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Cryptococcal antigenaemia in patients hospitalised with community acquired pneumonia at Chris Hani Baragwanath Academic Hospital

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CRYPTOCOCCAL ANTIGENAEMIA IN PATIENTS HOSPITALISED WITH COMMUNITY ACQUIRED PNEUMONIA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree

Of

Master of Medicine in the branch of Internal Medicine

Johannesburg, 2013

DECLARATION

I, Anneli Korb declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Anneli Korb

The 16th day of February, 2014

DEDICATION

This research report is dedicated to the memory of my grandfather

Jan Gideon Joubert

1924-2010

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Nil

ABSTRACT

Background

Cryptococcus is a life-threatening opportunistic infection; data is limited regarding early infection. Treatment of cryptococcal antigenaemia may impact on disease progression. Screening those most at risk for cryptococcal antigenaemia is necessary to be cost effective. The prevalence of cryptococcal antigenaemia in patients hospitalised with community acquired pneumonia (CAP) at Chris Hani Baragwanath Academic Hospital (CHBAH) was evaluated.

Methods

200 patients admitted to CHBAH with presumed CAP were enrolled. Clinical and laboratory data were collected and a Cryptococcal Lateral Flow Immunoassay was done on whole blood.

Results

Of the 200 patients, 185 (92.5%) were HIV-infected. Amongst the HIV-infected group, the median CD_4 cell count was 47 cells/mm^3 and 111 subjects (60%) had a CD_4 cell count < 100 cells/mm^3. The prevalence of cryptococcal antigenaemia was 0.5% (CI 0.01-2.75).

Conclusion

The prevalence of cryptococcal antigenaemia amongst inpatients with CAP was low. Routine screening of this group would not be cost-effective.

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Foremost, I would like to express my deepest appreciation to my supervisor, Dr Andrew Black for his patience, insight and continued guidance. He continually displayed an enthusiasm with regards to research and teaching. His encouragement and expertise guided me through this project.

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I further thank Chris Hani Baragwanath Academic Hospital for allowing me to conduct research at their facility. A special thanks to the wonderful patients of this community who are always willing to participate in our academic endeavours.

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ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
CM	Cryptococcal Meningitis
CNS	Central Nervous System
CrAg	Cryptococcal Antigen
CXR	Chest X-ray
TB	Tuberculosis
CAP	Community Acquired Pneumonia
EIA	Enzyme Immunoassay
LA	Latex Agglutination
LFA	Lateral Flow Assay
POCT	Point-of-care Test
WHO	World Health Organisation
ART	Antiretroviral Therapy
SAHIVSOC	Southern African HIV Clinician Society
NHLS	National Health Laboratory Services
CSF	Cerebrospinal Fluid

LP	Lumbar Puncture
NNT	Numbers Needed To Treat
CHBAH	Chris Hani Baragwanath Academic Hospital
EDTA	Ethylenediaminetetraacetic Acid
NICD	National Institute for Communicable Diseases
COTHI	Centre for Opportunistic, Tropical and Hospital Infections

1.0 Introduction

1.1 Cryptococcal disease

The systemic mycosis cryptococcosis is a life-threatening opportunistic infection occurring most frequently in immunocompromised patients. However, the disease has been described in patients with a normal immune system[1]. Among immunocompromised patients most of the burden falls on those infected with the Human Immunodeficiency Virus (HIV). Within this group, the majority of affected patients have a CD_4 cell count < 100 cells/mm^3[2, 3]. In a local review article other factors predisposing patients to cryptococcal infection were found to be: treatment with corticosteroids, organ transplantation and malignancies[2]. Systemic lupus erythematosus, sarcoidosis, chronic lung disease, sickle cell disease, Hepatitis B carrier state, diabetes mellitus, liver cirrhosis and Idiopathic CD_4 lymphocytopenia have also been described as immunosuppressive conditions predisposing patients to cryptococcal infection[4-6].

In response to the demand for fast and reliable detection recent advances in highly sensitive and specific antigen based cryptococcal tests have been made[7]. Despite recent national efforts in improved routine laboratory based cryptococcal testing little data exists on the utility of cryptococcal antigen detection in the acutely ill patient[8, 9].

1.1.1 Mycology of *Cryptococcus*

Cryptococcus neoformans is an encapsulated yeast-like fungus. Two varieties exist, namely *Cryptococcus var. neoformans* and *Cryptococcus var. grubii*. *Cryptococcus gattii* is now considered a distinct species. *Cryptococcus neoformans var. neoformans* and *Cryptococcus neoformans var. grubii* have been isolated in bird excreta, especially that of pigeons[10]. In 1990, Ellis and Pfeiffer found the primary ecological source of *Cryptococcus neoformans var. gattii* to be the plant debris under the river red gum (*Eucalyptus camaldulensis*) and other native Australian trees[11]. Humans acquire the disease by exposure to and inhalation of the infectious propagules found mainly in soil and vegetation. These infectious propagules are hypothesized to be small desiccated yeast cells facilitating deposition in terminal bronchioles and alveoli[12, 13].

Cryptococcus neoformans var. neoformans, known to cause life-threatening disease in immunosuppressed individuals, has been isolated worldwide and is known to be the cause of most clinical cases of cryptococcosis[14, 15]. Until as recently as 10 years ago it was thought that *Cryptococcus neoformans var. gattii* is a rare cause of fungal infection affecting mainly immunocompetent individuals in tropical and subtropical regions[16, 17]. However, in 1999 an outbreak of *Cryptococcus var. gattii* in the temperate region of British Colombia, Canada

showed that the infection is not limited to tropical and subtropical climates[18]. *Cryptococcus neoformans var. gattii* was later also found to cause disease in immunocompromised individuals[19].

1.1.2 Spectrum of cryptococcal disease

The spectrum of cryptococcal infection ranges from asymptomatic colonization to pulmonary cryptococcosis, cryptococcal meningitis (CM) and severe disseminated disease. Pulmonary cryptococcosis is caused by inhalation of the biodiospore and deposition in the terminal bronchioles and alveoli leading to an asymptomatic or symptomatic pneumonitis[15, 20]. Although pulmonary cryptococcosis is said to be a less common manifestation of illness, it is likely that the diagnosis of cryptococcosis is often missed due to the diagnosis of more common respiratory opportunistic infections and the minimal or nonspecific presenting symptoms[21].

Subsequent haematogenous spread to the central nervous system (CNS) causes a meningitis or meningoencephalitis. The lung can therefore be said to be the portal of entry for cryptococcal disease.

Early disease may be asymptomatic or present as an acute respiratory infection[22, 23]. In a retrospective, case-control

study by Visnegarwala *et al*, 29 out of 210 (13.8%) HIV-positive patients with a positive cryptococcal antigen (CrAg) test had acute respiratory failure as a result of cryptococcal disease. Other clinical pulmonary manifestations included influenza-like symptoms, cough, fever, dyspnoea, haemoptysis and pleural effusions. Radiological manifestations included asymptomatic pulmonary nodules, diffuse interstitial opacities, lymphadenopathy, alveolar opacities, cavitary lesions and pleural effusions[21, 23].

1.1.3 Burden of cryptococcal disease

The clinical problem is that cryptococcal testing is usually only performed when the patient presents with CM. In a study of the current global burden of CM among patients living with HIV/AIDS it was estimated that of the 957 900 global cases of CM in 2006, 720 000 occurred in sub-Saharan Africa. In sub-Saharan Africa 504 000 deaths were due to CM. There were an estimated 350 000 deaths due to Tuberculosis (TB) in 2006. This comparison clearly demonstrates the staggering global burden of CM[14]. CM is the most common cause of adult meningitis in South Africa accounting for 63% of microbiological diagnoses[24].

