Infections and malignancies associated with cryptococcal antigenemia among patients with CD_4 counts ≤100 cells/mm^3

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MMed Research Proposal

MC000-MASTER OF MEDICINE

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A research report submitted to the University of the Witwatersrand, Johannesburg in fulfilment for the requirements of the degree of Master of Medicine.

DECLARATION

I, **Mmabatho Ngoananoka Portia Ratau-Dintwe,** hereby declare that this dissertation presented to the University of Witwatersrand for the degree Master of Medicine in Internal Medicine, is my own work and has not been presented previously by me for a degree at any other tertiary institution.

_____ _____/_____/_____

Dr Mmabatho Ngoananoka Portia Ratau-Dintwe Date

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ABSTRACT

Background: Cryptococcal infection causes high morbidity and mortality in advanced retroviral disease**.** The South African government in 2016 instituted a nationwide screening program for cryptococcal antigenemia (CRAG) among human immunodeficiency (HIV) infected persons with CD_4 counts below 100 cells/mm^3. This program offers health workers an opportunity to detect cryptococcal disease early and pre-emptively treat patients to prevent progression from antigenemia to disseminated cryptococcal disease. In HIV disease CRAG is a forecaster of cryptococcal meningitis (CM) and a predictor of mortality.

Objective: To document the infectious and malignant conditions associated with CRAG antigenemia in patients routinely screened as part of the national screening program.

Method: A retrospective descriptive study including patients with CRAG accessing the teaching hospitals of the University of the Witwatersrand i.e. Chris Hani Baragwanath Academic Hospital; Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Academic Hospital. The study period was 54 months (01 October 2012 to 31 March 2017). The National Health Laboratory Service database was reviewed for eligible patients' records to determine concomitant infections and malignancies.

Results: The cohort had 272 patients, 58.8% were males. Cerebrospinal fluid analysis results were available for 224 (82.4%) patients of whom 199 had confirmed CM. CM was strongly associated with a younger age was (p=0.016). Cryptococcal fungemia was found in 125 of the 194 patients tested (64.4%). Lower CD_4 counts (≤50 cells/mm^3) were significantly associated with cryptococcal fungemia (p=0.001). There were 52 cases of confirmed tuberculosis (TB) with 30 pulmonary and 21 extra-pulmonary TB.

Malignancies and pre-malignant conditions were found in 20 (7%) patients. Kaposi sarcoma and high-grade cervical lesions were the most prevalent (11 and 6 cases respectively).

Conclusion: Our study confirms a high burden of cryptococcal meningitis and fungemia in patients with CRAG. Age <49 years is a strong predictor of CM and low CD_4 cell

count is a strong predictor of cryptococcal fungemia. Tuberculosis is a relatively common co-infection in patients with CM and should be actively excluded.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BD glucan	1,3-β-D-glucan
CHBAH	Chris Hani Baragwanath Academic Hospital
C-IRIS	Cryptococcal immune reconstitution inflammatory syndrome
CM	Cryptococcal meningitis
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CMV	Cytomegalovirus
CNS	Central nervous system
CrAg	Cryptococcal antigen
CRAG	Cryptococcal antigenemia
CSF	Cerebrospinal fluid
DALY	Disability-adjusted life year
DLBCL	Diffuse large B-cell lymphoma
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GUD	Genital ulcer disease
HGSIL	High-grade squamous intraepithelial lesion
HIV	Human immunodeficiency virus
HJAH	Helen Joseph Academic Hospital
KS	Kaposi Sarcoma
LFA	Lateral flow assay
NHLS	National Health Laboratory Service

NNT	Number needed to treat
OI(s)	Opportunistic infections
PJP	Pneumocystis jirovecii pneumonia
PTB	Pulmonary tuberculosis
SA	South Africa
TB	Tuberculosis
TBM	Tuberculous Meningitis
UNAIDS	United Nations Programme on HIV/AIDS
USD	United States dollar
WHO	World Health Organization

CHAPTER 1: PROTOCOL AND EXTENDED LITERATURE REVIEW

1.1 Introduction

Globally, there were 36.9 million people living with human immunodeficiency virus (HIV) in 2017 across all ages (35.1 million adults) (1). There were an estimated 21.7 million people receiving antiretroviral therapy (ART) worldwide, at the end of 2017 (1). Compared to a decade ago, ART is now reaching 5,5 times more patients worldwide (1). As access to ART improves, the incidence of opportunistic infections (OIs) associated with low CD_4 cell counts has decreased. This has been noted as a cumulative decrease in the total number of deaths globally from acquired immunodeficiency syndrome (AIDS)-related illnesses among people living with HIV (2). This benefit has extended across all age groups. Total attributable mortality has declined remarkably (50.5%) between 2004 and 2017 (2).

South Africa (SA) has one of the largest HIV epidemics in the world, with an estimated 7,2 million people living with HIV and AIDS in 2017. The HIV prevalence in SA among adults aged 15 to 49 years is estimated at 18.8 % (3, 4). SA has the largest ART program in the world, accounting for 20% of people receiving ART globally (4). This has resulted in a 42% decrease in the number of AIDS-related deaths between 2005 and 2017 (1, 4). Despite the scale of the ART program only 61% of HIV positive people aged 15 years and above were on ART in 2017 (4).

In adults advanced HIV disease is present when CD_4 cell counts are <200 cells/mm^3 or World Health Organization (WHO) stage 3 or 4 is present (5). Studies suggest that between 30–40% of people living with HIV starting ART in low- and middle-income settings have advanced HIV disease and 20% of these patients have a CD_4 cell count of less than 100 cells/mm^3 (6-8). The South African National department of health, 2019 ART clinical guidelines and WHO ART clinical guidelines have recommended that all patients with HIV infection should receive rapid ART initiation (within seven days or on the same day of HIV diagnosis if there are no contraindications) (5, 9). Patients with advanced HIV should be given priority for assessment and initiation of ART (5, 9). Notwithstanding the magnitude of the ART program in SA, a high proportion of patients still present with advanced HIV disease (6). Men who entered healthcare centers in SA were twice as likely as women to have CD_4 cell counts <200 cells/mm^3 (23.1% vs 12.6%) (6).

OIs are infections that occur when the patients' immune system is suppressed as in HIV infected patients, patients on immunosuppressive drugs, diabetic patients etc.

OIs drive morbidity and mortality in HIV infected people and this effect is more pronounced in patients with advanced HIV disease. The different climates and socio-economic conditions in various countries lead to the differences in the prevalence of OIs in those countries (10). Different CD_4 cell count levels predict the type of OIs an HIV infected patient may develop (11). See table 1.1 below. CD_4 cell counts are also a predictive factor for all malignancies except for anal cancer (12). Pang has shown that having a CD4 cell count <100 cells/mm^3 and not receiving ART are two risk factors for

in-hospital mortality in HIV infected patients (10). OIs are still seen in the ART era, even among populations with satisfactory access to medical care (13). The beneficial effect of ART in the first year was the greatest for oral candidiasis, cerebral toxoplasmosis, and Pneumocystis jirovecii pneumonia (PCP) with risk reduction in the range of 57% to 91% (14). The reduction for cryptococcal meningitis (CM), herpes zoster, Kaposi sarcoma (KS), esophageal candidiasis and genital ulcer disease (GUD) was not statistically significant (14). The leading causes of mortality among adults with advanced HIV disease include tuberculosis (TB), severe bacterial infections, CM, toxoplasmosis and PCP (5, 15). CM remains a major OI and is associated with high mortality and morbidity (16, 17).

The risk of OIs are strongly predicted by baseline CD_4 cell counts before initiation of ART (18). The Swiss HIV cohort study on AIDs related opportunistic illnesses occurring after initiation of ART has shown that the hazard ratio for developing OIs was 2.5 if the CD_4 cell count was between 51-200 cells/mm^3 and increased to 5.8 with CD_4 cell counts below 51 cells/mm^3 at baseline (19). The risk of developing OIs was high in the first months after initiating ART, reduced substantially beyond 6 months if the CD_4 cell count increased by at least 50 cells/mm^3 and viral load was undetectable (19). The decline in these OIs varies. Cytomegalovirus (CMV) disease, esophageal candidiasis and nontuberculous mycobacterium disease such as Mycobacterium avium were shown to have a slower decline in incidences after ART (19).

