CRYPTOCOCCAL INFECTION IN THE ERA OF ANTI-RETROVIRAL THERAPY (ART) AMONG HIV INFECTED PATIENTS WITH MENINGITIS AT A TERTIARY HOSPITAL IN A LOW-RESOURCE SETTING

BY

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Abstract

**Background:** Despite improved access to anti-retroviral therapy (ART) in Africa, HIV-associated cryptococcal meningitis (CM) appears to remain a cause of substantial morbidity and mortality in sub-Saharan African. This study determined the prevalence, associated factors and outcomes of CM among HIV positive meningitis patients at a tertiary hospital in Zimbabwe.

**Methods:** Eligible HIV positive patients admitted with clinical features of meningitis were recruited into the study. CM was diagnosed on the basis of a positive cerebrospinal fluid (CSF) culture for cryptococcus species, a positive CSF cryptococcal antigen test (CRAG) or a positive CSF India ink test. Patients’ demographic information, clinical features and laboratory test values were recorded.

**Results:** One hundred and forty-four participants were enrolled into the study. CM was diagnosed in 41% (59/144). Of the patients with CM, slightly more than half [54.2% (32/59)] were on ART and 53% (17/32) had initiated ART within one year prior to the diagnosis of CM. Median current CD4 counts were significantly lower among CM patients [32.0(IQR 10.5 – 64.0)] compared to non-CM patients [158.0(IQR: 47.0–324.0)] (p<0.001). In-hospital mortality associated with CM was 45%.

**Conclusions:** The proportion of cryptococcal meningitis cases and the associated in-hospital mortality were very high in the study. This suggests that cryptococcal disease burden is still significantly high despite improved access to ART. Expansion of prevention strategies, such as screening for asymptomatic cryptococcal infection among patients with low CD4 count (less than 200), has the potential to improve the morbidity and mortality associated with cryptococcal meningitis.
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<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
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<tr>
<td>AmB</td>
<td>Amphotericin B</td>
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<td>ART</td>
<td>Anti-retroviral therapy</td>
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<td>CRAG</td>
<td>Cryptococcal antigen</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CM</td>
<td>Cryptococcal meningitis</td>
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<td>ELISA</td>
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<td>FBC</td>
<td>Full Blood Count</td>
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<td>Non-CM</td>
<td>Non-cryptococcal meningitis</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>IRIS</td>
<td>Immune Reconstitution Inflammatory Syndrome</td>
</tr>
<tr>
<td>UZCHS</td>
<td>University of Zimbabwe College of Health Sciences</td>
</tr>
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<td>WHO</td>
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1.0 Introduction and background

1.1 Introduction

Cryptococcus neoformans and cryptococcus gatti are ubiquitous environmental fungal pathogens. They cause a life threatening infection in patients with intact and compromised immune systems. The significance of cryptococcal infection has changed dramatically in the last 30 years owing in large part to the expanding numbers of patients with acquired immune-suppression from HIV infection and medications, such as corticosteroids, cytotoxic treatments for malignancies and therapies to prevent organ transplantation rejection. Cryptococcal meningitis is the most common and severe form of cryptococcal infection. Among patients with HIV/AIDS, cryptococcal disease contributes to more than 600 000 deaths per year globally.\(^1\) Sub-Saharan Africa has the greatest burden of disease, contributing more than 75% of the cases and 80% of the deaths due to cryptococcal disease worldwide.\(^1\) In the United States cryptococcal infection is increasingly recognised as a major cause of invasive fungal infection in solid organ transplant recipients and has been identified as the third commonest fungal infection following candida and aspergillus species.\(^2\)

1.2 Background

Cryptococcal disease, often manifesting as cryptococcal meningitis (CM), is a leading cause of morbidity and mortality among patients with HIV infection. The HIV pandemic has changed the face of disease patterns especially in sub-Saharan Africa where the prevalence of HIV infection is highest. Consequently the highest global burden of cryptococcal disease is in Southern Africa with a median yearly incidence of 3.2% (720 000 cases; range, 144 000–1.3 million).\(^1\) A study of the causes of
meningitis in Zimbabwe in 1994 showed that the most common cause of meningitis in patients admitted to two central hospitals was CM, accounting for 45% of cases. Before antifungal therapy was widely available in Zimbabwe, the median survival after diagnosis of CM was 14 days and only 22% of patients survived beyond 30 days.