Prompt identification of asymptomatic antigenaemia and early initiation of antifungal agents in susceptible patients may

therefore better outcomes by initiating appropriate treatment before progression to meningitis[25]. Treating disease at an early stage will also decrease morbidity and severe complications of cryptococcal disease.

In a retrospective Thai study among patients hospitalised with pneumonia, *cryptococcus* was found to be the second most common pathogen associated with acute respiratory infections in HIV-infected individuals. Of the 704 HIV-infected samples tested, 92 (13.1%) were CrAg positive. 53% of patients with a positive CrAg test had no history of CM. 43% of patients had a positive CrAg test and had no evidence of another co-infection, indicating active cryptococcal disease as the cause of their pneumonia. They concluded that there is strong evidence for routine CrAg testing among HIV-infected patients hospitalised with pneumonia in Thailand and highlighted the need for a rapid point-of-care immunoassay in aiding the diagnosis of early cryptococcal disease in that population[26].

In 2013 a Ugandan study was published looking at CrAg testing in HIV positive patients hospitalised at a Kampala hospital with an unexplained cough ≥2 weeks. 32 (5.7%) of the 563 HIV positive patients had a positive CrAg test. The

CrAg positive patients had a median CD_4 cell count of 25 cells/mm^3[27].

Cryptococcus accounts for up to 44% of annual HIV/AIDS deaths in sub-Saharan Africa and CM is the most common disease manifestation of cryptococcal infection with a global yearly incidence estimate of 12% among HIV-infected individuals[14]. In a cohort study among HIV-infected miners in Southern Africa assessing the cause of demise and presence of pulmonary disease at autopsy, *cryptococcus* was found in 29 out of 202 HIV-infected individuals who died of natural causes. It is therefore an important cause of death in HIV-infected men, and in this study second only to TB[28].

Taking the above-mentioned studies into account, it poses the question whether prognosis could be improved by identification and targeted pre-emptive treatment in the pulmonary phase of cryptococcal disease. There is currently no published data looking at the prevalence of cryptococcal antigenaemia in patients hospitalised with community acquired pneumonia (CAP) in South Africa.

1.1.4 Cryptococcal testing

In the past clinicians have had to rely on microscopy and culture or laboratory based Cryptococcal Antigen (CrAg)

testing, such as the Enzyme Immunoassay (EIA) and Latex Agglutination (LA) for cryptococcal diagnosis. More recently the Lateral Flow Immunoassay (LFA), a point-of-care test (POCT), was introduced as an alternative to CrAg testing. In an evaluation done by Lindsley *et al*, the LFA was compared to blood cultures (the gold standard) and found to have 100% sensitivity when serum specimens were used. Their evaluation also pointed out that the LFA complies with the World Health Organization's ASSURED guidelines for POCTs (Affordable, Sensitive, Specific, User Friendly, Rapid, Equipment-free, Delivered to those who need it)[7, 29]. The LFA has the advantage of being an economical, highly sensitive and expeditious test that requires no additional laboratory equipment or expertise and is therefore an ideal diagnostic tool in a resource-limited setting.

1.1.5 Cryptococcal screening

The World Health Organisation (WHO) released their guideline for the Diagnosis, Prevention and Management of Cryptococcal Disease in HIV infected Adults, Adolescents and Children in Resource-limited Settings in December 2011 [30]. In this document they recommend routine screening for cryptococcal disease in Antiretroviral Treatment (ART) naïve adults with a CD_4 cell count <100 cells/mm^3 prior to ART

initiation in populations with a high prevalence of cryptococcal antigenaemia.

In 2013 the Southern African HIV Clinicians Society (SAHIVSOC) updated their Guideline for the Prevention, Diagnosis and Management of Cryptococcal Meningitis among HIV-infected Persons based on expert consensus and best clinical evidence[31]. In this document screening for cryptococcal antigenaemia is recommended for ART-naïve adult patients with a CD_4 cell count <100 cells/mm^3 who have no history of prior CM.

Phased laboratory-based screening for cryptococcal antigenaemia using the LFA is currently being conducted at over 500 National Health Laboratories Services (NHLS) laboratories in Gauteng and the Free State. All specimens submitted for CD_4 cell count found to a CD_4 cell count <100 cells/mm^3 will routinely be screened for CrAg using the LFA. The aim is to introduce pre-emptive antifungal treatment in all CrAg positive patients and thereby reduce morbidity and mortality associated with cryptococcal disease[9].

1.1.6 Management of cryptococcal disease

According to the SAHIVSOC Guideline for the Prevention, Diagnosis and Management of Cryptococcal Meningitis, all

patients with a positive CrAg result should be managed as follows:[31]

- Immediate evaluation for the symptoms,and signs of meningitis, followed by urgent cerebrospinal fluid (CSF) analysis for CM if no contra-indications to lumbar puncture (LP) exist.
- In order to exclude early CM, CSF analysis is recommended in patients lacking symptoms and signs of meningitis if immediately attainable.
- In patients with a CSF analysis excluding CM and those with no suspected meningitis, high dose oral Fluconazole (800mg/day) is recommended for 2 weeks, followed by consolidation treatment (400mg/day for 8 weeks) and thereafter maintenance treatment (200mg/day until the CD_4 cell count is >200 cells/mm^3 for at least 6 months and the most recent HIV viral load is suppressed) as recommended by the SAHIVSOC.
- In HIV positive patients not yet on ART, it is recommended to commence ART 2 weeks after initiating antifungal therapy.
- CrAg positive patients with a history of prior CM would only require full evaluation in the presence of new symptoms or signs of meningitis to rule out relapse CM. In the absence of these symptoms and signs, adequate Fluconazole maintenance therapy is

recommended. Following successful treatment of CM, the CrAg can remain positive in serum and CSF for a protracted period of time[32].

- Female patients of child-bearing age with a positive CrAg should have a pregnancy test. In light of a negative pregnancy test the patients should be offered appropriate contraception and advised to avoid pregnancy whilst on Fluconazole. Expert consultation should be sought for pregnant patients requiring antifungal therapy.
- Fluconazole therapy should be continued until the CD_4 cell count is >200 cells/mm^3 for at least 6 months and the most recent HIV viral load is suppressed.
- Patients with CM should be treated with 2 weeks of induction therapy, followed by consolidation and maintenance antifungal therapy as per the guideline.

1.1.7 Cost implications of cryptococcal disease

In a retrospective study conducted on 707 stored plasma samples obtained on enrolment in an ART cohort in South Africa 46 (7%) were CrAg positive[33].

In a Ugandan cohort 609 ART-naïve adults with AIDS initiating ART were prospectively enrolled to test serum CrAg. Of the 295 persons with CD_4 cell count ≤100 cells/mm^3

26(8.8%) were CrAg positive[34]. This cohort found the Number Needed to Treat (NNT) for screening was 11.3 (95% CI: 7.9–17.1) in those with CD_4 cell count ≤100 cells/mm^3 and 15.9 (95% CI: 11.1–24.0) people would need to be screened and treated to prevent one death. Despite the high cost of the undiluted CrAg test ($16.75 per test) in Uganda they concluded that CrAg screening would be cost-effective in persons with a CD_4 cell count ≤100 cells/mm^3. If one takes the high cost of their CrAg testing and the potential costs of hospital admission into consideration, they may well be underestimating the actual cost-effectiveness of cryptococcal screening.

In a cost-effectiveness analysis by Jarvis, JN *et al* 4 screening strategies for patients with CD_4 cell count ≤100 cells/mm^3 starting ART were compared. These strategies included no screening or prophylaxis, primary fluconazole prophylaxis, CrAg screening with high-dose fluconazole for CrAg positive patients and CrAg screening with LP in CrAg positive followed by amphotericin-B for those with CNS disease or fluconazole for those without. The most cost-effective strategy was CrAg screening followed by high dose fluconazole treatment in the CrAg positive group. In the group where positive CrAg screening was followed by LP for detection of CM the

additional costs of CSF analysis and amphotericin treatment outweighed the benefit[35].