Table 1.1: Opportunistic infections and malignancies in HIV infected patients.

Author[a]	Year published[b]	Place of study[c]	Mean/median CD_4 cell count (cells/mm^3) [d]	Opportunistic infections[e]	Malignancies[f]
Miro et.al (11)	1994	Spain	0-100	Systemic cryptococcosis, CMV retinitis, PML	Primary cerebral lymphoma, KS
			0-200	PCP Encephalic Toxoplasmosis Visceral leishmaniasis	Non-Hodgkin's lymphoma, KS
			>200	TB, Esophageal candidiasis. Enteritis by Isospora belli	KS
Vajpayee et.al (20)	2003	India	204	TB, Chronic diarrhea, Oral candidiasis,	KS
Losina et.al (21)	2006	Côte d'Ivoire	261	Bacterial infections, TB	
Holmes et.al (22)	2006	South Africa	≤50	Oral candidiasis, Chronic diarrhea, Esophageal candidiasis,	
			51-200	Oral candidiasis, Pulmonary TB, Extrapulmonary TB,	
			201-350	Oral candidiasis, Pulmonary TB, Extrapulmonary TB,	
			>350	Oral candidiasis, Pulmonary TB, Extrapulmonary TB,	
Manosuthi et.al (23)	2007	Thailand	26	TB, CMV retinitis, MAC infection,	
Kong et.al (24)	2007	Cambodia	15	TB, Cryptosporidiosis, Severe bacterial infections,	
Xiao et.al (25)	2013	China		Candidiasis, Pulmonary TB, PCP	Lymphoma, KS, Cervix carcinoma
Katano et.al (8)	2014	Japan	77.0 (ART exposed) 39.6 (ART naïve)	CMV, PCP	NHL, KS
Luo et. al (26)	2016	China	34	PCP, Bacterial infection, TB	KS, DLBCL, Unclassified lymphoma
Tanuma et.al (7)	2016	Vietnam	110	TB, Toxoplasmosis of brain, PCP	Cervical cancer, Primary CNS lymphoma, NHL
Pang et.al (10)	2018	China	137 ART exposed	Bacterial pneumonia, Candida infection, TB	NHL, KS
			94 ART naive	Bacterial pneumonia, Candida infection, TB,	

CMV, Cytomegalovirus infection; DLBCL, Diffuse large B-cell lymphoma, DLBCL, Kaposi sarcoma, KS; Non-Hodgkin's lymphoma, NHL; Pneumocystis jirovecii pneumonia, PCP; progressive multifocal leukoencephalopathy, PML, Tuberculosis, TB

[a] first author of the paper
[b] Year the paper was published
[c] Country in which the study was done
[d] Mean or median CD_4 cell count level as according to the article
[e] Most common three opportunistic infections described
[f] Most common three malignancies described

CD_4 cell counts are also an independent predictor of cancer (27). Lower CD_4 cell counts (<200 cells/mm^3) are associated with higher incidences of KS, lymphoma and human papillomavirus–related cancer (including cervical, anal, squamous cell oral cavity/pharynx, vaginal/vulvar, and penile cancer) (27). In patients with CD_4 cell counts <200 cells/mm^3 the incidence of KS in the first 6 months is high followed by a steep decline in incidences after ART initiation (19, 27). A low incidence of KS was seen in patients with CD_4 cell counts ≥200 cells/mm^3 after ART initiation (27). Patients with previous ART exposure had a lower incidence of lymphoma compared to ART-naive patients in the first year after ART initiation (27). AIDS defining malignancies add to the morbidity and mortality in patients with advanced HIV infection (8). Katano et. al performed an autopsy study of 277 cases of HIV infected patients from four Japanese hospitals. The mean CD_4 cell count in their study was 77 cells/mm^3. They documented Non-Hodgkin lymphoma and KS in 31.6% and 16.9% of their cases respectively. Non-aids defining malignancies were found in only 8.9% of the cases (8).

1.2 Cryptococcal disease/cryptococcosis

1.2.1. Definition

Cryptococcal disease or Cryptococcosis is Cryptococcal species infection impairing normal body function manifesting with abnormal clinical symptoms or signs (28, 29). Cryptococcal disease may be classified as either definite or probable. Definite cryptococcal disease is diagnosed when a patient is febrile with confirmed cryptococcal infection in the absence of any other demonstrable explanatory pathogenic process (28). A diagnosis of probable cryptococcal disease is made when a blood/serum sample

is cryptococcal antigen (CrAg) positive, in the absence of confirmatory clinical and laboratory information (28). Cryptococcosis is defined as the first isolation by culture of any Cryptococcus species from any clinical specimen, a positive India ink test, a positive cryptococcal latex agglutination test or a positive histopathological specimen (30).

1.2.2 Pathogenesis

Cryptococcal disease is predominantly caused by Cryptococcus neoformans variant grubii (serotype A), and to a lesser extent by Cryptococcus gatti infections (formerly Cryptococcus neoformans serotypes B and C) (16, 31). Cryptococcus neoformans has a worldwide distribution. It is a saprophyte that is found in soil contaminated with pigeon droppings and has also been isolated from the heartwood of several tree species (31, 32). After inhalation, there may be an initial pulmonary infection. Subsequent dissemination depends on the immune status of the host, and the number and virulence of the organisms inhaled. The initial infection may be cleared or contained within granulomata as a latent infection in the lungs in immunocompetent individuals whilst it can disseminate in immunocompromised individuals (17, 31, 32). Disseminated cryptococcal disease is associated with T-cell dysfunction (malignancy, immunosuppressive medication, autoimmune disease, sarcoidosis and HIV infection). Macrophages play a role in the dissemination of cryptococcus throughout the host, including dissemination across the blood brain barrier (17, 32). The central nervous system (CNS) is a predilected site for cryptococcus species (17).

1.2.3 Diagnosis

CrAg is a polysaccharide component of the cryptococcal capsule which is shed in biological fluids and is therefore a marker of the presence of the cryptococcus species within the host (33, 34). Detecting CrAg is an easy and relatively cost-effective method of diagnosing cryptococcosis (33). Cryptococcal antigenemia (CRAG) is defined as a positive CrAg in either blood or serum while detection of the CrAg in cerebrospinal fluid (CSF) indicates CM. Persistently elevated CSF CrAg titers in HIV-infected patients indicate ongoing production of viable cryptococcus neoformans and is a poor prognostic indicator which is associated with increased mortality in these patients (35).

Diagnosis of CM is based on microbiological techniques which include India ink microscopy, CSF fungal culture, CrAg latex agglutination and CrAg lateral flow assay (LFA) for CrAg testing (36, 37). Latex agglutination is used to detect CrAg by detecting polysaccharide antigens of cryptococcal capsule (36, 37). The CrAg latex agglutination test is done in a laboratory environment, by skilled laboratory workers, it also needs electricity, cold chain shipping and refrigeration of reagents (36, 37). These make CrAg latex agglutination tests expensive and labour intensive (36, 37). In well-resourced settings CrAg latex agglutination tests take approximately 5 hours from requisition to availability of results (36, 37). The CrAg latex agglutination test has a high sensitivity (97-98%) and specificity (86-100%) which varies depending on the manufacturer (36, 37).

CrAg LFA is an immunochromatographic assay that also detects antigen using a dipstick test (36, 37). CrAg found in serum, plasma or CSF bind to the gold-conjugated,

anticryptococcal antibodies on the test strip causing a visible line (36, 37). CrAg LFA is approved by the United States Food and Drug Administration (FDA) as a point of care dipstick test (36, 37). It does not require laboratory training and can therefore be used by semi-skilled healthcare workers (36). There is no refrigeration needed and results are available within 10 minutes making it an ideal investigation in poorly resourced settings (36, 37). LFA performance has shown a sensitivity of 99.3%,a specificity of 99.5% and a positive predictive value of 99.5% and a negative predictive value of 98.7% (37).

