# DURBAN UNIVERSITY OF TECHNOLOGY

The Impact of Cryptococcal Antigenaemia Screening Among Patients Attending King Dinuzulu Hospital

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# The Impact of Cryptococcal Antigenaemia Screening Among Patients Attending King Dinuzulu Hospital

By

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Durban University of Technology

I, Missi Emilienne Yohali, do declare that this dissertation is representative of my own work in both conception and execution (except where acknowledgements indicate to the contrary)

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# DEDICATION

Firstly, I dedicate this dissertation to God Almighty for seeing me through the hurdles of my Master's degree programme. Secondly, I dedicate this dissertation to my best friend, my husband. You are my supporter and my strength. I hope I have made you proud. To my parents for all the support they gave me throughout my education.

Finally, this dissertation is dedicated to all patients undergoing treatment for cryptococcal meningitis at King Dinuzulu Hospital whose health condition inspired this study.

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# ABSTRACT


## Introduction

The goal of this study was to give a general overview of *Cryptococcus* as a cryptococcosis causative agent. The morbidity, mortality and management of this type of infection are discussed, as well as the collaboration between the diagnostic laboratory service and the Department of Health.

## Aim

The goal of this study was to see how cryptococcal antigenemia screening affected patient care at KDH.

## Objectives

To determine whether screening for cryptococcus antigenemia leads to doctors necessary follow-up investigations. To report how often positive serum cryptococcal antigen screening results in cryptococcal meningitis treatment. To see if the current CD4 antigenemia threshold of less than 100 cells/µl is suitable for our patient population.

## Materials and Method

This study took samples from patients at King Dinuzulu Hospital in Durban, eThekwini, KwaZulu-Natal, South Africa. The Laboratory Information System was utilized to diagnose cryptococcal infections in patients' serum, plasma, and cerebrospinal fluid (CSF), as well as for normal patient management (Trakcare). Clinicians gathered specimens from patients as part of standard clinical care and forwarded them to the laboratory as data sources for this study. Cross-sectional, descriptive, and quantitative research were conducted on these specimens.

## Results

The data that satisfied the inclusion criteria of the study were collected and analysed. Assay of 724 CSF and serum/plasma cryptococcal antigen (CrAg) results, including CSF culture results (n = 264) and CD4 counts (n = 446), were analysed for this section of the study. The bulk of CD4 counts, 433 (97.1%), were < 100 cell/µl whereas 13 (2.9%) were > 100 cells/µL. The number of patients with a CD4 count who stuck

to their treatment was 22 out of 62. Eighty-five CrAg positive findings were obtained, with 62 receiving a CD4 count. There were 31 CrAg with a CD4 count of 0-49 cells/µL, 28 with a CD4 count of 50-99 cells/µL, one with a CD4 count of 100-149 cells/µL, one with a CD4 count of 150-199 cells/µL, and one with a CD4 count of more than 200 cells/µL. A higher percentage of patients had a follow up appointment. There was a higher proportion of patients who received a follow-up (10, 55.6%) than those who did not (3, 6.8%) $p < 0.001$. Furthermore, those who started treatment within seven days had a higher proportion of patients without follow up (81.8 percent, n = 36) than those who had a follow up (0 percent, n = 0).

**Conclusion**

In conclusion, there was no positive benefit of CrAg screening on HIV-positive patients attending KDH, as stated in the study's goal. In terms of the study's goal and objectives, the findings revealed that the majority of positive CrAg samples were nt followed up on.

**Keywords**: Impact, Cryptococcal antigenaemia, Screening, *Cryptococcus neoformans*, Cryptococcus meningitis.

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# ABBREVIATIONS

| | |
|---|---|
| **AIDS** | Acquired Immune Deficiency Syndrome |
| **ART** | Antiretroviral Therapy |
| **CM** | Cryptococcal Meningitis |
| **CrAg** | Cryptococcal Antigen |
| **CrAgLFA** | Cryptococcal Antigen Lateral Flow Assay |
| **CSF** | Cerebrospinal Fluid |
| **CT** | Computer Tomography |
| **DOH** | Department of Health |
| **HIV** | Human Immunodeficincy Virus |
| **IREC** | Institutional Research Ethics Committee |
| **KDH** | King Dinuzulu Hospital |
| **KZN** | KwaZulu-Natal |
| **LIS** | Laboratory Information System |
| **LP** | Lumbar Puncture |
| **MLST** | Multiple Locus Sequence Typing |
| **NDOH** | National Department of Health |
| **NHLS** | National Health Laboratory Service |
| **AARMS** | Academic Affairs & Research Management System |
| **NICD** | National Institute for Communicable Disease |
| **PHC** | Primary Health Care |
| **PS** | Polysaccharide |
| **SA** | South Africa |
| **SCrAg** | Serum Cryptococcal Antigen |
| **SSA** | Sub-Saharan Africa |
| **WHO** | World Health Organization |

# CHAPTER 1: INTRODUCTION

## 1.1 Introduction

This chapter provides an overview to *Cryptococcus*, the organism that causes cryptococcosis. It gives an overview of this type of infection in terms of morbidity, mortality, and illness management, as well as the diagnostic laboratory service's partnership with the national Department of Health (DoH). In addition, this chapter explains the study's purpose, objectives, and reasoning. Finally, an overview of the dissertation is offered.

## 1.2 Background

Cryptococcosis is a life-threatening fungal disease caused by pathogenic species-complexes of *Cryptococcus neoformans* (*C. neoformans*) and *Cryptococcus gattii (C. gattii)* from the family Cryptococcus (Binnicker et al. 2012). According to Rajasingham et al. (2017), 278 000 people have cryptococcus meningitis (CM), with 200 000 (71.9%) deaths occurring each year in sub-Saharan Africa (SSA).

Patients with acquired immune deficiency syndrome (AIDS) carry a disproportionate burden of disease, though the incidence of cryptococcal disease has been rising in organ transplant patients and other individuals in developed countries (Pyrgos et al. 2013). Cryptococcal meningitis is a devastating human immunodeficiency virus (HIV)-related opportunistic infection, estimated to affect nearly a million individuals per year, with a disproportionate number of infections (720 000 individuals per year) in SSA (Rajasingham et al. 2017).

Infections like cryptococcosis, an opportunistic illness, are still a major cause of mortality and morbidity in HIV-positive people in South Africa (SA) (Coetzee et al. 2018). Routine cryptococcal antigen (CrAg) testing is suggested in South African HIV treatment guidelines by the National Health Laboratory Service (NHLS) in partnership

with the DoH through the National Institute for Communicable Disease (NICD). Patients with a CD4 T-lymphocyte (CD4) count of less than 100 cells/µl, immune-compromised patients, those undergoing organ transplantation, and those with rheumatologic disorders requiring immunosuppressive medications should all be tested, according to this suggestion. According to NHLS and DoH standards, antigen screening tests are performed routinely on blood samples with low CD4 levels (National Department of Health 2019). In 2019, the guidelines were updated to recommend serum cryptococcal antigen (sCrAg) screening for all adults and adolescents with a CD4 count of less than 200 cells/µL (< 200 cells/µL) who are starting antiretroviral therapy (ART), switching therapy after ART failure, or re-entering care after a period of disengagement (Govender et al. 2019).

Screening for sCrAg could help identify people who are at the higher risk of developing disseminated cryptococcal illness. Cryptococcal antigen testing can be performed on Cerebrospinal fluid (CSF), serum, or urine sample as a quick and efficient approach for cryptococcosis diagnosis. As a fast diagnostic, Cryptococcal antigen detection has been extensively researched (Shah, Patel and Vegad 2011). Despite evidence that CrAg screening reduces the incidence of CM and associated mortality, there is still a link between CrAg positivity and death, even among individuals who take pre-emptive fluconazole therapy as recommended by the World Health Organization (WHO). This could be due to a lack of investigation into those who have antigenaemia and cases of CM that require more severe antifungal treatment. Another purpose for administering fluconazole alone is to avoid the development of life-threatening cryptococcal illness in a subset of CrAg-positive people (Wake et al. 2019). On the request of the attending clinicians, Cryptococcal antigen testing is performed on routine CSF samples. Both screening and diagnosis are done in the same way in the laboratory.

KwaZulu-Natal (KZN) is the most affected province with HIV in SA, with a prevalence of 27.0% compared with the Western Cape, which has a prevalence of 12.6% (Kharsany et al. 2019). Screening of plasma samples submitted for CD4 testing was proposed and implemented in 2016 as a strategy for the earlier diagnosis of cryptococcal disease in immune-suppressed HIV positive patients. Research is limited regarding early detection of CM (KwaZulu-Natal Provincial Department of

Health 2017). There is a need to establish the degree to which the screening of serum predicts CM in patients, and to determine if the result is timeous for patient management and to analyze whether this screening approach is appropriate for individuals attending KDH.

The key to lowering mortality from CM is early diagnosis and treatment. When it comes to persons with advanced HIV disease, health-care providers should have a low threshold for suspecting CM. Countries should make reliable access to quick diagnostics a top priority. For use on CSF, serum, plasma, or whole blood, CrAg tests, ideally CrAg lateral flow assays (CrAgLFA) (World Health Organization [WHO] 2018b).

## 1.3 Rationale of the study

### 1.3.1 Screening in the early detection of cryptococcal infection

In HIV-infected individuals with low CD4 cell counts, screening is critical for early diagnosis of cryptococcal infection. Individuals study in Uganda from the pre-antiretroviral therapy (pre-ART) era in 2002, where HIV-positive patients' serial serum samples were used, was one of the first to explain the natural history of cryptococcal antigenemia. These patients were tested for CrAg after they developed CM. According to this study, CrAg levels can be checked 22 days before the onset of meningitis. The same study found that early detection of cryptococcal illness and preventive treatment with the goal of preventing the development of meningitis is possible (French et al. 2002).

### 1.3.2 Cryptococcosis as a worldwide public health challenge

Cryptococcosis has become a major public health concern around the world. Cryptococcal infection affects people who aren't immune-compromised as well as those who don't appear to be immune-compromised (Park et al. 2009). As a result, cryptococcosis should no longer be regarded as a sporadic infection. Understanding the severity of cryptococcal disease is critical for public health professionals planning

and prioritizing essential resources for disease prevention and control. To prevent, diagnose, and treat cryptococcosis, more research is needed to better characterize the scope of the disease and follow the infection's epidemiology (Rajasingham et al. 2017).

### 1.3.3 Management of patients with cryptococcosis

The management of CM remains challenging due to the high acute mortality of the disease and the limited number of effective antifungal options available. Research in recent years has made great strides in understanding how adjunctive therapies and supportive care can improve outcomes. Multiple clinical trials have either recently been completed or are currently underway to establish improved antifungal regimens, focusing primarily on maintaining efficacy while increasing accessibility and decreasing toxicity (WHO, 2019).

All patients who screen CrAg-positive for the first time should have a lumbar puncture (LP) performed. The absence of symptoms of meningitis does not exclude CM as approximately one third of patients with asymptomatic cryptococcal antigenaemia have concurrent CM. The three different phases in the management of CM, namely induction, consolidation and maintenance will be briefly discussed (Govender et al. 2019).

#### 1.3.3.1 Induction therapy

Induction therapy is the first stage in the management of HIV-associated CM where patients are hospitalized, placed on treatment for the rapid killing of the fungus and the management of intracranial pressure for two weeks (WHO 2019). First week: amphotericin B deoxycholate (1mg/kg/day) and flucytosine (100mg/kg/day) divided into four doses per day. Second week: fluconazole (1200mg daily for adults; 12mg/kg/day for children and adolescents up to a maximum of 800mg daily). Induction therapy usually takes two weeks (Govender et al. 2019).

#### 1.3.3.2 Consolidation therapy

The consolidation phase occurs after acute hospitalization and includes a period of two weeks when antifungals are de-escalated and ART is initiated. Fluconazole (800 mg daily is given to the adults, 12 mg/kg/day for children and adolescents up to a maximum of 800 mg daily) therapy usually takes eight weeks (Govender et al. 2019).

#### 1.3.3.3 Maintenance therapy

Immunocompromised persons convalescing from recent CM remain at risk for disease until a critical level of immune reconstitution has been achieved (WHO 2019). Maintenance therapy of fluconazole (200 mg daily for adults, 6 mg/kg/daily for children and adolescents up to a maximum of 200 mg daily) continues until a single CD4 count of more than 200 cells/µL and a suppressed HIV viral load have been maintained for at least 12 months (Govender et al. 2019).

## 1.4 Aim of the study

Based on the described rationale for this study, the aim of the study was to investigate the impact of cryptococcal antigenemia screening on patient management at KDH.

## 1.5 Research objectives

### 1.5.1 Objective one

To determine whether screening for Cryptococcus antigenemia leads to appropriate follow-up investigations by clinicians.

### 1.5.2 Objective two

To describe the degree to which positive serum cryptococcal antigen screening results lead to treatment of cryptococcal meningitis.

### 1.5.3 Objective three

To determine whether the current antigenemia threshold of CD4 less than 100 cells/µl is appropriate for our patient population.

## 1.6 Outline of chapters in this dissertation

Chapter 2 will provide an overview of the literature, which was obtained through Google scholar, in order to facilitate a better understanding of cryptococcal antigenaemia screening. Chapter 3 will describe the research design and methodology used in this study. Chapter 4 and 5 will present the results and discussion of this study.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

As the HIV epidemic peaked, cryptococcosis was initially detected with a single case report in 1895 and developed to its current global explosion of disease with a million cases per year (Park et al. 2009). As a sugarcoated polysaccharide (PS) capsule yeast, Cryptococcus has established a significant presence in clinical mycology. Despite proper treatment, it can efficiently kill a susceptible host (Park et al. 2009).

It also serves as a prominent fungal model system for the research and development of fungal pathogenesis, diagnostic, and treatment concepts. This yeast has a well-developed molecular biology background, with over 100 genetic loci associated to its virulence composition, and numerous genomic, transcriptomic, and proteomic studies (Park et al. 2009). Routine culture procedures, a well-described histopathology, and the finest validated serological assays in all medical mycology all contribute to its identification. Finally, some of the most through clinical research in the field of invasive fungal illnesses backs up its therapy ideas (Park et al. 2009).

This chapter is a review of the literature pertaining to this study. It describes the genus *Cryptococcus*, different species in this genus, cryptococcosis, the contribution of WHO, CrAg screening, the impact of cryptococcosis in Africa and its impact in the province of KZN.

## 2.2 Definition of *Cryptococcus*

*Cryptococcus* (Greek for hidden sphere) is a fungus genus that grows in culture as yeasts in culture. *Cryptococcus* species' sexual forms, or teleomorphs, are filamentous fungi belonging to the genus *Filobasidiella* (Hong et al. 2017). When referring to this fungus' yeast stage, the name *Cryptococcus* is used (Sugita, Cho and Takashima 2017).

## 2.3 *Cryptococcus* species

The *Cryptococcus* genus has a large number of species, majority of which live in the soil and are not pathogenic to people (Arendruo et al. 2014). They are found in decaying organic matter, soil, and bird all over the planet, and they are sometimes associated with trees (Pyrgos et al. 2013). *Cryptococcus* exists as a simple yeast that only rarely finds a sexual partner to turn it into *Filobasidiella*, a fungal form (Feynman 2019).

For *C. neoformans*, contaminated pigeon droppings are the primary environmental source, whereas rotting hollows in living trees are the primary reservoir for *C. gattii* (Salehei, Mahmoudabadi and Zarrin 2015). *C. neoformans* is found all over the world, although *C. gattii* is more commonly found in tropical and subtropical areas (Dixit, Carroll and Qureshi 2009). A thin layer of glycoprotein capsular material with a gelatin-like consistency covers the cells of these species. It aids in the extraction of nutrients from the soil (Ragupathi and Reyna. 2015).

According to one theory, climatic changes allowed these cryptococcal strains to compete in the environmental microbiome and infect susceptible hosts as a result of greater exposure (Harris et al. 2013).

### 2.3.1 Cryptococcus neoformans

*C. neoformans* is a fungus that causes meningitis in AIDS patients and is a leading cause of death in these individuals (Bicanic et al. 2009). CM affects over a quarter of a million people worldwide each year, resulting in 181 000 fatalities. If infections are not treated, the mortality rate is 100% (Loyse et al. 2019). In 2014, it was projected that 278 000 persons worldwide have cryptococcal antigenaemia each year, with SSA accounting for 73 percent of the 223 100 cases (Rajasinham et al. 2017).

Following *C. neoformans* activation, Meningitis is the most prevalent symptom (Public Health Agency of Canada 2018). Among *Cryptococcus* species, this yeast is the most common human and animal infection. It has a O-acetylated capsule that is high in glucuronic acid and mannose, its capsule is the most important virulence component

(Endo et al. 2011). The polysaccharide (PS) capsule of the human pathogenic fungus *C. neoformans* appears as a translucent “halo” in an India ink preparation. Capsules protect yeast from oxidative stress while also increasing capsule size, resulting in increased protection from host immune defenses (Kumar et al. 2011).