All the above studies therefore concluded that CrAg screening would be a cost-effective strategy to identify individuals at risk for CM and to prevent CM related mortality in those patients initiating ART in a resource limited setting, specifically those with a CD_4 cell count ≤100 cells/mm^3. They also state that ART alone is insufficient and recommend integration of CrAg screening and pre-emptive antifungal therapy into routine HIV care.

1.2 Aim of the study

The aim of the study was to determine the prevalence of cryptococcal antigenaemia among patients admitted to Chris Hani Baragwanath Academic Hospital (CHBAH) with Community Acquired Pneumonia (CAP) and to determine whether routine CrAg testing would be beneficial in patients presenting with CAP. This strategy would allow screening of patients prior to obtaining their CD_4 cell counts which may well be lower than their baseline prior to their acute illness.

1.3 Objectives

a. To determine the prevalence of cryptococcal antigenaemia among adult patients admitted to CHBAH with Community Acquired Pneumonia.

b. To compare CrAg positive patients with CrAg negative patients with regards to the following variables: age, sex, HIV status, respiratory rate, oxygen saturation and blood pressure.

c. To do a sub-analysis of the prevalence of CrAg positivity by CD_4 cell count stratum in the HIV positive subset of patients.

2.0 Materials and methods

2.1 Definition of terms

Community Acquired Pneumonia was defined as symptoms suggestive of a respiratory tract illness (fever, cough with or without sputum production, pleurisy, dyspnoea) or clinical evidence of a respiratory tract infection (pyrexia, tachypnoea, tachycardia, audible crackles) and radiological findings consistent with an acute respiratory infection (infiltrates on chest X-Ray)[2].

2.2 Study design

This is a prospective, quantitative cross-sectional study with descriptive elements.

2.3 Study setting

The study was conducted in the medical wards at CHBAH, a level 3 state hospital that provides healthcare services to patients across the Gauteng province. Consent to collect data at this facility was obtained from the Head of the Department of Internal Medicine, Prof KRL Huddle and the medical superintendent Dr KA Mustafa on 25 January 2012. (Appendix B)

2.4 Study population and sample

Cryptococcus antigen testing was performed on the first 200 patients admitted to the medical admissions ward at CHBAH meeting the inclusion and exclusion criteria.

Purposive (non-random) convenience sampling using consecutive patients that met the inclusion criteria allowed for selection of individuals from the spectrum we were interested in.

2.4.1 Inclusion criteria

a. Patients aged 18 years or older;
b. Patients who met more than two of the following clinical criteria as per the definition of CAP: fever, cough, pleurisy, dyspnoea, crackles on chest auscultation and infiltrates on CXR.

2.4.2 Exclusion criteria

a. Confused patients;
b. Patients not willing to consent to participation in the study;
c. Patients with a history of prior cryptococcal disease;
d. Patients currently taking fluconazole treatment.

2.4.3 Informed consent

Written informed consent for access to clinical data and all laboratory results for research purposes was obtained from all participants. The data was collected on numbered data sheets and an identifying code book kept by the principal investigator. Access to clinical data and laboratory results was limited to the principal investigator who informed the attending physician of any positive results and the decision to treat patients with positive CrAg results rested upon the attending physician. Attending physicians of patients with positive CrAg results were also advised to do a lumbar puncture on patients with positive CrAg results to rule out CM.

2.4.4 Ethical considerations

Ethical approval was granted by University of the Witwatersrand Human Research Ethics Committee. (Clearance number M120249; Appendix D)

2.5 Methodology

Adult patients admitted to the medical admissions ward at CHBAH with CAP were screened for inclusion into the study. Patients were enrolled in the study prospectively and clinical and laboratory data were obtained by direct observation at the time of informed consent

and phlebotomy. 5ml of venous blood was collected from a peripheral site in an Ethylenediaminetetraacetic acid (EDTA) tube and tested for CrAg by the principal investigator.

Cryptococcus antigen testing was performed using the Lateral Flow Immunoassay (Immune-mycologics, Norman, OK, USA) on blood collected in EDTA tubes. All testing and data collection was done by the principle investigator thus avoiding interobserver bias. Test validation was performed as per manufacturer instruction for each batch of tests.

All clinical information was recorded on a numbered questionnaire and transferred to a numbered data sheet in Microsoft Excel, Version 14.0.6112.5000.

2.5.1 Variables measured

Age, sex, HIV status, respiratory rate, oxygen saturation and blood pressure were recorded from admission notes. The patient's CD_4 cell count was obtained from laboratory records. CXR changes observed on admission CXR were also recorded. Results of the LFA test were recorded.

2.5.2 Sample size

From published data, 13.1% of 704 HIV positive patients with respiratory symptoms were CrAg positive[18]. Using 3% CrAg

positivity as a cut-off for cost-effectiveness we considered 10% to be a clinically significant difference[31]. α was set at 0.05. Funding was available for 200 tests. Calculated power using a sample size of 200 and the above assumptions was 0.96. Assuming approximately 60% of patients admitted with respiratory symptoms in the medical wards at CHBAH are HIV positive we can expect approximately 120 HIV positive patients amongst the 200 patients entered into the study giving a power of 0.82.

2.5.3 Comparative analysis

I compared the proportion of positive CrAg results I obtained with the number of CrAg positive patients in the study conducted in Thailand by Harris JR et al[26].

There is currently a large surveillance study being conducted by the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS). Monthly data is published on a provisional basis to provide information regarding communicable diseases. As part of this surveillance the NICD's Centre for Opportunistic, Tropical and Hospital Infections (COTHI) in conjunction with the Department of Health has begun the first phase of laboratory-based cryptococcal antigen screening using the LFA on samples sent for CD_4 cell count testing found to have a CD_4

cell count of <100 cells/mm^3. In the July 2013 report it was stated that 7171 samples have thus far been screened, of which 287 (4%) tested CrAg positive[8]. I compared the proportion of positive CrAg results I obtained with their provisional CrAg results from the NICD in order to ascertain whether the prevalence of CrAg positivity in a population of patients with CAP admitted to CHBAH is similar or significantly different to theirs.

2.6 Statistical analysis

Data was initially collected on data collection sheets and later transferred to a Microsoft Office ® Excel 2010 spread sheet. Data was analysed using Intercooled STATA version 10 (Stata-Corp, LP, College Station.TX).

Parametric data was described using Mean and Standard Deviation (SD) and non-parametric data described using Median and Interquartile range with a 95% Confidence Interval (CI).

For comparative statistics, continuous data was analysed using the two-tailed unpaired t test. Discreet data was analysed using Fisher's exact test. A P value of <0.05 was considered significant. Non-parametric data was analysed using the Mann-Whitney test.

2.7 Measurement instruments

2.7.1 Lateral Flow Assay

Cryptococcus Antigen testing was performed using the Lateral Flow Immunoassay (Immune-mycologics, Norman, OK, USA) on bloods collected in EDTA tubes. Test validation was performed as per manufacturer instruction for each batch of tests.

2.8 Limitations of the study

Consecutive patients meeting the inclusion criteria were enrolled into the study, these patients were referred to the investigator by the admitting doctors; it is possible that referral bias existed. Due to the low number of positive results I was unable to do comparative statistics. Based on the literature at the time of protocol development the expected frequency of positive results was estimated to be between 10 and 15% and a 10% difference was considered clinically significant. Based on these assumptions a sample size of 200 was determined to be adequate (α=0.05; power=0.96) and would have provided sufficient numbers to proceed with the intended comparative analyses.