Microscopy with India ink may be used to detect cryptococcus in the CSF. India ink staining is a type of stain that is used to fill the background field but it is not taken up by the thick cryptococcus capsule, forming a halo of light that can be visualized using a light microscope (36). This technique is referred to as negative-field microscopy. The sensitivity of India Ink testing is 86% and is dependent both on fungal burden and the skill of the microscopist (36, 37). CSF culture is the gold standard for the diagnosis of CM but may take as long as 2 weeks (36). Difficulties in making the diagnosis of CM have previously contributed to the delay in treatment and the high mortality associated with CM in HIV infected patients (36). The use of CrAg LFA in the detection of CrAg in CSF is the most efficient diagnostic investigation in confirming CM (36, 37).

1.2.4 Clinical presentation

Clinical presentation of cryptococcal infection varies from asymptomatic colonization to symptomatic infection, and includes the clinical syndromes of pneumonia, respiratory failure and meningitis. Meningitis is the predominant clinical presentation of

disseminated cryptococcal infection in HIV infected patient (17). There is a strong association between the epidemiology of cryptococcal disease and the HIV pandemic (16). In 2014, CM accounted for 15% of global HIV-related deaths (38). According to the 2014 Joint United Nation Program on HIV and AIDS, the annual global estimate of deaths due to CM was 181100 and 135 900 (75%) of these occurred in sub-Saharan Africa (38). HIV-associated CM usually presents as a subacute meningo-encephalitis in patients with CD_4 cell counts <100 cells/mm^3 (17). The median duration from symptom onset to presentation is 2 weeks (17). Patients with CM typically present with malaise, headache and fever which may be followed by visual disturbances and altered mental status. Signs may include meningism, seizures, papilledema, cranial nerve palsies, reduced level of consciousness and eventually coma (17, 31, 39). HIV associated CM is associated with high rates of neurological complications, which include cranial nerve palsies, papilledema, seizures, hemiplegia, blindness and deafness (39). CM and tuberculous meningitis (TBM) are the commonest causes of adult meningitis in South Africa (40). Jarvis et al studied the results of 820 lumbar punctures (between 2006 and 2008) at a public sector referral hospital in SA, a setting of high HIV burden (40). Of all the microbiological CSF results obtained, cryptococcus accounted for 63% of the diagnoses, TB for 28% and bacterial meningitis for 8% (40). They found that 99% of CM patients were HIV positive with a median CD_4 cell count of 39 cells/mm^3 (40).

Pulmonary cryptococcosis in HIV infected patients is underdiagnosed, due to its nonspecific and subtle manifestations such as chronic cough, dyspnea, chest pain, fever and night sweats (41, 42). A South African study on 589 patients reported the prevalence of cryptococcal pneumonia to be 7% (43). This study evaluated the

histological results of miners' autopsies between1996-2000. Antepartum cryptococcal pneumonia had been diagnosed in only 7/589 cases (1.2%) (43). In immunocompetent patients pulmonary cryptococcosis is localized and self-limited; presenting as a single nodule, multiple nodular lesions or mass-like consolidation (41, 44). Radiological manifestations in immunocompromised patients are varied and nonspecific. In a retrospective study of medical records of 80 patients with cryptococcosis (both immunocompetent and immunocompromised), it was found that radiological presentations are dependent on patients' CD_4 cell count; nodule-like radiographic findings were found more in patients with normal CD_4 cell counts (45). Pulmonary nodules, masses, and halo signs were more common in patients with CD_4 cell counts ≥ 691 cells/mm^3 and pulmonary cavities within the nodules/masses were more frequently in patients with CD_4 ≤378 cells/mm^3 (45). A retrospective study on computed tomography chest scans in 60 HIV infected patients with median CD_4 cell counts of 20 cells/mm^3 revealed mainly nodular lesions 56/60 (93.3%). Predominant presentations were solitary nodules 39/56 (69.6%) and cavities with a cavitation rate of 44/56 (78%) (46). These findings have unfortunately not translated into improved diagnoses of pulmonary cryptococcosis in HIV infected patients.

1.3 Cryptococcal antigenemia

1.3.1 Prevalence and Screening

The average global CRAG prevalence was 6.0% among people with CD_4 cell counts <100 cells/mm^3 in 2014 (38) and 7.2 % among 36 cohorts with CD_4 cell counts <100 cells/mm^3 in Africa (47). It was subsequently postulated that implementing a screening

and pre-emptive treatment strategy as part of HIV care practice among patients with CD_4 cell counts <100 cells/mm^3 could prevent the incidence of often fatal CM in the setting of the HIV pandemic (38, 47). CrAg screening of individuals initiating ART and pre-emptive fluconazole treatment of CrAg positive patients has been shown to decrease the cases of CM compared with historic unscreened cohorts (48). As a result, the WHO and many national guidelines for cryptococcal disease management have recommended CrAg screening in HIV-infected persons in blood, and pre-emptive treatment for CrAg positive persons (49, 50).

In South Africa, routine laboratory-initiated screening of patients' blood samples in whom the CD_4 cell count is <100 cells/mm^3 has been initiated. This program has been implemented at healthcare facilities in the Gauteng and Free State provinces since 2013 (34, 50). In November 2016, the South African national CrAg LFA screening program coverage by the NHLS using CrAg LFA was around 96% (18) and the prevalence of CrAg positive samples was 5.4% (51).

The most recent South African guidelines (2019) recommend screening in individuals with CD_4 cell count is <200 cells/mm^3 (52).

1.3.2 Cryptococcal antigenemia and cryptococcal meningitis

CrAg positivity is a sensitive predictor of incident CM during the first year of ART in patients with a CD_4 cell count of <100 cells/mm^3 without prior history of the disease and is known to be an independent predictor of mortality (36, 53, 54). The presence of CrAg in blood precedes cryptococcal symptoms by about 3 weeks (28). Jarvis et. al showed

that in HIV infected patients with positive CrAg and CD_4 cell counts <100 cells/mm^3, 28% developed new or relapsed clinically apparent CM within 1 year (53).

In a study of 1201 HIV infected hospitalized patients with suspected meningitis in Uganda, 725/1201 (60%) patients had CRAG, but negative CSF studies were described (55). This study found that 54/725 (7.2%) patients did not have CM detected by CSF CrAg test (55). Of these patients 5/54 (9%) had cryptococcus isolated on CSF culture or PCR and 6/54 (11%) had confirmed TBM (55). An enhanced diagnostic work up for all immunocompromised patients with suspected CNS infection to include blood CrAg testing was therefore recommended (55).

1.3.3 Cryptococcal antigenemia screening and prophylaxis

The presence of CrAg positivity in the serum/blood of patients with advanced HIV prior to the development of symptoms of cryptococcal disease allows the use of pre-emptive fluconazole as prophylactic treatment before ART is started**.** This decreases the number of CM cases, therefore decreasing overall CM morbidity (48). Longley et.al demonstrated that only 40% of patients who agreed to undergo lumbar puncture had CM after receiving pre-emptive fluconazole treatment for CRAG (48). Patients with advanced HIV infection were screened and given pre-emptive treatment for cryptococcal infection before the initiation of ART. This resulted in a substantial reduction in mortality (24-28%) compared to standard of care (56, 57). A cost-benefit study has shown that the number needed to test and treat (NNT) with CrAg screening and pre-emptive fluconazole treatment to prevent 1 CM case is 11.3 at a cost of United states dollar (USD)190 while NNT to save 1 life is 15.9 at a cost of USD266. The cost

per disability-adjusted life year (DALY) saved is USD21 in a patient with advanced HIV infection with positive CrAg at risk of CM or CM related death (58). CrAg screening in patients with advanced HIV infection with CD_4 cell counts <100 cells/mm^3 remains a cost-effective strategy with the potential to decrease cryptococcal morbidity and mortality (58, 59).

