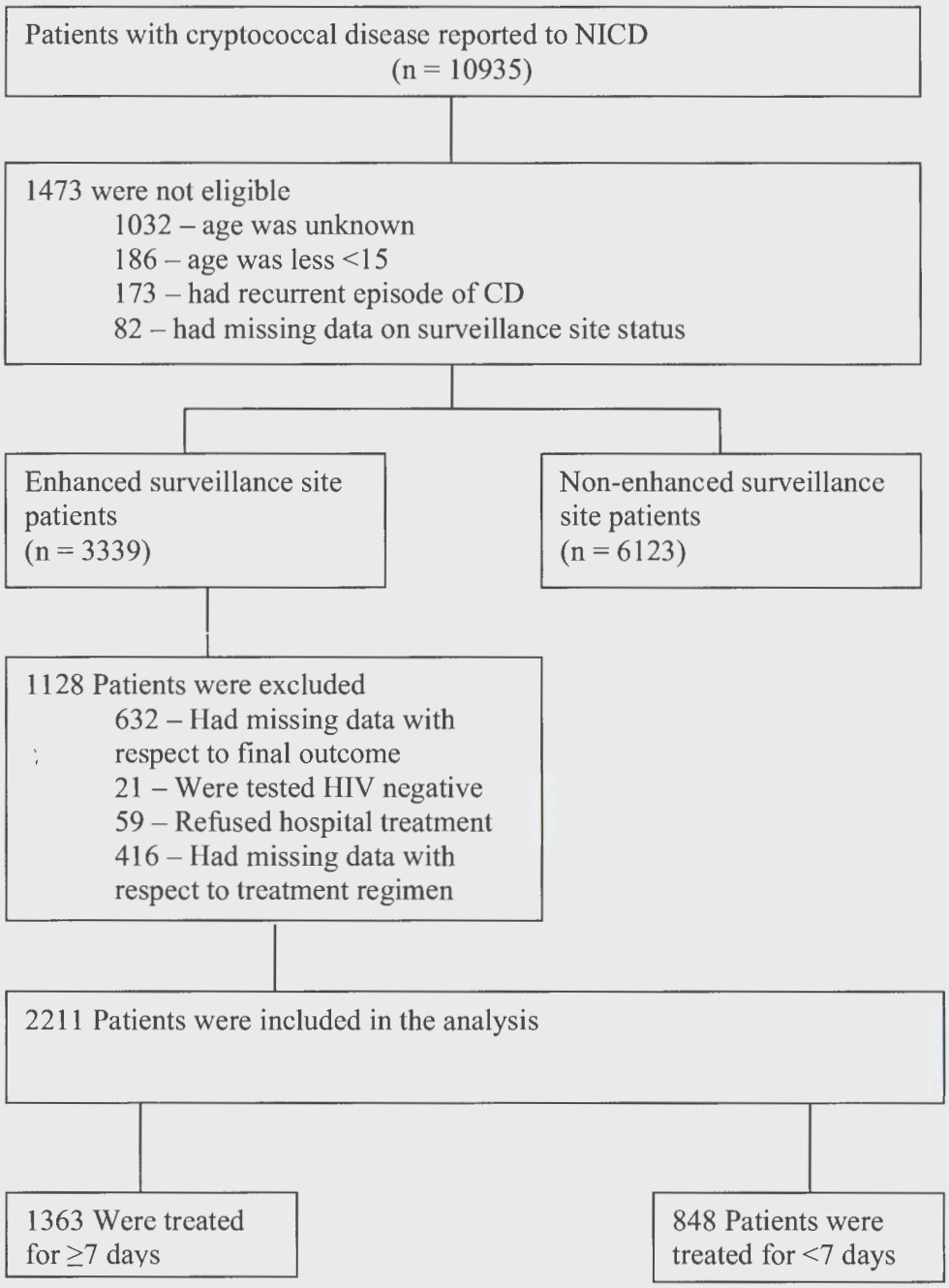


Figure 1: Flow chart indicating numbers of patients with cryptococcal disease reported to NICD and patients included in the current study for the period 2005 through 2006

3.2 Comparison of cases presenting to enhanced and non-enhanced sites

The characteristics of patients diagnosed at enhanced surveillance sites versus non-enhanced surveillance sites are compared in table 3.1.

Of the total number of cases reported to NICD, a high proportion of cases were reported from non-enhanced surveillance sites. The age and gender distribution of patients did not differ between enhanced and non-enhanced surveillance sites.

There was a significant difference in the proportion of cases reported between provinces comparing enhanced to non-enhanced surveillance sites. Patients from KwaZulu-Natal were 1.3 times likely to be reported from enhanced surveillance sites compared to patients from Gauteng province. Patients from the Eastern Cape were 75% less likely to be diagnosed at an enhanced surveillance site compared to patients from Gauteng. Blood specimens were 1.2 times likely to be submitted from enhanced surveillance sites.

Table 3.1: Demographic and microbiologic characteristics of patients with cryptococcal disease from GERMS-SA enhanced and non-enhanced surveillance sites, South Africa, 2005-2006

Characteristic	Enhanced surveillance sites n (%)	Non-enhanced surveillance sites n (%)	Odds ratio	95% Confidence Intervals	P-value
Gender	**n = 3322**	**n = 6011**			
Male	1483 (37)	2577 (63)	Reference		
Female	1839 (35)	3434 (65)	0.93	0.85 – 1.01	0.09
Age in group years	**n = 3339**	**n = 6123**			
<20	25 (28)	65 (72)	Reference		
20-39	2374 (36)	4246 (64)	1.45	0.91 – 2.31	0.14
40-59	887 (34)	1696 (66)	1.36	0.85 – 2.17	
≥60	53 (31)	116 (69)	1.19	0.66 – 2.09	
Province	**n = 3339**	**n = 6122**			
Gauteng	1410 (45)	1730 (55)	Reference		
KwaZulu-Natal	1005 (51)	955 (49)	1.29	1.15 – 1.44	<0.001
Limpopo	85 (27)	233 (73)	0.45	0.35 – 0.58	
Eastern Cape	263 (17)	1280 (83)	0.25	0.22 – 0.29	
Western Cape	166 (24)	512 (76)	0.39	0.33 – 0.48	
Mpumalanga	240 (36)	430 (64)	0.68	0.58 -0.81	
Free State	62 (13)	427 (87)	0.17	0.13 – 0.23	
North West	59 (11)	501 (89)	0.14	0.11 – 0.19	
Northern Cape	49 (48)	54 (52)	1.11	0.75 – 1.65	
Specimen	**n = 3339**	**n = 6168**			
CSF	3092 (34)	5972 (66)	Reference		
Blood	239 (56)	187 (44)	1.24	1.18 – 1.29	<0.001
Other *	3 (60)	2 (40)	NA		
Not specified	5 (42)	7 (58)	NA		
India Ink	**n = 2096**	**n = 4649**			
Positive	1978 (31)	4342 (69)	Reference		
Negative	118 (28)	307 (72)	0.84	0.67 – 1.05	0.12
Cryptococcal antigen latex	**n = 1933**	**n = 2099**			
Positive	1920 (48)	2080 (52)	Reference		
Negative	13 (41)	19 (59)	0.74	0.36 – 1.50	0.40

* Biopsy, bone, fluids and pus, # diagnosis was performed by cryptococcal antigen latex or India ink only, β patients were not reported to NICD but were identified through laboratory audits conducted by NICD. NA – not applicable.

3.3 Description of all patients with cryptococcal disease at enhanced surveillance sites

3.3.1 Frequency of distribution of demographic and microbiologic characteristics of cases with cryptococcal disease at enhanced surveillance sites

Table 3.2 demonstrates the characteristics of the patients from enhanced surveillance sites. In addition the characteristics of patients treated for <7 days and ≥7 days are compared. Female patients constituted 55% of the study population, 70% of the patients were in the 20-39 year age group, and 48% of the patients were reported from Gauteng province. The overall case fatality ratio was 32%. A high proportion of patients received a fluconazole containing regimen i.e. fluconazole alone: 62% (1,369/2211) and mixed treatment with fluconazole and amphotericin B: 20% (433/2211). A high proportion (86%) had CD4+ T-cell counts ≤100 cells/µl with the majority of patients with CD4+ T-cell counts less than 50 cells/µ (0-50 in 64%, 51-100 in 22%). Thirty six percent presented with an altered mental status.