2.9 Strengths of the study

A complete data set was obtained with minimal missing variables. All tests were performed by a single operator in the field eliminating inter-observer variability. The data was collected from patients with a wide range of age and immune status. Amongst immunocompromised patients there were a sufficient number of patients with CD_4 cell counts <100 cells/mm^3. This enabled comparison to a similar study by Harris JR *et al* and provisional data being collected by the NICD for the development of the new national cryptococcal screening guidelines[8, 26].

3.0 Results

Data from two hundred patients admitted to the CHBAH medical wards with a diagnosis of CAP was analysed after initial screening for inclusion and exclusion criteria. No subjects were excluded from the initial data collection.

3.1 Demographics

3.1.1 Sex and Age

Table 3.1 indicates that the study group comprised 115 males (57.5%) and 85 females (42.5%).

Table 3.1: Sex of Subject in Data Set

Sex	Result
Male	85 (42.5%)
Female	115 (57.5%)
Total	200

Figure 3.1 illustrates the age profile of individuals in the data set. The mean age of the group was 43 years (SD ± 14.4 years). The range was 20-79 years with an interquartile range (IQR) of 30 – 50.75.

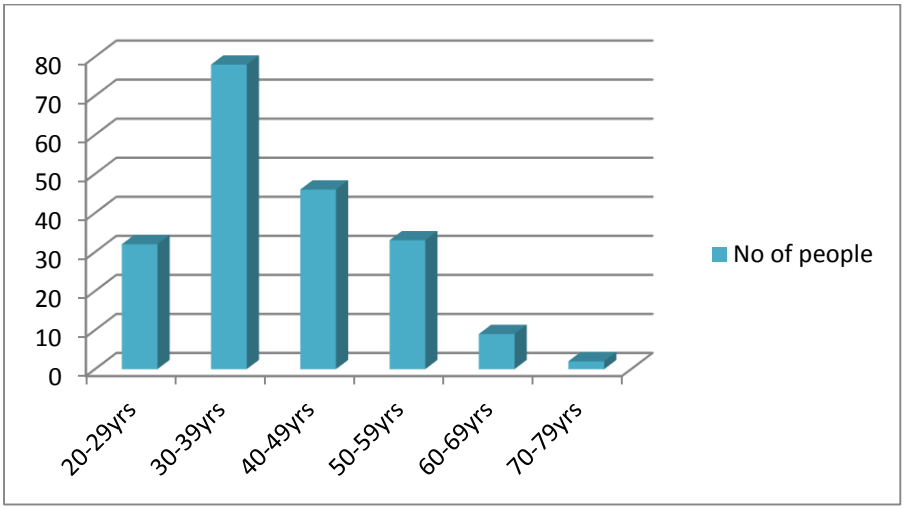


Figure 3.1: Age distribution of data set

3.1.2 HIV status

Of the 200 subjects included in the data set 185 were HIV positive, 13 HIV negative and 2 patients weren't aware of their current HIV status as illustrated in Table 3.2.

Table 3.2: HIV Status of Subjects in Data Set

HIV Status	Result
HIV Positive	185 (92.5%)
HIV Negative	13 (6.5%)
Unknown	2 (1%)
Total	200

3.1.3 CD_4 cell count stratification and Antiretroviral therapy

Among the HIV positive subjects, the median CD_4 cell count was 47 cells/mm^3 (range:0-1439 cells/mm^3, IQR20.75 – 170.75 cells/mm^3). 111 (60%) of 185 HIV positive patients had a CD_4 cell count of <100 cells/mm^3.

137 (74.05%) had a CD_4 cell count of ≤200 cells/mm^3 and 156 (84.32%) had a CD_4 cell count of ≤350 cells/mm^3. 49 (26.5%) were on ART. Based on the new national ART guidelines implemented by the Department of Health on 1 April 2013, 156 (84.32%) subjects were eligible for ART[36]. 137 (74.05%) subjects were eligible for ART based on the former ART guidelines[37].

Table 3.3: CD_4 cell count Stratification of HIV positive subjects

CD_4 cell count (cells/mm^3)	Result
<100	111 (60%)
101-200	26 (14%)
201-350	19 (10%)
>350	16 (8%)
Unknown	13 (8%)
Mean	128.5

IQR	20.75 - 170.75
Median	47

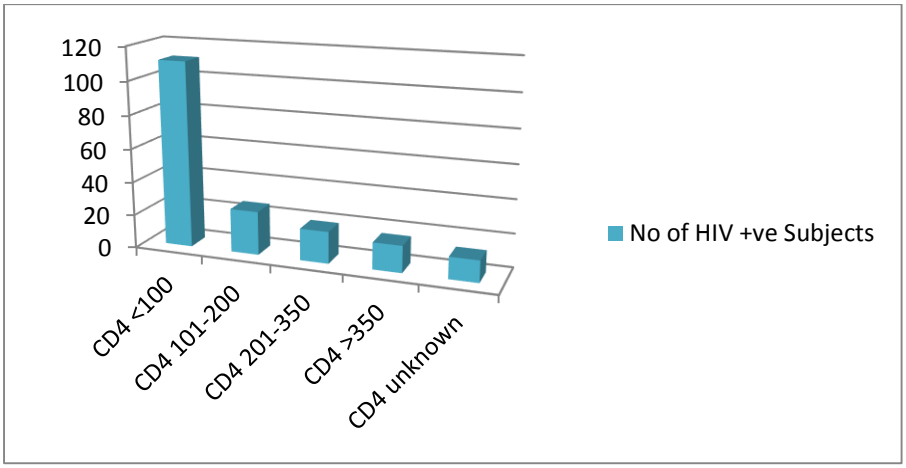


Figure 3.2: CD_4 cell count distribution

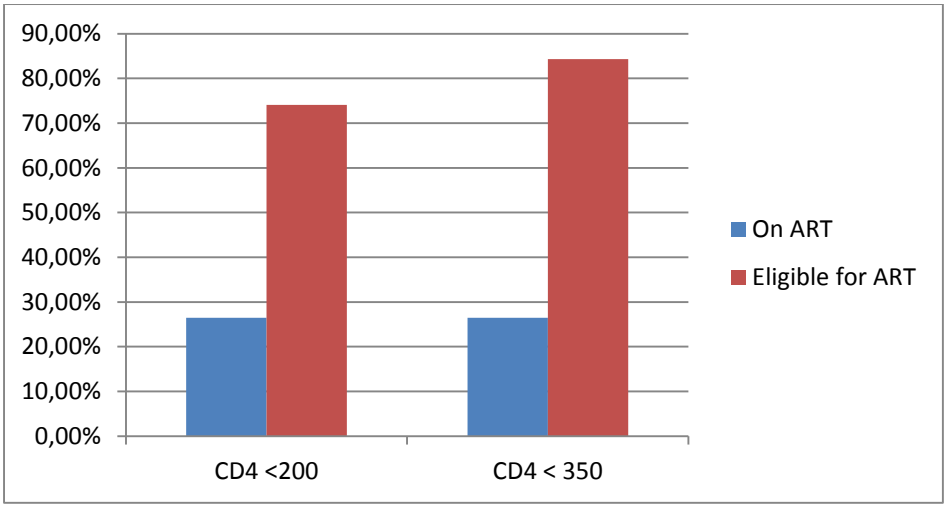


Figure 3.3: Comparison of subjects eligible for ART based on old and new guidelines vs. patients currently on ART

3.1.4 Smoking habits

Of the 200 subjects in the study group, 32 (16%) were smokers, 16 (8%) were ex-smokers and 152 (76%) were non-smokers. Of the 185 HIV positive patients, 24 (12.9%) were current smokers and 15 (8.1%) were ex-smokers.

3.1.5 Tuberculosis history

Table 3.4 illustrates the TB history within our cohort.