1.4 Cryptococcal immune reconstitution inflammatory syndrome

ART initiation results in the restoration of immune responses to both viable and non-viable organisms as well as to shed antigen. In the setting of cryptococcosis, this may lead to a paradoxical clinical deterioration called cryptococcal immune reconstitution inflammatory syndrome (C-IRIS). This syndrome is characterized by either the emergence of previously sub-clinical cryptococcal disease (unmasking IRIS) or clinical worsening of symptoms of CM whilst on treatment (paradoxical worsening IRIS) (31). The following risk factors have been associated with C-IRIS: a poor baseline inflammatory response, higher fungal burden or CrAg titer, rapid immune reconstitution from a low baseline CD_4 cell count on ART and ART initiation within eight weeks of diagnosis and CM therapy (60).

1.5 Challenges

Despite the change in policy to offer ART to all people living with HIV infection, advocating for earlier access to ART and ART regimens with improved drug efficacy and tolerability; there remains a persistent burden of patients who present late for care with advanced HIV disease (6, 61). In a study done in Cape Town (SA), cryptococcosis cases remained above 400 cases per year from 2005 to 2015 (61). This lack of decline

in high incidence is due to patients presenting with very advanced HIV disease despite the successful scaling up of ART (61). Poor adherence to ART and failure to retain patients in HIV care (i.e. lost to care/follow-up) are factors contributing to this problem (17, 61). Osler et.al. has further shown that 51.8% of ART experienced patients had CD_4 cell counts <50 cells/mm^3 in 2016, 76% of these patients were poorly adherent to ART or had recent detectable viremia in the same year (61). ART experienced patients with a CD_4 cell count <50 cells/mm^3 in 2016 were predominantly men (61). Increasing HIV-associated morbidity is now seen in ART treatment-experienced patients who default treatment or are lost to follow-up and subsequently are not virologically suppressed (61).

In a Ugandan study, patients who had CRAG with CD_4 cell counts <100 cells/mm^3 and TB received pre-emptive fluconazole and standard TB treatment two weeks prior to ART initiation (62). Death rates were significantly higher among subjects who were both TB and CrAg positive compared to subjects without this co-infection (62). Despite CrAg screening and pre-emptive antifungal therapy decreasing the incidence of CM; the mortality among CrAg positive patients remains higher compared to CrAg negative patients (48, 57, 62). Factors contributing to this increased mortality among CrAg positive patients need to be explored.

Though cryptococcal disease is associated with high mortality and morbidity, the predominant presentation is that of CM with pulmonary cryptococcosis underdiagnosed in HIV infected patients. There is a dearth of published literature describing the infections and co-morbidities in HIV infected patients with CRAG (identified on routine

screening) although opportunistic infections and malignancies associated with advanced HIV are well described (see table 1.1). Our study aims to retrospectively document the associated conditions (infections and malignancies) in HIV infected patients with CRAG (as identified on routine screening).

1.6 Study objective

To describe the infections and malignancies associated with positive CrAg (detected on routine screening in HIV infected patients with CD_4 cell counts of <100 cell/mm^3).

1.7 Methods

1.7.1 Study design

Retrospective, descriptive study.

1.7.2 Sites of study

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Helen Joseph Academic Hospital (HJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH) in Gauteng province.

1.7.3 Study population

Patients with CrAg positive (as identified on routine screening) at the CMJAH; CHBAH and HJAH during the study period (01 October 2012 to 31 March 2017)

a. Inclusion criteria

Patients whose results were available via the National Health Laboratory Services (NHLS) database with the following:

- HIV infected with CD_4 cell count <100 cell/mm^3
- Age ≥18 years old
- CrAg positive on serum/blood

b. Exclusion criteria

Age <18 years; incomplete records.

1.7.4 Sample size

An estimated sample size of 230 patients.

1.7.5 Data collection

Data collection was on associated infections and malignancies of patients with CRAG and advanced HIV infection (CD_4 cell count <100 cell/mm^3). Tuberculosis laboratory diagnosis was based on a positive Gene-X-pert, acid fast bacilli identified, positive culture results or tuberculous granulomata reported within six months of CRAG diagnosis. CM was diagnosed on CSF with either a positive cryptococcal latex antigen test, positive India ink on microscopy or a positive CSF cryptococcal culture results within six weeks of CRAG diagnosis. Cryptococcus on blood culture also called cryptococcal fungemia was a laboratory diagnosis within six weeks of CRAG diagnosis based on a positive cryptococcal serum/blood culture results. Fungal cell wall antigen

1→3-β-d-glucan (BD Glucan), is a soluble marker for the presumptive diagnosis and therapeutic monitoring of invasive fungal infections in patients with advanced HIV infection (63). Serum BD Glucan is also a useful supporting diagnostic tool to quantitative PCR on sputum in the diagnosis of suspected PJP (64). Beta D glucan was considered positive if the value was >80 pg/ml (65) within six weeks of CRAG diagnosis. Bacterial infections were based on blood culture results within six weeks of CRAG diagnosis. Malignancies were based on tissue biopsy results any time within the data collection period. Data collection period was from 01 October 2012 to 31 March 2017, over a period of 54 months. The CD_4 cell count level at the time of diagnosis of CRAG was used, further CD_4 cell count changes were not considered. Data collection sheet was used. See appendix A

1.8 Data Analysis

Data was interpreted using frequencies, percentages, mean and standard deviation. The statistical software, STATA version 13.0 (StataCorp; College Station, Texas) was used for the data analysis. Comparison was performed using the chi-square test for categorical variables. The p-values of less and/or equal to 0.05 were considered statistically significant.

1.9 Ethics

The proposal was submitted to the faculty of Health Sciences Research Ethics Committee of the University of Witwatersrand to evaluate, see appendix B. Permission was obtained from the NHLS laboratory to access and use their data, see appendix F.

Permissions from the superintendents at CMJAH, HJAH and CHBAH were sought, see appendices C-E.

The data generated from the study is considered confidential by the investigator, except to the extent that it is included in a publication as agreed in the publication policy of this protocol. To protect subjects' privacy, all research results was labelled with a code that only the principal investigator had access to and was able to link data with the subjects' details. Any identifying information about the subjects was kept confidential to the extent permitted by law. Samples were tracked using a computer system on the NHLS laboratory system.

1.10 Schedule

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Literature review												
Preparing protocol												
Protocol assessment												
Ethics application												
Collecting data												
Data analysis												
Writing up thesis												
Writing up paper												

1.11 Funding

	Estimated cost
Photocopying (paper, cartridge)	R3000
Transport	R1000
Data bundle	R2000
Total	R6000

1.12 References

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CHAPTER 2: SUBMISSIBLE ARTICLE

TITLE: INFECTIONS AND MALIGNANCIES ASSOCIATED WITH CRYPTOCOCCAL ANTIGENEMIA AND LOW CD_4 CELL COUNTS

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Short Title: Infections and malignancies associated with cryptococcal antigenemia and low CD_4 cell counts

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2.1 Abstract

Background: Cryptococcal infection causes high morbidity and mortality in advanced retroviral disease**.** The South African government in 2016 instituted a nationwide screening program for cryptococcal antigenemia (CRAG) among human immunodeficiency (HIV) infected persons with CD_4 cell counts below 100 cells/mm^3 This program offers health workers an opportunity to detect cryptococcal disease early and pre-emptively treat patients to prevent progression from antigenemia to disseminated cryptococcal disease. In HIV disease CRAG is a forecaster of cryptococcal meningitis (CM) and a predictor of mortality.

Objective: To document the infectious and malignant conditions associated with CRAG in patients routinely screened as part of the national screening program.

Method: A retrospective descriptive study including patients with CRAG accessing the teaching hospitals of the University of the Witwatersrand i.e. Chris Hani Baragwanath Academic Hospital; Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Academic Hospital. The study period was 54 months (01 October 2012 to 31 March 2017). The National Health Laboratory Service database was reviewed for eligible patients' records to determine concomitant infections and malignancies.

Results: The cohort had 272 patients, 58.8% were males. Cerebrospinal fluid analysis results were available for 224 (82.4%) patients of whom 199 had confirmed CM. CM was strongly associated with a younger age was (p=0.016). Cryptococcal fungemia was found in 125 of the 194 patients tested (64.4%). Lower CD_4 counts (≤50 cells/mm^3) were significantly associated with cryptococcal fungemia (p=0.001). There were 52 cases of confirmed tuberculosis (TB) with 30 pulmonary and 21 extra-pulmonary TB.