There was a sizeable amount of missing data on the rest of the parameters. Out of 2211 patients from the enhanced sites 7% of data on mental status was missing, 55% on CD4+ T-cell count, 22% on ART, 31% on cotrimoxazole prophylaxis, 26% on TB treatment and 70% on wasting. For those in whom data were available, only 11% were on treatment with ART, and 24% were on concurrent anti-TB treatment. Comparison by duration of treatment showed significant differences between the groups of patients treated for <7 days as compared to those treated for ≥7 days. Among those treated for <7 days, 59% were from Gauteng province as compared to only 41% among those treated for

≥7 days. The death rate was higher in the <7 days group (41%) compared to the group treated for ≥7 days (26%), p<0.001. A high proportion of the <7 day treatment group was treated with fluconazole alone, 78% vs. 52% in the ≥7 day treatment group. A smaller proportion in the <7 day treatment group received TB treatment: 19% compared to 27% in the ≥7 day group.

Table 3.2: Distribution of demographic and microbiologic characteristics of patients with cryptococcal disease from GERMS-SA enhanced surveillance sites by duration of treatment, 2005-2006

Characteristic	n (%)	<7days treatment n (%)	≥7days treatment n (%)	P value*
Gender (n=2206)				
Male	998 (45)	382 (45)	616 (45)	0.95
Female	1208 (55)	464 (55)	744 (55)	
Age group in years (n=2211)				
<20	14 (1)	3 (1)	11 (1)	0.28
20-39	1549 (70)	597 (70)	952(70)	
40-59	617 (28)	240 (28)	377 (28)	
≥60	31 (1)	8 (1)	23 (1)	
Province (n=2211)				
Gauteng	1055 (48)	500 (59)	555 (41)	<0.001
KwaZulu-Natal	603 (27)	197 (23)	406 (30)	
Limpopo	66 (3)	15 (2)	51 (4)	
Eastern Cape	109 (5)	21 (2)	88 (6)	
Western Cape	126 (6)	30 (4)	96 (7)	
Mpumalanga	154 (7)	61 (7)	93 (7)	
Free State	56 (2)	18 (2)	38 (3)	
North West	25 (1)	4 (1)	21 (1)	
Northern Cape	17 (1)	2 (0)	15 (1)	
Outcome (n=2211)				
Recovered	1505 (68)	501 (59)	1004 (74)	<0.001
Died	706 (32)	347 (41)	359 (26)	
Treatment (n=2211)				
Amphotericin B	409 (18)	129 (15)	280 (21)	<0.001
Fluconazole	1369 (62)	660 (78)	709 (52)	
Amphotericin B + fluconazole	433 (20)	59 (7)	374 (27)	
Mental status (n=2061)				
Alert	1309 (64)	486 (63)	823 (64)	0.64
Altered	752 (36)	287 (37)	465 (36)	
CD4+ T-cell count (n=1003)				
0-100	863 (86)	345 (89)	518 (84)	0.11
101-200	106 (11)	33 (8)	73 (12)	
>200	34 (3)	10 (3)	24 (4)	
ART© (n=1717)				
Yes	193 (11)	64 (11)	129 (12)	0.64
No	1524 (89)	531 (89)	993 (88)	
Cotrimoxazoleprophylaxis (n=1536)				
Yes	401 (26)	131 (24.)	270 (27)	0.19
No	1135 (74)	412 (76)	723 (73)	
TB treatment■(n=1639)				
Yes	391 (24)	106 (19)	285 (27)	<0.001
No	1248 (76)	462 (81)	786 (73)	
Wasted (n=655)				
Yes	652 (99)	274 (99)	378 (100)	0.39
No	3 (1)	2 (1)	1 (0)	

* Comparing patients treated for <7 days vs. those treated for ≥7 days, †KZN- KwaZulu-Natal, © anti-retroviraltreatment, ■ anti-tuberculosis treatment

3.4 Description of cases included in analysis of risk factors for mortality

3.4.1 Frequency distribution of demographic and microbiologic characteristics of patients with cryptococcal disease treated for at least 7 days

More than seventy percent of cases were reported from Gauteng and KZN (41% and 30% respectively), 55% of the cases were female, 70% were in the age group 20-39 years, 21% were treated with amphotericin B, 52% treated with fluconazole and 27% were treated with amphotericin B plus fluconazole. There were incomplete data for other characteristics but for the subset in whom a reasonable amount of data were available; 64% were admitted with altered mental status, and the CD4+ T-cell count was ≤100 cells/μl in 84%. Only a small proportion of patients (27%) were receiving cotrimoxazole prophylaxis, and 27% were receiving concurrent TB treatment (Table 3.2).

3.4.2 Comparison of characteristics of patients on treatment with amphotericin B, fluconazole and mixture of amphotericin B plus fluconazole in patients treated for at least 7 days

Table 3.3 shows a comparison of the characteristics of the patients in the treatment groups. A high proportion of patients in the amphotericin B group were from KwaZulu-Natal (59%) compared to the fluconazole group with a high proportion of cases coming from Gauteng (63%), $p<0.001$. The proportion of cases with an altered mental status was lower in the amphotericin B group compared to the other treatment groups; 26% vs. 38% in fluconazole alone and 39% for the combined treatment group; amphotericin B plus fluconazole, $p=0.001$. The amphotericin B plus fluconazole group had a higher proportion of

patients receiving cotrimoxazole prophylaxis, 35% compared to 26% in the amphotericin B group and 23% in the fluconazole alone group.

Table 3.3: Comparison of the characteristics of patients receiving treatment with amphotericin B, fluconazole and amphotericin B plus fluconazole in patients treated for at least 7 days

Characteristic	AmphotericinB n (%)	Fluconazole n (%)	Amphotericin B and Fluconazole n (%)	Chi^2	P-value
Gender					
Male	142 (51)	307 (43)	167 (45)	4.64	0.09
Female	137 (49)	401 (57)	206 (55)		
Age group in years					
<20	4 (1)	4 (1)	3 (1)	4.33	0.63
20-39	203(73)	494 (69)	255 (68)		
40-59	70 (25)	199 (28)	108 (29)		
≥60	3 (1)	12 (2)	8 (2)		
Province					
Gauteng	13 (5)	449 (63)	93 (25)	503.67	<0.001
KwaZulu-Natal	166 (59)	134 (19)	106 (28)		
Limpopo	21 (7)	2 (0)	28 (7)		
Eastern Cape	12 (4)	35 (5)	41 (11)		
Western Cape	33 (12)	6 (1)	57 (15)		
Mpumalanga	19 (7)	61 (9)	13 (4)		
Free State	8 (3)	6 (1)	24 (6)		
North West	2 (1)	13 (2)	6 (2)		
Northern Cape	6 (2)	3 (0)	6 (2)		
Outcome					
Recovered	198 (71)	521 (74)	285 (76)	2.51	0.28
Died	82 (29)	188 (26)	89 (24)		
Mental status					
Alert	195 (74)	411 (61)	217 (62)	13.65	0.001
Altered	70 (26)	262 (39)	133 (38)		
CD4+ T cell count					
0-100	70 (79)	313 (86)	135 (84)	2.23	0.69
101-200	13 (15)	41 (11)	19 (12)		
>200	5 (6)	12 (3)	7 (4)		
ART*					
Yes	30 (13)	51 (9)	48 (15)	7.63	0.02
No	208 (87)	514(91)	271 (85)		
Cotrimoxazole					
Yes	57 (26)	115 (23)	98 (35)	11.64	0.003
No	161 (74)	377 (77)	185 (65)		
TB treatment■					
Yes	64 (27)	129 (25)	92 (29)	1.86	0.39
No	172 (73)	390 (75)	224 (71)		
Wasted					
Yes	65 (100)	212 (100)	101 (99)	2.72	0.26
No	0	0	1 (1)		

*Anti-retroviraltreatment, ■ anti-tuberculosis treatment

3.4.3 Characterization of fluconazole dosing among patients treated for at least 7 days

The dosage of fluconazole was recorded for 708 patients treated with fluconazole alone (708/709, >99%) (Table3.4). Six percent (45/708) were treated with a dose of 200mg/d, 57% (402/708) were treated with the standard dose of 400mg/d, and 37% (261/708) were treated with a dose of 800mg/d considered to be high dose. Mortality was not significantly different among the various dose regimens: 29%, 27% and 25% respectively, p=0.73.

Table 3.4: Comparison of case fatality ratio among patients with cryptococcal disease patients treated with different fluconazole dose regimens *

Fluconazole dose	Died	Discharged	Chi^2	P-value
100-200mg	13 (29)	32 (71)	0.625	0.73
400-600mg	110 (27)	292 (73)		
800mg	65 (25)	196 (75)		

*Only patients treated with fluconazole alone for at least 7 days included

3.5 Outcome

The overall case fatality ratio (CFR) among patients diagnosed at the enhanced surveillance site was 706/2211 (32%). The median time to death was 7 days (interquartile range [IQR] 3-12). The CFR varied by age group with the lowest case fatality in the 20-39 years age group, p=0.002.