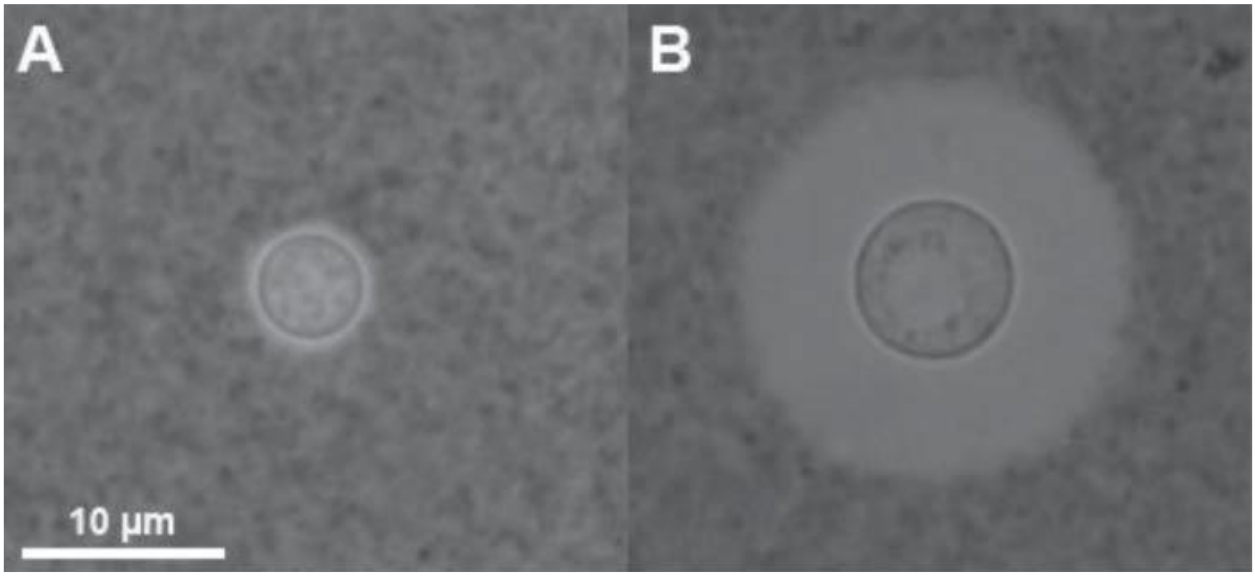



**Figure 2.1Capsules of *C. neoformans* with India ink staining**.
A: Yeast cells fermented in an environment with optimal nutrition. B. The increased cell body, generation of what appears to be a large vacuole, and drastic increase in capsule size are noted (Araujo et al. 2016).

*Cryptococcus neoformans* yeast cells can be observed by India ink under phase contrast microscopy. A dense PS capsule excluding India ink particles as shown in Figure 2.1.

### *2.3.2 Cryptococcus gattii*

*C. gattii*, formerly known as *C. neoformans* var *gattii*, is a tropical and subtropical *Cryptococcus* species. This geographically limited fungus has been detected in soil debris regularly, particularly in areas with certain trees such as oaks and eucalyptus (Springer et al. 2014). *C. gattii* unlike *C. neoformans*, is not only connected with HIV infection or other immunodeficiency illnesses, but it can also infect health people (Ma et al. 2009).

AIDS is the most common risk factor for *C. neoformans* cryptococcosis, but infections caused by *C. gattii* are more commonly documented in immunocompetent patients with unknown risk than in the immunocompromised patients. *C. neoformans* and *C. gattii* are distinguished by the degree of mannose backbone replacement. Antigenic variations in the PS have been used to classify cryptococcal strains into five serotypes: as A, B, C, D, and AD, with A, D and AD grouping with *C. neoformans* and B and C grouping with *C. gattii* (O'Meara and Alspaugh 2012).

### 2.3.3 *Cryptococcus laurenti* and *Cryptococcus albidus*

In humans with reduced immunity due to HIV infection, cancer chemotherapy or metabolic immunosuppression, *Cryptococcus laurenti* and *Cryptococcus albidus* have been found to induce moderate-to-severe illness (Larson et al. 2016).

## 2.4 Cryptococcosis

Cryptococcosis is a life-threatening fungus caused by pathogenic species-complexes of the *Cryptococcus* genus, specifically *C. neoformans* and *C. gattii* (Binnicker et al. 2012). Other cryptococcal species have only been found to cause human disease in extremely rare instances. These two species' taxonomy and population genetics are still being studied. Our understanding of strain ancestry has improved thanks to the widespread and effective use of multiple locus sequence typing (MLST) studies and whole genome sequencing (Perfect and Bicanic 2015). These two *Cryptococcus* species are documented in one of the most comprehensive MLST fungal databases, as well as with whole genome sequencing tools (Beal et al. 2015). The cryptococcal genome's adaptability and micro evolutionary characteristics have been highlighted in genomic investigation (Hu et al. 2011). The cryptococcal genome structure can after as a result of strain passage *in vitro* or *in vivo* (Janbon et al. 2014).

Furthermore, there are genomic differences noted even between clinical strains isolated from different infected patients. Certain specific genotypes have been associated with a poor clinical prognosis (Wiesner et al. 2012). The cryptococcal genome is differentiated by capsule and melanin production, growth at high

temperature, and phospholipase/urease activity. These observations challenge medical mycologists to characterize the precise complete genetic virulence composite of these strains (Tesei, Sterflinger and Marzban 2019). Patients with AIDS carry a disproportionate burden of disease, though the incidence of cryptococcal disease has been rising in organ transplant patients and other individuals in developed countries (Pyrgos et al. 2013). Strain passage in vitro or in vivo in the stress environment of the human subarachnoid space can cause these modifications in cryptococcal genome structure.

Pneumonia, meningitis, or involvement of skin, bones, or viscera, headache due to raised intracranial pressure, vomiting, confusion, altered level of consciousness, sixth cranial nerve palsies with double vision (diplopia) and visual impairment, swollen optic disc (papilledema), fever, pustules and nodules are some of the symptoms and signs of cryptococcosis (Govender and Dlamini 2014). Cryptococcosis is a well-known opportunistic illness that affect individuals with a lack of cell-mediated immunity, such as those with untreated HIV, organ transplantation recipients, and those with rheumatologic disorders that require immunosuppressive medications. cryptococcosis can also affect people who have no evident immunological deficiencies (Rajasingham et al. 2017).

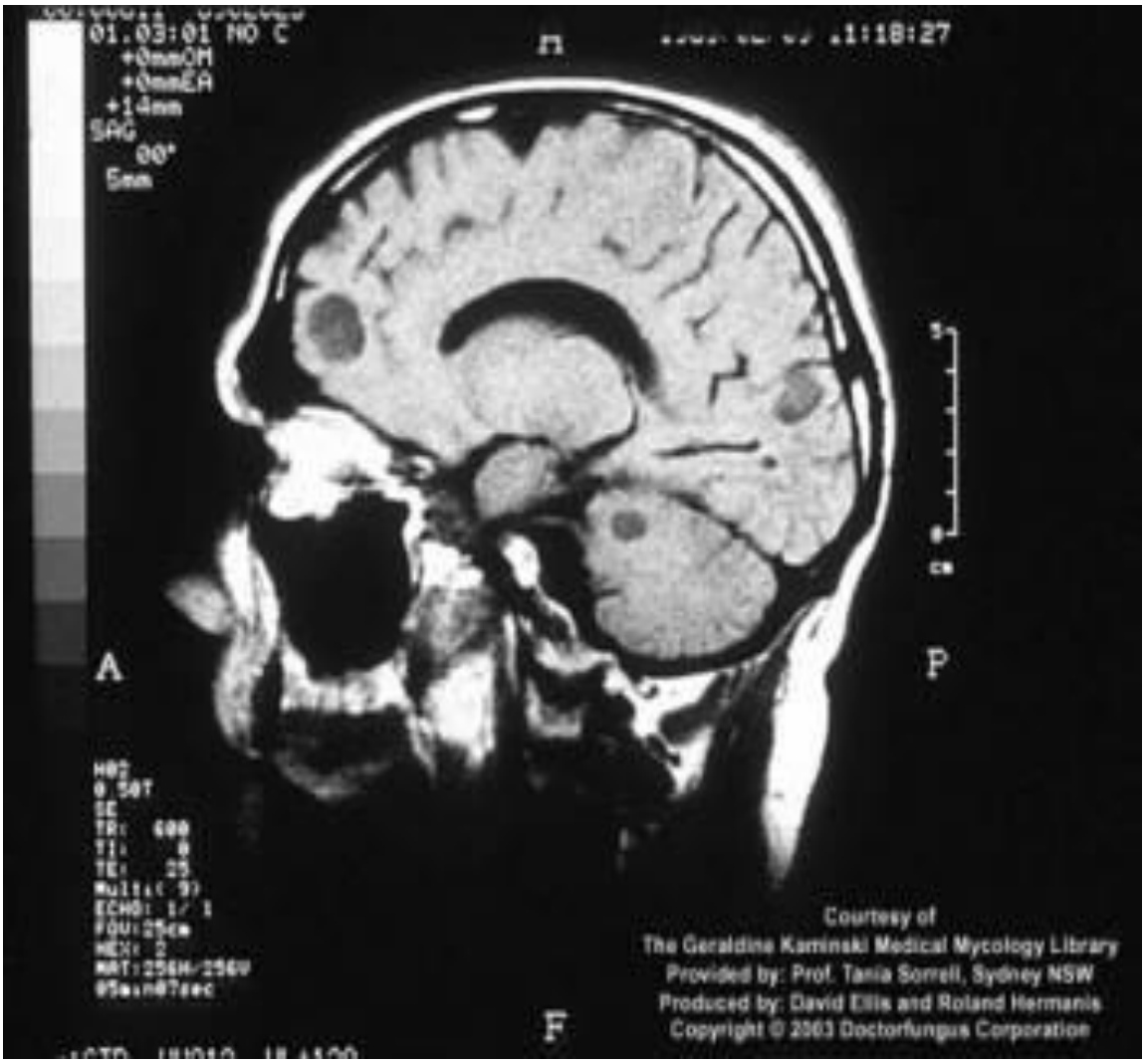



**Figure 2.2**: **Cryptococcosis in** c**entral** n**ervous** s**ystem from computer tomography (CT) scan**
Source: (Skipper, Abassi and Boulware (2019).

The majority of CM symptoms which are usually nonspecific, are caused by cerebral oedema (e.g., headache, blurred vision, confusion, depression, agitation, and other behavioural changes). Aside from ocular or facial palsies, focused indications arise later in the disease' progression. Cerebral oedema can also cause blindness if the optic nerves are involved (MacDougall et al. 2011).

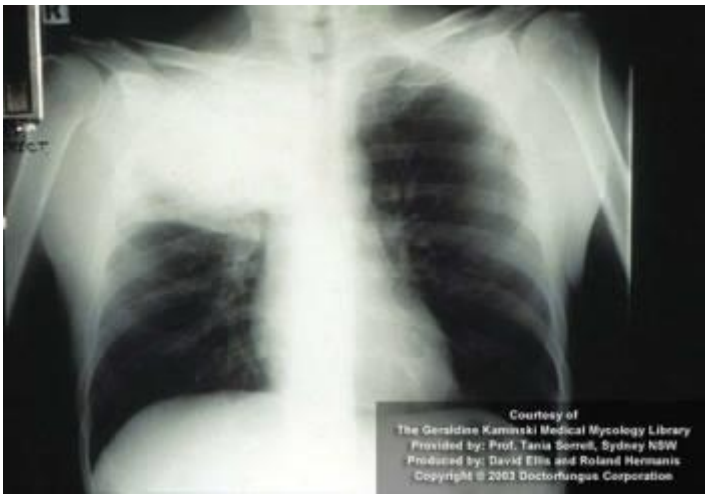



**Figure 2.3**: **Image of right upper lobe in patient with cryptococcal pneumonia**
Source: Setianingrum, Rautemaa-Richardson and Denning (2019)

Many cryptococcal pulmonary infection patients are asymptomatic. A cough and other nonspecific respiratory symptoms are common in people who have pneumonia. However, as shown in Figure 2.3, AIDS-related cryptococcal pulmonary infection can present as severe and progressive pneumonia with acute dyspnea and an x-ray pattern suggestive of pneumocystis infection (Messina et al. 2017).

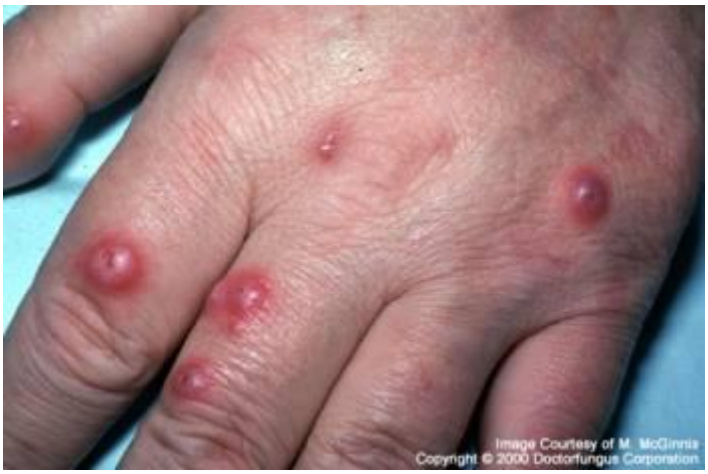



**Figure 2.4**: **Disseminated cryptococcosis**
Source: Hartati (2017)

As shown in Figure 2.4, disseminated cryptococcosis can cause pustular, papular, nodular, or ulcerated skin lesions. Acne, molluscum contagiosum, and basal cell cancer can all cause similar symptoms (MacDougall et al. 2011).

## 2.5 Pathophysiology

Cryptococcosis is spread through the lungs by inhaling infectious propagules (either poorly encapsulated yeast cells or basidiospores) from environmental reservoirs which then deposit in the alveoli (Christianson, Engber and Andes 2003). Many individuals have asymptomatic, self-limiting initial lung lesions when they first come in. even without antifungal medication, isolated pulmonary lesions in immunocompetent patients frequently cure spontaneously without propagating (Duarte et al. 2017).

*Cryptococcus* may propagate after inhalation, most commonly to the brain and meninges, where it manifests as microscopic multifocal intracerebral lesions (Carod-Artal 2018). Granulomas in the meninges and bigger focalized brain lesions may be visible. Although pulmonary involvement is rare, cryptococcal meningitis is life-threatening and necessitates prompt treatment (Schwartz et al. 2018). Pyelonephritis is a rare complication of renal papillary necrosis. Acute inflammatory alterations are mild or nonexistent in infected tissues, which generally include cystic masses of yeast that seem gelatinous due to the accumulated cryptococcal capsular polysaccharide (MacDougall et al. 2011).

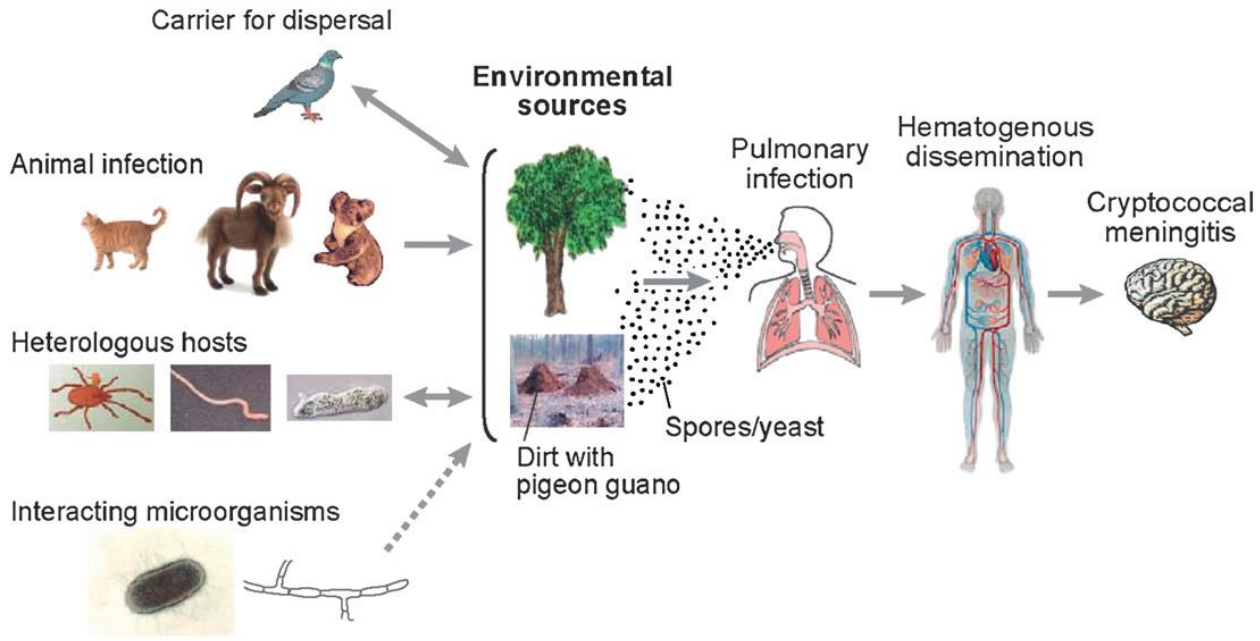



**Figure 2.5**: **Diagram showing the transmission Cycle of *C. neoformans***
Source: Eisenman, Casadevall and McClelland (2007).

Pyrgos et al. (2013) pointed out AIDS patients bear a disproportionate burden of disease, despite the fact that cryptococcal disease is becoming more common in organ transplant patients and other people in industrialized countries. CM is a deadly devastating HIV-related opportunistic infection that affects almost a million people per year in SSA, with a disproportionate number of infections (720 000) each year (Park et al. 2009).