Table 3.4: Tuberculosis history of subjects in data set

TB History	Result
Current TB	41 (20.5%)
Previous TB	38 (19%)
No history of TB	121 (60.5%)
Total	200

3.1.6 LFA Results

Of the 200 patients tested for LFA only 1 tested positive giving a prevalence of 0.5% (CI 0.01 – 2.75%); This one patient was HIV positive and had a CD_4 cell count < 100cells/mm^3. When excluding patients with CD_4 cell counts above 100 cells/mm^3 there was a 0.9% prevalence (CI 0.02 -4.92%).

My data did not allow for statistical comparison between CrAg positive and CrAg negative patients within my sample owing to the low rate of CrAg positivity in the sample population.

As my number of positive patients was less than 5, I was unable to perform standard parametric testing for differences of proportions between my sample and those of Harris JR *et al* and the provisional figures reported by the NICD, therefore an exact test was required and the limitations of the statistical test in this situation is recognised[8, 26].

Differences in prevalence between the sample populations were tested using the Fisher's exact test and were found to be significantly different as illustrated in Tables 3.5 and 3.6.

Table 3.5: 2x2 table for the LFA positive and LFA negative patients in my study and the NICD provisional figures

	My study	NICD figures	Total
LFA positive	1	287	288
LFA negative	199	6884	7083
Total	200	7171	7371

The two-tailed Fisher's exact P value equals 0.0049.

Table 3.6: 2x2 table for LFA positive and LFA negative patients in my study and results found by Harris JR *et al*

	My study	Harris JR *et al*	Total
LFA positive	1	92	93
LFA negative	199	704	903
Total	200	796	996

The two-tailed Fisher's exact P value equals 0.0001.

When comparing only patients with a CD_4 cell count < 100 cells/mm^3 from my sample with the NICD provisional data (all patients in subset had a CD_4 cell count < 100 cells/mm^3) no significant difference was found as illustrated in Table 3.7. CD_4 cell count data was not available from the Harris study.

Table 3.7: 2x2 table for LFA positive and LFA negative patients with CD4<100 cells/mm^3 in my study and the NICD provisional figures

	My study	NICD figures	Total
LFA positive & CD_4<100 cells/mm^3	1	287	288
LFA negative & CD_4<100 cells/mm^3	110	6884	6994
Total	111	7171	7282

The two-tailed P value equals 0.1340.

4.0 Discussion

With regards to the demographic profile of my sample there was a female predominance and the majority of patients were younger than 40 years of age. This is in keeping with the profile of the HIV epidemic in Sub-Saharan Africa[38].

In this non-randomised sample of consecutive patients presenting with CAP 92.5% were HIV-infected which was above our estimate of 60%. Reasons for the high proportion of HIV-infected patients may include potential referral bias as ward staff may have preferentially referred HIV-infected patients for inclusion in my CrAg study. CHBAH is a tertiary hospital and due to bed shortages only the sickest patients are admitted which may have increased proportions of patients with advanced HIV disease.

The median CD_4 cell count was 47cells/mm^3. However, patients were acutely ill and the CD_4 cell count prior to acute illness was likely higher. In patients with acute bacteraemic streptococcal CAP the difference between median CD_4 cell count at presentation and 1 month post resolution was 158 cells/mm^3[39]. Even when this acute decrease in CD_4 cell count is taken into account the majority of my cohort would still be severely immunocompromised and would still have been eligible for ART prior to their admission to hospital.

The very low number of eligible patients on ART 9 years post ART roll-out in the public sector is of concern. Despite best efforts to increase access to ART in South Africa 8-26% of patients die within the first year of ART initiation largely due to very low CD_4 cell counts and advanced symptomatic disease[40]. The addition of routine cryptococcal screening to the national program may cause additional strain on a system that appears to be failing. Perhaps more emphasis should be placed on early identification of HIV infection within the population and timeous initiation of ART.

A fifth of patients in my study were on TB treatment at the time of data collection. A similar number of patients had a history of prior TB. This reflects the high burden of tuberculosis in our community.

I report a prevalence of cryptococcal antigenaemia of 0.5% amongst patients presenting to the CHBAH medical wards with a diagnosis of CAP. This was significantly lower than previous studies looking at cryptococcal antigenaemia in patients presenting with respiratory symptoms[26, 27]. This result was surprising given the high percentage of HIV infected patients with CD_4<100cells/mm^3 in my study. This difference in the prevalence of cryptococcal antigenaemia may be due to:

- Possible genetic susceptibility to cryptococcal infection (although different genetic susceptibility to infection has not been described, a recent study has shown polymorphisms of

phagocytic Fc gamma receptors as potential markers of increased risk for cryptococcal disease)[41].

- Geographic variation may play a role in the prevalence of *cryptococcus* in the environment. Despite the Red Eucalyptus being endemic on the Highveld, the cold, dry winters and high altitude may result in a lower number of environmental sources [11, 16-18].
- A high burden of other respiratory opportunistic infections (e.g. Tuberculosis) may result in underrepresentation of the less pathogenic *Cryptococcus* in this population.
- Cryptococcal respiratory disease usually presents with no or minimal symptoms not requiring tertiary inpatient management and a higher prevalence may be found in patients with less severe illness. However, the study by Visnegarwala *et al* showed that a high percentage of respiratory failure in their cohort was due to cryptococcal disease suggesting that severe respiratory illness does not exclude a diagnosis of cryptococcal disease[25].
- CD_4 count at the time of acute respiratory illness is not necessarily representative of the patient's true immune status as demonstrated by Schleicher GK *et al*[39].

Owing to the low prevalence of cryptococcal antigenaemia in my study I was unable to do comparative analysis between CrAg positive and negative patients or CD_4 count stratification.

The prevalence of cryptococcal antigenaemia detected in my study in patients with CAP independent of CD_4 count was low and the 95% CI (0.01 – 2.75%) did not cross the proposed cut-off of 3% for cost-effectiveness of cryptococcal screening. When looking at only those patients with CD_4<100cells/mm^3 the 95% CI (0.02 -4.92%) did cross the proposed threshold of 3%. My study does not support the findings of previous researchers that used respiratory symptoms as a screening tool for cryptococcal antigenaemia in other resource-limited settings[26]. However, even with the low prevalence of cryptococcal antigenaemia found in this population CrAg screening in patients with CD_4<100cells/mm^3 would still be cost-effective. The cost-benefit analysis was a conservative calculation and savings from reduced inpatient management and avoidance of further expensive antifungal treatment would add to the cost-effectiveness. Furthermore, decreased cost per unit due to increased scale would further enhance cost-effectiveness. Therefore, routine CrAg screening should be performed in this subgroup.

5.0 Conclusion

Cryptococcal disease accounts for the majority of deaths among HIV-infected patients in the developing world[14].

In patients admitted to CHBAH with CAP during June-July 2013 a prevalence of cryptococcal antigenaemia of 0.5% was found. This prevalence was lower than expected given prior results from similar studies.

Screening all patients admitted with CAP at CHBAH independent of HIV status and CD_4 count would not be cost-effective. However, when limited to patients with CD_4 cell counts < 100cells/mm^3 CrAg screening would be cost-effective, especially considering availability of the recently developed highly sensitive and specific LFA.

In conclusion, I therefore advocate the use of CrAg screening in AIDS patients presenting with respiratory symptoms and a CD_4 cell counts < 100cells/mm^3. Given the high prevalence of untreated advanced HIV disease in this subset improved early diagnosis of HIV infection and timeous ART initiation should be a priority.

5.1 Recommendations

This is the only study to date looking at cryptococcal antigen testing in patients admitted with CAP in South Africa. More studies are needed to confirm or refute my findings. Although cryptococcal infection is not common amongst the general population, it is one of the most devastating opportunistic infections in immunocompromised individuals. Challenges remain and more work is required to determine the true burden of cryptococcal respiratory disease, especially amongst immunocompromised patients in our community.