Malignancies and pre-malignant conditions were found in 20 (7%) patients. Kaposi sarcoma and high-grade cervical lesions were the most prevalent (11 and 6 cases respectively).

Conclusion: Our study confirms a high burden of cryptococcal disease (meningitis and fungemia) in patients with CRAG. Age <49 years is a strong predictor of CM and low

CD_4 cell count is a strong predictor of cryptococcal fungemia. Tuberculosis is a relatively common co-infection in patients with CM and should be actively excluded.

2.2 Introduction

Globally, there were 36.9 million people living with human immunodeficiency virus (HIV) in 2017, across all ages (35.1 million adults) (1). The HIV prevalence in South Africa (SA) among adults aged 15 to 49 years is estimated at 18.8 % (2, 3). SA has one of the largest HIV epidemics in the world, with an estimated 7,2 million people living with HIV and acquired immunodeficiency syndrome (AIDS) in 2017 (2, 3).

Studies suggest that between 30–40% of people living with HIV starting ART in low- and middle-income settings have advanced HIV disease, and 20% of these patients have a CD_4 cell count <100 cells/mm^3 (4-6). Notwithstanding the magnitude of the ART program in SA, a high proportion of patients still present with advanced HIV disease (4).

Opportunistic infections (OIs) drive morbidity and mortality among adults with advanced HIV disease. These include tuberculosis (TB), severe bacterial infections, cryptococcal meningitis (CM), toxoplasmosis and Pneumocystis jirovecii pneumonia (PCP) (7, 8). AIDS defining malignancies are also seen in advanced HIV infected patients (6). The risk of OIs and AIDS defining malignancies are strongly predicted by baseline CD_4 cell counts before initiation of ART (9).

Cryptococcal disease is predominantly caused by Cryptococcus neoformans var. grubii (serotype A) (10, 11). Cryptococcosis is defined as the first isolation by culture of any Cryptococcus species from any clinical specimen, a positive India ink test, a positive cryptococcal latex agglutination test or a positive histopathological specimen (12).

Cryptococcal antigen (CrAg) is a polysaccharide component of the cryptococcal capsule which is shed in biological fluids, a marker of the presence of the cryptococcus species within the host (13, 14). Detecting CrAg is an easy and relatively cost-effective method of diagnosing cryptococcosis (13). Cryptococcal antigenemia (CRAG) reflects the presence of CrAg in either blood or serum while the detection of CrAg in cerebrospinal fluid (CSF) implies cryptococcal meningitis (CM).

Diagnosis of CM is based on microbiological techniques which include India ink microscopy, CSF fungal culture, CrAg latex agglutination and CrAg lateral flow assay (LFA) for CrAg testing (15, 16). CSF culture is the gold standard for the diagnosis of CM but may take as long as 2 weeks (15). CSF culture is the gold standard for the diagnosis of CM but may take as long as 2 weeks (15). Difficulties in making the diagnosis of CM have previously contributed to the delay in treatment and the high mortality associated with CM in HIV infected patients (15). LFA CrAg testing on CSF is the most efficient diagnostic investigation in the diagnosis of CM (16). It has a sensitivity and specificity of >99%, a high positive predictive value of 99.5% with a negative predictive value of 98.7% (16).

Clinical presentation of cryptococcal infection varies from asymptomatic colonization to symptomatic infection, and includes the clinical syndromes of pneumonia, respiratory failure and meningitis. Meningitis is the predominant clinical presentation of disseminated cryptococcal infection in HIV infected patients and usually presents as a subacute meningo-encephalitis in patients with CD_4 cell counts <100 cells/mm^3 (17). In

2014, CM accounted for 181 100 deaths (15% of global AIDS-related deaths) and the majority (75%) of these occurred in sub-Saharan Africa (18).

CrAg positivity is a sensitive predictor of incident CM during the first year of ART in patients with a CD_4 cell count of <100 cells/mm^3 without prior history of the disease and is known to be an independent predictor of mortality (15, 19, 20). Jarvis et. al showed that in HIV infected patients with positive CrAg and CD_4 cell counts <100 cells/mm^3, 28% developed new or relapsed clinically apparent CM within 1 year (19). The presence of CrAg in blood precedes cryptococcal symptoms by about 3 weeks (21). CrAg screening of individuals initiating ART and pre-emptive fluconazole treatment of CrAg positive patients has been shown to decrease the cases of CM compared with historic unscreened cohorts (22). As a result, the WHO and many national guidelines for cryptococcal disease management have recommended CrAg screening in HIV-infected persons in blood, and pre-emptive treatment for CrAg positive persons (23, 24). In South Africa, routine laboratory-initiated screening of patients' blood samples in whom the CD_4 cell count is <100 cells/mm^3 has been initiated. This program has been implemented at healthcare facilities in the Gauteng and Free State provinces since 2013 (14, 23) and nationwide since 2016. The most recent South African guidelines (2019) recommend screening in individuals with CD_4 cell count is < 200 cells/mm^3 (25).

In 2014 the average global CRAG prevalence was 6.0% among people with a CD_4 cell count <100 cells/mm^3 in 2014 (18) and 7.2% among 36 cohorts with CD_4 cell counts <100 cells/mm^3 in Africa (26). In November 2016, the South African national CrAg

screening program coverage by the National Health Laboratory Services (NHLS) using LFA was around 96% (18) and the prevalence of CrAg positive samples was 5.4% (27).

The presence of CrAg positivity in the serum/blood of patients with advanced HIV infection prior to the development of symptoms of cryptococcal disease allows the use of pre-emptive fluconazole as prophylactic treatment before ART is started**.** Pre-emptive fluconazole treatment of CrAg positive patients decreases the number of CM cases, therefore decreasing overall CM morbidity (22). This also resulted in a substantial reduction in mortality by 24-28% compared to standard of care (28, 29). Longley et. al demonstrated that only 40% of patients who agreed to undergo lumbar puncture had CM after receiving pre-emptive fluconazole treatment for CRAG (22). Lumbar puncture is therefore recommended by guidelines in patients with CRAG to exclude CM.

Despite CrAg screening and pre-emptive antifungal therapy in CrAg positive patients decreasing the incidence of CM; the mortality among CrAg positive patients remains higher compared to CrAg negative patients (22, 29, 30). Factors contributing to this increased mortality among CrAg positive patients need to be explored.

Our study intents to retrospectively describe the infections and malignancies associated with CRAG (detected on routine screening in patients with CD_4 counts of <100 cells/mm^3).

2.3 Materials and Methods

Study design

Retrospective description of the laboratory-recorded infections and malignancies of HIV infected patients with CD_4 cell counts of <100 cell/mm^3 with CRAG whose results were available on the National Health Laboratory Services (NHLS) database from Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Helen Joseph Academic Hospital (HJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH) in Gauteng province, SA .

Study population and sampling strategy

HIV infected patients, ≥18 years old with a CD_4 cell count <100 cell/mm^3 with CRAG detected on routine screening. Patients should have had further investigations depending on the clinical presentation after diagnosis of CRAG. Patients with incomplete laboratory records to fulfil the inclusion criteria were also excluded. A minimum sample size of 230 was calculated based on the 95% confidence level, 5% sampling error, non-response rate of 10%, design effect of 1.5 and national average CrAg prevalence of 10.1% among people with a CD_4 cell count <100 cells/mm^3 in 2014 (27). Data from the NHLS system was reconciled and verified using NHLS-labtrack results viewer, patients with incomplete record or without further investigations were excluded.