The disease cryptococcosis remains a major cause of mortality in HIV-infected individuals worldwide (second only to tuberculosis), despite the widespread use of ART (Worodria et al. 2011). Cryptococcosis is thought to be responsible for 13-44% of AIDS deaths (Kozel and Bauman 2012). In immune-compromised patients with meningitis, pneumonia, or molluscum-like skin lesions, cryptococcal infection should be investigated as a differential diagnosis. The high (2.4%) early mortality rate in ART regimens in low-resource settings is largely due to CM (Jarvis et al. 2013).

## 2.6 Contribution of the World Health Organization

In advanced AIDS patients starting ART, The WHO recommends routine CrAg screening (WHO 2019). Patients enrolled in ART programs may be screened for CrAg which could help identify those at risk of CM and allow for focused pre-emptive medication (Bratton et al. 2013). NHLS in partnership with the South African Department of health, recommends routine CrAg testing for all patients with a CD4 count of fewer than 200 cells/u. Immuno-compromised patients, organ transplant recipients, and rheumatologic conditions requiring immunosuppressive drugs are also included in the guidelines (National Department of Health 2019).

## 2.7 Serum screening

In HIV-infected individuals with low CD4 counts, serum cryptococcal screening is critical for early diagnosis of cryptococcal infection, especially in those with CD4 counts of fewer than 200 cells/µL. In these patients, sCrAg should be done on a regular basis. Some patients with a negative sCrAg test may acquire cryptococcal

infection. To validate this, more research will be needed to find the best screening frequency until individuals acquire a higher CD4 cell count (Pongsai, Atamasirikul and Sungkanuparph 2010).

The cryptococcal screen and treat intervention was applied prospectively at a large urban hospital affiliated HIV clinic in S.A, and patients at risk of dying from CM identified. Patients with a CD4 count fewer than 100 cells/µL had a 2.8% chance of developing incident antigenaemia (Jarvis et al. 2013). Screening for cryptococcal antigenaemia and pre-emptive antifungal treatment of individuals with a CD4 count of < 200cells/µL is a promising technique for preventing mortality in a targeted way. This campaign supports the government's initiatives to prioritize primary health care (PHC), with a move towards early cryptococcal illness detection at PHC clinic level rather than delayed CM diagnosis at hospital level (SA AIDS Council. 2012).

Significant "losses throughout the continuum of care," as widely documented in SSA contexts, are likely to have the biggest influence on the efficacy of a screen-and-treat intervention method (Kranzer et al. 2012). Individuals who are severely immunocompromised have a CD4+ T-lymphocyte (CD4) count < 100 cells/µL are at the highest risk of developing CM (Pongsai, Atamasirikul and Sungkanuparph 2010). Hospitalization, intravenous antifungal therapy, access to LP, and adequate monitoring are all required for the treatment of CM. Because of the severity of the disease, access to treatments and diagnostics are still limited in resource-limited areas, mortality rates from CM remain high (Park et al. 2009). Patients who do survive often have long-term serious neurological consequences as a result of poor treatment of increasing intracranial pressure through their illness (Bothwell, Janigro and Patabendige 2019).

Cryptococcus organisms have been found in HIV patients up to 234 days before meningitis symptoms appear (Oyella et al. 2012). Its existence is correlated with the onset of CM within a year (Jarvis et al. 2009). In the first few months of ART, serum CrAg positive is linked to an increased risk of death (Ganiem et al. 2014). Any HIV treatment that includes a daily combination of two or more medications is known as ART. In patients with untreated systemic cryptococcal infection, this is thought to be partly attributable to immunological reconstitution inflammatory syndrome. For these

reasons, screening for sCrAg in HIV-infected people with advanced HIV infection (e. g. those with CD4 counts below 100 cells/μL) and rapid treatment of CrAg-positive people with anti-fungal, followed by ART, has avoid CM and minimize *Cryptococcus* infection (Nelson, Manabe and Lucas 2017).

In 2011, the WHO published Rapid Advice with a conditional suggestion that CrAg screening followed by treatment with high-dose fluconazole in people with advanced HIV infection be considered in areas where cryptococcal antigenaemia is common (WHO 2011). At the same time in 2011, high prevalence was characterized as > 3%, however subsequent assessments have found that screening can be cost-effective even at 0.6% prevalence (Jarvis et al. 2013). In SSA and Southeast Asian countries, prevalence of sCrAg in people with advanced HIV infection ranges from 1% to 16% (Pongsai Atamasirikul and Sungkanuparph 2010).

CrAg screening and early therapy have piqued the interest of several countries. These programs have already been implemented in a few countries, including SA, Rwanda, and Mozambique. However, disseminated cryptococcal disease prevention is only one of several areas of HIV care that governments must consider. In the face of competing demands and limited resources, countries must prioritize HIV care and support initiatives (Vijay et al. 2019).

## 2.8 Impact of cryptococcosis in South Africa

Cryptococcosis, an opportunistic illness, is still a leading cause of death and morbidity in HIV-positive people in SA (National Department of Health 2015). In a retrospective research conducted in SA in 2009, 6% CrAg ART naive individuals with CD4 count fewer than 100 cells/μL had CrAg. Moreover, a quarter (28%) of those who tested positive did not receive pre-emptive fluconazole medication and went on to develop CM. Those who tested CrAg negative had a majority rate of 11%, while 34% of those who tested positive had a mortality rate of 34% (Jarvis et al. 2009).

## 2.9 Impact of cryptococcosis in KwaZulu-Natal

With a frequency of 27.0%, KwaZulu-Natal (KZN) is the most HIV-affected province with in SA, compared to 12.6% in Western Cape (Kharsany et al. 2019). In 2016, a procedure for screening plasma samples submitted for CD4 testing as an approach for earlier identification of cryptococcal illness in immune-suppressed HIV positive individuals was proposed and adopted. Early detection of CM is still a work in progress (KwaZulu-Natal Provincial Department of Health 2017).

The goal of this study was to see how well serum screening predicts Cryptococcus meningitis in patients attending KDH, a district hospital in Durban, KwaZulu-Natal. It also wanted to see if the results were timely for patient care and if CD4 fewer than 100 cells/µL screening was appropriate for those who came to KDH.

## 2.10 Conclusion

In this Chapter, the relevant literature on the subject was reviewed. The definition of *Cryptococcus*, the different *Cryptococcus* species, cryptococcosis, the role of the WHO, cryptococcal screening, the impact of cryptococcosis in Africa, and its impact on the province of KZN were all discussed in chapter 2. The methods employed in this investigation will be described in the following chapter.

# CHAPTER 3: THREE: MATERIALS AND METHODOLOGY

## 3.1 Introduction

This chapter includes a description of the study design, study setting and the study population. It further outlines the inclusion/exclusion criteria, data collection for pilot and main studies, ethical considerations and statistical analysis.

## 3.2 Ethical considerations

After the researcher submitted gatekeeper permissions from NHLS Academic Affairs and Research Management System (AARMS), King Dinuzulu Hospital, and KwaZulu-Natal Provincial DOH, the Institutional Research Ethics Committee (IREC) at Durban University of Technology granted full ethical approval (Appendix I) to conduct this study. All permission or application letters are attached as Appendices A through H to this dissertation.

Invasive techniques such as blood and CSF collection were deemed to pose a moderate risk to patients. All such investigations, on the other hand, were considered standard of care and were entirely at the discretion of the attending physicians. The patients' identity and confidentiality were preserved by employing codes rather than names or other identities that could link each sample to a specific patient. Furthermore, anonymity was ensured by storing the completed data collecting tools, as well as any other related papers, in a secure, locked location only accessible by the researcher and supervisors.

## 3.3 Research design

This was a cross-sectional, descriptive and quantitative study. Patients' serum, plasma and CSF specimens were used to diagnose cryptococcal infections.

## 3.4 Research Setting

Specimens from patients attending KDH in KZN, SA, were used in this study. This hospital is a district hospital with 665 beds and is located in Asherville, Durban, in the eThekwini municipality. It serves the community of Sydenham and surrounding areas. In addition to general district level health care services, it also offers psychiatric, drug-resistant tuberculosis, and dental care services.

## 3.5 Population of the study

There was no recruitment of human participants in this study. Specimens were collected as part of routine clinical care by clinicians from patients attending King Dinuzulu Hospital in KwaZulu-Natal, South Africa.

## 3.6 Pilot study

### 3.6.1 Background

The primary goal of a pilot study is to assess the viability of a method that will be used in a bigger investigation (Leon, Davis and Kraemer 2012). A retrospective analysis of laboratory results was used in pilot trial. The researcher collected data from serum cryptococcal antigen screening, CD4 counts (less than 100 cells/µl), CSF cryptococcal antigen screening results, microscopy and culture using TrakCare, NHLS' laboratory information system (LIS).

The results of the pilot study were used to assess the feasibility of the main investigation by identifying whether each specimen was followed up on (serum or plasma, CSF). For example, if a patient with a positive CrAgLFA-result and a CD4 of less than 100 cells/µL, a CSF culture was performed on a patient who had a positive serum CrAgLFA result and a CD4 count of <100 cells/µL. all specimens between May to July 2019 were subjected to a retrospective analysis. The findings of pilot study informed the researcher on the following topics:

- Serum or plasma antigen positive specimens that had CSF culture done and the total number of those that yielded positive culture results.
- For all patients where *Cryptococcus* was cultured from their CSF, the researcher determined how many of them had serum CD4 count results as well.

### 3.6.2 Inclusion and exclusion criteria

The inclusion and exclusion criteria for the pilot study were as follows:

#### 3.6.2.1 Inclusion criteria

Patients with the following results were included:

- Serum cryptococcal antigen screening results
- CD4 count less than 100 cells/µL
- CSF cryptococcal antigen screening, microscopy and culture results.

#### 3.6.2.2 Exclusion criteria

Patients without the following results were excluded:

- Serum/plasma cryptococcal antigen screening,
- CD4 count less than 100 cells/uL,
- CSF microscopy and culture results.

### 3.6.3 Data collection procedure

After ethical approval for the study and all letters for permission and gatekeeper permission were granted, the researcher used TrakCare to collect all the results. These were obtained for serum cryptococcal antigen screening, CD4 count less than 100 Cells/µL, CSF cryptococcal antigen screening, microscopy and culture and were recorded on a table as indicated on Table 3.1.

**Table 3.1: Data collection**

| Code Number | CrAg | | Culture | CD4 |
|---|---|---|---|---|
| | CSF | Serum/Plasma | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## 3.7 Prospective study

The prospective component of the investigation began when the pilot study was completed and the Institutional Research Ethics Committee gave its full permission. The CSF, serum/plasma specimens used in this study were sent to the laboratory for normal examinations at the request of the attending clinician.

The sample size for this study was calculated statistically, using a chi-square test, to be 722.

The input parameters were as follows:

| | |
|---|---|
| Effect size w | 0.15 |
| α err prob | 0.05 |
| Power (1-β err prob) | 0.8 |
| Df | 10 |
| Total sample size | 722 |
| Actual power | 0.8001173 |

The inclusion and exclusion criteria specified in 3.6.1 and 3.6.2 were used to select the patients' samples for the study. Until the required sample size was reached, all specimens that met the inclusion criteria were included in this investigation.

### 3.7.1 Inclusion and exclusion criteria

The following criteria were used to decide on the specimens and lab result data from patients to include or exclude for analysis.

#### 3.7.1.1 Inclusion

The CSF and serum/plasma specimens were included if they were one of the following:

- Serum/plasma and CSF specimens that had cryptococcal antigen screening tests requested.
- Plasma CD4 counts less than 100 cells/µl.
- CSF samples that were brought to the laboratory for microscopy and culture requests.

#### 3.7.1.2 Exclusion

All specimens that were not for cryptococcal antigen screening and CSF microscopy and culture were excluded from the study.

### 3.8 Data collection

Testing was part of the routine management of patients.

### 3.8.1 Cryptococcal antigen screening using lateral flow assay (CrAg LFA) Kit

a) One drop of specimen diluent was added to a test tube for each test.
b) 40 µL of plasma or CSF was added to the same tube and contents were mixed.
c) Submerge strip in that tube.
d) Bands were observed after 10 minutes.
e) Test was interpreted as positive or negative, according to package insert.

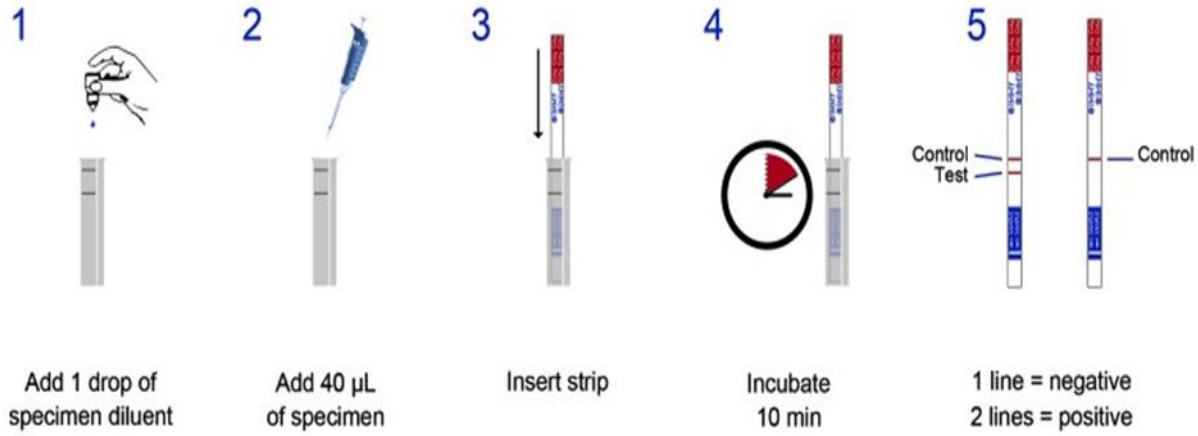



**Figure 3.1**: **The five steps of the cryptococcal antigen lateral flow assay**
Source: Gates-Hollingsworth and Kozel (2013)

### 3.8.2 Cell count CSF

a) Nine drops of neat CSF were added into a tube and mixed with one drop of CSF stain.
b) Neubauer counting chamber loaded and viewed microscopically for the presence and counting of cells.

### 3.8.3 Gram stain

a) The CSF specimen was centrifuged at 3000 revolutions per minutes for 5 minutes and 50 µL of sediment was used to prepare a smear.
b) Gram stain was performed for microscopic identification of yeast cells.

### 3.8.4 CSF Culture

Culture is a confirmatory test of Cryptococcus meningitis.

a) 5% blood agar, chocolate agar and Sabouraud's agar plates were inoculated with CSF sediment.
b) Plates were incubated at 35 °C for seven days.
c) Plates were observed daily for colony growth, for the duration of the incubation period.

Results were tabulated using Table 3.2.

**Table 3.2: Data collection**

| Number | Patient's code | CrAg | | Culture | CD4 | Clinician's comments |
|---|---|---|---|---|---|---|
| | | CSF | Serum/Plasma | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

The sample table in Table 3.2 was used as a daily record activity. The researcher checked for each CrAg-positive specimen as follows:

- If the culture was done, and if the CD4 count was done or could be done for each positive culture. She made touch with the clinicians and suggested that the unfished studies be completed.
- Clinician feedback was recorded. All patients who had positive cryptococcal screening results were followed up with by attending physicians to ensure that confirmatory testing was completed and that they were receiving appropriate treatment.
- If the patient had previously been diagnosed with cryptococcal disease, the attending clinician ensure that the patient continued antifungal treatment.
- If this is a new diagnosis, the patient should be checked for signs and symptoms of disseminated cryptococcal illness, including meningitis, by attending clinician
- A lumbar puncture should be performed on symptomatic patients to rule out meningitis while asymptomatic patients should be put on antifungals. For further information on how to manage CrAg-positive patients, consult the national consolidated HIV guidelines.

### 3.8.5 CD4 count

In HIV-positive people, the CD4 count is a good predictor of mortality (WHO 2017). In person with advanced HIV disease, Pre-ART CD4 count assessment is still the best tool to determine the need for screening and prophylactic measurements against common opportunistic infections, such as cotrimoxazole chemoprophylaxis and cryptococcal antigen screening (WHO 2018b).

### 3.8.6 Validity of results

Cryptococcal antigen screening was done using plasma with CD4 counts less than 100 cells/µL as well as serum specimens and CSF received in the laboratory for routine clinical investigation from patients with suspected meningitis. Testing was done according to ISO 15189-aligned protocols and inter-operator variability was therefore within internationally acceptable ranges. Quality controls were used for:

- Gram stain: ATCC strain of *Staphylococcus aureus* (ATCC25923) as positive control and *Escherichia coli* (ATCC25922) as negative control.
- Cryptococcal antigen lateral flow assay: a standardized positive control and normal saline as negative control.

## 3.9 Statistical analysis

Descriptive and inferential statistics were utilized using a p-value of less than 0.05 is considered as being statistically significant.

## 3.10 Conclusion

This chapter outlined the research methodology and materials used in this study. It also presented the data analysis in the manner relevant to the descriptive and quantitative research. Chapter 3 described the ethical considerations as well as sampling and statistical methods. Chapter 4 will present the results obtained from this study.

# CHAPTER 4: RESULTS

## 4.1 Introduction

The results of the statistical analysis of the data collected are presented in this chapter. The outcomes of routine cryptococcal screening at an NHLS laboratory were examined in this study. The outcomes of both the pilot and main studies are presented in this chapter. To fulfill the study's objectives, the results acquired from patients' specimens sent for routine analysis were collected and analysed. The goal of the study was to see how cryptococcal antigenemia screening affected patient care at KDH.