I recommend a larger sample size in future studies given the low prevalence of cryptococcal antigenaemia found in this population. Further studies including patients with acute respiratory infections not requiring tertiary care is recommended for earlier recognition of cryptococcal antigenaemia.

The large number of HIV positive patients eligible for ART but not yet on ART suggests that more focus be placed on early diagnosis and treatment initiation.

Complete Data Capture Sheet

Appendix A

	Age	Sex	HIV	CD_4	ART	Prev. PTB	Smoking	Resp R	Sats %	BP	Mening.	CXR	CrAg
1	50	M	-ve	n/a	n/a	x1 current	5 py - quit	19	96	84/58	N	2/3/4/6/B/D	-ve
2	47	M	+ve	162	N	x 3	7 py - quit	34	56	112/68	N	2/3/4/6/B	-ve
3	36	F	+ve	181	Y- 9m	x1 current	N	34	98	116/85	N	1/3/4/A	-ve
4	25	F	-ve	n/a	n/a	N	1 py-current	18	91	129/80	N	2/A/C	-ve
5	70	F	-ve	n/a	n/a	N	N	24	96	171/80	N	2/A/C	-ve
6	31	F	+ve	7	N	x1 current	N	50	89	115/60	N	4/B/D	-ve
7	48	F	-ve	n/a	n/a	N	27py- current	30	77	162/98	N	2/B/D	-ve
8	27	M	+ve	459	N	N	2 py - quit	24	81	118/81	N	1/3/B/D	-ve
9	67	M	-ve	n/a	n/a	N	15py-current	16	98	111/77	N	4/B/D	-ve
10	45	M	-ve	n/a	n/a	N	5py-current	22	96	110/62	N	1/4/B/D	-ve
11	30	F	+ve	?	N	x1	N	45	84	103/60	N	0/B	-ve
12	36	F	+ve	173	N	N	N	21	85	139/98	N	4/B/D	-ve
13	60	F	+ve	75	N	N	3py-current	34	90	135/96	N	2/3/A/C	-ve
14	36	M	+ve	7	N	x1 current	N	42	94	118/57	N	2/4/A/D	-ve
15	35	M	+ve	122	y-6m	x1 current	1py-current	22	92	90/59	N	4/5/B/C	-ve
16	50	M	+ve	?	Default	x1 current	5py-current	22	99	120/72	N	1/2/3/4/B/D	-ve
17	30	F	+ve	19	Default	x1	3py-current	30	89	116/60	N	4/6/B/D	-ve
18	25	F	+ve	1245	Y-1y	N	N	31	95	123/84	N	2/B/D	-ve
19	23	F	+ve	9	N	N	N	40	86	119/76	N	2/A/C	-ve
20	21	M	-ve	n/a	n/a	N	1py-current	26	91	120/76	N	2/A/C	-ve
21	60	F	-ve	n/a	n/a	N	N	24	85	167/101	N	2/A/C	-ve
22	21	F	+ve	?	N	N	N	32	99	121/69	N	2/A/C	-ve
23	29	F	+ve	49	N	N	N	36	92	104/54	N	2/A/C	-ve
24	34	M	+ve	?	N	N	12py-quit	32	96	110/54	N	2/B/C	-ve
25	48	M	+ve	32	N	x1	4py-current	25	89	111/65	N	2/6/B/C	-ve
26	63	M	?	n/a	N	N	10py-current	52	98	98/66	N	2/A/C	-ve
27	49	F	+ve	260	Y-9y	N	N	32	98	104/74	N	2/A/C	-ve
28	29	F	+ve	56	N	x1	N	18	99	112/75	N	4/6/B/D	-ve
29	53	F	+ve	1439	N	N	7py-quit	21	95	110/85	N	2/A/C	-ve
30	42	F	+ve	166	N	N	N	22	94	100/54	N	4/B/D	-ve
31	61	F	-ve	n/a	n/a	N	N	41	88	154/42	N	2/A/C	-ve
32	70	F	?	n/a	N	N	N	38	92	145/97	N	4/6/B/D	-ve
33	47	F	+ve	29	N	x1 current	N	24	92	110/62	N	4/B/D	-ve
34	44	M	+ve	17	N	x3 current	5py-current	47	93	118/87	N	2/4/B/D	-ve
35	36	M	+ve	11	N	x1 current	6py-current	33	96	115/73	N	4/B/D	-ve
36	44	f	+ve	3	y-4m	N	N	24	99	101/93	N	2/A/C	-ve
37	33	F	+ve	7	N	N	N	26	95	103/65	N	2/4/B/D	-ve
38	41	F	+ve	400	N	N	N	36	93	104/82	N	2/3/A/C	-ve
39	48	F	+ve	296	Y-3m	N	N	31	95	70/38	N	2/A/C	-ve
40	26	F	+ve	5	N	x1 current	N	42	94	106/77	N	1/B	-ve
41	34	F	-ve	n/a	n/a	x1 current	N	28	85	65/53	N	1/4/B/D	-ve
42	36	F	+ve	25	N	N	N	18	92	104/75	N	4/B/D	-ve
43	50	F	+ve	178	Y-3y	x1 current	N	21	87	143/88	Y	3/4/B/D	-ve
44	36	F	+ve	23	N	N	N	36	96	92/66	N	4/B/D	-ve
45	32	F	+ve	4	Y-5y	x1	N	24	97	78/58	N	2/A/C	-ve
46	54	F	-ve	n/a	n/a	x1 current	N	34	97	114/70	N	4/B/C	-ve
47	42	F	+ve	185	Y-5y	x1	N	16	98	113/70	N	6/B/C	-ve
48	53	F	+ve	223	N	N	N	30	89	154/104	N	1/2/4/B/D	-ve
49	47	F	+ve	15	N	N	N	18	87	87/47	N	4/B/D	-ve