Other African HIV cohorts conducted showed a devastating mortality among CM patients that approached 100% at 6 months with or without treatment. Factors associated with the high mortality include abnormal mental status, high organism load (culture or antigen titer), raised cerebrospinal fluid (CSF) opening pressure, low CSF white cell count and low patient Karnofsky performance status.

Management of patients diagnosed with CM has evolved over the years but has generally remained disappointing. Mortality still remains high despite antifungal treatment. In a study done in South African where patients were treated with amphotericin B (AmB) and/or fluconazole (antifungal drugs), there was a 14 day and 90 day survival of 68% and 41% respectively.

Studies have shown that CM largely develops in individuals with CD4 counts less than 200 cells/µL, and usually less than 100 cells/µL. Therefore, getting many HIV patients onto anti-retroviral therapy (ART) and immune reconstituted is a means of avoiding the development of CM and alleviating the morbidity and mortality due to CM. Many African countries have scaled up provision of ART to patients over the past 10 years. According to a 2013 Zimbabwe National AIDS Council third quarter report, there were 644,214 individuals on ART out of an estimated total of 962,779 individuals in need of ART. This represents overall coverage of 66.9% (Adults 68.7%, Children= 51.9%) against a set target of 87% for adults and 62% for children.
Despite the scaling up of ART provision in Africa, some investigators have noted an on-going high burden of CM.\textsuperscript{13} This may be partly due to the fact that in many African settings, the numbers of patients presenting to health services with advanced immune-suppression still exceeds the capacity of ART programs and large numbers of patients die without accessing ART.\textsuperscript{13}

It is therefore of interest to define the current contribution of CM to morbidity and mortality among HIV patients in Zimbabwe. A similar study was done 20 years ago and the public health programme to improve access to ART started 10 years ago. Antifungal therapy has also become increasingly available for patients with CM.

1.3 Problem Statement

Cryptococcal meningitis is a common infection in severely immune-suppressed patients in Zimbabwe as well as sub-Saharan Africa and accounts for one third of all HIV-related deaths.\textsuperscript{6, 14} Despite improved access to ART in Africa, reports suggest that the burden of cryptococcal disease is still high (compared to the pre-ART era). There is limited availability to rapid and accurate diagnosis of CM in resource limited settings and mortality from the infection remains unacceptably high with currently available treatment regimens.

2.0 Literature Review

The cryptococcus genus has been divided into four serotypes using capsular agglutination assays. These are cryptococcus neoformans var. grubii (formerly serotype A), cryptococcus neoformans var. neoformans (formerly serotype D) and cryptococcus gatti (formerly serotypes B and D). Cryptococcus neoformans var. grubii has a worldwide distribution and its importance as a human pathogen has
increased substantially secondary to the expanding numbers of patients with profound immune-suppression. The vast majority of HIV-related cryptococcal infection is caused by var. grubii strains.\textsuperscript{15} The widespread adoption of effective anti-retroviral therapy has led to a decrease in the incidence of cryptococcosis and a diagnosis of CM in western countries is now considered a marker of poor access to health care.\textsuperscript{16} However, similar improvements have not been achieved in the developing world. In parts of Africa, cryptococcus neoformans var. grubii is the first or second most common cause of culture-proven meningitis.\textsuperscript{3} Cryptococcus neoformans var. grubii is also an important cause of disease in patients without HIV infection.\textsuperscript{17} Most of the patients will have an identifiable risk factor for cryptococcal infection e.g. steroid therapy, solid organ transplant, chronic renal or hepatic failure, a rheumatologic disorder, chronic lung disease or malignancy but others will have apparently normal immune systems. Interestingly cryptococcosis is rarely seen in cancer patients and in bone marrow transplant recipients, despite the high incidence of other fungal infections in this population.\textsuperscript{18} Cryptococcus neoformans var. neoformans is found predominantly in Western Europe and it usually causes disease in immune-compromised hosts. Studies indicate that cryptococcus neoformans var. neoformans may cause less severe disease compared with cryptococcus neoformans var. grubii.\textsuperscript{19} Cryptococcus gatti infections frequently occur in hosts with seemingly intact immune systems. This was illustrated in a study in Australia and New Zealand where both cryptococcus neoformans var. grubii and cryptococcus gatti are endemic. Isolated lung or brain lesions (cryptococcomas) were more commonly seen in cryptococcus gatti infection, especially among immune-competent hosts.\textsuperscript{20}
2.1 Clinical features and diagnosis of cryptococcal meningitis