The purpose of this investigation was to answer the following study objectives. The first goal was to see if screening for cryptococcal antigenemia leads to appropriate follow-up investigations by physicians, and the second goal was to see how much positive CrAg screening results lead to CM therapy. The final goal was to see if the existing antigenaemia cutoff of CD4 counts of <100 cells/µl was adequate for our patient population. The findings reported in this chapter will be examined in Chapter 5 in order to establish a direct link between them and the above-mentioned goals.
To graphically depict the data, bar graphs and charts are employed, with a brief narrative accompanying each graph or table.

## 4.2 Analysis of the results of the pilot study

The results of pilot study informed the larger study by demonstrating the number of specimens analysed in the laboratory over time. The data was needed to check the number of CrAg tests performed over time, to guide data collection methods and total numbers received and, finally, to determine whether the proposed research project was feasible.

There 609 CrAg values that included both CSF and serum/plasma, with 521 being CSF CrAg and 88 being serum/plasma CrAg. CrAg-positivity, culture-positivity and

whether the CD4-count was above or below 100 cells/uL. Were all used to further characterize the samples. When clinicians requested these standard tests as per the guidelines for follow-up, there was no particular order in which they did so. All (100%) Of the 65 positive CSF CrAg patients had a culture performed, and 19 of them had CD4 counts performed within a month of the culture or during the data collecting period. Twenty-eight S/P positive CrAg had no culture done on them, whereas 17 had CD4 count done (Table 4.1).

**Table 4.1: Pilot study results**

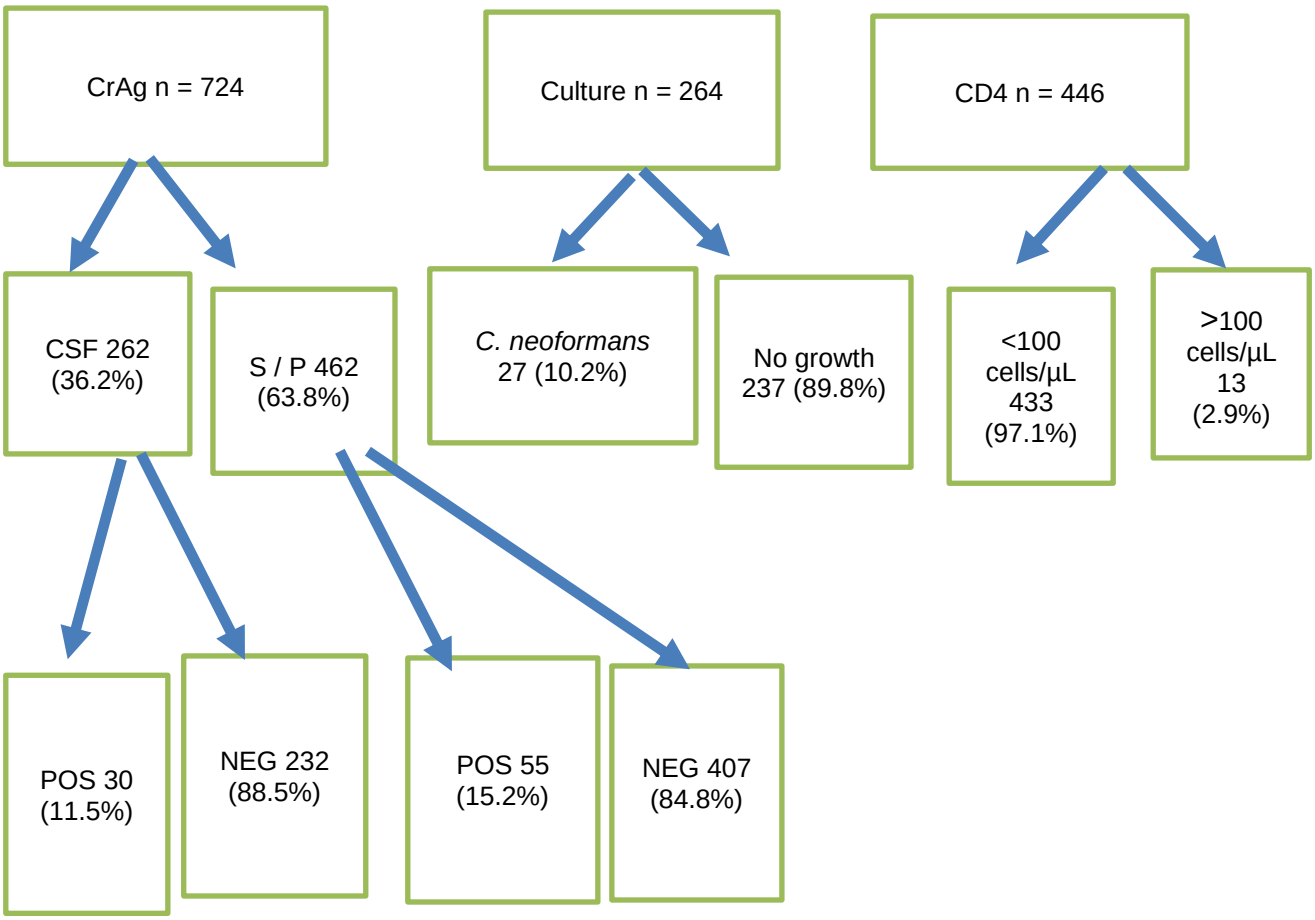
| | **Cryptococcal Antigen** | **Culture** | | **CD4 (cells/µl)** | |
|---|---|---|---|---|---|
| | | ***C.neoformans*** | **No growth** | **<100** | **>100** |
| | **CSF Positive :** 65 | 48 | 17 | 16 | 3 |
| | **CSF Negative :** 456 | - | 456 | 103 | - |
| | **Serum/Plasm Positive :** 28 | - | - | 17 | - |
| | **Serum/Plasm Negative :** 60 | - | - | 11 | - |
| **Total** | **609** | **521** | | **150** | |

## 4.3 Main study

The researcher collected the data over a period of three months from January to March 2021. Data was obtained from CSF and serum/plasma specimens and this information was used to answer the three objectives of this study. Results that were collected included CrAg testing, CSF culture, CD4 count, CD4 count categories, CrAg follow up, time lapse before treatment was started.

### 4.3.1 Sample size and response rate

The data that satisfied the inclusion criteria of the study were collected and analysed. Seven hundred and twenty-four results were collected for the main study. Data analysed for this part of the study included results obtained from a total of 724 CSF and serum/plasma CrAg results combined, including CSF culture results (n = 264) as and CD4 counts (n = 446), as shown in Figure 4.1.




*S/P= CrAg test on serum/plasma
CSF= CrAg test on CSF

**Figure 4.1: CrAg, culture results and CD4 count**

### 4.3.2 CrAg, culture results and CD4 count

An analysis was carried out based on the 262 CSF CrAg test results that were received. The majority of the CSF culture results were negative 232/262 (88.5%) and the minority positive 30/262 (11.5%). The sCrAg investigations demonstrated 407 (88.1%) negativity rate and 55 (11.9%) positivity rate in 462 tests.

Of 264 cultures carried out on CSF samples, 27 (10.2%) yielded *C. neoformans* positive culture and 237 (89.8%) showed no growth.

A total of 446 CD4 counts were analysed in this study, comprising either CSF or serum/plasma CrAg or both. The majority of the CD4 counts, 433 (97.1%), had < 100 cell/µl, while 13 (2.9%) had CD4 count of > 100 cells/µL.

## 4.4 CrAg screening follow up

### 4.4.1 Follow up from CrAg screening, CD4 count, culture to treatment

Out of 724 CrAg test requests received, 85 tested positive and 639 tested negative. From the 85 samples that tested CrAg positive, 62/85 samples were further tested for CD4 count and 59/62 samples resulted in a CD4 count of less than 100 cells/µL and only 3/62 (122,187,342) showed a CD4 count greater than 100 cells/µL.

Of the 59 samples with CD4 count < 100 cell/µL, only 19 had CSF culture requested and 18/59 yielded *C. neoformans* positive culture and one showed no growth. All three that tested CD4 count > 100 cells/µL showed growth of *C. neoformans.*

The number of patients with a CD4 count results who started antifungal treatment was 22/62. Treatment started after CrAg positive results were received by clinicians, where ten patients started treatment after one day (1 Day), ten patients started after two days (2 Days), one after three days (3 Days) and one after seven days (7 Days), as shown in Figure 4.2.


*n = total number

**Figure 4.2: CrAg screening, CD4 count, culture and treatment**

### 4.4.2 Results analysis in the order: CrAg, culture, CD4 count and treatment

Of the 85 samples that tested CrAg positive, 33 had culture done and 52 did not. Among the 33 that had culture done on them, 22 had CD4 count tested and 11 did not. Among the 22 that tested for CD4 count, 19 had < 100 cells/µL whereas three had CD4 count of > 100 cells/µL.

All 19 patients that had < 100 cells/µL CD4 count started antifungal treatment, ten patients started their treatment after one day, eight started after two days and one

started after one day. All three patients that had > 100 cells/µL CD4 count received treatment, one after one day, another one after two days, the last one after three days. Among the 11 patients that had no CD4 count result, eight started treatment after one day and one after two days. Of the 52 that had no culture, tested positive for CrAg and started treatment, one was started on treatment after two days, one after three days and one after seven days.

Out of 52 patients that had no culture, 50 were treated. Eleven started their treatment after one day, three after two days and 36 after seven days, as shown in Figure 4.3

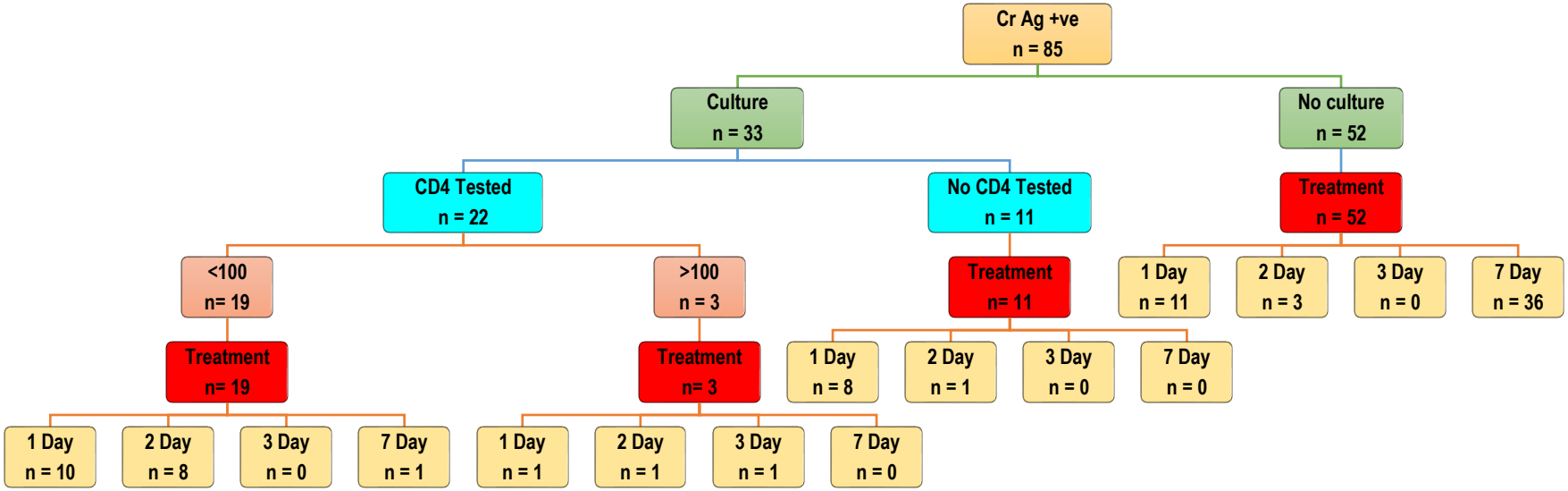



**Figure 4.3: Positive CrAg, culture, CD4 count and treatment**

## 4.5 Positive S/P leading to treatment

### 4.5.1 Description of S/P CrAg leading to treatment

Four hundred and eighty-one samples were analysed for serum/plasma CrAg; of these, 74 tested positive and 407 tested negative. Of the patients with positive CrAg results, 72 patients adhered to treatment with the following details: 23 after one day followed by 12 after two days, and one after three days followed by 36 after seven days. This indicates that majority of patients were introduced to treatment after seven days (36/74) and 23/74 after one day of receipt of CrAg results, as detailed in Figure 4.4.

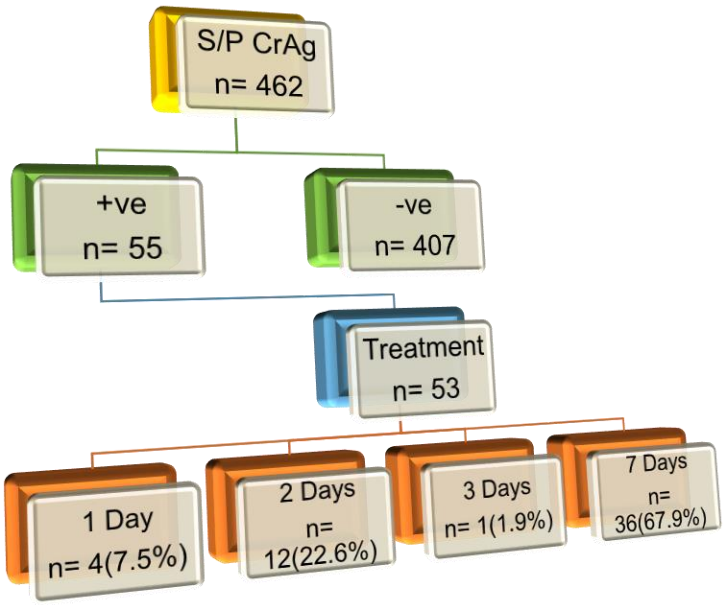



**Figure 4.4: Serum/plasma CrAg results leading to treatment**

### 4.5.2 Description of positive CrAg (CSF and Serum/plasma combined) leading to treatment

Seven hundred and twenty-four CrAg samples were processed which comprised both serum/plasma and CSF. Of the 724 results, 262 were CSF CrAg samples from which 30 were CrAg positive and 232 were negative. All 30 patients that tested positive for CSF CrAg received treatment, 19 patients received treatment after one day, nine after two days, one after three days and one after seven days.

Four hundred and eighty-one S/P CrAg out of 724 CrAg were done, the majority of which 407 were negative and 74 were positive.

From the 74 S/P CrAg positives, 72 patients were given treatment as follows: 23 after one day followed by 12 after two days, one after three days and 36 after seven days. The majority of the S/P CrAg positive patients (n=36) were started on treatment after seven days and 23 after one day of receipt of CrAg results, as detailed in Figure 4.5.

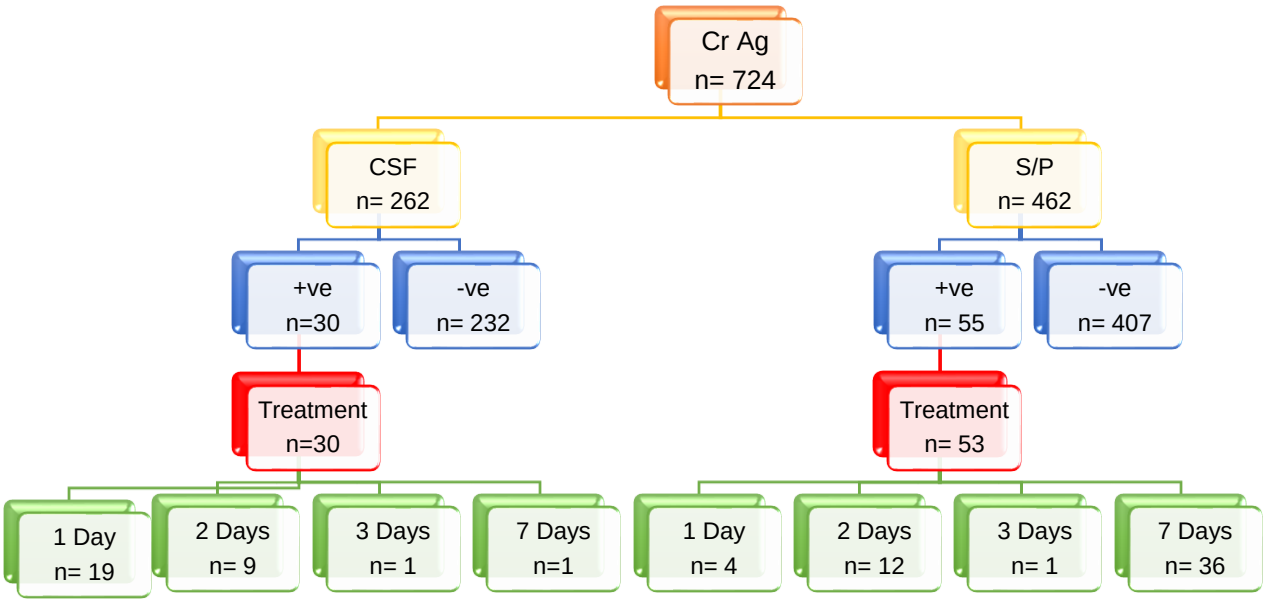



**Figure 4.5: CSF and S/P CrAg to treatment**

### 4.5.3 Details of CSF culture leading to treatment

A total of 264 CSF samples was processed for culture from which 26 were tested for CD4 count and 238 were not tested for CD4 count. From the 26 tested for CD4 count, 19 had < 100 cells/µL and seven had > 100 cells/µL CD4 count. All 19 patients that tested positive for CrAg started treatment, with 10 patients starting after one day and nine starting after two days. Among the 26 patients that had CD4 count results, seven showed more than 100 cells/µL, out of which three tested positives for CrAg and started treatment. Of the three that tested positive for CrAg and started treatment,

one was started on treatment after two days, one after three days and one after seven days.