50	57	F	+ve	91	N	N	N	24	96	124/80	N	2/A/C	-ve
51	42	M	+ve	118	N	x2	N	16	75	119/74	N	4/6/A/D	-ve
52	45	M	+ve	36	N	N	10py-quit	30	88	82/50	N	4/5/B/D	-ve
53	39	M	+ve	15	N	x1 current	N	43	83	95/66	N	4/5/B/D	-ve
54	44	M	+ve	13	N	x1	N	32	82	104/74	N	4/B/D	-ve
55	32	M	+ve	89	N	N	4py-quit	32	88	121/78	N	3/4/B/D	-ve
56	52	M	+ve	433	Y-4m	x1	N	24	89	98/59	N	5/B/D	-ve
57	39	M	+ve	337	Y-5m	N	3py-current	22	91	132/87	N	4/B/D	-ve
58	35	M	+ve	170	Y-2w	N	N	22	90	135/83	N	1/2/4/B/D	-ve
59	58	M	+ve	42	N	x1	N	26	87	134/85	N	2/A/C	-ve
60	34	M	+ve	?	Default	x1 current	N	31	81	89/52	N	2/5/B/D	-ve
61	60	M	+ve	38	N	N	15py-current	26	98	119/90	N	2/A/C	-ve
62	33	M	+ve	444	Y-6y	N	N	54	80	151/98	N	2/5/A/C	-ve
63	55	M	+ve	27	N	N	N	32	94	121/64	N	4/B/D	-ve
64	46	M	+ve	?	Y-2w	x1 current	N	24	100	66/45	N	1/4/B/D	-ve
65	34	M	+ve	21	N	x1 current	7py-quit	18	96	105/70	N	4/B/D	-ve
66	42	M	+ve	132	Y-1y	N	5py-current	22	58	132/66	N	2/A/C	-ve
67	45	M	+ve	2	N	x1 current	N	18	93	137/90	N	1/4/B/D	-ve
68	60	F	+ve	?	Y-2w	N	N	24	84	138/90	N	4/B/D	-ve
69	63	F	+ve	27	N	N	N	42	77	93/69	N	3/4/A/C	-ve
70	29	F	+ve	228	N	N	N	22	92	107/79	N	3/A/C	-ve
71	45	F	+ve	72	Y-5y	x1	N	22	100	151/111	N	2/A/C	-ve
72	37	F	+ve	300	N	N	N	25	94	135/83	N	3/4/A/C	-ve
73	58	M	-ve	n/a	n/a	N	N	26	72	101/68	N	2/4/B/D	-ve
74	42	M	+ve	38	N	N	N	32	88	97/65	N	1/5/B/D	-ve
75	57	M	+ve	34	N	N	N	22	98	97/62	N	4/B/D	-ve
76	40	M	+ve	?	N	x3 current	5pyquit	40	88	99/66	N	2/6/B/D	-ve
77	24	M	+ve	36	N	N	N	26	75	104/62	N	4/B/D	-ve
78	34	M	+ve	40	N	N	N	37	93	93/79	N	1/2/A/C	-ve
79	55	M	+ve	32	Y-8m	x1	N	32	92	159/79	N	2/A/C	-ve
80	37	M	+ve	94	Y-13m	N	7.5py-quit	35	89	106/86	N	4/B/D	-ve
81	39	M	+ve	3	N	x1	N	22	77	104/66	N	4/B/C	-ve
82	33	M	+ve	10	N	N	N	18	90	109/68	N	1/4/B/D	-ve
83	41	M	+ve	508	N	N	10py-current	22	99	96/62	N	2/A/C	-ve
84	21	M	+ve	20	Y-4y	x1	N	18	97	120/79	N	1/B/D	-ve
85	26	M	+ve	140	Y-2w	x1 current	N	16	90	77/49	N	2/A/C	-ve
86	38	M	+ve	2	Y-1m	N	N	23	98	121/70	Y	4/B/D	+ve
87	37	M	+ve	65	N	x1 current	N	32	94	89/46	N	1/4/B/C	-ve
88	35	F	+ve	1	Default	x1 current	N	18	97	99/64	N	2/A/C	-ve
89	21	F	+ve	47	N	x1	N	33	79	97/67	N	1/4/B/D	-ve
90	31	F	+ve	161	Y-6m	x1	N	25	94	134/90	N	2/6/A/C	-ve
91	24	F	+ve	208	N	N	N	18	94	102/68	N	4/B/D	-ve
92	25	F	+ve	61	Y-1m	x1 current	N	16	96	117/84	N	1/4/B/D	-ve
93	32	M	+ve	89	N	N	10py-quit	22	88	109/84	N	3/4/A/C	-ve
94	54	M	+ve	38	N	x1 current	N	23	96	74/43	Y	1/B/C	-ve
95	53	M	+ve	102	N	x2	24py-current	26	90	111/91	N	4/6/B/D	-ve
96	32	F	+ve	28	N	x1	N	26	88	97/58	N	2/4/B/D	-ve
97	40	M	+ve	54	Y-1m	N	2py-quit	18	94	142/85	N	2/A/C	-ve
98	44	F	+ve	40	Default	x1	N	22	98	144/64	N	2/3/A/C	-ve
99	43	F	+ve	328	Y-3y	x1	N	30	83	149/87	N	2/B/C	-ve

100	34	F	+ve	184	Y-4m	N	N	33	87	74/54	N	2/4/B/D	-ve
101	29	F	+ve	?	Default	x1	N	28	96	115/80	N	2/3/A/C	-ve
102	36	M	+ve	53	N	x1 current	N	32	88	118/65	N	3/A/C	-ve
103	39	M	+ve	34	N	N	N	24	99	94/66	N	2/A/C	-ve
104	33	M	+ve	254	6Y	x1	N	18	100	151/99	N	2/4/B/D	-ve
105	31	M	+ve	6	N	x1 current	N	22	96	141/94	N	1/2/B/C	-ve
106	27	M	+ve	79	N	N	5py-current	41	91	106/76	N	4/B/C	-ve
107	30	M	+ve	32	N	N	N	17	99	123/78	N	2/A/C	-ve
108	43	M	+ve	230	2M	N	N	24	98	161/92	N	2/6/B/C	-ve
109	51	M	+ve	452	N	N	10py-current	26	85	128/61	N	3/A/C	-ve
110	40	M	+ve	14	N	x1	N	24	56	113/76	N	4/B/D	-ve
111	39	M	+ve	12	N	N	N	22	91	100/68	N	4/A/B	-ve
112	52	M	+ve	285	N	x2	15py-current	22	86	127/86	N	2/6/B/D	-ve
113	32	M	+ve	32	N	N	N	24	94	88/68	N	4/B/D	-ve
114	32	M	+ve	193	N	N	N	30	100	104/47	Y	2/A/C	-ve
115	60	M	+ve	38	N	N	N	21	97	119/90	N	6/A/C	-ve
116	32	M	+ve	28	Default	N	N	32	98	97/59	N	2/3/A/C	-ve
117	55	M	+ve	55	N	N	N	24	94	121/64	N	4/B/D	-ve
118	50	M	+ve	30	Default	N	12py-quit	24	97	95/64	N	2/A/C	-ve
119	42	M	+ve	55	N	N	N	42	85	135/81	N	2/4/A/D	-ve
120	30	M	+ve	3	N	N	N	32	84	79/54	N	5/B/D	-ve
121	47	M	+ve	15	Default	N	N	28	92	58/38	N	5/B/D	-ve
122	35	M	+ve	46	N	N	N	26	93	110/73	N	4/B/D	-ve
123	55	M	+ve	25	N	N	N	36	92	99/56	N	5/A/D	-ve
124	28	M	+ve	552	N	x1 current	2py-current	34	88	105/54	N	2/3/A/D	-ve
125	49	M	+ve	176	3Y	N	N	31	90	113/75	N	4/B/D	-ve
126	39	M	+ve	24	Default	x1	N	28	78	108/74	N	2/3/4/A/C	-ve
127	46	F	+ve	87	13Y	N	N	21	92	102/65	N	5/B/D	-ve
128	36	F	+ve	68	N	N	N	35	95	147/97	N	4/B/D	-ve
129	39	F	+ve	284	N	N	N	32	90	129/90	N	1/2/A/C	-ve
130	53	F	+ve	224	N	x1 current	N	30	89	154/104	N	2/4/B/D	-ve
131	40	F	+ve	425	16M	N	N	40	94	107/91	N	2/A/C	-ve
132	51	F	+ve	46	14M	x1	N	25	97	118/45	N	2/A/C	-ve
133	28	F	+ve	8	Default	x1	N	32	92	98/61	N	1/4/B/D	-ve
134	29	F	+ve	41	9Y	N	N	28	93	106/70	N	4/B/D	-ve
135	40	F	+ve	332	N	N	N	42	87	94/82	N	2/A/C	-ve
136	30	M	+ve	11	N	N	3py-current	30	95	130/78	N	1/4/B/D	-ve
137	30	M	+ve	3	N	x1 current	N	32	69	79/54	N	1/5/B/D	-ve
138	24	M	+ve	47	N	N	N	22	98	130/68	N	2/A/C	-ve
139	35	M	+ve	46	N	N	N	26	93	110/73	N	5/B/C	-ve
140	42	M	+ve	4	N	N	12py-quit	33	84	109/78	N	4/B/D	-ve
141	59	M	-VE	n/a	N	x1 current	20py-current	42	66	94/66	N	4/B/D	-ve
142	39	M	+ve	545	N	N	N	22	92	100/62	N	2/A/C	-ve
143	54	M	+ve	238	N	x1 current	15py-quit	18	92	105/69	N	2/A/C	-ve
144	38	M	+ve	237	6Y	x2	10py-current	23	96	182/111	N	4/B/D	-ve
145	56	M	+ve	21	10M	x1	N	40	91	89/52	N	2/A/C	-ve
146	27	M	+ve	0	N	N	N	44	66	149/68	N	5/B/D	-ve
147	35	F	+ve	362	3Y	x2	N	42	91	93/62	N	1/4/B/D	-ve
148	31	F	+ve	73	N	x1	N	40	90	115/75	N	1/2/A/C	-ve
149	39	F	+ve	617	11Y	N	N	28	77	105/84	N	4/B/D	-ve