Symptoms typically begin indolently over a period of one to two weeks. The most common symptoms are fever, malaise, and headache.\textsuperscript{21} Neck stiffness, photophobia, and vomiting are seen in one-fourth to one-third of patients. Clinical disease is rarely fulminant but in such cases the patient may present with coma and rapid progression to death.

The initial physical examination may not reveal much. In one report, 24 percent of patients had altered mental status on presentation and 6 percent presented with focal neurologic deficits.\textsuperscript{21} Other manifestations of disseminated disease may be evident, including tachypnoea and skin lesions resembling molluscumcontagiosum.\textsuperscript{22} Visual and hearing loss have also been reported.\textsuperscript{23, 24}

The definitive diagnosis of cryptococcal meningo-encephalitis is made by culture of the organism from the cerebrospinal fluid (CSF). The diagnosis can be difficult given the sub-acute onset of symptoms and non-specific presentation. A high index of suspicion should be maintained in patients with advanced HIV infection who have signs and symptoms referable to the central nervous system (CNS).

Lumbar puncture with measurement of the opening pressure and examination of the CSF with India ink staining should suggest the diagnosis in most cases. Because the burden of organisms is usually high in AIDS patients, the India ink preparation and culture of the CSF are frequently positive in these hosts. The opening pressure may be markedly elevated in patients with AIDS. Almost 70 percent of reported patients have opening pressures greater than 20 cm of water on the initial spinal tap.\textsuperscript{25}
Examination of the CSF with India ink will show the typical encapsulated yeast forms in approximately 75 percent of patients. White blood cell (WBC) counts in the CSF are characteristically low (<50/µL) with a mononuclear predominance. Protein levels are usually only slightly abnormal; glucose concentrations can be low. In a study of the management of increased intracranial pressure in 381 patients who were stratified into five groups based on their baseline opening pressures, CSF glucose concentrations ranged from 2.4 to 2.7 mmol/L.\textsuperscript{25} Approximately 25 to 30 percent of AIDS patients with cryptococcal meningo-encephalitis have normal CSF profiles on initial examination.\textsuperscript{26}

Cryptococcal antigen can be detected in serum and CSF through immunodiagnostic techniques, such as latex agglutination or sandwich enzyme-linked immunosorbent assay (ELISA). A positive antigen test can suggest the presence of infection well before the cultures become positive. The antigen test is very sensitive and specific in the CSF. In a study, 4 latex agglutination test assays and one enzyme linked immunoassay all performed well with sensitivities ranging from 93 to 100 percent and specificities from 93 to 98 percent.\textsuperscript{27} False positive tests can result from infection due to the fungus Trichosporon asahii (formerly Trichosporon beigeli) or bacteria of the Stomatococcus and Capnocytophaga genera.\textsuperscript{28, 29} These false positive results are usually present with low titers. False positive CSF cryptococcal antigen (CRAG) results have been reported rarely following exposure of samples to disinfectants or soap, or after samples were placed into an anaerobic transport vial.\textsuperscript{30, 31} Reports of false negative cerebrospinal fluid latex agglutination with culture positive CM is noted in literature.\textsuperscript{32} Reasons for this include low CSF CRAG titer and type of antigen test kit used. Such patients may pose a diagnostic challenge.
Testing for cryptococcal antigen in serum can also be performed. In patients with AIDS, the sensitivity of serum antigen testing is comparable to CSF testing, and is a useful screening test in patients who cannot undergo lumbar puncture. The use of serum cryptococcal antigen for screening may be of high clinical utility in areas where cryptococcal infection is endemic. Antigen titers generally correlate with organism burden. However, titers are not helpful in management of acute disease in HIV-infected patients since changes in titer do not correlate with clinical response. Also the slope of decline is not helpful in predicting those patients who may relapse.