Out of 264 CSF culture, 238 had no CD4 count done but had CrAg done; 11 were positive and all were treated. Seven started their treatment after one day, one after two days and one after seven days. Two did not received treatment, as described in Figure 4.6.

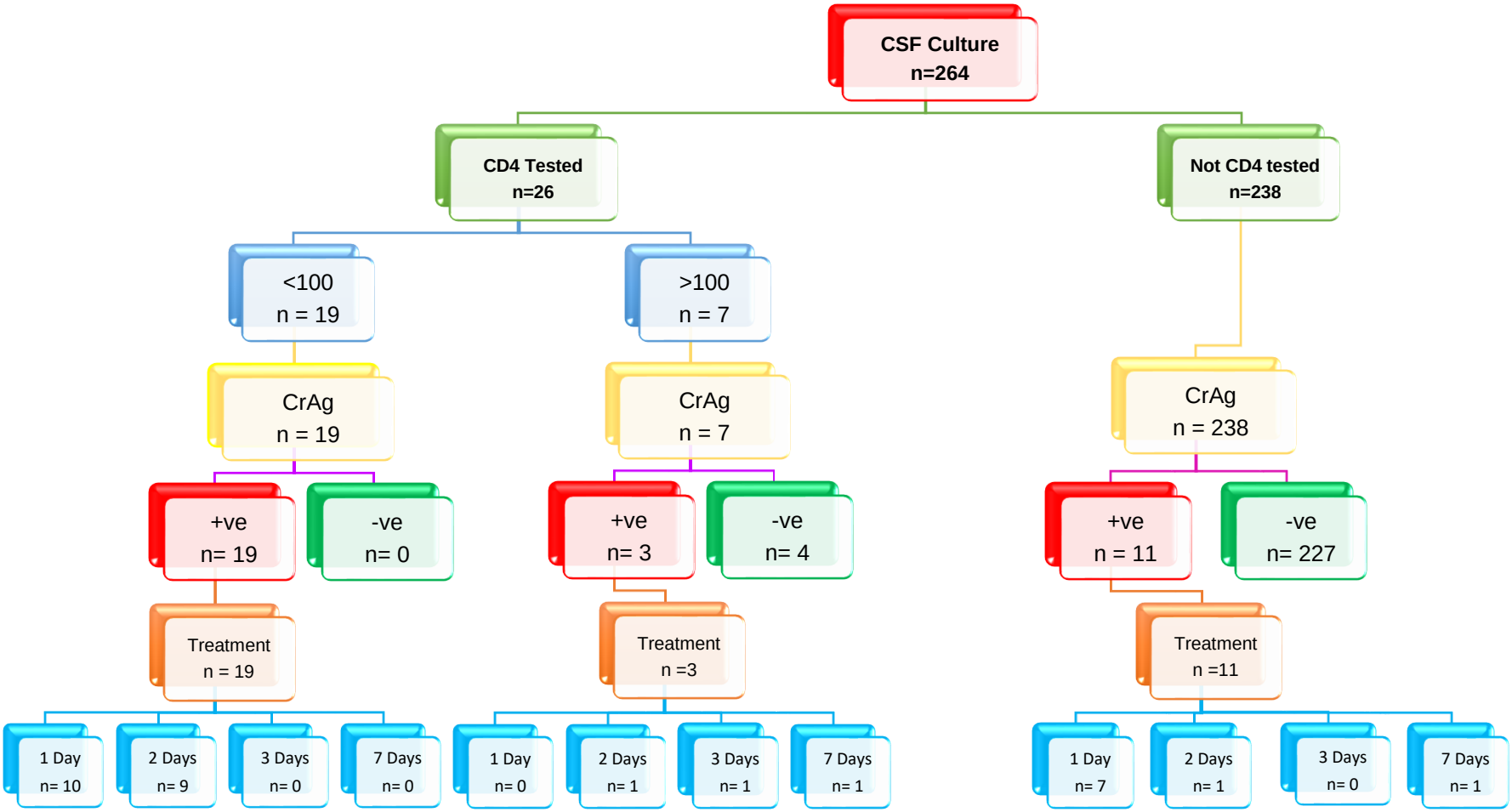



**Figure 4.6: CSF culture, CrAg, CD4 count and treatment**

## 4.6 CD4 threshold appropriate to our population

There were 85 positive CrAg, 62 patients had CD4 count done, the remaining 23 were not tested for CD4 count. Of the 62 CrAg, 31 had CD4 count between 0-49 cells/µL, 28 between 50-99 cells/µL, one between 100-149 cells/µL, one was between 150-199 cells/µL, and one more than 200 cells/µL, as shown in Figure 4.7.

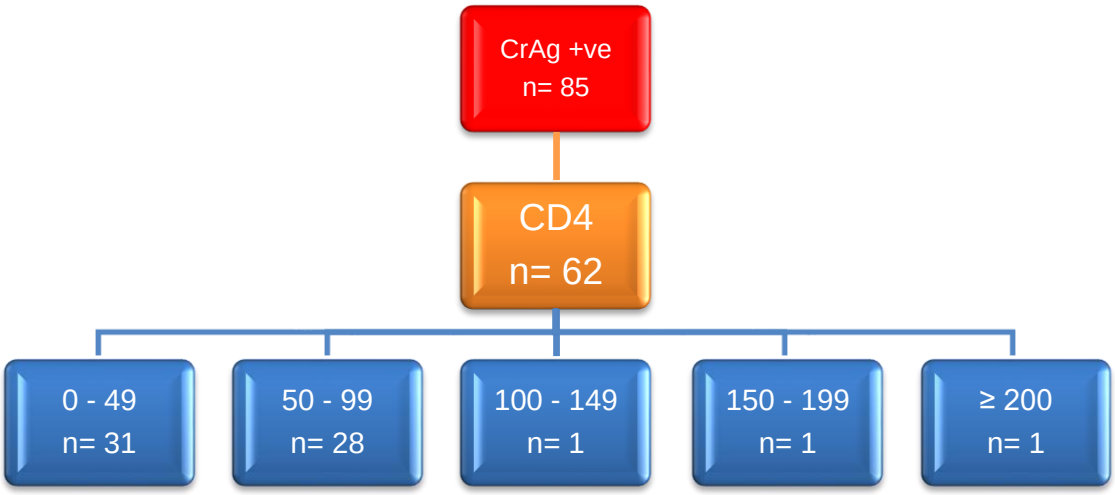



**Figure 4.7: Positive CrAg to the population threshold**

## 4.7 CD4 count categories

The results that were obtained from all the CD4 counts included in the study were categorized into four groups (Figure 4.8). A total of 446 CD4 counts that were analysed were broken down into different levels of CD4 count categories. Two hundred and thirty-six (52.9%) fell in the CD4 count category of between 0-49 cells/µL, 197 (44.2%) between 50-99 cells/µL, 11 (2.5%) in more than 150 cells/µL, and two (0.4%) between 100-149 cells/µL.

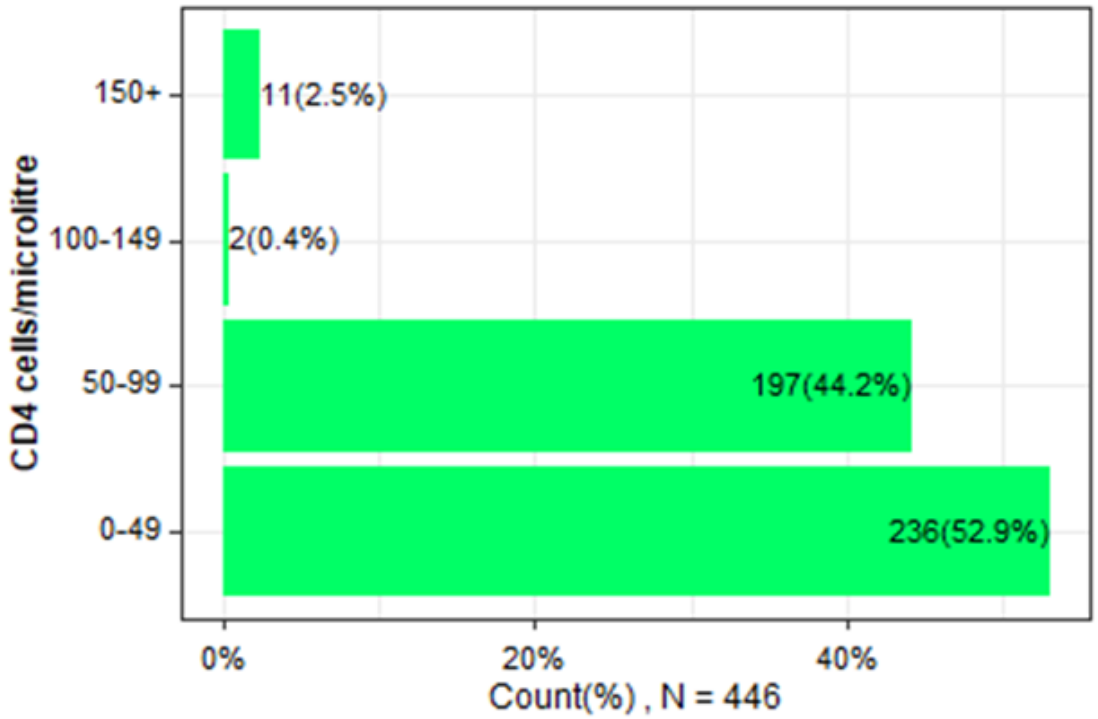



**Figure 4.8: CD4 count categories**

## 4.8 CrAg follow up

The full investigations of CrAg positive require CSF culture as a confirmatory test and CD4 count. Out of 81 (CrAg) positives, 57 (70.4%) had no follow-up done, 21 (25.9%) had follow-up done, two (2.5%) had not been requested, and one (1.2%) had a follow up done which was not suggested by the researcher.

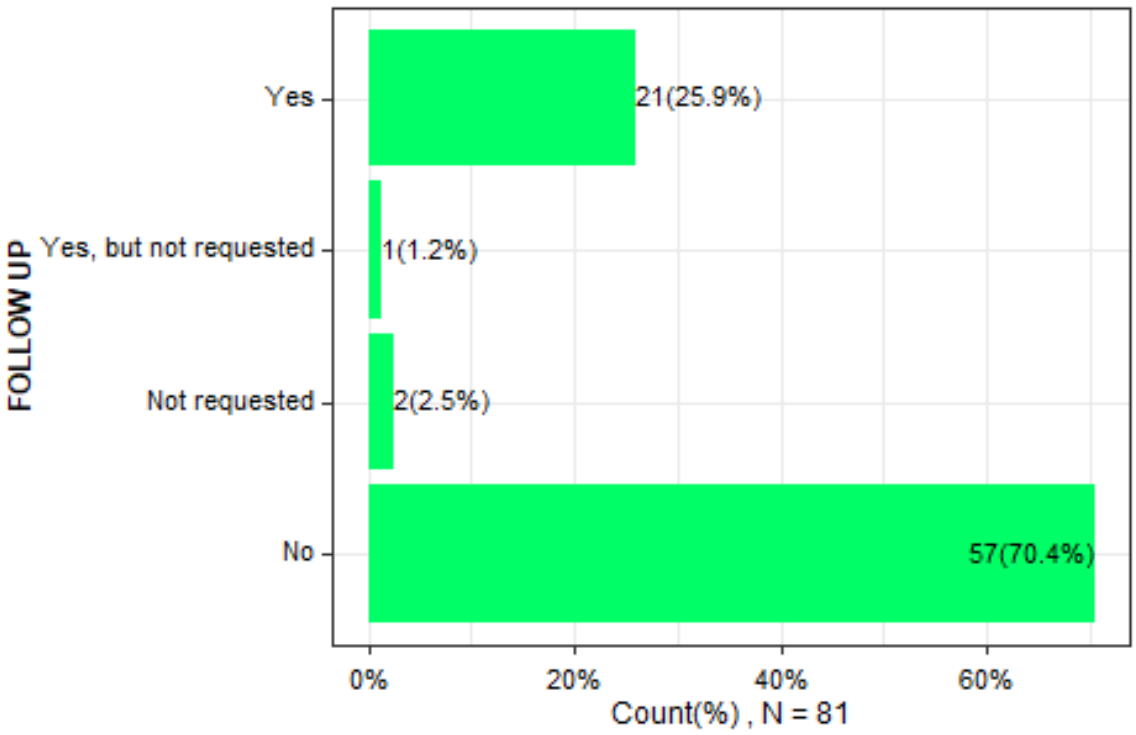



**Figure 4.9: Cryptococcal antigen follow up**

## 4.9 Classification of CSF CrAg and CD4 group

In Figure 4.10, the CSF CrAg results represented had a CD4 count. They were classified according to the grouping of CD4 count. Most of these CSF CrAg were positive and ranged between 0-49 cells/µl and 50-99 cells/µL, both with 9 positive CrAg (42.9%). Followed by more than 150 cells/µL with four (100%) negative and two (9.5%) positive, and between 100-149 cells/µL with one (4.8%).

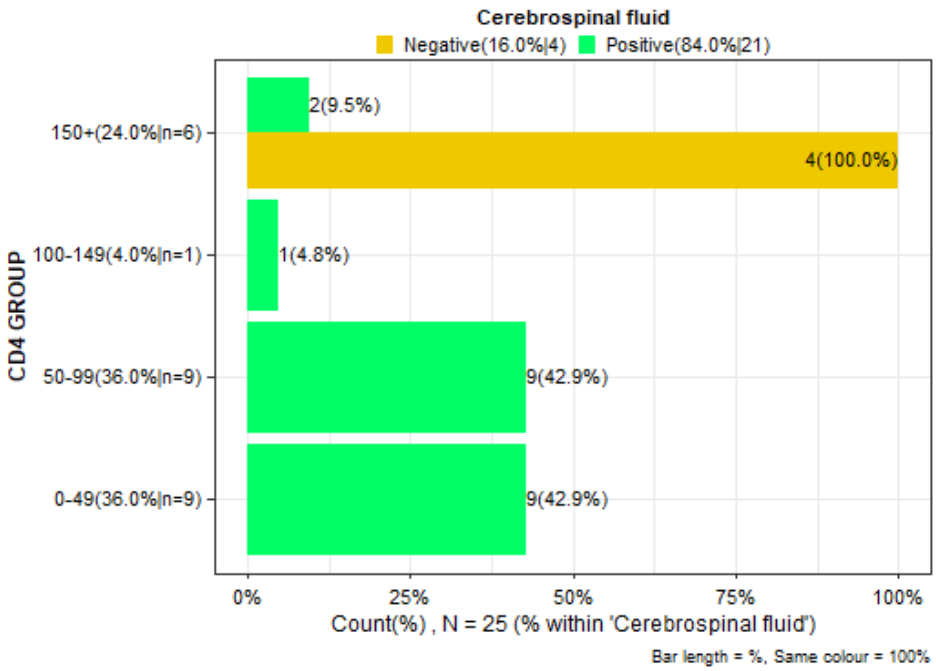



**Figure 4.10: Classification of CSF CrAg and CD4 group**

## 4.10 Classification of serum/plasma CrAg and CD4 group

Figure 4.11 shows the S/P CrAg results that had a CD4 count, and were classified according to the grouping of CD4 count. Most CrAg tests found between 0-49 cells/µL, with 205 (53.9%) negative and 30 positive (50.8%). This was followed by a CD4 count of between 50-99 cells/µL with 169 (44.5%) negative and 27 (45.8%) positive CrAg results. The next CD4 count group was 150 cells/µL and comprised 2 (3.4%) positive and 5 (1.3%) negative CrAg results, and 1 (0.3%) CD4 count between 100-149 cells/µL had a negative CrAg result.