150	39	F	+ve	50	N	N	N	28	86	132/77	N	5/B/D	-ve
151	38	F	+ve	166	N	N	N	48	88	120/77	N	4/B/D	-ve
152	37	F	+ve	25	N	N	N	26	95	111/67	N	2/A/C	-ve
153	33	F	+ve	19	N	N	N	53	70	131/84	N	5/B/D	-ve
154	29	F	+ve	6	N	x1 current	N	32	88	100/54	N	4/B/D	-ve
155	50	M	+ve	211	2Y	x1 current	N	18	91	146/87	N	1/4/B/C	-ve
156	35	M	+ve	36	N	N	2py-current	22	95	120/75	N	4/5/B/D	-ve
157	37	M	+ve	?	N	N	N	20	70	108/68	N	2/A/C	-ve
158	30	M	+ve	12	N	N	N	48	88	111/69	N	5/B/D	-ve
159	41	M	+ve	716	N	N	14py-current	16	95	134/70	N	4/B/D	-ve
160	26	M	+ve	11	N	x1 current	N	50	70	145/87	N	4/B/D	-ve
161	54	M	+ve	24	4Y	x1	N	38	73	135/101	N	1/5/B/C	-ve
162	28	M	+ve	23	N	N	N	32	91	99/44	N	4/B/D	-ve
163	40	F	+ve	108	N	x1 current	N	42	86	79/40	N	2/A/C	-ve
164	34	F	+ve	200	11Y	x1	N	26	91	88/52	N	4/A/D	-ve
165	36	F	+ve	68	N	N	N	52	92	120/79	N	4/B/D	-ve
166	40	F	+ve	332	N	N	N	26	91	145/78	N	2/A/C	-ve
167	31	F	+ve	34	N	x1	N	32	83	110/63	N	4/B/D	-ve
168	28	F	+ve	8	N	x1 current	N	22	91	98/61	N	4/B/D	-ve
169	23	F	+ve	13	N	N	N	24	88	126/92	N	5/B/D	-ve
170	51	F	+ve	46	N	x1	N	34	89	118/45	N	3/4/B/D	-ve
171	46	M	+ve	25	N	x1 current	N	22	96	88/68	N	4/B/C	-ve
172	32	M	+ve	86	N	N	2py-current	28	95	102/65	N	1/4/A/C	-ve
173	35	M	+ve	?	N	N	N	26	97	95/61	N	4/6/A/D	-ve
174	35	M	+ve	8	N	N	N	16	98	116/64	N	4/B/D	-ve
175	45	M	+ve	542	N	N	N	28	96	101/54	N	4/B/D	-ve
176	46	M	+ve	53	3Y	N	N	18	99	81/56	N	5/B/D	-ve
177	42	M	+ve	8	N	x1	N	35	82	96/52	N	2/6/A/D	-ve
178	32	M	+ve	24	Default	N	N	28	95	97/59	N	2/3/A/C	-ve
179	37	M	+ve	128	N	N	N	22	96	106/56	N	1/2/A/C	-ve
180	34	F	+ve	1	Default	N	N	32	97	111/77	N	5/B/D	-ve
181	25	F	+ve	60	1M	x1 current	N	24	96	117/84	N	5/B/C	-ve
182	47	F	+ve	?	N	x1 current	N	32	78	148/82	N	1/4/A/C	-ve
183	39	F	+ve	106	2w	N	N	28	96	85/65	Y	5/B/D	-ve
184	46	F	+ve	?	N	N	N	32	94	107/53	N	1/2/A/C	-ve
185	50	F	+ve	150	N	N	N	32	85	130/80	N	2/A/C	-ve
186	47	F	+ve	138	1M	x1	N	34	87	89/58	N	4/B/C	-ve
187	29	F	+ve	8	N	N	N	44	73	89/55	N	5/B/D	-ve
188	37	F	+ve	167	N	N	N	30	98	105/65	N	2/A/C	-ve
189	30	M	+ve	125	4Y	N	N	32	90	113/70	N	1/2/A/C	-ve
190	28	M	+ve	13	N	N	6py-current	22	97	115/63	N	2/A/C	-ve
191	54	M	+ve	24	4Y	N	3py-quit	24	89	135/101	N	2/6/A/D	-ve
192	25	M	+ve	11	N	x1 current	N	50	62	145/87	N	4/B/D	-ve
193	51	M	+ve	452	N	N	10py-current	22	93	117/86	N	2/3/A/D	-ve
194	37	M	+ve	15	N	x1	N	33	90	110/84	N	5/B/D	-ve
195	59	M	+ve	29	N	x1 current	25py-current	26	91	90/60	N	5/B/D	-ve
196	38	M	+ve	35	N	N	N	18	93	150/98	Y	5/B/D	-ve
197	47	M	+ve	54	6M	x1 current	N	22	91	115/97	N	3/A/C	-ve
198	30	M	+ve	11	N	N	N	30	96	130/78	N	2/A/C	-ve
199	39	M	+ve	161	8M	N	N	34	95	134/53	N	2/A/C	-ve

200	54	M	+ve	238	N	N	15py-current	36	90	105/69	N	2/A/C	-ve

Appendix B

health and social development
Department: Health and Social Development
GAUTENG PROVINCE

PERMISSION FOR RESEARCH

DATE: 25 January 2012

NAME OF RESEARCH WORKER: Anneli Korb

TITLE OF RESEARCH PROJECT: Prevalence of Cryptococcal Antigenaemia in patients hospitalised with Community Acquired Pneumonia at Chris Hani Baragwanath Academic Hospital

OBJECTIVES OF STUDY (Briefly or include a protocol):

See attached protocol

METHODOLOGY (Briefly or include a protocol): See attached protocol

CONFIDENTIALITY OF PATIENTS MAINTAINED: See attached informed consent

COSTS TO THE HOSPITAL: Nil

APPROVAL OF HEAD OF DEPARTMENT: Prof. Hoosdie 25/1/12

APPROVAL OF CRHS OF WITS UNIVERSITY:

SUPERINTENDENT PERMISSION:

Signature: K. A. Mahomed Date: 25.01.12

Subject to any restrictions:

Appendix C

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011)717-2075/6

Reference: Ms Salamina Segole
E-mail: Salamina.segole@wits.ac.za
11 April 2012
Person No: 521220
PAG

Dr A Korb
PO Box 44512
Linden
2104
South Africa

Dear Dr Korb

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled "*Cryptococcal antigenaemia in patients hospitalised with community acquired pneumonia at Chris Hani Baragwanath Academic Hospital*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Ethics Clearance certificate **Appendix D**

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Anneli Korb

CLEARANCE CERTIFICATE	**M120249**
PROJECT	Prevalence of Cryptococcal Antigenaemia in Patients hospitalised with Community Acquired Pneumonia at Chris Hani Baragwanath Academic Hospital
INVESTIGATORS	Anneli Korb.
DEPARTMENT	Department of Internal Medicine
DATE CONSIDERED	24/01/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 22/11/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Andre Black

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

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