2.2 Treatment of cryptococcal infection

Worldwide, guidelines for the treatment of cryptococcal meningo-encephalitis are largely adopted from the Infectious Disease Society of America (IDSA) practice guidelines and World Health Organization (WHO). Patients should be managed with induction courses of amphotericin B deoxycholate (AmB, 0.7-1mg/kg/day) and flucytosine (100mg/kg/day) for 2 weeks followed by consolidation therapy with high dose fluconazole (400mg/day) for 8 weeks in those patients with initial clearance of the CNS infection, as documented by a negative CSF culture at 2 weeks of therapy. Subsequent discontinuation of fluconazole therapy in immune-suppressed patients leads to unacceptably high relapse rates, therefore maintenance therapy with lower dose fluconazole (200mg/day) is recommended for an indefinite period of time, depending on immune reconstitution. In a randomised trial, 40% and 28% of patients had positive CSF cultures for cryptococcus neoformans at 2 and 10 weeks of therapy, respectively and inadequate CSF fungal clearance was associated with poorer outcomes. Mortality rates as high as 26% have been reported after
successful completion of 10 weeks of therapy. Resource-limited settings (RLS) often have erratic supplies of AmB. Through the Diflucan Partnership Program, fluconazole has been made available in RLS and many times this drug is the only option available for patients. Several studies have demonstrated that clearance of cryptococcus neoformans from the CSF is significantly faster with AmB and flucytosine than with AmB alone, and the lack of flucytosine during therapy may be associated with mycological failure at 2 weeks of therapy. In-vivo synergy has been demonstrated with AmB and flucytosine. However AmB and flucytosine are toxic compounds and their use in developing countries is often impractical owing to the inability to manage intravenous infusions and adequately monitor for drug toxicities. AmB may cause acute infusion-related hypersensitivity reactions but is more commonly associated with chronic infusion-related nephrotoxicity. This is manifested by a rise in serum creatinine and the loss of electrolytes such as potassium and magnesium through the kidneys. The renal impairment is often reversible but some patients may go on to require renal replacement therapy. As a consequence of nephrotoxicity, antifungal treatment may be stopped despite a life-threatening fungal infection. The introduction of liposomal AmB (LAmB) has helped in reducing the risk and incidence of AmB-related toxicities. LAmB is at least as efficacious as AmB deoxycholate. However LAmB is not available in RLS. Because of the limitations regarding use of AmB, more efficacious and better tolerated antifungal treatments are desired. The newer azoles such as voriconazole and posaconazole have not been extensively investigated but indications are that they are unlikely to have a role in the management of cryptococcal infection. The novel echinocandin class of antifungal agents such as micafungin and capsofungin is not effective against cryptococcal infection.
An important aspect of treatment of CM is the management of raised intracranial pressure (ICP) which is an important contributor to morbidity and mortality. The most likely mechanism for raised ICP is that the fungal polysaccharide antigen interferes with CSF absorption through the arachnoid villi. Up to 75% of patients had an elevated baseline ICP (≥20cm of water) in one study. An increase in ICP among HIV-infected patients after 2 weeks of treatment was associated with a poorer clinical response. Hydrocephalus is often inconsistently managed even in resource-rich settings. In a United States audit, ‘major deviations’ from the IDSA treatment guidelines regarding elevated ICP management were observed, i.e. failure to measure opening pressure and/or failure to lower the opening pressure within 72hrs. Treatment options for managing elevated ICP include sequential lumbar punctures to drain CSF, insertion of a lumbar drain and placement of a ventriculo-peritoneal shunt. Medical therapy has no proven efficacy.

2.3 Prevention of cryptococcal disease

Prevention of cryptococcal disease is a new and exciting area. Thus far attempts at treating CM have been disappointing especially in sub-Saharan Africa. The development of latex agglutination and enzyme immunoassay tests for detection of cryptococcal antigen (CRAG) in CSF and serum has markedly improved sensitivity and specificity in diagnosis of CM. This test has also found value in screening for asymptomatic infection. Studies have shown that overt cryptococcal disease is often preceded by a period of asymptomatic cryptococcal antigenaemia in the serum (or CSF) and this provides an opportunity for preventing development of disease. Presence of CRAG in serum is a strong independent risk factor for the development of CM and death after initiation of ARVs. A positive serum CRAG is the most important risk factor for the development of CM.
Syndrome (IRIS). A study from Uganda demonstrated that positive serum CRAG preceded the onset of clinical symptoms by a median of 22 days (5 – 234 days). In a study done in South Africa, the prevalence of asymptomatic positive serum CRAG in HIV infected patients was 7% and two studies in Uganda each showed a prevalence of 5.8% and 8.2%. Studies have also demonstrated that the greatest benefit in screening for serum CRAG was among patients with a CD4 count less than or equal to 100 cells/µL.