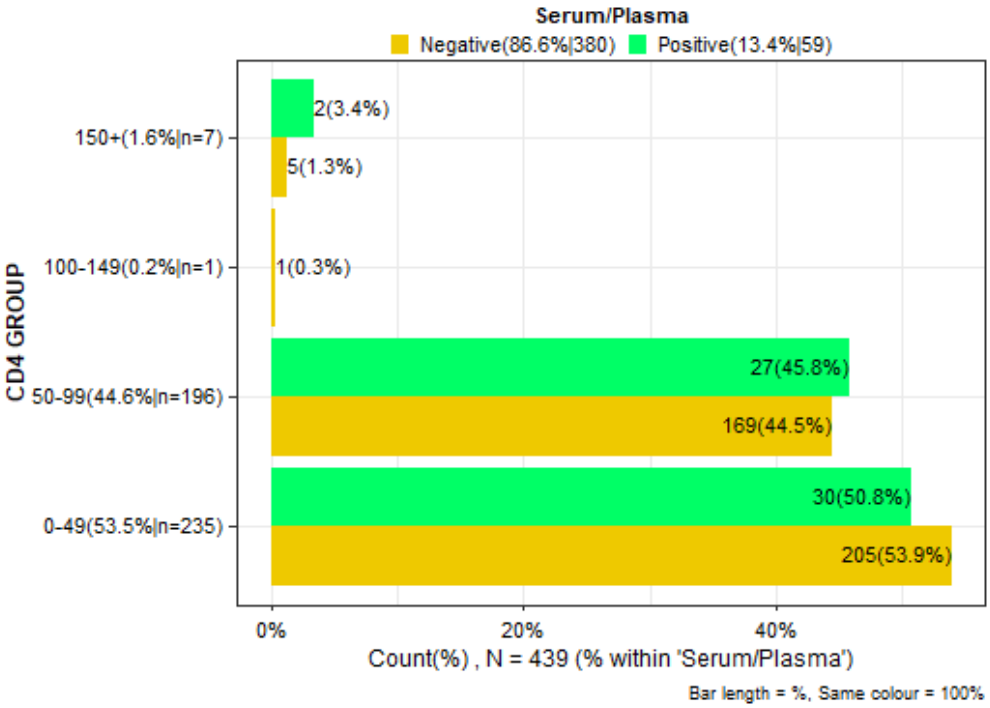



**Figure 4.11: Serum/plasma CrAg and CD4 group Classification**

## 4.11 An analysis of culture, CD4, treatment starting time, CD4 groups and CD4 threshold follow up

Follow up was done in this study and analysis is shown in Table 4.2. There was no statistically significant (p = 0.136) difference between CSF culture with follow up and those without follow up. However, there was a smaller proportion 10.3% (n = 3) of specimens with no growth.

Treatment starting time duration was associated with the follow up; there was a statistically significant association (p < 0.001) between treatment starting time and the follow up for patients that had started treatment two days after results were received by the attending clinician. That is, among those that were followed up, there was a significantly (p < 0.001) higher proportion 55.6% (10/24) of patients whose treatment started after two days of receiving the results when compared to just 6.8% (2/57) that was observed within the group that was not followed up.

The results showed that there was an association between CD4 threshold and follow up. However, there was higher proportion of < 100 cells/µL CD4 count from those without follow up and only 86% (n = 19) of < 100 cells/µL CD4 count had follow up.

In addition, > 100 cells/µL CD4 count had a significantly higher proportion of no follow up compared to >100 cells/µL CD4 count with follow up.

Another observation was that the CD4 count showed no significant difference between patients with follow up and those without follow up. In addition, there was no statistical significance in association between CD4 group and follow up (p = 0.121).

**Table 4.2: Summary of culture, CD4, treatment starting time, CD4 groups or from CD4 threshold follow up**

| | Follow up No (N=57) | Yes (N=24) | p-value | Overall (N=81) |
|---|---|---|---|---|
| **Culture** | | | Fisher's, p = 0.136 | |
| No growth | 2 (28.6%) | 1 (4.5%) | | 3 (10.3%) |
| *C. Neoformans* | 5 (71.4%) | 21 (95.5%) | | 26 (89.7%) |
| **CD4 count** | | | p = 0.154 | |
| Median(Q1-Q3) | 42.0(24.5-69.0) | 56.5(35.3-71.5) | Ranksum | 50.0(30.0-70.0) |
| Min-Max | 1.00-99.0 | 12.0-342 | | 1.00-342 |
| **Treatment starting time** | | | Fisher's, p<0.001 | |
| 1 day | 5 (11.4%) | 7 (38.9%) | 0.115 | 12 (19.4%) |
| 2 days | 3 (6.8%) | 10 (55.6%) | <0.001 | 13 (21.0%) |
| 3 days | 0 (0.0%) | 1 (5.6%) | 1.000 | 1 (1.6%) |
| 7 days | 36 (81.8%) | 0 (0.0%) | <0.001 | 36 (58.1%) |
| **CD4 group** | | | Fisher's, p = 0.121 | |
| 0-49 | 21 (53.8%) | 9 (40.9%) | | 30 (49.2%) |
| 50-99 | 18 (46.2%) | 10 (45.5%) | | 28 (45.9%) |
| 100-149 | 0 (0.0%) | 1 (4.5%) | | 1 (1.6%) |
| 150+ | 0 (0.0%) | 2 (9.1%) | | 2 (3.3%) |
| **CD4 threshold** | | | Fisher's, p = 0.043 | |
| <100 | 39 (100.0%) | 19 (86.4%) | | 58 (95.1%) |
| 100+ | 0 (0.0%) | 3 (13.6%) | | 3 (4.9%) |

| % and p-values based on non-missing cases | Ranksum test | Kruskal-Wallis test | Chisq. test | Fisher's exact test |

## 4.12 Conclusion

This chapter presented the results of the study. The following chapter will present the discussion of the results.

# CHAPTER 5: DISCUSSION OF RESULTS AND CONCLUSION

## 5.1 Introduction

*Cryptococcus* is an important participant in clinical mycology because it is protected by a polysaccharide capsule that allows it to kill the susceptible host both with and without therapy (Park et al. 2009). The yeast genome contains more than 100 genetic loci associated to its virulence composite and organism reconstruction, giving it a solid molecular base (Park et al. 2009). Cryptococcosis is a life-threatening fungal disease caused by *Cryptococcus neoformans* and *Cryptococcus gattii*, two pathogenic species-complexes from the family *Cryptococcus* (Binnicker et al. 2012).

CM is not acquired by people who have a healthy immune system. Those with a weakened immune system, on the other hand, are vulnerable to *c*ryptococcal infection, which has a special affinity for the central nervous system and can result in a deadly CM. In Southeast Asia and SSA, *C. neoformans* is a common prevalent opportunistic infection. This illness still the leading cause of HIV-related death worldwide, with SSA bearing the brunt of the disease's burden because to insufficient health-care access (Adeyemi and Ross 2015). Serum cryptococcal antigen can help identify people who are most at risk for disseminated *c*ryptococcal illness.

Cryptococcosis is an opportunistic infection that is still leading cause of death and morbidity in HIV-positive people in SA (NDOH 2013). South Africa has the world's greatest HIV epidemic, with roughly 7.06 million South Africans (12.6% of the total population) living with the virus in 2017 (Stats SA, 2017). With a frequency of 27.0%, KwaZulu-Natal (KZN) is the most HIV-affected province in SA, compared to 12.6% in the Western Cape (Kharsany et al. 2019). Despite the targets set forth in the SA National Strategic Plan and increasing ART coverage in SA, the prevalence of HIV-associated CM remains high, with a case-fatality rate ranging from 30% to 50% (Govender and Dlamini 2014).

The NHLS, in partnership with the DOH and NICD, recommends routine CrAg testing in the South African HIV treatment guidelines (National Department of Health 2018). All patients with CD4 counts below 200 cells/µL, as well as other immuno-compromised patients, organ transplants recipients, and rheumatologic disorders requiring immunosuppressive medications, should follow this guidance.

Antigen screening is performed on blood samples with a CD4 count of below 200 cells/µL on a regular and automatic basis. This is in accordance with NHLS and DOH recommendations (National Department of Health 2018). Antigen testing on CSF samples is done on a regular basis when the attending clinicians request it.

KwaZulu-Natal is the province in SA with the highest HIV prevalence. In 2016, a screening technique of plasma samples submitted for CD4 testing was proposed and used as a solution for more effective identification of cryptococcal illness in immune-suppressed HIV positive people, leading to the start of treatment. Early detection of the infection is still a work in progress. The goal of this study was to see how cryptococcal antigenaemia screening patient care at King Dinuzulu Hospital. This was accomplished by first determining if cryptococcal antigenaemia screening leads to practitioners conducting suitable follow-up studies. Second, determining the extent to which positive serum cryptococcal antigen screening findings result in CM treatment, and finally, determining whether the current antigenaemia threshold of CD4 < 100 cells/µL is appropriate.

The goal of this study was to see how cryptococcal antigenaemia screening affected patient care at King Dinuzulu Hospital. This was accomplished by first determining if cryptococcal antigenaemia screening leads to practitioners conducting suitable follow-up studies. Second, to see how often positive serum cryptococcal antigen screening findings lead to CM treatment, and third, to see if the current antigenaemia threshold of CD4 < 100 cells/µL is appropriate for the KDH patient population.

This chapter will therefore be discussing the results as presented in Chapter 4, linking these to the aim and objectives of this study. It will further outline the challenges that were encountered during the study and thereafter outline the recommendations for

further studies, arising from the data, and finally to give concluding statements to the study.

To meet the objectives of this research study, the following questions had to be answered from the data analysis:

- What are the outcomes of follow up investigations done by clinicians?
- Is the treatment of CM appropriate to the antigen screening results?
- Is the testing of the CrAg at the threshold of < 100 cells/µL appropriate for the KDH patient population?

## 5.2 Screening for Cryptococcus antigenaemia and appropriate follow-up investigations by clinicians

All plasma samples with a CD4 count of < 100 cells/µL are subjected to a reflex CrAg analysis in another NHLS referral laboratory. Local clinicians are either unaware of the positive CrAg results or fail to request follow-up studies, as advised by the South African recommendations for the management of cryptococcal illness, according to the laboratory technologists.

“For a suspected initial episode of CM, CSF should be submitted to the laboratory for a CrAg test and CSF culture,” according to these guidelines. “To diagnose CM, lumbar puncture (LP) is suggested for all patients with a new positive CrAg screening test result” (Govender et al. 2019). This is clearly not the case when looking at the findings of the current study. Only 33 of the 85 CrAg positive individuals had a CSF fungal culture performed. This practice of not following up on all CrAg positive cases could be harmful to the care of cryptococcal-infected patients. The loss of follow-up, according Kranzer et al. (2012), can have a significant impact on the effectiveness of screening, diagnosis and patient treatment.

Only 2.6% (5 out of 190) of CrAg positive patients received an LP as part of the screening program, according to a study conducted at the Prince Mshiyeni Memorial Hospital (PMMH) laboratory (Ndayishimiye and Ross 2018). Another study in Gauteng province found that 41% (99 out of 244) of CrAg positive individuals were

symptomatic, with 56 (57%) having an LP and 59% (33 out of 56) being diagnosed with CM. Furthermore, CM on LP was seen in 26% (8 out of 31) of asymptomatic CrAg positive individuals in a research project in Gauteng Province (Walaza 2014). All positive CrAg patients should be offered an LP to rule out CM, according to Srikanta et al. (2014). In our local situation, despite these recommendations, effective follow up does not appear to be adhered to by attending clinicians. Given the high rate of CM in asymptomatic CrAg positive patients, the challenges of early detection of subclinical CM, the cost effectiveness of the screen-and-treat intervention (estimated to be cost-effective at a CrAg threshold of 0.6%), and the resources available in SA, serious consideration should be given to recommending LP for all patients with a low CD4 count threshold (Ndayishimiye and Ross 2018).

### 5.3 Treatment of CM and its appropriateness to the antigen screening results

With positive surveillance sCrAg results, the researcher contacted the attending clinicians directly to find out if the patients were being treated for CM and to promote appropriate follow-up investigations. Part of this project was to record the time that elapsed between LIS reporting of the CrAg result and (a) the time when the doctor started seeking follow-up studies and (b) the initiation of treatment. In absence of a clear clinical diagnosis of CM, clinician usually rely heavily on laboratory results before commencing. The turn-around-time for CrAg testing is less than one day, but if it only follows a positive CD4 screening procedure, then it may take up to two days before the clinician will have a laboratory result. This may result in a significant delay in the start of treatment of CM. Patients taking ART visit the hospital's outpatients' department on a regular basis for follow up assessments. Treatment choices are based on the precise diagnosis, the patient's availability (particularly if the sample is taken from an outpatient facility), and past treatment regiments.

Patients in this study, with CrAg positive results were given antifungal medication. This is in accordance with the South African cryptococcal disease management guidelines and recommendations (Govender et al. 2019). All patients with a low CD4 count (< 100µL should be put on ART so as soon as possible to minimize the chance of developing CM. More research is needed to determine the effectiveness of the guidelines in reducing CM mortality. This includes oral fluconazole in individuals with

CrAg but not CM, as well as high-dose fluconazole in combination with amphotericin B for the treatment of CM (Ndayishimiye and Ross 2018).

The majority of patients were treated one day after testing positive for CSF CrAg, but this figure increased to seven days following a positive S/P antigen test. From this study, treatment starts times of one day, two days, three days, and seven days reveal that most patients may theoretically begin treatment before the manifestation of CM. CM symptoms typically last one to two weeks in HIV patients and six to 12 weeks in non-HIV patients from onset to presentation (Williamson et al. 2017).

### 5.4 CrAg testing and its appropriateness for the KDH patient population

There were 62 S/P CrAg positive patients that had CD4 counts done, 59 had a CD4 count < 100 cells/µL, two had CD4 count < 200 cells/µL, and one was more than 200 cells/µL. Except for the oneCD4 count that was higher than 200 cells/µL, these findings are consistent with the guidelines by Govender et al. (2019) which state that CrAg screening is now recommended for all adults or adolescents with a CD4 count less than 200 cells/µL who are either (1) initiating ART for the first time, (2) switching therapy after ART failure, or (3) re-entering into care after prior disengagement.

The one patient with a CrAg positive test (1/85,1.2%) and a CD4 count of > 200 cells/µl (342 cells/µl), may imply that the CD4 count threshold of 200 cells/µL may not identify all patients in our local population that are at risk of CM. Future studies should consider a higher CD4 count threshold than what this study looked at to establish which CD4 count threshold is appropriate for the KDH patient population. This view is also supported by the study performed by the NDOH in March 2015, which incorporated CrAg screening into the national consolidated HIV guidelines, along with other changes, which include initiating ART with a CD4 count threshold of 500 cells/µL, an increase from the previous threshold of 350 cells/µL (National Department of Health, South Africa. 2015). This indicates a need to consider a higher threshold for CD4 count.

Before the main study commenced, a pilot study was carried out to evaluate the feasibility of the main study, by means of indicating if follow up was done for each

patient with positive CrAg, and determining the impact of the threshold of <100 cells/µL for those with a positive CrAg result. The pilot study involved a retrospective analysis of laboratory results while the main study was a prospective study. For the pilot study as well as for the main study, there was no particular order that was followed by clinicians when requesting for these routine tests as per the guidelines for follow-up. Of the 93 CrAg-positive patients from the pilot study, 65 (69.9%) had a CSF culture done and 33 (50.8%) of these had CD4 counts done on them. The main study results showed that of the 85 CrAg-positive patients, 33 (38.8%) had culture, and 62 (76.5%) had CD4 count done on them. The pilot study results agree with the main study; in both the pilot and main studies, follow up with CSF culture was pursued in less than half of the patients, when the CD4 count was of > 100 cells/µL.

The findings of this study showed that of the 62 CrAg positive patients, 59 had CD4 count 100 cells/µL, one between 100-200 cells/µL, one was > 200 cells/µL, and 23 had no CD4 count. The audit of the screen-and-treat interventions to reduce CM in HIV-positive patients with low CD4 count study carried out by Ndayishimiye and Ross (2018) at PMMH showed 80 positive CrAg patients. It found that 57 (71.3%) had a CD4 count ≤ 100 cells/µL, 10 patients had a CD4 count > 100 cells/µL and 13 had no CD4 count. The findings of the two studies indicate that most positive CrAg results have a CD4 count of < 100 cells/µL, but also that CrAg can be positive for a patient with > 100 cells/µL. This could indicate that consideration should be given to increasing the CD4 count threshold to < 200Cells/µL or even more for CrAg screening testing at KDH.

In SA, reductions in numbers of people with CD4 counts have recently stalled when over 15% of those with very advanced disease have a CD4 count of < 100 cells/µL and over 30% of people seeking care with advanced HIV disease, have a CD4 count of < 200 cells/µL (Carmora et al. 2018). The findings of the present study at KDH found that most patients with a positive CrAG results had a CD4 count of <100 cells/µL, but a substantial number of patientshad a CD4 count between 100 and 200 cells/µL. this study therefore, largely supports the Southen African HIV Clinician Guideline’s recent increased of the CD4 count screening threshold to < 200 cells/µL.

## 5.5 Challenges

It was observed during the study that there were challenges around follow-up with patient results. Challenges such as the following were noticed:

- Patients being discharged early in light of the recent Covid-19 pandemic, meaning that when positive CrAg result were received by the attending clinician, the patient had to be called to come back.
- Difficulty to contact the patient.
- Patients were reluctant to come to the hospital thinking that they might get Covid-19 by exposing themselves to sick individuals at the hospital.

This together with COVID-19 lockdown delayed the data collection process.

## 5.6 Conclusion

In conclusion, it seems like there was only marginal benefit to the patients atteinding KDH from the CrAg screening program. The results showed significant lack of follow up by attending clinicians on most positive CrAg samples 64 (75.3%). Only 21 (24.7%) out of a total of 85 CrAg positive results had follow up done. The reasons for failure to do appropriate follow-up are not known as this was outside the scope of this study. It can only be speculated that reasons may have been due to:

- The doctors failed to implement the current cryptococcal disease management guidelines.
- King Dinuzulu laboratory is still using the previous Southern African guideline recommending CrAg screening for patients with CD4 count less than 100 cells/µl. This failed to detect a substantial proportion three (3.5%) of cases that occur in patients with CD4 counts between 100 cells/µL and 200 cells/µL.