In the group treated for at least 7 days, mortality was 359/1,363 (26%) and the median time to death was 12 days (IQR 9-18). The death rate was higher in the <7 day treatment group, 347/848 (41%) compared to the group treated for ≥7 days

359/1,363 (26%), p-value <0.001. There was no significant difference in CFR by treatment regimen among patients treated for 7 days: 29% for amphotericin B, 27% fluconazole and, 24% for amphotericin B plus fluconazole group (p-value 0.28).

3.6 Risk factors associated with mortality in patients with cryptococcal disease treated for at least 7 days

3.6.1 Univariate analysis

In the univariate analysis for demographic and clinical factors associated with in-hospital mortality (Table 3.5 and 3.6), age group, province and altered mental status were significantly associated with in-hospital mortality. The lowest CFR was in the 20-39 years age group. The highest CFR was in Limpopo, Mpumalanga and North West Province. Patients with altered mental status were 2.7 times more likely to die.

The treatment regimen (the exposure of interest in this study) was not significantly associated with mortality (p=0.285).

Table 3.5: Univariate analysis of demographic factors associated with in-hospital mortality among patients with cryptococcal disease treated for at least 7 days

Risk factor	Recovered	Died	Odds ratio	95% Confidence interval	P-value
Gender					
Male	454 (74)	162 (26)	Reference		0.970
Female	549 (74)	195 (26)	0.99	0.78 – 1.27	
Age group in years					
20-39	728 (76)	224 (24)	Reference		0.002
<20	5 (45)	6 (55)	3.90	1.17 – 12.89	
40-59	256 (68)	121 (32)	1.54	1.18 – 1.99	
≥60	15 (65)	8 (35)	1.73	0.73 - 4.14	
Province					
Gauteng	420 (76)	135 (24)	Reference		0.02
KZN	312 (77)	94 (23)	0.94	0.69 – 1.27	
Limpopo	30 (59)	21 (41)	2.18	1.21 – 3.93	
Eastern Cape	58 (66)	30 (34)	1.61	0.99 – 2.60	
Western	78 (81)	18 (19)	0.72	0.42 – 1.24	
Cape	58 (62)	35 (38)	1.88	1.18 – 2.98	
Mpumalanga	23 (61)	15 (39)	2.03	1.03 – 4.00	
Free State	13 (62)	8 (38)	1.91	0.78 – 4.72	
North West	12 (80)	3 (20)	0.78	0.21 – 2.79	
Northern Cape					

*KZN- KwaZulu-Natal

Table 3.6: Univariate analysis of clinical factors associated with in-hospital mortality in patients with cryptococcal disease treated for at least 7 days

Risk factor	Recovered	Died	Odds ratio	95% Confidence interval	P-value
Treatment					
Amphotericin B	198 (71)	82 (29)	Reference		
Fluconazole	521 (74)	188 (26)	0.87	0.64 – 1.18	0.285
Amphotericin B + fluconazole	285 (76)	65 (24)	0.75	0.53 - 1.07	
Mental status					
Alert	670 (81)	153 (19)	Reference		
Altered	288 (62)	177 (38)	2.69	2.08 – 3.48	<0.001
CD4+ T cell count					
0-100	385 (74)	133 (26)	Reference		
101-200	59 (81)	14 (19)	0.69	0.37 – 1.27	0.467
>200	18 (75)	6 (25)	0.96	0.38 – 2.48	
ART					
Yes	97 (75)	32 (25)	Reference		
No	739 (74)	254 (26)	1.04	0.68 – 1.59	0.849
Cotrimoxazole prophylaxis					
Yes	539 (75)	184 (25)	Reference		
No	205 (76)	65 (24)	0.93	0.67 – 1.28	0.655
TB treatment					
Yes	586 (75)	200 (25)	Reference		
No	215 (75)	70 (25)	0.95	0.69 1.31	0.450
Wasted					
Yes	259 (68)	119 (32)			
No	1 (100)	0	Undefined	Undefined	

* Anti-retroviraltreatment
■ Anti-tuberculosis treatment

3.6.2 Multivariate analysis

On multivariate analysis amongst patients receiving at least 7 days of treatment, treatment regimen was not independently associated with outcome. After adjusting for confounding factors, age-group, province and altered mental status were found to be independent risk factors for in-hospital mortality in this study (Table 3.7). Patients in the age group 40–59 were 1.5 times more likely to die. Patients from Limpopo, Eastern Cape and Mpumalanga provinces were 2.3, 1.9 and 2.3 times likely to die.

Table 3.7: Multivariate analysis of factors associated with in-hospital mortality in patients with cryptococcal disease treated for at least 7 days

Risk factor	Odds ratio adjusted	95% Confidence interval	P-value
Treatment			
Amphotercin B	1	Reference	0.156
Fluconazole	0.77	0.52 – 1.15	
Amphotercin B and fluconazole	0.68	0.45 – 1.01	
Age group (years)			
20-39	1	Reference	0.044
<20	2.20	0.63 – 7.69	
40-59	1.47	1.11 – 1.96	
≥60	1.29	0.49 – 3.33	
Province			
Gauteng	1	Reference:	<0.001
KwaZulu-Natal	0.99	0.68 – 1.43	
Limpopo	2.30	1.18 – 4.51	
Eastern Cape	1.94	1.15 – 3.27	
Western Cape	0.55	0.28 – 1.07	
Mpumalanga	2.32	1.41 – 3.79	
Free State	1.16	0.50 – 2.69	
North West	1.79	0.60 – 5.33	
Northern Cape	0.49	0.10 – 2.30	
Mental status			
Alert	1	Reference:	<0.001
Altered	2.90	2.22 – 3.83	

3.7 Risk factors associated with mortality among patients with cryptococcal disease treated for less than 7 days

3.7.1 Univariate analysis

In the univariate analysis for demographic and clinical factors associated with in-hospital mortality (Table 3.8), province, treatment regimen and altered mental status were significantly associated with in-hospital mortality. Patients in Limpopo, Eastern Cape and Western Cape were 12.9, 6.4 and 3.9 times likely to die. Treatment with fluconazole only reduced the odds of death by 85%. Patients with an altered mental status were 7.3 times likely die than patients who were alert. Patients not taking anti-tuberculosis treatment were 1.6 times likely to die, although the association was borderline (CI 1.05 – 2.46).

Table 3.8: Univariate analysis of demographic and clinical factors associated with in-hospital mortality among patients with cryptococcal disease treated for less than 7 days

Risk factor	Recovered	Died	Odds ratio	95% confidence interval	P-value
Gender					
Male	225(59)	157(41)	Reference		0.914
Female	275(59)	189(41)	0.98	0.75 – 1.29	
Age group (years)					
20-39	361(60)	236(40)	Reference		0.504
<20	1(33)	2(67)	3.06	0.28 – 33.92	
40-59	135(56)	105(44)	1.19	0.88 – 1.16	
≥60	4(50)	4(50)	1.53	0.38 – 6.18	
Province					
Gauteng	333(67)	167(33)	Reference		<0.001
KwaZulu-Natal	113(57)	84(43)	1.48	1.06 – 2.08	
Limpopo	2(13)	13(87)	12.96	2.89 – 58.1	
Eastern Cape	5(24)	16(76)	6.38	2.29 – 17.70	
Western Cape	10(33)	20(67)	3.98	1.83 – 8.71	
Mpumalanga	31(51)	30(49)	1.93	1.13 – 3.29	
Free State	7(39)	11(61)	3.13	1.19 – 8.23	
North West	0	4(100)	Undefined		
Northern Cape	0	2(100)	Undefined		
Treatment					
Amphotericin B	31(24)	98(76)	Reference		<0.001
Fluconazole	446(68)	214(32)	0.15	0.09 – 0.23	
AmphoterinB+fluconazole	24(41)	35(59)	0.46	0.24 – 0.89	
Mental status					
Alert	374(77)	112(23)	Reference		<0.001
Altered	90(31)	197(67)	7.31	5.27 – 10.13	
CD4+ T cell count					
0-100	219(63)	126(37)	Reference		0.151
101-200	20(61)	13(39)	1.13	0.54 – 2.34	
>200	9(90)	1(10)	0.19	0.02 – 1.54	
ART*					
Yes	44(69)	20(31)	Reference		0.258
No	327(62)	204(38)	1.37	0.79 – 2.39	
Cotrimoxazole prophylaxis					
Yes	82(63)	49(37)	Reference		0.916
No	260(63)	152(37)	1.02	0.68 – 1.53	
TB Treatment■					
Yes	297(64)	165(36)	Reference		0.029
No	56(53)	50(47)	1.61	1.05 – 2.46	
Wasted					
Yes	0	2(100)	Reference:		
No	159(58)	115(42)	Undefined		

*Anti-retroviraltreatment, ■ anti-tuberculosis treatment

3.7.2 Multivariate analysis

On multivariate analysis for factors associated with in-hospital mortality in the subgroup of patients treated for less than 7 days (Table 3.9), treatment regimen was found to be a risk factor for mortality. Treatment with fluconazole reduced the odds of death by 82% compared to treatment with amphotericin B. Compared to Gauteng province, patients from the Eastern Cape were 4.3 times likely to die. Altered mental status was also found to be a risk factor for death.