The World Health Organisation (WHO) now recommends screening for CRAG among patients enrolling for ART with CD4 less than 100 because of the potential for preventing overt cryptococcal disease by implementing targeted pre-emptive therapy. Cost-effectiveness of CRAG screening has been evaluated in a prospective study in Kampala, Uganda. Among patients who tested positive for serum CRAG, pre-emptive fluconazole use was associated with better survival. At analysis of cost of screening for CRAG versus cost of AmB treatment, the authors found that, at antigen prevalence greater than 3%, screening was more cost-effective than AmB treatment. The ideal management of patients with positive serum CRAG at screening is not known as there are no randomised studies that have been done. According to the WHO recommendations, patients with positive serum CRAG should be tested for CSF infection. Asymptomatic patients with positive CSF CRAG are treated as CM patients. Patients without disseminated infection or CSF involvement are treated with fluconazole for 10 weeks.

An exciting development in cryptococcal disease is the Lateral Flow Assay (LFA) which is a simple point-of-care antigen-antibody test akin to the malaria rapid diagnostic test or pregnancy test strip. This test has great promise to enable rapid and accurate diagnosis of cryptococcal infection and can be applied as a cheap point
of care test for use in the most remote setting. The LFA test kit does not require refrigeration or special specimen preparation; can be used by non-laboratory staff and gives a result in 15 minutes. The LFA has been tested on CSF, serum and urine shown to be very sensitive when compared to traditional latex agglutination and enzyme-immunoassay. \textsuperscript{49, 50} Studies are underway to determine its sensitivity when used on whole blood and plasma. The WHO endorses the use of the LFA as an alternative to latex agglutination or enzyme immunoassay in screening patients for cryptococcal infection.\textsuperscript{37} However the LFA is not yet available for use in Zimbabwe.

With the promising developments in screening and diagnosis of cryptococcal infection, prevention should become a practical and useful strategy to improve the morbidity and mortality of cryptococcal meningitis. Rapid diagnosis of symptomatic disease enables prompt initiation of treatment. RLS still have a lot of challenges in dealing with cryptococcal infection.

3.0 Justification, research question and objectives

3.1 Justification

J. G. Hakim et al. looked at the impact of HIV infection on meningitis in Zimbabwe almost 20 years ago.\textsuperscript{3} Since then ART has become widely available, as well as antifungal therapy. Zimbabwe has been credited with significant achievements in ART coverage and HIV prevalence.\textsuperscript{12, 51} A cryptococcal prevalence study would define the effect of ART on HIV associated meningitis in Zimbabwe. Two prospective cohorts of HIV-infected patients in Uganda (one before and the other after the availability of ART) concluded that significant CM mortality persisted despite amphotericin B and HIV therapy.\textsuperscript{52}
Prevalence data enables development of local management algorithms that help to rapidly identify and risk stratify CM patients. This is often necessary in resource-limited settings where rapid access to reliable diagnostic tests is limited. The aspect of possible differences in the way CM patients present depending on whether they are taking ART is also of interest. This may necessitate new approaches to diagnosis and treatment. In the prior Zimbabwean study, headache was significantly associated with CM compared to other causes of meningitis.\textsuperscript{3} Neck stiffness and fever were more common in other forms of meningitis, and lower Glasgow Coma Scale (GCS) was more common in tuberculous and pyogenic meningitis compared to CM. The use of ART may modify these clinical features.

This is also an opportunity to strengthen strategies for prevention of disease. Since the strongest risk factor for developing CM is severe immune-suppression, the information obtained from this study may be an indicator of the progress of Zimbabwe’s ART programme.

3.2 Research Question
What is the proportion of cryptococcal meningitis cases compared to non-cryptococcal meningitis among HIV patients admitted with clinically suspected meningitis at a tertiary institution in Harare, Zimbabwe?