Data collection

Infections and malignancies were collated for all patients in whom CRAG was positive Data collection on associated infections and malignancies of patients with CRAG and advanced HIV infection (CD_4 cell count <100cell/mm^3). Tuberculosis laboratory diagnosis was based on a positive Gene-X-pert, acid fast bacilli identified, positive culture results or tuberculous granulomata reported within six months of CRAG diagnosis. CM was diagnosed on CSF with either a positive cryptococcal latex antigen test, positive India ink on microscopy or a positive CSF cryptococcal culture results within six weeks of CRAG diagnosis. Cryptococcus on blood culture also called cryptococcal fungemia was a laboratory diagnosis within six weeks of CRAG diagnosis based on a positive cryptococcal serum/blood culture results. Fungal cell wall antigen 1→3-β-d-glucan (BD Glucan), is a soluble marker for the presumptive diagnosis and therapeutic monitoring of invasive fungal infections in patients with advanced HIV infection (31). Serum BD Glucan is also a useful supporting diagnostic tool to quantitative PCR on sputum in the diagnosis of suspected PJP (32). Beta D glucan was considered positive if the value was >80 pg/ml (33) within six weeks of CRAG diagnosis. Bacterial infections were based on blood culture results within six weeks of CRAG diagnosis. Malignancies were based on tissue biopsy results any time within the data collection period. Data collection period was from 01 October 2012 to 31 March 2017, over a period of 54 months. The CD_4 cell count level at the time of diagnosis of CRAG

was used, further CD_4 cell count changes were not considered. Data collection sheet was used. See appendix A

Data Analysis

Data was interpreted using frequencies, percentages, mean and standard deviation. The statistical software, STATA version 13.0 (StataCorp; College Station, Texas) was used for the data analysis. Comparison was performed using chi-square test for categorical variables. The p-values of ≤0.05 were considered statistically significant.

Ethics

The proposal was submitted to the faculty of Health Sciences Research Ethics Committee of the University of Witwatersrand for evaluation and approval (the clearance number M170477), see appendix B. Permission was obtained from the NHLS laboratory to access and use their data, see appendix F. The superintendents at CMJAH, HJAH and CHBAH all gave permission letters; see appendices C-E.

2.4 Results

2.4.1 Baseline characteristics

Two hundred and seventy-two patient records were reviewed, more than half (58.8%) were men and 38.6% were women while in 2.6% the gender was unspecified. The mean age of the patients was 38.8±9.4 years (range 20-71 years). Nearly half (45%) of the patients were in the age group 30-39 years and almost one third (30%) were aged 40-49 years. Approximately three quarters (77%) of the patients had CD_4 counts ≤50 cells/mm^3 while the rest (23%) had CD_4 cell counts >50 cells/mm^3, see table 2.1 below.

Table 2.1: Baseline characteristics in patients with advanced HIV infection and cryptococcal antigenemia (N=272)

	n	**%**
Age (years)		
20-29	33	12.1
30-39	123	45.2
40-49	82	30.1
50-59	24	8.8
60+	10	3.7
Gender		
Male	160	58.8
Female	105	38.6
Unspecified	7	2.6
CD_4 T-cell count (cells/mm^3)		
≤50	208	76.5
51-99	64	23.5

2.4.2 Infections and malignancies documented in CRAG positive patients

The most common infection documented was CM. It was identified in 199 patients (88.8%) of the 224 patients in whom cerebrospinal fluid analysis was performed. Cryptococcal fungemia was detected in 125 patients (64.4%) of the 194 patients who had fungal cultures done.

Table 2.2: Infections and malignancies in patients with advanced HIV infection and cryptococcal antigenemia

Infections	**Number tested (%)**
Cryptococcal meningitis	199/224 (88.8)
Cryptococcal fungemia	125/194 (64.4)
Tuberculosis	52
Pulmonary tuberculosis	30
Extrapulmonary Tuberculosis	21
Fungemia (>80 pg/ml)	10
Bacterial infections	6
Mycobacterium Avium	1
Malignancies	20
Kaposi's Sarcoma	11
HGSIL	6
DBLCL	2
Adenocarcinoma Breast	1

HGSIL, high grade squamous intraepithelial lesions; DLBCL, diffuse large B-cell lymphoma

2.4.3 Cryptococcal Meningitis and Fungemia

Cerebrospinal fluid analyses were performed on 224 (82.4%) patients of whom the majority (199 patients) were diagnosed with cryptococcal meningitis. Bivariate analysis of CM by age showed a significant association between age and CM (p=0.016). Young patients below the age of 49 years were more likely to have CM. All patients between the age of 20 and 29 years while 83% of the patients between 30 and 39 years had CM in this cohort. Of all the meningitis patients (199 patients) were cryptococcal antigen positive, CSF culture and India ink positive samples were almost similar with 164 patients (82.4%) and 162 patients (81.4%) respectively. Blood cultures within six weeks of CRAG diagnosis were done in 194 patients. Cryptococcal fungemia was found in 125 patients (64.4%) who had blood culture done while only 2 patients had cryptococcosis identified both on blood culture and skin biopsy. Twelve patients with cryptococcal fungemia had tuberculosis, 7 patients had pulmonary tuberculosis, while 5 had extrapulmonary tuberculosis. In patients with fungemia there was a significant association with CD_4 counts below 50 cells/mm^3 (p-value=0.001). See table 2.3 below

Table 2.3: Cryptococcosis by gender, age, CD_4 cell count and concomitant tuberculosis in patients with advanced HIV infection and cryptococcal antigenemia

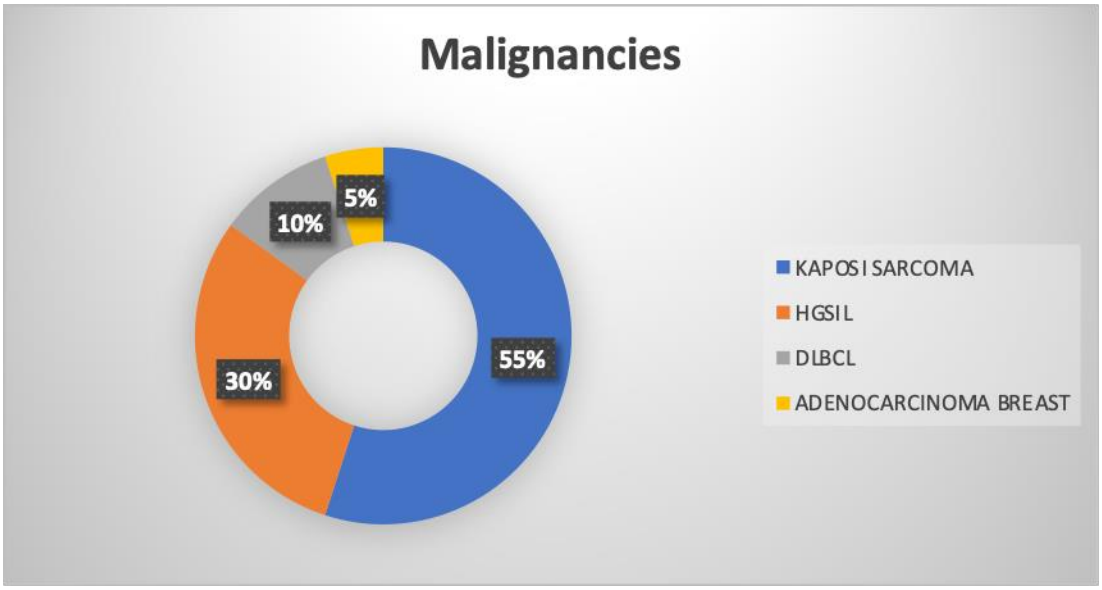
	Cryptococcal meningitis (%) [a]	**Cryptococcal Fungemia (%) [b]**
Number	199 (88.8)	125 (64.4)
Gender		
Male	122 (91.7)	114 (58.8)
Female	71 (84.5)	73 (37.6)
Unspecified	6 (85.7)	7 (3.6)
p-value	0.168	0.113
Age (years)		
20-29	28 (100)	17 (68.0)
30-39	83 (83)	58 (69.9)
40-49	65 (94.2)	34 (59.0)
50+	23 (85.2)	14 (56.0)
p-value	0.016	0.428
CD_4 (cells/mm^3)		
<50	155 (89.1)	111 (69.8)
>50	44 (88)	14 (40.0)
p-value	0.800	0.001
Concomitant Tuberculosis		
Pulmonary TB	15	7
Extra-pulmonary TB	13	5
Mycobacterium Avium	1	0

[a] Percentage of patients who had lumbar puncture
[b] Percentage of patients who had blood culture done

Fifty-two of patients tested positive for tuberculosis. Of these, PTB was diagnosed in 30 patients, extrapulmonary tuberculosis in 21 patients and one patient had Mycobacterium Avium on both blood culture and bone marrow results.