Table 3.9: Multivariate analysis of factors associated with in-hospital mortality among patients with cryptococcal disease treated for less than 7 days

Risk factor	Odds ratio adjusted	95% Confidence interval	P-value
Treatment			
Amphotericin B	1	Reference	<0.001
Fluconazole	0.18	0.10 – 0.32	
Amphotericin B+ fluconazole	0.51	0.24 – 1.11	
Province			
Gauteng	1	Reference	0.026
KwaZulu-Natal	0.98	0.62 – 1.57	
Limpopo	4.31	0.79 – 23.33	
Eastern Cape	4.33	1.32 – 14.24	
Western Cape	1.42	0.47 – 4.29	
Mpumalanga	1.64	0.84 – 3.19	
Free State	2.42	0.54 – 10.76	
North West	-	-	
Northern Cape	-	-	
Mental status			
Alert	1	Reference	<0.001
Altered	7.49	5.25 – 10.70	

■ Anti-Tuberculosis treatment

CHAPTER 4

4 Discussion

4.1 Introduction

We examined factors that were associated with in-hospital mortality among patients with HIV-associated CD who were treated with amphotericin B alone, fluconazole alone and mixed treatment with amphotericin B and fluconazole. The overall mortality among patients treated for at least 7 days was 26% and this is substantially higher than the mortality observed in the developed world setting: ranging from 3.9% to 15% and has been reported to be decreasing in the post-ART era [5, 20, 27, 29]. Our results are however similar to other series in sub-Saharan Africa where mortality associated with HIV-associated cryptococcal meningitis has been estimated to be between 25%-64% in the pre-ART era [18, 22, 34].

After adjusting for potential confounders in a multivariate logistic regression analysis, the following factors were significantly associated with in-hospital mortality: patients between the age group of 40–59 years, province in which the patients resided and altered mental status. In-hospital mortality was significantly higher in Limpopo, Eastern Cape and Mpumalanga compared to Gauteng province.

There was no association between type of treatment regimen and in-hospital mortality among patients who received treatment for at least 7 days.

There was however a significant difference in mortality by treatment regimen among patients who received treatment for less than 7 days. Patients treated with fluconazole were 82% less likely to die than patients treated with amphotericin B. The reason for this finding is unclear and we could not find studies with similar

findings. Selection bias with clinicians electing to treat patients with poor prognostic factors at presentation with amphotericin B cannot be excluded.

4.2 Representativeness of the study sample

Patients from enhanced surveillance sites with completed CRFs and available data on treatment and outcome were included in the multivariate analysis. We compared cases from enhanced surveillance sites to those from non-enhanced surveillance sites to assess whether the population from enhanced surveillance sites eligible to be included in the final analysis, were comparable to those at non-enhanced sites. The results showed a significant difference in the proportion of patients reported from enhanced and non-enhanced surveillance sites. A higher proportion of patients from Gauteng and KZN province were reported from enhanced surveillance sites. These two provinces have the largest share of the population as well as a high burden of the HIV disease [49-50]. In addition most enhanced sites are located in urban areas where there are tertiary and referral hospitals. The Eastern Cape however had more of their patients coming from the non-enhanced sites. While this is the third largest province in the country, it is more rural than the other two provinces [50]. The difference by reporting site may be a reflection of different health system networks, the accessibility to different levels of health care and the levels of compliance with the surveillance programme by province.

Because of the significant amount of missing data among patients in non-enhanced sites no further comparison was made other than the demographic characteristics. Overall, a large proportion of the patients (65%) reported to the surveillance

programme were from non enhanced surveillance sites and this may limit the generalizability of our results because we do not know how comparable these two groups are for other factors. The generalizability to other provinces may also be limited.

Since the primary objective of the study was analysis of in-hospital mortality among the treatment regimens for HIV-associated CD, we therefore focused on data from enhanced sites with completed CRFs and available data on outcome and treatment. A lot of data were missing from patients with incomplete case report forms and therefore unreliable.

4.3 Systematic overview of study findings

4.3.1 Description of the adult population with a laboratory confirmed incident episode of HIV-associated cryptococcal disease

The incidence of CD in the general population (8-11/100 000 population) in this study was lower than that reported from previous surveillance data in SA [23]. Previous estimates of CD incidence were based on active surveillance done in Gauteng province only and the incidence in our study may have been slightly underestimated due to passive surveillance [23]. The trend of an increase incidence of CD around this period of the study (2005-2006) is however similar to the earlier observation at the beginning of cryptococcal surveillance in SA [8]. More than half of the adult population with CD consisted of females (57%) and the majority of infections occurred in the 20-39 year old age group. This is consistent with the epidemiology of HIV in SA where HIV prevalence is highest

among women aged 15-39 years [49-50]. Most of the patients with cryptococcal disease were reported from the Gauteng and KZN provinces, the two provinces with the largest share of the population as well as a high burden of the HIV disease [49-50].

CD and especially cryptococcal meningitis commonly occurs among HIV infected patients [1, 5-6]. In this study, 99% of those who were tested for HIV were positive and the majority of CD was meningitis (97%). This is in keeping with the earlier surveillance data by McCarthy et al in the Gauteng province of SA where an overwhelming majority of patients with CD were HIV-infected and presented with cryptococcal meningitis [23]. Other series have also reported high proportions of HIV among CD patients. A prospective multicenter study by the cryptococcal study group in France also found that 77% of the patients enrolled with CD in their study were HIV positive [56]. Only 4% in this study were recorded to have non-meningeal presentation. However, other population based series pre-HAART in the USA and France reported larger proportions of patients with extra-meningeal presentations (40 and 22% respectively) [57-58]. It is likely that the non-meningeal manifestations such as pulmonary CD were missed in our study. Autopsy series of AIDS deaths in South Africa have shown that pulmonary CD is common but often misdiagnosed as smear negative tuberculosis [59].

4.3.2 Description of the adult population from enhanced sites with a laboratory confirmed incident episode of HIV-associated cryptococcal disease

Although guidelines for the treatment of HIV-associated CD advocate the use of amphotericin B, a high proportion of patients (62%) in this study were treated with fluconazole alone (18% amphotericin B alone and 20% amphotericin B and fluconazole). Earlier findings by McCarthy et al also showed a similar trend (fluconazole alone 72%, amphotericin B alone 18% and 9% amphotericin B and fluconazole) [23]. The higher proportion of amphotericin B and fluconazole group in our study compared to the findings by McCarthy et al may be an indication that clinicians are considering use of amphotericin B more than in the past. Amphotericin B requires intravenous infusion and monitoring for toxicity, which is often a challenge in many South African health care settings, overburdened by the high patient load and limited resources while fluconazole is more readily available, can be given orally and is well tolerated [6, 39, 43]. More recent data however is showing a trend toward an increase in prescription of amphotericin B [15].

Comparison by duration of treatment showed significant differences in the groups treated for at least 7 days compared to <7 days. The death rate was higher in the <7 days group (41%). It is likely that patients who die before receiving at least 7 days of induction phase treatment may have died due to advanced CD such as presenting with neurological complications and altered mental status or due to co-morbidity such as tuberculosis, the outcome of which would not have been modified greatly by antifungal treatment [34-35]. Among

those treated for <7 days, 59% were from Gauteng province. The reasons might be that Gauteng is more urbanized, densely populated and has the biggest hospitals compared to most provinces. Our observation may thus be a reflection of the high patient load in the hospitals resulting in the need to discharge patients early to make hospital beds available for new admissions. A high proportion of the <7 days group were treated with fluconazole, 78%. This may be a reflection of the ease of administration of fluconazole which is largely administered orally, allowing the patients to be discharged early from hospital, while amphotericin B can only be administered intravenously.