## 5.7 Recommendations

After careful analysis of results and observations during the study, the following recommendations can be made:

- Clinicians must be encouraged to abide by the DOH and NHLS guidelines of proper follow up investigations should a CrAg positive test result be received.
- A change of CD4 threshold for testing antigenaemia from < 100cells/μL to < 200cells/μL or even more for the KDH patient population should be strongly considered.
- More studies need to be conducted to assess and improve clinical action following receipt of positive CrAg results in order to prevent, diagnose, and treat CM.

# LIST OF REFERENCES

Adeyemi, B. O. and Ross, A. 2015. Profile and acute mortality outcome of patients admitted with cryptococcal meningitis to an urban district hospital in KwaZulu-Natal, South Africa. *South African Family Practice*, 57(2): 131–135. 10.1080/20786190.2014.976962.

Araujo, G. R., Fontes, G. N., Leao, D., Rocha, G. M., Pontes, B., Anna, C.S., DeSouza, W. and Frases, S. 2016. Cryptococcus neoformans capsular polysaccharides form branched and complex filamentous networks viewed by high-resolution microscopy. *Journal of Structural* Biology, 193(1): 75–82. Available: doi:10.1016/j.jsb.2015.11.010. (Accessed 10 November 2020).

Arendruo, M. C., Boekhout, T., Akova, M., Meis, J. F., Cornely, O. A., Lortholary, O. and ESCMID EFISG study group and ECMM. 2014. ESCMIB and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clinical Microbiology and Infection*, 20(suppl. 3): 76-98.

Beal, M. A., Sabiti, W., Robertson, E. J., Fuentes-Cabrjo, K. M., O'Hanton, S. J., Jarvis, J. N., Loyse, A., Meintjes, G., Harrison, T. S., May, R. C., Fisher, M. C. and Bicanic, T. 2015. Genotypic diversity is associated with clinical outcome and phenotype in cryptococcal meningitis across Southern Africa. *PLoS Neglected Tropical Diseases*, 9(6):c0003847. Available: https://doi.org/10.1371/journal.pntd.0003847 (Accessed 02 August 2021).

Bicanic, T., Meintjes, G., Rebe, K., Williams, A., Loyyse, A., Wood, R., Hayes, M. and Harrison, T. 2009. Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. *Journal of Acquired Immune Deficiency Syndrome*, 51(2): 130-4.

Binnicker, M. J., Jespersen, D. J., Bestrom, J. E. and Rollins, L. O. 2012. Comparison of four assays for the detection of cryptococcal antigen. *Clinical Vaccine Immunology,* 19 (12): 1988-1990.

Bothwell, S. W., Janigro, D. and Patabendige, A. 2019. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids and Barriers of the CNS*, 16(1): 1-18.

Bratton, E. W., Husseini, E. l., Chastain, N., Lee, C.A., Poole, M. S., Stürmer, C., Weber, T., Juliano, D.J. and Perfect, J. J. 2013. Approaches to antifungal therapies and their effectiveness among patients with cryptococcosis. *Antimicrobial Agents Chemotherapy*, 57(6): 2485–2495.

Carmora, S., Bor, J., Nattey, C., Maugahan-Brown, B., Maskew, M., Fox, M. P., Glencross, D. K., Ford. N. and MacLeod, W. B. 2018. Persistent high burden of advanced HIV disease among patients seeking care in South Africa's national HIV program: data from a nationwide laboratory cohort. *Clinical Infectious Diseases*, 4:66(suppl. 2) 5111-5117.

Carod-Artal, F. J. 2018. Fungal infections of the central nervous system. In: García-Moncó, J. C. ed. *CNS infections*. Cham: Springer, 129-156.

Christianson, J., Engber, W. and Andes, D. 2003. Primary cutaneous Cryptococcosis in immunocompetent and immunocompromised hosts. *Medical Mycology,* 41(3): 177-188.

Coetzee, L. M, Cassim, N., Govender , N. P. and Glencross, D. 2018. CrAg positivity rates reported from a national CD4 reflexed screening programme identify high-risk regions of co-existent HIV/cryptococcal disease, requiring urgent programmatic focus into care. Presented at the XXII International AIDS Conference, Le Palais des Congres de Paris, Paris, France. Available: https://www.ias2017.org/ (Accessed 05 March 2018).

Dixit, A., Carroll, S. F., and Qureshi, S. T. 2009. Cryptococcus gattii: an emerging cause of fungal disease in North America. *Interdisciplinary Perspectives on Infectious Diseases,* Available: DOI: 10.1155/2009/840452 (Accessed 10 June 2020).

Duarte, S. B. L., Oshima, M. M., Mesquita, J. V. D. A., Nascimento, F. B. P. D., Azevedo, P. C. D. and Reis, F. 2017. Magnetic resonance imaging findings in central nervous system cryptococcosis: comparison between immunocompetent and immunocompromised patients. *Radiologiabrasileira*, 50(6): 359-365.

Eisenman, H. C, Casadevall, A. and McClelland, E. E. 2007. New insights on the pathogenesis of invasive Cryptococcus neoformans infection. *Current Infectious Disease Reports*, 9(6): 457-464.

Endo, J. O., Klein, S. Z., Pirozzi, M., Prozzi, C. and Hull, C. M. 2011. Generalized Cryptococcus albidus in an immunosuppressed patient with palm pustular psoriasis. *Cutis*, 88(3): 129-32.

Feynman, R. 2019. Unstable taxonomies. In: Berman, J.J. *Taxonomic guide to infectious diseases: Understanding the biologic classes of pathogenic organisms.* London: Academic Press, 341-344.

French, N., Gray, K., Watera, C., Nakiyingi, J., Lugada, E., Moore, M., Lalloo, D., Whitworth, J. A. G. and Giks, C.F. 2002. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS*, 16(7): 1031–1038.

Gates-Hollingsworth, M. A. and Kozel, T. R. 2013. Serotype sensitivity of a lateral flow immunoassay for cryptococcal antigen. *Clinical and Vaccine Immunology,* 20(4): 634–635. Available: DOI: 10.1128/CVI.00732-12 (Accessed 12 June 2020).

Ganiem, A. R., Indrati, A. R., Wisaksana, R., Meijerink, H., Van der Ven, A., Alisjahbana, B. and Van Crevel, R. 2014. Asymptomatic cryptococcal antigenemia is associated with mortality among HIV-positive patients in Indonesia. *Journal of the International AIDS Society,* 17(1): 18821. Available: doi: 10.7448/IAS.17.1.18821 (Accessed 12 June 2020).

Govender, N. and Dlamini, S. 2014. Management of HIV-associated cryptococcal disease in South Africa. *South African Medical Journal*, 104(12): 896.

Govender, N. P., Meintjes, G., Mangena, P., Nel, J., Potgieter, S., Reddy, D., Rabie, H., Wilson, D., Black, J. and Boulware, D. 2019. Southern African HIV Clinicians Society guideline for the prevention, diagnosis and management of cryptococcal diseases among HIV-infected persons. *Southern African Journal of HIV Medicine,* 20(1), a1030. Available: https://doi.org/10.4102/sajhivmed.v20i1.1030 (Accessed 29 July 2021).

Harris, S. R., Lockhart, S. R., Sondermeyer, C., vugia, D. J., Crist, M. B., D'Angelo, M. T., Sellers, B., Franco-Paredes, C., Makvandi, M., Smelser, C., Green, J., Stanek, D., Signs, K., Nett, R. J., Chiller, T. and Park, B. J. 2013. Cryptococcus gattii infections in multiple state outside the US Pacific Northwest. *Emergency Infection Diseases*,19(10): 1620-1626.

Hartati, R. F. 2017. Identifikasi jamur Trichophytonrubrum pada petani yang terinfeksi tinea pedis. Diploma Thesis, STIKES Insan Cendekia Medika Jombang.

Hong, N., Chen, M., Fang, W., Al-Hatmi, A. M. S., Boekhout, T., Xu, J., Zhang, L., Liu, J., Pan, W. and Liao, W. 2017. Cryptococcosis in HIV-negative patients with renal dialysis. A retrospective analysis of pooled cases. *Mycopathologia*, 182(9-10): 887-896.

Hu, G., Wang, J., Choi, J., Jung, W. H., Liu, I., Litvintseva, A. P., Bicanic, T., Aurora, R., Mitchell, T. G., Perfect, J. R. and Kronstad, J. W. 2011. Variation in chromosome copy number in influences the virulence of Cryptococcus neoformans and occurs in isolates from AIDS patients. *BMC Genomics*, 12: 526.

Janbon, G., Ormerod, K. L., Paulet, E. J., Byrns 3rd, E. J., Yadav, V., Chatterjee, G., Mullapudi, N., Hon, C. C., Billmyre, R. B., Brunel, F., Bahn, Y. S., Chen, W., Chen, Y., Chow, E. W. L., Coppee, J. Y., Floyd-Averette, A., Gaillardin, C. Gerik, K. J., Goldberg, J., Gonzalez-Hilarion, S., Guja, S., Hamlin, J. L., Hsueh, Y. P., Laniri, G., Jones, S., Kodira, C. D., Kozubowski, L, Larn, W., Marra, M., Mesner, L. D., Mieczkowski, P. A., Moyrand, F., Nielsen, K., Proux, C., Rossignol, T., Schein, J. E., Sun, S., Wollschlaeger, C., Wood, I. A., Zeng, Q., Neuveglise, C., Newlon, C. S., Perfect, J. R., Lodge, J. K., Idnum, A., Stajich, J. E., Kronstad, J. W., Sanyal, K.,

Heitman, J., Fraser, J. A., Cuomo, C. A. and Dietrich, F. S. 2014. Analysis of the genome and transcriptome of Cryptococcus neoformans var, grubil reveals complex RNA expression and microevolution leading to virulence attenuation. *PloS Genetics*, 10: e1004561.

Jarvis, J. N., Lawn, S. D, Vogt, M., Bangani, N., Wood, R. and Harrison, T. S. 2009. Screening for cryptococcal antigenaemia in patients accessing an antiretroviral treatment program in South Africa. *Clinical and Infectious Diseases*, 48(7): 856–862.

Jarvis, J. N., Harrison, T. S., Lawn, S. D., Meintjes, G., Wood, R. and Cleary, S. 2013. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. *PLoS One*, 8(7): e69288.17.

Kharsany, A. B. M., Cawood, C., Lewis, L., Yende-Zuma, N., Khanyile, D., Puren, A., Madurai, S., Baxter, C., George, G., Govender, K., Beckett, S., Samsunder, N., Toledo, C., Ayalew, K. A., Diallo, K., Glenshaw, M., Herman-Roloff, A., Wilkinson, E., Oliveira, T., Salim, S., Karim, A. and Larim, Q. A. 2019. Trends in HIV prevention, treatment, and incidence in a hyperendemic area of KwaZulu-Natal, South Africa. *JAMA Network Open*, 2(11): e1914378.

Kozel, T. R. and Bauman, S. K. 2012. Cryptococcal antigen lateral flow assay for cryptococcosis. *Expert Opinion Medical Diagnostics,* 6(3): 245-251.

Kranzer, K., Govindasamy, D., Ford, N., Johnston, V. and Lawn, S. D. 2012. Quantifying and addressing losses along the continuum of care for people living with HIV infection in sub-Saharan Africa: a systematic review. *Journal of the International AIDS Society*, 15(2): 17383.

Kumar, P., Yung, M., Haynes, B. C., Skowyra, M. L. and Doering, T. L. 2011. Emerging themes in cryptococcal capsule synthesisi. *Current Opinion in Structural Biology,* 21(5): 597-602.

KwaZulu-Natal Provincial Department of Health. 2017. Universal test and treat: a game changer in HIV Prevention. Pietermaritzburg, South Africa: KwaZulu-Natal

Provincial Department of Health. Available: http://www.kznonline.gov.za/hivaids/councils/Provincial-Councils onAIDS/2016/Universal%20Test%20&%20Treat%20Presentation.pdf. (Accessed 09 March 2021).

Larson, B. A., Rockers P. C., Bonawitz, R., Sriruttan, R., Glencross, D. K., Cassim, N., Coetzee, L. M., Greene, G. S., Chiiler, T. M., Vallabhaneni, S. and Long, L. 2016. Screening HIV-infected patients with low CD4 counts for cryptococcal antigenaemia prior to initiation of antiretroviral therapy: cost effectiveness of alternative screening strategies in South Africa. *PLoS One*, 11(17): e0158986.

Leon, A., Davis, L. L. and Kraemer, H. C. 2012. The role and interpretation of pilot studies in clinical research. *Journal of Psychiatric Research,* 45(5): 626-629.

Loyse, A., Burry, J. Cohn, J., Ford, N., Chiller, T.,Ribeiro, I., Koulla-Shiro, S., Mahamba, J., Ramadhani, A., Nyirenda, R., Aliyu, A.H., Wilson, D., Le, T., Oladele, R., Lesikari, S., Muzoora, C., Kalata, N., Temfack, E., Mapoure, Y., Sini, V., Chanda, D., Shimwela, M., Lakhi, S., Ngoma, J., Gondwe-Chunda, L., Perfect, C., Shroufi, A., Andrieus-Meyer, I., Chan, A., Schutz, C., Hosseinipour, M., Van der Horst, C., Klausner, J. D., Boulware, D. R., Heyderman, R., Lalloo, D., Day, J., Jarvis, J. N., Roderigues, M., Jaffar, S., Denning, D., Migone, C., Doherty, M., Lortholary, O., Dromer, F., Stack, M., Molloy, S. F., Bicanic, T., Van Oosterhout, J. Mwaba, P., Kanyana, C., Kouanfack, C., Mfinanga, S., Govender, N. and Harrison, T. S. 2019. Leave no one behind: response to new evidence and guidelines for the management of cryptococcal meningitis in low-income and middle-income countries. *The Lancet Infectious Diseases*, 19(4): e143–e147. Available: doi: 10.1016/S1473-3099(18)30493-6 (Accessed 10 June 2020).

Ma, H., Hagen, F., Stekel, D. J., Johnston, S. A., Sionov, E., Falk, R., Polacheck, I., Boekhout, T. and May, R. C. 2009. The fatal fungal outbreak on Vancouver Island is characterized by enhanced intracellular parasitism driven by mitochondrial regulation. *Proceedings of the National Academy of Sciences of the United States of America,* 106(31): 12980-5.

MacDougall, L., Fyfe, M., Romney, M., Starr, M. and Galanis, E. 2011. Risk factors for *Cryptococcus gattii* infection, British Columbia, Canada. *Emerging Infectious Diseases*, 17(2): 193–199. Available: doi: 10.3201/eid1702.101020.

Messina, J. A., Maziarz, E. K., Spec, A., Kontoyiannis, D. P. and Perfect, J. R. 2017. Disseminated cryptococcosis with brain involvement in patients with chronic lymphoid malignancies on ibrutinib. *Open Forum Infectious Diseases,* 4(1).

National Department of Health, South Africa. 2013. *The 2012 National Antenatal Sentinel HIV & Herpes Simplex Type-2 Prevalence Survey in South Africa*. Pretoria: Government Printer.

National Department of Health, South Africa. 2015. *National consolidated guidelines for preventing of mother-to-child transmission of HIV and the management of HIV in children, adolescents, and adults*. Pretoria: Government Printer.

National Department of Health, South Africa. 2019. *Essential drugs programme: primary healthcare standard treatment guidelines and essential medicines list*. Pretoria: Government Printer.

Ndayishimiye, E. and Ross, A. J. 2018. An audit of the screen-and-treat intervention to reduce cryptococcal meningitis in HIV-positive patients with low CD4 count. *African Journal of Primary Health & Family Medicine,*10(1): 1779. Available: doi: 10.4102/phcfm.v10i1.1779 (Accessed 15 November 2021).

Nelson, A. M., Manabe, Y. C. and Lucas, S. B. 2017. Immune reconstitution inflammatory syndrome (IRIS): what pathologists should know? *Seminars in Diagnostic Pathology,* 34(4): 340-351.

Oyella, J., Meya, D., Bajunirwe, F. and Kamya, M. R. 2012. Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda: a cross-sectional study. *Journal of the International AIDS Society*, 15: 15. Available: doi: 10.1186/1758-2652-15-15 (Accessed 10 August 2020).

O'Meara, T. R., and Alspaugh, J. A. 2012. The Cryptococcus neoformans capsule: a sword and a shield. *Clinical Microbiological Reviews,* 25(3): 387–408.

Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G. and Chiller, T. M. 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*, 23(4): 525–530.

Perfect, J. R. and Bicanic, T. 2015. Cryptococcosis diagnosis and treatment: what do we know now. *Fungal Genetics and Biology,* 78: 49-54.

Pongsai, P., Atamasirikul, K. and Sungkanuparph, S. 2010. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. *The Journal of Infection,* 60: 474–477.

Public Health Agency of Canada. 2018. Cryptococcus neoformans, pathogen safety data sheet-infectious substances. Available: htpp://www.phac-asp.gc.ca/lab-bio/res/psds-ftss/Cryptococcus=eng.php. (Accessed 11 December 2021).

Pyrgos, V., Seitz, A. E., Steiner, C. A., Prevots, D. R. and Williamson, P. R. 2013. Epidemiology of cryptococcal meningitis in the US. *PloS* One, 8(2): e56269.

Ragupathi, L. and Reyna, M. 2015. Case report of Cryptococcus albidusperitonis in a peritoneal dialysis patient and a review of the literature. *Peritoneal Dialysis International*, 35(4): 421-427.