BD glucan was positive (>80 pg/ml) in 10 patients. Six patients were found to have bacterial infection on blood culture.

Malignancies and pre-malignant conditions were found in 20 patients (7%). Kaposi Sarcoma (KS) and high grade squamous intraepithelial lesions (HGSIL) were the most frequent at 11 and 6 patients respectively. Two patients had diffuse large B-cell lymphoma (DLBCL) and one patient had an adenocarcinoma of the breast. See figure 2.1.



HGSIL, high grade squamous intraepithelial lesions; DLBCL, diffuse large B-cell lymphoma

Figure 2.1: Doughnut chart on malignancies in patients with advanced HIV infection and cryptococcal antigenemia

2.5 Discussion

South Africa has an extensive population of people with advanced HIV disease who succumb to cryptococcal meningitis. The mortality from the disease remains high even in the presence of optimal therapy. In 2016 this country became the first country in the world to implement a national routine screening program for cryptococcal disease. Screening for CRAG among HIV infected patients with CD_4 counts below 100 cells/mm^3 offers an opportunity to identify cryptococcal disease early and proactively treat patients to prevent evolution from antigenemia to meningitis.

We identified 272 patients who were CRAG positive and had associated infections and/or malignancies. The most common infections identified were cryptococcal meningitis (88.8%); cryptococcal fungemia (64.4%) and tuberculosis (52 patients). Malignancies were only identified in 20 patients, the majority (11) of whom had KS with a notable 6 patients having HGSIL. The strength of this study is that it was conducted at academic hospitals which are likely to be more diligent in their work-up of patients. The weakness is that due to the retrospective nature some of our data was incomplete.

An interesting finding in this study has been the predominance of CRAG in patients in the age groups 30-39 years and 40-49 years respectively. These patients accounted for three quarters of the cohort. This finding is consistent with the results of Vallabhaneni et. al (34).They reviewed the data of 4,395 patients screened for CRAG in the Western Cape. The median age of the patients in their study was 34 years (interquartile range: 29-41 years). A recent CRAG screening surveillance report by Greene et al described the demography of 34534 patients who tested positive (35). The largest numbers in

their cohort were between the ages of 30 and 44 years (35). This has been a notable factor in HIV patients with CRAG, younger patients seem to be carrying the burden of the disease.

In our cohort there were more males than females (1.5:1) which is consistent with the data from Greene et. al (35) In their study the ratio of CRAG positive tests was 1.26:1 (35). Almost three quarters (76.5%) of our CRAG positive patients had CD_4 cell counts below 50 cells/mm^3. It has previously been shown in the South African setting, that the number of CRAG positive tests could be inversely correlated with CD_4 cell counts (35).

Our study confirms that CrAg is highly effective in identifying patients at risk of CM in advanced HIV as shown by Jarvis et al., and may therefore be an important factor in decreasing AIDs related CM morbidity and mortality(19). CRAG screening prior to the initiation of ART could potentially reduce the burden of CM and decrease the significant mortality associated with it (30). The antigen is detectable in peripheral blood approximately 22 days prior to the development of symptoms associated with CM (36). The mortality of patients with CD_4 cell counts ≤100 cell/mm^3 admitted in hospital with CM was reported to be almost 30% in a study done in Kwazulu Natal, SA (37).

In this study CM was detected in 199 patients (88.8% of those tested). This figure is alarming on two accounts; the first one being that 48 patients (17.6%) of patients with detectable CRAG did not have LPs performed, contrary to published guidelines (25) and secondly that 199 patients of those tested (88.8%) had meningitis within 3 months of CRAG detection. Of all patients who had lumbar punctures done the majority (82.3%) had CM within six weeks of detection of CRAG.

The high number of patients with CM is consistent with previous information which has shown that cryptococcosis is the foremost cause of adult meningitis in South Africa (38, 39). The proportion of patients with meningitis in this study (88.8% of patients who had lumbar puncture done) is much higher than in previously published data. In a 2018 study done in Cameroon the prevalence of CM was 45.5% in 14 CRAG positive patients (40). Letang et al.(20) detected CM in 39% (11 of 28) of CRAG-positive patients. Their study was based in rural Tanzania (20). Our finding is however consistent with the prediction of Rajasingham et al (18).They estimated that 70% of people positive for CRAG would progress to develop cryptococcal disease or die without diagnosis, unless treated with ART or pre-emptive fluconazole(18). It is possible that many in our cohort did not receive the necessary therapy due to the high rates of loss to follow-up following HIV diagnosis (41). Low CD_4 cell counts (<200 cells/mm^3) are an additional factor contributing to low follow up rates and our patients were all in this category (42).

Younger patients were more likely to have CM, all patients aged ≤29 years and 83% of those between 30-39 years had CM. Age was a significant and independent variable of CM in patients with CRAG. (p-value =0.016) This finding is in keeping with the results of Britz et al. who studied the epidemiology of meningitis in Gauteng province (39). In their study, the overall incidence of cryptococcal meningitis was highest in the 30–39 years age group (39). This data also concurred with that of McCarthy et al who showed the age-adjusted incidence of CM peaking at the category 35-44 years in Gauteng (12, 43). Baseline CD_4 cell count is an independent predictor on CM (44). In this study there was no correlation between patients with CD_4 cell counts <50 cell/mm^3 as previously shown in the study by Jarvis et al. (38).

Cryptococcal fungemia was identified in 125 patients (64.4% of those tested). The presence of the organism in the blood is indicative of disseminated infection and in this study was significantly associated with a CD_4 cell count <50 cells/mm^3 (p-value <0.05). Cryptococcal infection has previously been identified as an important cause of blood stream infections in advanced HIV disease. Qi et al. showed a correlation between fungemia and low CD_4 cell count (<50 cell/mm^3) (45). Cryptococcal neoformans was the most common bloodstream pathogen found in 22.7% of their HIV infected patients and the median CD_4 cell count (14 cell/mm^3) was lower in subjects with cryptococcal fungemia compared to those with mycobacteria or other bacteria on blood culture (45) In the study by Kiertiburanakul et al. patients with fungal infections had a mortality rate of 17.2%, a feature which has implications for future management of these patients (46). There were 3 patients in whom fungemia was detected but no evidence of CM. Meningoencephalitis due to CNS cryptococcosis, may present in the brain parenchyma without CSF involvement (47). Blood cultures may assist in the identification of these patients who carry an increased risk of cryptococcal-related death.

Tuberculosis was diagnosed within 6 months in 52 (19.1%) patients with detectable CrAg. Jarvis et al. has shown that there is a robust and temporal relationship between patients with a recent history of TB and CM (44). Majority of patients (76.9%) with TB had CD_4 cell counts <50 cell/mm^3. In a previous Ugandan study documenting CM and TB co-infection patients were similarly severely immunosuppressed and had a higher risk of death (48). There were no cases of co-infection with CM and tuberculous meningitis. Cases of co-infection have previously been described but are rare (49).

Malignancies in this cohort were similar to those described in patients with advanced HIV CD_4 cell count <100 cell/mm^3 found to have increased risk of Kaposi Sarcoma (KS), human papillomavirus–related cancer/precancer and lymphoma (50, 51).

It is well recognized that people with advanced HIV infection have an elevated risk for cancer, more specifically AIDS-defining cancers which include KS; specific lymphomas and cervical carcinoma (52). In our cohort there were 20 patients (7.4%) with confirmed malignancies the most common of which was KS (11 patients). The incidence of KS has increased dramatically in the HIV era and remains the most common malignancy in advanced infection (52). Malignancies and pre-malignant conditions documented were HGSIL (6); DLBCL (2) and adenocarcinoma of the breast in 1 patient. Dhokotera et al. studied the burden of cancers associated with HIV in the South African public health sector and determined that HIV-infected patients were at a higher risk of KS, cervical cancer and lymphoma (53). Stein et al similarly found a significantly increased risk for KS, lymphoma and cervical cancer in a local population (53). The association between HIV and carcinoma of the breast is controversial and we are unable to make a meaningful contribution as we had only 1 case.