4.3.3 Risk factors of in-hospital mortality

4.3.3.1 Treatment regimen not associated with in-hospital mortality

There was no association between the treatment regimen used and in-hospital mortality. Treatment with fluconazole alone or mixed treatment with amphotericin B and fluconazole was not associated with increased mortality compared to amphotericin B alone. We hypothesized that treatment of patients with CD with amphotericin B for a minimum of 7 days of the induction period was associated with lower in-hospital mortality than in patients with CD treated with fluconazole or a mixture of amphotericin B and fluconazole.

The finding of a high mortality in the context of treatment with amphotericin B is similar to the other studies in Africa. Kambugu et al in Uganda reported 20%-42% 2 week mortality with HIV-associated CM among patients treated with amphotericin B alone [18]. Lightowler et al in a more recent prospective study

in KZN where 80% of the patients were treated with high dose amphotericin B also reported 28% of deaths occurring within the first 14 days of admission [55]. In this study however they were able to demonstrate that patients treated with fluconazole alone had worse outcome than patients treated with amphotericin B alone. Bicanic et al reported that amphotericin B alone was superior over fluconazole alone but this was a small study [17]. In both studies however they had more patients with severe disease receiving fluconazole and thus potential bias resulting in underestimating the efficacy of fluconazole [17, 55].

Randomized therapeutic studies done in the developed countries have shown the use of high dose amphotericin B plus 5-flucytocine to be associated with increased rate of CSF sterilization and decreased mortality at 2 weeks compared to amphotericin B alone and fluconazole alone [20, 40]. Dromer et al in an observational cohort concluded that the combination of amphotericin B and 5-flucytocine was the best regimen for induction therapy among patients with meningoencephalitis, all patients with high fungal burden and abnormal neurology [47]. The regimen was associated with significantly less frequent mycological failure at 2 weeks than any other regimen (23% vs. 47% $p<0.001$), including patients with severe disease [47].

Treatment with amphotericin B was not combined with 5-flucytocine in our study and the absence of 5-flucytocine in the initial phase of treatment has been found to be independently associated with treatment failure and death [20, 56].

Fluconazole is not the recommended primary drug for treatment of CD but is commonly used in our setting. It is fungistatic at doses of 200-400 mg and is

associated slower sterilization of the CSF compared to amphotericin B and 5-flucytocine and amphotericin B alone [39-41]. Higher-dose fluconazole has been proposed as the alternative for induction phase in the treatment for CD [25]. While they have demonstrated a significant increase in early fungicidal activity when fluconazole dosage was increased from 800mg/d to 1200mg/d this has not translated into improved patient outcomes [25]. The proportion of patients in our study who were treated with a higher dose of fluconazole did not have a better outcome compared to those treated with a lower dose. Given the superiority of amphotericin B over fluconazole in fungicidal activity, rapid sterilization of the CSF it is possible that some factors in our study may have confounded and diluted the effect of amphotericin B.

Comparison of patients by treatment regimen however showed that patients in the fluconazole group and the fluconazole plus amphotericin B group had a higher proportion of patients with an altered mental status compared to those in the amphotericin B group. An altered mental status is a marker of severity of the disease and this suggest that patients in the fluconazole group had more severe disease than those in the amphotericin B group [28, 56]. Altered mental status continued to be a significant risk factor for mortality in the multivariate analysis. In a setting where treatment is available, including amphotericin B, it is possible that other factors such as late presentation of patients for health care and possibly suboptimal treatment during the initiation phase such as fluconazole monotherapy and unavailability of 5-flucytocine contribute to the high mortality.

4.3.3.2 Age a risk factors for in-hospital mortality

To our knowledge age has not been previously found to be risk factor for mortality within the adult population. In this study patients in the 40–59 year old age group were 1.5 times likely to die compared the 20–39 year age group. However within the 40–59 year old age group there are likely to be individuals with underlying morbidity such as uncontrolled diabetes mellitus and other vascular diseases that may complicate the presentation of CD. Data on underlying disease were not captured in our study.

4.3.3.3 Gender not risk factors for in-hospital mortality

Gender was not associated with in-hospital mortality. We included this factor in our analysis as a priori. While studies in Europe have reported a higher in incidence of CD among men compared to females, gender has not been shown to be a risk factor for death [47].

4.3.3.4 In-hospital mortality differed by province

Analysis in this study showed that in hospital mortality differed by provinces. In-hospital mortality was significantly higher in Limpopo, Eastern Cape and Mpumalanga compared to Gauteng province. The odds of dying in the other provinces were twice that of being in the Gauteng province and this was statistically significant at $p<0.001$. The availability of diagnostics, drugs, trained medical and nursing staff and other health systems, which may influence access

to health care and therefore timely diagnosis, differs among provinces. While we are unable to confirm this due to the nature of our data, these offer possible explanations to our observation.

4.3.3.5 Altered mental status associated with in-hospital mortality

Altered mental status was an independent risk factor significantly associated with increased in-hospital mortality. The odds of dying were three times likely if the patients had an altered mental status compared to an alert mental status. This is consistent with the findings in studies that have described pretreatment factors associated with early death during treatment in patients with HIV-associated CD [18, 28, 55]. Altered mental status is a marker of severe disease and is associated with raised intracranial pressure, which has also been cited as a risk factor of mortality [60]. Patients in our study did not have recordings of opening intracranial pressure during lumber puncture and it is not general practice to measure in many health care centers.

Acute management and monitoring of raised intracranial pressure is considered to be one of the interventions that are associated with improvement of early outcomes of cryptococcal meningitis [21]. In a study by Graybill ct.al repeated lumbar punctures to drain CSF and to reduce pressure were performed [60]. After receiving antifungal therapy those patients whose CSF reduced by >10 mm or did not change had more frequent clinical response at 2 weeks than those whose pressure increased >10 mm. Patients with pretreatment opening pressure

<250mmH_2O had increased short term survival compared to those without a higher pressure.

4.3.3.6 Cotrimoxazole prophylaxis not associated with in-hospital mortality

There was no association between the administration of cotrimoxazole prophylaxis and in-hospital mortality. We included cotrimoxazole prophylaxis in our analysis because use of cotrimoxazole has been associated with a reduction in all cause mortality by decreasing other opportunistic infections among patients infected with HIV [53-54]. The presence of other opportunistic infections may contribute to early mortality in patients with CD.

4.3.3.7 Concurrent tuberculosis treatment not a risk factor for in-hospital mortality

There was no association between concurrent tuberculosis treatment and mortality. We used it as a marker of underlying tuberculosis, which as a serious co-morbid illness could adversely influence the outcome. Lightowler et al were the first to describe the association of concurrent tuberculosis treatment with an increased risk of mortality in CD [55]. They attributed this to the high rate of ongoing transmission in the community and possibly drug resistant tuberculosis in a small number of those who were on tuberculosis treatment. None of their patients were thought to have active tuberculous meningitis and treatment compliance was unlikely to be the problem in their assessment.

4.3.3.8 CD4+ T-cell count not a risk factor for in-hospital mortality

There was no association between CD4+ T-cell count and in-hospital mortality. To our knowledge CD4+ T-cell count has not been associated with increased mortality in HIV-associated CM. However, a CD4+ T-cell count level below 100 cells /µl is a risk factor for developing CD in particular cryptococcal meningitis [6]. More than 80% of our study patients had a CD4+ T-cell count below a 100 cells/µl.

4.3.3.9 Antiretroviral therapy not a risk factor for in-hospital mortality

There was no association between use of antiretroviral treatment and in-hospital mortality. There is uncertainty on whether short-term outcomes are better in those already on ART at presentation when compared to ART naïve patients [16]. Jarvis et al reported that about 20% of adults with a first episode of HIV-associated CD presented shortly after ART initiation and that although they had favorable prognostic signs the outcomes were poor and no better than in the ART naïve patients [36]. Makadzange et al found that ART initiated within 3 days of presentation with CD was associated with increased mortality in HIV-associated CD [61]. It is suggested that these patients might be experiencing unmasking of CD after initiation of ART as a result of immune reconstitution and studies on the optimal timing of ART are ongoing. Only a small fraction of our study patients were on ART and our study may not have been powered to show the difference between the ART experienced and ART naïve.

4.3.3.10 Wasting not a risk factor for in-hospital mortality

Wasting was not associated with in-hospital mortality. It was included in the analysis because wasting with more than 10% of body weight is generally a finding of advanced HIV disease [62]. Antifungal treatment may therefore not influence the outcome of CD.

4.4 Potential study biases

4.4.1 Collection of exposure and outcome data

Data collection using objective data sources such as medical record review was standard practice. Patient interviews were also conducted on clinical factors missing on medical records such as current use of ART, cotrimoxazole prophylaxis and concurrent treatment for tuberculosis. Systemic differences might have arisen due to differences in interviewing skills between surveillance officers.