Rajasingham, R., Smith, R. M., Park, B. J., Jarvis, J. N., Govender, N. P. and Chiller, T. M., Denning, D. W., Loyse, A. and Boulware, D. R. 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *The Lancet Infectious Diseases,* 17(8): 873–881.

Salehei, Z., Mahmoudabadi, A. Z. and Zarrin, M. 2015. Lack of Cryptococcus gattii from Eucalyptus in Ahvaz. *Current Medical Mycology*, 1(1): 1–3.

Schwartz, S., Kontoyiannis, D. P., Harrison, T. and Ruhnke, M. 2018. Advances in the diagnosis and treatment of fungal infections of the CNS. *The Lancet Neurology,* 17(4): 362-372.

Setianingrum, F., Rautemaa-Richardson, R. and Denning, D. W. 2019. Pulmonary cryptococcosis: a review of pathobiology and clinical aspects. *Medical Mycology*, 57(2): 133-150.

Shah, H. S., Patel, D. A. and Vegad, M. M. 2011. Evaluation of conventional and Serological methods for rapid diagnosis of cryptococcal meningitis in HIV seropositive patients at tertiary care hospital. *National Journal of Community Medicine*, 2(3): 354-357.

Skipper, C., Abassi, M. and Boulware, D. R. 2019. Diagnosis and management of central nervous system cryptococcal infections in HIV-infected adults. *Journal of Fungi*, 5(3): 1-17.

South Africa National AIDS Council. National strategic plan on HIV, STIS and TB, 2012-2016, Pretoria: South Africa National AIDS council, 2012. Available: http:// www.org.za/index.phl/resources/national-strategic.plan. (Accessed 12 September 2021).

Springer, D. J., Billmyre, R. B., Filler, E. E., Voelz, K., Pursall, R., Mieczkowski. P. A, Larsen, R. A., Dietrich, F. S., May, R. C. Filler, S. G. and Heitman, J. 2014. Cryptococcus gattii VGIII isolates causing infections in HIV/AIDS patients in Southern California: identification of the local environmental source as arboreal. *PLoS Pathogens*, 10 (8): e1004285.

Srikanta, D., Santiago-Tirado, F. H., Doering, T. L. 2014. Cryptococcus neoformans: historical curiosity to modern pathology. *Yeast*, 31(2): 47-60.

Stats SA. 2017. Statistical release. Mid-year population estimates for 2017. Available: http://www.statssa.gov.za/publications/P0302/P03022017.pdf. (Accessed 14 November 2021).

Sugita, T., Cho, O. and Takashima, M. 2017. Current status of taxonomy of pathogenic yeasts. *Medical Mycology Journal*, 58(3): J77-J81.

Tesei, D., Sterflinger, K. and Marzban, G. 2019. Mission accomplished? In: Tiquia-Arashiro, S. M. and Grube, M. eds. *Fungi in extreme environments: ecological role and biotechnological significance*. Cham: Springer, 205.

Vijay, S., Ingole, N., Wanjare, S. and Mehta, P. 2019. Prevalence of cryptococcaemia in HIV seropositive patients in an Indian setting. *Journal of Clinical & Diagnostic Research,* 13(6): 1-4.

Wake, R. M., Govender, N. P., Omar, T., Carolina, N., Mazanderani, A. H., Karat, A. S., Ismail, N. A., Tiemessen, C. T., Jarvis, J. N. and Harrison, T. S. 2019. Cryptococcal-related mortality despite fluconazole pre-emptive treatment in a cryptococcal antigen screen-and treat programme. *Clinical Infectious Diseases*, 70(8): 1683-1690.

Walaza, S. 2014. *Cryptococcal screen-and-treat in Gauteng province, South Africa: Update from the first two years of implementation, 2012–2014.* Pretoria: Gauteng Province.

Wiesner, D. L., Moskalenko, O., Corcoran, J. M., McDonald, T., Rolfes, M. A., Meya, D. B., Kajumbula, H. A. K., Bohjanem, P. R., Knighyt, J. F. and Boulware, D, R. K. N. 2012. Cryptococcal genotype influences immunologic response and human clinical outcome after meningitis. *mBio*, 3: e00196-12.

Williamson, P. R., Jarvis. J. N., Panackal, A. A., Fisher, M. C., Molloy, S. F., Loyse, A. and Harrison, T. S. 2017.Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. *Nature Reviews Neurology*, 13(1): 13-24.

World Health Organization (WHO). 2011. *Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children*. Geneva (Switzerland).

World Health Organization (WHO). 2017. *Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy*. Geneva (Switzerland).

World Health Organization (WHO). 2018b. *Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy*. Geneva (Switzerland).

World Health Organization (WHO). 2019. *Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children*. Geneva (Switzerland).

Worodria, W., Massinga-Loembe, M., Mayanja-Kizza, H., Namaganda, J., Kambugu, A., Manabe, Y. C., Kestens, L. and Colebunders, R. 2011. Antiretroviral treatment-associated tuberculosis in a prospective cohort of HIV-infected patients starting Antiretroviral therapy. *Clinical and Developmental Immunology*. 758350. Available: https:dx.doi.org/10.1155/2011/758350. (Accessed 11 December 2021).

# APPENDICES

## Appendix A: Letter of permission – KZN Department of Health


## Letter of Permission

**To**: KwaZulu Natal Provincial Department of Health

**From**: Mrs Missi Emilienne Yohali

**Date**:

**RE**: Permission to perform research project

---

Dear Madam / Sir

I am currently working at King Dinuzulu National Health Laboratory Service. As part of my Master's degree program at D.U.T. I am required to conduct a full master's research project. I hereby request permission to collect data and do data analysis from your department.

**Project title**: The impact of Cryptococcal Antigenemia screening among patients attending King Dinuzulu Hospital.

**The aim of this study**: To investigate the impact of cryptococcal antigenemia screening on patient management at King Dinuzulu Hospital.

There will be no recruitment of human participants in this study however, specimens collected from patients attending King Dinuzulu Hospital in Durban, KwaZulu-Natal province, South Africa received in the laboratory will be sources of data for this research.

This study will involve human specimens. It is undertaken to establish the degree to which the screening of serum predicts *Cryptococcus* meningitis in patients.

**Principal Investigator**: Missi Emilienne Yohali

National Health Laboratory Service at King Dinuzulu Hospital Laboratory

**Degree registered for:** Master of Health Sciences Degree in Medical Laboratory Science at DUT.

Telephone: 0732086672 Email address: missiemilienne@yahoo.fr

**Supervisor:** Dr JN Mbatha (DUT)

PhD in Medical Science: Medical Microbiology, University of KwaZulu-Natal, 2017

Telephone: 0313735280 Email: nonhlanhlam@dut.ac,za

**Co-Supervisor:** Dr Abraham J. Niehaus (NHLS and UKZN)

PhD in Medicine (Medical Microbiology), University of KwaZulu-Natal, 2019

Fellow of the College of Pathologists (Microbiology), College of Medicine of South Africa, 2011

M.B; Ch.B. (University of Free State), 2003. Telephone: 0833778206

Email address: abrahamniehaus@gmail.com

Please contact the researcher or supervisors if you require any additional information or clarification.

Kind regards

---------- ----------

M. E. Yohali Date

---------- ----------

Dr A. J. Niehaus Date

---------- ----------

Dr J. N. Mbatha Date

## Appendix B: Letter of permission – King Dinizulu Hospital


# Letter of Permission

**To**: King Dinuzulu Hospital - Human Resources Department Ethics Committee

**From**: Mrs. Missi Emilienne Yohali

**Date**:

**RE**: Permission to perform research project

---

Dear Madam / Sir

I am currently working at King Dinuzulu National Health Laboratory Service. As part of my Master's degree program at D.U.T. I am required to conduct a full master's research project. I hereby request permission to collect data and do data analysis from your department.

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National Health Laboratory Service at King Dinuzulu Hospital Laboratory

**Degree registered for:** Master of Health Sciences Degree in Medical Laboratory Science at DUT.

Telephone: 0732086672 Email address: missiemilienne@yahoo.fr

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M.B; Ch.B. (University of Free State), 2003. Telephone: 0833778206

Email address: abrahamniehaus@gmail.com

Please contact the researcher or supervisors if you require any additional information or clarification.

Kind regards

---------- ----------

M. E. Yohali Date

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Dr A. J. Niehaus Date

---------- ----------

Dr J. N. Mbatha Date

## Appendix C: Letter of permission – King Dinuzulu Hospital Ethics Committee


# Letter of Permission

**To**: King Dinuzulu Hospital - Human Resources Department Ethics Committee

**From**: Mrs Missi Emilienne Yohali

**Date**:

**RE**: Permission to perform research project

---

Dear Madam / Sir

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M.B; Ch.B. (University of Free State), 2003. Telephone: 0833778206

Email address: abrahamniehaus@gmail.com

Please contact the researcher or supervisors if you require any additional information or clarification.

Kind regards

---------- ----------

M. E. Yohali Date

---------- ----------

Dr A. J. Niehaus Date

---------- ----------

Dr J. N. Mbatha Date

## Appendix D: Letter of permission – NHLS

# Letter of Permission

**To**: NHLS - Academic Affairs & Research Management System

**From**: Mrs Missi Emilienne Yohali

**Date**:

**RE**: Permission to perform research project

---

Dear Madam / Sir

I am currently working at King Dinuzulu National Health Laboratory Service. As part of my Master's degree program at D.U.T. I am required to conduct a full master's research project. I hereby request permission to collect data and do data analysis from your department.

**Project title**: The impact of Cryptococcal Antigenemia screening among patients attending King Dinuzulu Hospital.

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This study will involve human specimens. It is undertaken to establish the degree to which the screening of serum predicts *Cryptococcus* meningitis in patients.

**Principal Investigator**: Missi Emilienne Yohali

National Health Laboratory Service at King Dinuzulu Hospital Laboratory

**Degree registered for:** Master of Health Sciences Degree in Medical Laboratory Science at DUT.

Telephone: 0732086672 Email address: missiemilienne@yahoo.fr

**Supervisor:** Dr JN Mbatha (DUT)

PhD in Medical Science: Medical Microbiology, University of KwaZulu-Natal, 2017

Telephone: 0313735280 Email: nonhlanhlam@dut.ac,za

**Co-Supervisor:** Dr Abraham J. Niehaus (NHLS and UKZN)

PhD in Medicine (Medical Microbiology), University of KwaZulu-Natal, 2019

Fellow of the College of Pathologists (Microbiology), College of Medicine of South Africa, 2011

M.B; Ch.B. (University of Free State), 2003. Telephone: 0833778206

Email address: abrahamniehaus@gmail.com

Please contact the researcher or supervisors if you require any additional information or clarification.

Kind regards

---------- ----------

M. E. Yohali Date

---------- ----------

Dr A. J. Niehaus Date

---------- ----------

Dr J. N. Mbatha Date

## Appendix E: Letter of approval from King Dinizulu Hospital

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Physical Address: 75 Dr RD Naidu Driveway,Sydenham,Durban
Postal Address: P O DORMERTON, 4015
Tel: 031 271 1181 Email: Tumelo.Mabesa2@kznhealth.gov.za
www.kznhealth.gov.za

Senior Manager: Medical Services
King Dinuzulu Hospital Complex

Enquiries: Dr TP Mabesa

24th November 2020

Dear Ms M.E Yohali

**RE: PERMISSION TO CONDUCT RESEARCH "The impact of cryptococcal antigenaemia screening among patients attending KDHC".**

I have pleasure in informing you that permission to conduct the above study has been granted to you by King Dinuzulu Hospital Complex.

Please note the following:

1. Please ensure that you adhere to all policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. Please ensure that this office is informed before you commence your research.
3. Neither the District Office nor KDHC will provide any resources for this research.
4. Your attention is drawn to the maintenance of confidentiality with respect to staff records/files and may not be removed from this Institution.
5. You will be expected to provide feedback on your findings to KDHC.

Yours sincerely

**Dr TP Mabesa**
**Senior Manager: Medical Services**
**King Dinuzulu Hospital Complex**

## Appendix F: Letter of approval from KZN Department of Health

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email: [redacted]
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management

NHRD Ref: KZ_202011_002

**Dear Ms E. Yohali**
(DUT)

**Approval of research**

1. The research proposal titled **'The Impact of cryptococcal antigenaemia screening among patients attending King Dinuzulu Hospital.'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Dinuzulu Hospital Complex.

2. You are requested to take note of the following:
   a. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   b. *Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to* ***HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200*** *and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

**Dr E Lutge**
Chairperson, Health Research Committee
Date: 11/12/2020

Fighting Disease, Fighting Poverty, Giving Hope

## Appendix G: Letter of approval – NHLS

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

**09 February 2021**

**Applicant:** Missi Emilienne Yohali
**Institution:** NHLS / DUT
**Department:** Biomedical and Clinical Technology
**Email:** emilienne.yohali@nhls.ac.za
**Tel:** 031 242 6077
**Cell:** 073 208 6672

**CC:** Abraham Niehaus
Medical Microbiology

**Re: Provisional Approval to access National Health Laboratory Service (NHLS) Data**

Your application to undertake a research project **"The Impact of cryptococcal antigenaemia screening among patients attending King Dinuzulu Hospital, ref no: PR2010421"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been provisionally approved ***without patient names***. For full approval to be granted and for you to be given access to the data you have requested, you need to satisfy the following conditions:

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please send any requested documents through to academic.research@nhls.ac.za. Once your application has received full final approval, we will confirm with you in writing. Should you wish to speak with us, please contact us on 011 555 0367.

Yours sincerely

**Dr Babatyi Malope-Kgokong**
**National Manager: AAR**

## Appendix H: Provisional approval DUT IREC

**Institutional Research Ethics Committee**
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

**www.dut.ac.za**

14 September 2020

Mrs M E Yohali
Private Bag X03
Cato Manor
Mayville
Durban
4058

Dear Mrs Yohali

**The Impact of cryptococcal antigenaemia screening among patients attending King Dinuzulu Hospital.**

I am pleased to inform you that **PROVISIONAL APPROVAL** has been granted to your proposal subject to:

- Piloting of the data collection tools. *Please note that should there be any changes to the data collection tools, in a letter signed by the researcher and supervisor, list the changes to the documents and submit to IREC with the final data collection tools. Even when there are no changes to the data collection tools, IREC has to be notified.*
- Obtaining and submitting the necessary gatekeeper permission/s to Institutional Research Ethics Committee (IREC).

PLEASE NOTE THAT THIS IS NOT A FINAL APPROVAL LETTER. KINDLY SUBMIT THE ABOVE MENTIONED DOCUMENTS WITHIN THREE MONTHS TO THE IREC OFFICE. DATA COLLECTION CAN ONLY COMMENCE WHEN IREC ISSUES FULL APPROVAL

The Proposal has been allocated the following Ethical Clearance number **IREC 026/20**. Please use this number in all communication with this office.

Approval has been granted for a period of **ONE YEAR**, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the IREC. This form must be submitted to the IREC at least 3 months before the ethics approval for the study expires.

Yours Sincerely

Prof J K Adam
Chairperson: IREC

## Appendix I: Full approval DUT IREC

**Institutional Research Ethics Committee**
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

**www.dut.ac.za**

3 March 2021

Mrs M E Yohali
Private Bag X03
Cato Manor
Mayville
Durban
4058

Dear Mrs Yohali

**The Impact of cryptococcal antigenaemia screening among patients attending King Dinuzulu Hospital.**
**Ethical Clearance number IREC 026/20**

The Institutional Research Ethics Committee acknowledges receipt of your notification regarding the piloting of your data collection tool.

Kindly ensure that participants used for the pilot study are not part of the main study.

In addition, the IREC acknowledges receipt of your gatekeeper permission letters.

Please note that FULL APPROVAL is granted to your research proposal. You may proceed with data collection.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Yours Sincerely

Professor J K Adam
Chairperson: IREC

## **Appendix J:** Data collection tables

Section A

**Table 3.1 Data collection**

| Code Number | CrAg | | Culture | CD4 |
|---|---|---|---|---|
| | CSF | Serum/Plasma | | |
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Section B

**Table 3.2: Data collection**

| Number | Patient's code | CrAg | | Culture | CD4 | Clinician's comments |
|---|---|---|---|---|---|---|
| | | CSF | Serum/Plasma | | | |
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## Appendix K: Editing certificate

**DR RICHARD STEELE**
BA HDE MTech(Hom)
**HOMEOPATH**
Registration No. A07309 HM
Practice No. 0807524
**Freelance academic editor**
**Associate member: Professional Editors' Guild, South Africa**

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**EDITING CERTIFICATE**

Re: **Missi Emilienne Yohali**
Master's dissertation DUT: **The Impact of *Cryptococcal Antigenemia* Screening Among Patients Attending King Dinuzulu Hospital**

I confirm that I have edited this dissertation and the references for clarity, language and layout. I returned the document to the author with track changes so correct implementation of the changes and clarifications requested in the text and references is the responsibility of the author. I am a freelance editor specialising in proofreading and editing academic documents. My original tertiary degree which I obtained at the University of Cape Town was a B.A. with English as a major and I went on to complete an H.D.E. (P.G.) Sec. with English as my teaching subject. I obtained a distinction for my M.Tech. dissertation in the Department of Homoeopathy at Technikon Natal in 1999 (now the Durban University of Technology). I was a part-time lecturer in the Department of Homoeopathy at the Durban University of Technology for 13 years and supervised many master's degree dissertations during that period.

Dr Richard Steele
**05 January 2022**
*per email*