Limitations

Our study was based on available laboratory data. No clinical information or exposure to ART was known.

Information on parasitic and viral infections was not readily available as these are not performed unless there are clinical indications.

Patients' inpatient or outpatient information at the time of testing could not be verified.

A further limitation of this study is that it was conducted at 3 university teaching hospitals in Johannesburg. HIV-infected patients in other urban areas and rural areas may have different characteristics and the distribution of causative pathogens may differ among particular areas.

Despite these limitations, we believe that our study provides valuable insights and could with further research contribute to the management of patients in whom CRAG has been identified.

Conclusion

Our study confirms a high burden of cryptococcal disease in patients with advanced HIV (as evidenced by CD_4 cell counts <100 cell/mm^3) and CRAG. These patients are at a very high risk for CM and/or cryptococcal fungemia. Age <49 years is a strong predictor of CM and a CD_4 cell count <50 cells/mm^3 was associated with fungemia. We therefore suggest that fungal blood cultures be considered as an adjunct investigation tool to identify disseminated cryptococcal infections among HIV infected patients with CRAG and low CD_4 cell count, specifically in patients in whom CM has been excluded.

Tuberculosis is a relatively common co-infection in patients with CM and should be actively excluded. CRAG is associated with AIDS related malignancies and clinicians need to maintain a high index of suspicion.

Further research is warranted to assess the viability of routine screening for tuberculosis in patients with CRAG.

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CHAPTER 3: APPENDICES

A: Data collection Sheet

INFECTIONS AND MALIGNANCIES ASSOCIATED WITH CRYPTOCOCCAL ANTIGENEMIA, AMONG PATIENTS WITH CD_4 COUNTS <100 CELLS/MM^3

PATIENT ID	……

INSTRUCTION: TICK THE RELEVANT BOX (√) WERE APPLICABLE					
1.1	GENDER	MALE	1		
		FEMALE	2		
1.2	AGE	……	YEARS		
1.3	CD_4 COUNT	<100CELL/MM^3	1		
		<50CELL/MM^3	2		
2.1	CRYPTOCOCCAL MENINGITIS	CSF NEGATIVE	0		
		CRAG ON CSF	1		
		INDIA INK	2		
2.2	DISSEMINATED CRYPTOCOCCAL INFECTION	NEGATIVE	0		
		CULTURE	1		
		HISTOLOGY	2		
3.1	MYCOBACTERIUM TUBERCULOSIS				
		TB NEGATIVE	0		
		ACID FAST BACILLI	1		

		TB CULTURE	2		
		HISTOLOGY	3		
		PCR	4		
3.2	MYCOBACTERIUM TUBERCULOSIS	PULMONARY TB	1		
		EXTRAPULMONARY TB	2		
		DISSEMINATED TB			
3.3	MTB RESISTANCE	NONE	0		
		RIF SENSITIVE	1		
		RIF/INH MONORESISTANCE	2		
		MDR TB	3		
		XDR TB	4		
4.1	PCP	NEG	0		
		SPUTA	1		
		BD GLUCAN	2		
OTHER INFECTIONS					
5.1	CMV	PCR NEG	0		
		PCR POS	1		
6.1	MAC	NEG	0		
		CULTURE STERILE AREA	1		
		SPUTA X2	2		
7.1	TOXOPLASMOSIS	NEGATIVE	0		
		IGM	1		
		IGG	2		
8.1	PML	JC VIRUS NEG	0		

		JC VIRUS POS	1		
9.1	HISTOPLASMOSIS	NEG	0		
		CULTURE	1		
		SEROLOGY	2		
		ANTIGEN TEST	3		
		PCR	4		
		HISTO/CYTOLOGY	5		
10.1	COCCIDIOIDOMYCOSIS	NEG	0		
		HISTO/CYTOLOGY	1		
11.1	CANDIDIASIS	NEG	0		
		CULTURE (STERILE SITE)	1		
12.1	MALIGNANCIES	NEG	0		
		CYTOLOGY	1		SPECIFY……
		HISTOLOGY	2		SPECIFY……
13.1		BLOOD CULTURE			……..
13.2		SPUTUM			……..
13.3		URINE			……..
13.4		CSF			……
13.5		STOOL			……
13.6		PLEURAL FLUID			……
13.7		ASCITIC FLUID			……
					……

B: Ethics Clearance Certificate

R14/49 Dr Mmabatho Ngoananoka Portia Dintwe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170477

NAME: (Principal Investigator)	Dr Mmabatho Ngoananoka Portia Dintwe
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital Helen Joseph Hospital Chris Hani Baragwanath Academic Hospital National Health Laboratory Service
PROJECT TITLE:	Infectious and Malignancies Associated with Cryptococcal Antigenemia, among Patients with CD4 Counts <100Cells/MM³
DATE CONSIDERED:	05/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr K. Hari
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	28/06/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

C: Helen Joseph Hospital approval letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. M.R. Billa
Chief Executive Officer
Tel :(011) 489-0306/1087
Fax :(011) 726-5425
E mail: Raymond.Billa@gauteng.gov.za
Date: 05 July 2017

Dr.M.R.Billa
Chief Executive Officer
Helen Joseph Hospital

Dear Dr.Billa

STUDY: Infection and malignancies associated C count <100 cells / mm3.

RESEARCHERS: Dr. Mmabatho Portia

Ethic: M170477

The above was discussed at the Research Committee Meeting. We recommend that permission be granted for Helen Joseph Hospital to be used as a site for the above research. However, since this is a research project involving voluntary participation. We cannot guarantee participation of individuals/patients.
Upon completion of the study, a copy thereof should be submitted to Helen Joseph Hospital.

Thank you

DR. Murimisi Mukansi
CHAIRPERSON
DATE:

Approved

DR. M.R. BILLA
CHIEF EXECUTIVE OFFICER
DATE: 17.07.2017

D: Chris Hani Baragwanath Academic Hospital approval letter

GAUTENG PROVINCE
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 29th March 2017

TITLE OF PROJECT:

Infections and Malignancies Associated with Cryptococcal Antigenemia, Among Patients with CD4 Counts <100cells/mm^3

UNIVERSITY: Witwatersrand

Principal Investigator: Dr Dintwe

Department: Internal Medicine

Supervisor : Dr K Hari

Permission Head Department (where research conducted): Yes

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

.....
Recommended
(On behalf of the MAC)
Date: 29/03/2017

.....
Approved/Not Approved
Hospital Management
Date: 04/04/17

E: Charlotte Maxeke Johannesburg Academic Hospital approval letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Mr. Johannes Maepa
Office of the Clinical Director
Tell: (011) 488-3365
Email:johannes.maepa@gauteng.gov.za
31 March 2017

Dear Dr. Mmabatho Ngoananoka Portia Dintwe

STUDY TITLE: infections and malignancies associated with cryptococcal antigenemia, among patients with CD4 counts <100cells/mm3.

Permission to conduct the above mentioned study is provisionally approved. Your study can only commence once Ethics approval is obtained. Please forward a copy of your ethics clearance as soon as the study is approved by the Ethics committee for the CEO's to give you the final approval to conduct the study.

Supported/not supported

Dr M.I. Mofokeng
Clinical Director
DATE: 3/4/2017

Approved/not approved

Ms G Bogoshi
Chief Executive Officer
Date: 4-4-2017

F: National Health services laboratory approval letter

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

03 July 2017

Applicant: Dr Portia Dintwe
Institution: University of the Witwatersrand
Department: Internal Medicine
Email: Portia.dintwe@gmail.com
Cell: 082 781 4057

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

Your application to undertake a research project "**Infectious and Malignancies Associated with Cryptococcal Antigenemia, among Patients with CD4 Counts <100 Cells/MM^3**" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- 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 note that this letter constitutes approval by the NHLS Academic Affairs and Research. Any data related queries may be directed to the NHLS Corporate Data Warehouse, Tel: (011) 386 6074 email: zarina.sabat@nhls.ac.za

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research