4.4.2 Underestimation of incidence

Surveillance for HIV-associated CD uses laboratory based surveillance system [8]. Patients who died at home, before hospital admission or those in whom lumbar puncture was not performed would not have been captured by the surveillance system. In addition the compliance of laboratories in submitting isolates to the surveillance programme may differ and prior to the implementation of audits for laboratories some cases may have been missed

resulting in underestimation of the incidence reported in this study [63]. Thus surveillance bias might exist in our study.

4.4.3 Bias in risk factor analysis

Underestimation of disease burden might have introduced an erroneous estimation of in-hospital mortality and associated risk factors in our study. Patients who died at home or palliative care centers were not included in our study.

4.5 Residual confounding

All factors of interest were initially examined in a univariate model to determine if they were associated with increased risk of mortality. Significant factors at $p \leq 0.1$ were included in the multivariate logistic regression model where each factor controlled for confounding effect of the other. Factors significant at $p \leq 0.05$ were considered significant risk factors for mortality.

Additional factors that have been described as risk factors for early death among patients with HIV-associated CD include: raised intracranial pressure, abnormal brain imaging, presence of fungaemia, high CSF antigen titre and high serum antigen titre [26, 56]. Although data was available on whether some patients in this study had fungaemia, we were unable to evaluate this variable as a risk factor for mortality. This is because we only had information on patients who tested positive on blood cultures but did not have any information on how many patients

had a blood culture test and which patients tested negative on blood culture for comparison. Data on other variables was also not available. Residual confounding due to these factors cannot be excluded. We did not examine all factors that could be risk factors for in-hospital mortality such as socioeconomic factors and access to health.

4.6 Generalizability

The difference in the distribution of cases by province might limit generalizability of our study results to other provinces. Not knowing the comparability regarding beyond demographic factors between patients from enhanced and non-enhanced sites might also limit our generalizability.

4.7 Study strengths

- Our study used national data of laboratory confirmed cases of HIV-associated CD to determine if treatment regimen influenced in-hospital mortality.
- The large numbers of patients included in our study gave enough power to detect difference (no difference) between treatment regimens in the initial phase of treatment in our setting.

4.8 Study limitations

- Detailed data on other clinical indicators of severity of illness such as the cryptococcal antigen titers, opening intracranial pressure and CSF cell count

was not available and as such we could not make an in depth analysis of other factors associated with in-hospital mortality

- We did not evaluate if patients treated with amphotericin B were prescribed appropriate doses as per weight and evaluate minimum/maximum dose at time of outcome. Thus could not evaluate if dose was associated with mortality
- Our study was a retrospective analysis of data from the surveillance database. The data was not collected specifically for this purpose, thus some measurements of interest were captured in manner that limited the information e.g. CD4 count was captured as a range rather than the exact count
- There was insufficient data on ART regimen; when treatment was started in relation to the current illness with CD and as such we were unable to examine the association with mortality fully
- This was an in-hospital mortality study making the assumption that discharged patients lived. Patients who died shortly after hospital discharge or discharged to die in palliative care facilities were not considered and we might have underestimated early mortality

4.9 Suggestions for further studies

In view of findings discussed in this chapter, further studies are required to determine the optimal alternative treatment to combination therapy with amphotericin B and 5-flucytocine that will be suitable to the settings in developing countries.

In addition the association of province in which the patient resided with mortality needs further investigation to tease out factors that are amenable to intervention to improve health outcomes in these provinces.

Studies to explore management and monitoring of raised intracranial pressure as one of the interventions that can improve early outcomes of cryptococcal meningitis should be considered.

5.1 Conclusion

In conclusion we found that in-hospital mortality among patients treated with an amphotericin B or fluconazole containing regimen for 7 days was 26%. In-hospital mortality was similar among patients treated with amphotericin B or fluconazole or amphotericin and fluconazole. High dose fluconazole was not associated with better outcomes compared to treatment with a lower dose. Altered mental status, province where patients resided and age-group 40–59 years were found to be high risk factors for death. Only a small fraction of our study patients were on ART thus our study was not powered to show the difference in mortality between the ART experienced and ART naïve patients. While hypothesis of this study was not proven, the significant reduction in mortality among patients treated with fluconazole for <7 days is an important finding that needs further exploration. It may be that amphotericin B only is not superior but equivalent to fluconazole in the first 7 days of treatment and the use of 5-flucytosine may be the intervention required to improve outcome in the first 7 days. Or it may be that patients are reaching care too late for a significant impact in disease outcome to be observed and that prevention of CD is required; namely early initiation of ART and primary prophylaxis for CD. Finally the most likely reason for the lower mortality in the fluconazole treated group is selection bias in that they are less sick than in the amphotericin B group.

5.2 Summary and recommendations

In summary, the study findings that treatment with amphotericin B versus fluconazole does not lead to improvement in the outcome of in-hospital mortality

among patients with HIV-associated CD in South Africa are valid and unlikely to be due to chance. While fluconazole is not the primary drug of choice for treatment of CD in the initial phase, it is used widely in our setting. To our knowledge no studies have been done nationally to compare the outcome among patients treated with fluconazole versus the drug of choice in the initial phase; amphotericin B. The findings of a high mortality among all patients with CD while on antifungal treatment, as well as lack of association of treatment with amphotericin B with improved outcomes underscores the need to procure 5-flucytosine in this part of the world. Studies on the management of HIV-associated CD emphasize the importance of the 5-flucytocine and amphotericin B combination in the initial phase. Lack of 5-flucytocine in the treatment regimen has been associated with death and the combination with amphotericin B has been associated with improved outcomes among patients with high risk factors for death. Our study has broader implications on the public health policy and government to commit to improving access to health in all provinces, adequate management of HIV disease and up-scaling of ART. Optimal acute management of CD improves the patient's chances of initial survival thus presenting an opportunity for subsequent entry into the ART program. Our findings thus call for more standardization of the optimum treatment available as well as advocacy for the availability of 5-flucytocine. These could be achieved with retraining of doctors and more vigorous audits of mortality data.

APPENDIX A

Clinical Case Report for Surveillance of Invasive *Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.

RESPIRATORY AND MENINGEAL PATHOGENS RESEARCH UNIT (RMPRU) TEL: 011 489 9710 / FAX: 011 489 9716

ENTERIC DISEASES REFERENCE UNIT (EDRU) TEL: 011 489 9333 / FAX: 011 489 9361

MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9361

Regional Laboratory Specimen Number: ☐☐☐☐☐☐☐☐☐☐ Laboratory Name:

Hospital Name:

Hospital Number: | Ward: | Gender: M ☐ F ☐ Unk ☐ | Race: Asian ☐ Coloured ☐ Black ☐ White ☐ Unk ☐ | Date of Birth: d d m m y y y y DOB Unk ☐ | Age: ☐☐☐ Units: days ☐ months ☐ yrs ☐

Name of Patient: surname: first name: middle initial:

Address: Town/City: Province:

Tel: (H) (W) (Cell) (Neighbour)

Have you stayed in SA for the last month: Yes ☐ No ☐ Unk ☐ If no, which country have you come from? _____

Date of admission to Acute Hospital: d d m m y y y y

Outcome at Acute Hospital: Transferred ☐ Discharged ☐ Died ☐ RHT/Absconded ☐ Unk ☐

If patient was transferred, name of hospital transferred to: _____ Date of Transfer: d d m m y y y y

Final outcome of patient at place of discharge: Discharged ☐ Died ☐ RHT/Absconded ☐ Unk ☐ Date of final outcome: d d m m y y y y

DIAGNOSIS

Meningitis ☐ LRTI ☐ Dysentery ☐ Diarrhoea ☐ Fungaemia/Bacteraemia without focus ☐ Other ☐ Specify _____

Date of specimen collection d d m m y y y y

Site of collection CSF ☐ Blood Culture ☐ Joint Fluid ☐ Other _____

SPECIES ISOLATED

Haemophilus sp. ☐ *N. meningitidis* ☐ *S. pneumoniae* ☐

Salmonella sp. ☐ *Shigella* sp. ☐ *Cryptococcus* sp. ☐

Number of children living with you: (<18 years) None ☐ Number ☐ Place of safety ☐ Unk ☐

Have any of these children been hospitalised recently? (last 3 months) Yes ☐ No ☐ Unk ☐

SEVERITY OF ILLNESS (On the day the positive specimen was taken)

Temperature: ____ °C Fever: Yes ☐ No ☐ Unk ☐ BP: ____/____ Unk ☐ Mechanical Ventilation: Yes ☐ No ☐ Unk ☐ Cardiac Arrest: Yes ☐ No ☐ Unk ☐

Mental Status: Alert ☐ Disorientated ☐ Stuporous ☐ Comatosed ☐ Unk ☐ GCS ____/15 ___E ___M ___V ___ Unk ☐

UNDERLYING DISEASES

HIV STATUS PRIOR TO THIS ADMISSION Positive ☐ Negative ☐ Unknown ☐

HIV STATUS AT THIS ADMISSION Positive ☐ Negative ☐ Unknown ☐

Pre & Post test counselling offered by SO Yes ☐ No ☐

If yes, was HIV consent given to SO Yes ☐ No ☐

If NO HIV taken, is there clinical suspicion of HIV? Yes ☐ No ☐ Unk ☐

Most recent CD4 count: Absolute ____ ; ____ % Date taken d d m m y y y y Unk ☐

Most recent viral load: _____ Date taken d d m m y y y y Unk ☐

OTHER IMMUNOCOMPROMISE: *(Tick all that apply)* ☐ None ☐ Unknown

- ☐ TB
- ☐ Oral candidiasis
- ☐ PCP
- ☐ Wasting secondary to HIV
- ☐ Chronic diarrhoea > 10 days
- ☐ Kaposis Sarcoma
- ☐ Current smoker
- ☐ Sickle cell anaemia
- ☐ Splenectomy/asplenia
- ☐ Immunoglobulin deficiency
- ☐ Immunosuppressive therapy (Steroids,Chemotherapy,Radiation)
- ☐ Kwashiokor/marasmus
- ☐ Asthma
- ☐ Emphysema/COPD
- ☐ Diabetes mellitus
- ☐ Nephrotic syndrome
- ☐ Chronic renal failure
- ☐ Systemic Lupus Erythematosus(SLE)
- ☐ Cirrhosis/Liver failure
- ☐ Alcohol dependancy
- ☐ Coronary artery disease
- ☐ Valvular disease
- ☐ Heart failure
- ☐ Burns
- ☐ Cerebral vascular accident (CVA)/ Stroke
- ☐ History of head injury/ head surgery
- ☐ Hydrocephalus with VP shunt
- ☐ Malignancy (specify) _____
- ☐ Organ transplant (specify) _____
- ☐ Other (specify) _____

PREVIOUS ADMISSIONS in last 12 months: Yes ☐ No ☐ Unk ☐ Number of admissions ☐☐

APPENDIX A

Clinical Case Report for Surveillance of Invasive *Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.

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MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9361

Regional Laboratory Specimen Number: ☐☐☐☐☐☐☐☐☐☐

VACCINATION STATUS FOR *STREPTOCOCCUS PNEUMONIAE*

If <15 years of age did patient receive pneumococcal conjugate vaccine? Yes ☐ No ☐ Unk ☐ If YES, please complete the list below

DOSE	DATE GIVEN (d d m m y y y y)	NAME OF CLINIC
1	☐☐☐☐☐☐☐☐	
2	☐☐☐☐☐☐☐☐	
3	☐☐☐☐☐☐☐☐	

Has patient received 23-valent pneumococcal polysaccharide vaccine?
Yes ☐ No ☐ Unk ☐

If YES, list date most recently given and vaccine name
(d d m m y y y y) ☐☐☐☐☐☐☐☐ _ _ _ _ _ _ _ _ _ _ _ _

HAEMOPHILUS INFLUENZAE

If <15 years of age did patient receive *Haemophilus influenzae* type b vaccine? Yes ☐ No ☐ Unk ☐

DOSE	DATE GIVEN (d d m m y y y y)	NAME OF CLINIC
1	☐☐☐☐☐☐☐☐	
2	☐☐☐☐☐☐☐☐	
3	☐☐☐☐☐☐☐☐	

Was there documented proof of vaccination for:
Haemophilus influenzae type b (Hib) vaccine?
Yes ☐ No ☐ Unk ☐

OTHER VACCINATIONS

Meningococcal vaccine: A/C ☐ A/C/Y/W135 ☐ *Salmonella typhi* vaccine ☐ Date of vaccination (d d m m y y y y) ☐☐☐☐☐☐☐☐

ANTIBIOTICS PRIOR TO THIS ADMISSION

Cotrimoxazole prophylaxis Yes ☐ No ☐ Unk ☐	Dose: Route:		Date initiated: (d d m m y y y y) ☐☐☐☐☐☐☐☐	Compliant in last month: Most ☐ Some ☐ None ☐
ABX in 24 hours before specimen: Yes ☐ No ☐	Names: 1 2 3 4	Dose:	Route:	Date initiated: (d d m m y y y y) ☐☐☐☐☐☐☐☐
Other ABX in 2 months Yes ☐ No ☐ Unk ☐	Names: 1 2	Dose:	In the last 30 days: Yes ☐ No ☐	In the last 30 to 60 days: Yes ☐ No ☐
TB Rx Yes ☐ No ☐ Unk ☐	Drugs: 1 2	Dose:	Date initiated: (d d m m y y y y) ☐☐☐☐☐☐☐☐	Compliant in last month: Most ☐ Some ☐ None ☐

ANTIBIOTIC USE IN HOSPITAL DURING THIS ADMISSION (excluding TB therapy)

Name of antimicrobial	Dose	Route	Date initiated (d d m m y y y y)	Total doses given/no. of days
1			☐☐☐☐☐☐☐☐	
2			☐☐☐☐☐☐☐☐	
3			☐☐☐☐☐☐☐☐	
4			☐☐☐☐☐☐☐☐	
5			☐☐☐☐☐☐☐☐	

ANTIRETROVIRAL USE

Any antiretroviral use? Yes ☐ No ☐ Unk ☐ If Yes: Current ☐ Previous ☐ Perinatal ☐ Unk ☐

Current Antiretroviral therapy: 3TC ☐ D4T ☐ Efavirenz ☐ Nevirapine ☐ AZT ☐ DDI ☐ Kaletra ☐ Unk ☐ Duration: Months _ _ _ _ _ _ .

APPENDIX A

Clinical Case Report for Surveillance of Invasive
***Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.**

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ENTERIC DISEASES REFERENCE UNIT (EDRU) TEL: 011 489 9333 / FAX: 011 489 9361

MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9361

ANTIFUNGALS PRIOR TO THIS ADMISSSION (For crypto isolates ONLY)

			Date initiated	Dose:
Fluconazole:	Yes ☐ No ☐ Unk ☐	If yes	d d m m y y y y	
Amphotericin B	Yes ☐ No ☐ Unk ☐	If yes	d d m m y y y y	

ANTIFUNGALS DURING THIS ADMISSSION

	Dose	Route	Date initiated	Total doses given/no. of days
Fluconazole:			d d m m y y y y	
Amphotericin B			d d m m y y y y	

On discharge was the patient given fluconazole? Yes ☐ No ☐ Unk ☐

Sources of data: Patient/Guardian ☐ Clinician ☐ Medical Records ☐

Has regional laboratory sent isolate to RMPRU/EDRU/MRU Yes ☐ No ☐ **Date isolate sent** d d m m y y y y

SURVEILLANCE OFFICER NAME: .....

APPENDIX B

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Xoliswa Poswa

CLEARANCE CERTIFICATE	**M090233**
PROJECT	School of Public Health Disease in Patients Treated with Amphotericin B Virus versus Fluconazole
INVESTIGATORS	Dr Xoliswa Poswa.
DEPARTMENT	School of Public Health
DATE CONSIDERED	09.02.27
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.03.02 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr C Cohen

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....

REFERENCES

1. Perfect, J., *Cryptococcus neoformans: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 7th Edition ed.
2. Kwon-Chung, K.J., et al., *(1557) Proposal to conserve the name Cryptococcus gattii against C. honduri-anus and C. bacillisporus (Basidiomycota, Hymenomycetes, Tremellomycet-idae).* Taxon, 2002. **51**: p. 804-806.
3. Garcio-Hermoso, D., Janbon, G., Dromer, F., *Epidemiological evidence of dormant Cryptococcus neoformans infection* J. Clin. Microbiol., 1999. **37**: p. 3204-3209.
4. Lim, TS, Murphy, M.J., *Transfer of immunity to cryptococcosis by T-enriched splenic lymphocytes from Cryptococcus neoformans-sensitized mice.* Infection and Immunity 1980. **30**(1): p. 5-11.
5. Park, B.J., et al., *Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS.* AIDS, 2009. **23**(4): p. 525-30.
6. Crowe, S.M., et al., *Predictive value of CD4 lymphocyte numbers for the development of opportunistic infections and malignancies in HIV-infected persons.* J Acquir Immune Defic Syndr, 1991. **4**(8): p. 770-6.
7. Pappas, P.G., et al., *Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy.* Clin Infect Dis, 2001. **33**(5): p. 690-9.
8. *GERMS South Africa Annual Report*. 2007, NICD.
9. *UNAIDS. Report on the Global HIV/AIDS Epidemic*. 2008, Geneva.
10. Jarvis, J.N., Harrison, T.S. , *HIV-associated cryptococcal meningitis.* AIDS, 2007. **21**: p. 2119-2129.
11. Mirza, S.A., et al., *The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000.* Clin Infect Dis, 2003. **36**(6): p. 789-94.
12. Dromer, F., et al., *Epidemiology of HIV-associated cryptococcosis in France (1985-2001): comparison of the pre- and post-HAART eras.* AIDS, 2004. **18**(3): p. 555-62.
13. DOH, *Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa 2003.* . 2003, Pretoria: South Africa Department of Health, 2003.
14. WHO, *Towards Universal Access: Scaling Up Priority HIV/AIDS Interventions in the Health Sector: Progress Report.* 2008, Geneva: WHO Press, 2008
15. Govender, N., et al., *Trends in Treatment of Adults with Incident Cryptococcosis, South Africa, 2005-2008*. 2010, NICD.
16. Jarvis, J.N., et al., *High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out.* AIDS, 2009. **23**(9): p. 1182-3.
17. Bicanic, T., et al., *Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole.* Clin Infect Dis, 2007. **45**(1): p. 76-80.

18. Kambugu, A., et al., *Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy.* Clin Infect Dis, 2008. **46**(11): p. 1694-701.
19. Saag, M.S., et al., *Practice guidelines for the management of cryptococcal disease. Infectious Diseases Society of America.* Clin Infect Dis, 2000. **30**(4): p. 710-8.
20. van der Horst, C.M., et al., *Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group.* N Engl J Med, 1997. **337**(1): p. 15-21.
21. *Guidelines for the prevention diagnosis and managment of cryptococcal meningitis and disseminated cryptococcosis in HIV-Infected patients.* The South African Journal of HIV Medicine, 2007: p. 1-14.
22. Schaars, C.F., et al., *Outcome of AIDS-associated cryptococcal meningitis initially treated with 200 mg/day or 400 mg/day of fluconazole.* BMC Infect Dis, 2006. **6**: p. 118.
23. McCarthy, K.M., et al., *Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence.* AIDS, 2006. **20**(17): p. 2199-206.
24. Haubrich, R.H., et al., *High-dose fluconazole for treatment of cryptococcal disease in patients with human immunodeficiency virus infection. The California Collaborative Treatment Group.* J Infect Dis, 1994. **170**(1): p. 238-42.
25. Longley, N., et al., *Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda.* Clin Infect Dis, 2008. **47**(12): p. 1556-61.
26. Chuck, S.L. and M.A. Sande, *Infections with Cryptococcus neoformans in the acquired immunodeficiency syndrome.* N Engl J Med, 1989. **321**(12): p. 794-9.
27. Robinson, P.A., et al., *Early mycological treatment failure in AIDS-associated cryptococcal meningitis.* Clin Infect Dis, 1999. **28**(1): p. 82-92.
28. Saag, M.S., et al., *Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group.* N Engl J Med, 1992. **326**(2): p. 83-9.
29. McNaghten, A.D., et al., *Effects of antiretroviral therapy and opportunistic illness primary chemoprophylaxis on survival after AIDS diagnosis. Adult/Adolescent Spectrum of Disease Group.* AIDS, 1999. **13**(13): p. 1687-95.
30. *Update: trends in AIDS incidence - United States.* 1996, CDC.
31. *Update: trends in AIDS incidence*, in *Morbidity and Mortality Weekly Report.* 1997. p. 861-867.
32. *Update: Trends in AIDS incidence, Deaths, and Prevalence*, in *Morbiditiy and Mortality Weekly.* 1997. p. 165-173.
33. Gulick, R.M., et al., *Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy.* N Engl J Med, 1997. **337**(11): p. 734-9.
34. Moosa, M.Y. and Coovadia, Y.M., *Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for*

human immunodeficiency virus (HIV)-positive and HIV-negative patients. Clin Infect Dis, 1997. **24**(2): p. 131-4.
35. Mwaba, P., et al., *Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions.* Postgrad Med J, 2001. **77**(914): p. 769-73.
36. Jarvis, J.N., G. Meintjes, and T.S. Harrison, *Outcomes of cryptococcal meningitis in antiretroviral naive and experienced patients in South Africa.* J Infect, 2010. **60**(6): p. 496-8.
37. Powderly, W.G., et al., *A controlled trial of fluconazole or amphotericin B to prevent relapse of cryptococcal meningitis in patients with the acquired immunodeficiency syndrome. The NIAID AIDS Clinical Trials Group and Mycoses Study Group.* N Engl J Med, 1992. **326**(12): p. 793-8.
38. Lortholary, O., et al., *Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy.* AIDS, 2006. **20**(17): p. 2183-91.
39. Rex, H.J., Stevens, D.A., *Systemic antifungal agents: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 7th Edition ed.
40. Bennett, J.E., et al., *A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptoccal meningitis.* N Engl J Med, 1979. **301**(3): p. 126-31.
41. Brouwer, A.E., Rajanuwong, A., Chierakul, W., Griffin, G.E., Larsen, R.A., White, N.J., Harrison, T.S. , *Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial.* Lancet 2004. **363**: p. 1764 - 1767.
42. Tansuphaswadikul, S., et al., *Comparison of one week with two week regimens of amphotericin B both followed by fluconazole in the treatment of cryptococcal meningitis among AIDS patients.* J Med Assoc Thai, 2006. **89**(10): p. 1677-85.
43. DeMuria, D., et al., *Pharmacokinetics and bioavailability of fluconazole in patients with AIDS.* Antimicrob Agents Chemother, 1993. **37**(10): p. 2187-92.
44. Smith, J. and D. Andes, *Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations.* Ther Drug Monit, 2008. **30**(2): p. 167-72.
45. Nussbaum, J.C., et al., *Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi.* Clin Infect Dis, 2010. **50**(3): p. 338-44.
46. Bicanic, T., et al., *High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial.* Clin Infect Dis, 2008. **47**(1): p. 123-30.
47. Dromer, F., et al., *Major role for amphotericin B-flucytosine combination in severe cryptococcosis.* PLoS One, 2008. **3**(8): p. e2870.
48. Bicanic, T., et al., *Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures.* AIDS, 2009. **23**(6): p. 701-6.
49. *Mid-year population estimates, South Africa*. 2005, Statistics South Africa.
50. *Mid-year population estimates, South Africa*. 2006, Statistics South Africa.
51. Tanner, D.C., Weinstein, M.P., Fedorciw, B., Joho, K.L., Thorpe, J.J., and Reller, L.B., *Comparison of commercial kits for detection of cryptococcal antigen.* J. Clin. Microbiol., 1994. **32**(7): p. 1680-1684.

52. Singh, E., et al., *A description of HIV testing strategies at 21 laboratories in South Africa.* Communicable Diseases Surveillance Bulletin, 2008: p. 16-19.
53. Hardy, W.D., et al., *A controlled trial of trimethoprim-sulfamethoxazole or aerosolized pentamidine for secondary prophylaxis of Pneumocystis carinii pneumonia in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trials Group Protocol 021.* N Engl J Med, 1992. **327**(26): p. 1842-8.
54. Walker, A.S., et al., *Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort.* Lancet, 2010. **375**(9722): p. 1278-86.
55. Lightowler, J.V., et al., *Treatment of cryptococcal meningitis in KwaZulu-Natal, South Africa.* PLoS One, 2010. **5**(1): p. e8630.
56. Dromer, F., et al., *Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study.* PLoS Med, 2007. **4**(2): p. e21.
57. Hajjeh, R.A., et al., *Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. Cryptococcal Active Surveillance Group.* J Infect Dis, 1999. **179**(2): p. 449-54.
58. Friedman, G.D., et al., *Cryptococcosis: the 1981-2000 epidemic.* Mycoses, 2005. **48**(2): p. 122-5.
59. Wong, M.L., et al., *Cryptococcal pneumonia in African miners at autopsy.* Int J Tuberc Lung Dis, 2007. **11**(5): p. 528-33.
60. Graybill, J.R., et al., *Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups.* Clin Infect Dis, 2000. **30**(1): p. 47-54.
61. Makadzange, A.T., et al., *Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa.* Clin Infect Dis, 2010. **50**(11): p. 1532-8.
62. *WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children* 2007, WHO Press. p. 39.
63. *GERMS South Africa Annual Report.* 2009, NICD.