3.3 Hypothesis
In a cross-sectional study at a tertiary hospital, the proportion of patients with clinically suspected meningitis who will be positive for cryptococcal meningitis will be less than or equal to 45%.
3.4 Study Objectives

3.4.1 Primary Objective

1. To determine proportion of cryptococcal meningitis cases among patients with suspected or confirmed meningitis

3.4.2 Secondary Objectives

1. To determine the short-term in-hospital outcome of patients with cryptococcal meningitis. Outcomes of interest are death and survival to discharge
2. To identify factors associated with a diagnosis of CM and compare features between CM and non-CM patients
3. To compare CSF and serum CRAG latex agglutination positivity among patients with suspected or confirmed meningitis

4.0 Research Methodology

4.1 Study design

This was a descriptive prospective cohort study

4.2 Study setting

The study was conducted at Parirenyatwa Hospital, a large referral hospital in Harare, the capital city of Zimbabwe. Parirenyatwa hospital and two other referral hospitals within Harare probably cater for at least two-thirds of the country’s referrals to central hospitals. Parirenyatwa hospital also provides district health services to the local Harare community.
4.3 Study Population

Consecutive patients admitted with a clinical suspicion of acute or chronic meningitis to Parirenyatwa hospital adult medical wards were eligible for screening.

4.4 Inclusion criteria

The following were included in the study:

- Patients 18 years and above, male or female
- Patients who were HIV positive. Documented HIV positive status was sought before inclusion. Pre-test counseling was done and HIV testing offered to patients whose HIV status was unknown. This was done with the assistance of the nursing staff caring for the patient.
- Patients who were admitted with a clinical or suspected diagnosis of acute or chronic meningitis as determined by the admitting physicians

4.5 Exclusion criteria

- Patients with a previous known diagnosis of cryptococcal meningitis were excluded.

4.6 Sample size determination and sampling plan

According to Hakim et al.\textsuperscript{3} cryptococcal meningitis accounts for 45% of meningitis cases in HIV patients. Using the Dobson formula with 95% confidence interval and 5% precision, the required sample size was 381 participants.

4.7 Method of data collection

Definitions

Suspected meningitis: Patient admitted with symptoms and signs suggestive of meningitis as determined by the admitting physicians. Symptoms and signs included
some or all of the following - headache, vomiting, photophobia, seizures, altered mental status/coma, fever, neck stiffness and focal neurological deficits.

**Confirmed meningitis:** Patient with suspected meningitis and CSF biochemical and/or microbiology features consistent with meningitis. This included demonstration/evidence of a typical causative organism in the CSF

**Cryptococcal meningitis:** The presence of cryptococcus neoformans in the CSF of a suspected meningitis patient. The definition of CM was on the basis of a positive CSF culture for cryptococcus species and/or a positive CSF CRAG test and/or a positive CSF India Ink test.

**Non-cryptococcal meningitis:** suspected or confirmed meningitis patient with negative CSF tests for cryptococcus species.

**Patient recruitment**

After screening, written informed consent was sought from eligible patients in English or Shona which is a local language for the majority of Zimbabweans. Due to severe illness and altered mental status, some patients could not provide consent. In these cases consent was sought from the closest relative of the participant. For confidentiality purposes, the relatives were not informed of the patient’s HIV status (if they did not already know) and the relevant consent form did not include issues of HIV status. When the patient regained consciousness, the study was explained to them and they were free to give their consent and continue to participate or to withdraw from the study. Patients who presented with disorientation and unknown HIV status were enrolled if a suitable relative was available to give consent. However the patient’s HIV status was not disclosed. For patients meeting eligibility criteria and
were willing to participate, a history of presenting complaints and past history was obtained, either from participant or relative, followed by examination of the patient. A data collection form was used to collect the relevant information. Enrolled patients were initiated on meningitis treatment by the treating physicians on the wards, pending laboratory tests, according to the standard guidelines.

**Laboratory measurements**

Enrolled participants had the following procedures done: lumbar puncture to obtain a CSF specimen for cryptococcal tests. At the same time a CSF specimen was given to the treating physicians for routine non-cryptococcal tests. CSF opening pressures were not measured at the time of lumbar puncture because of unavailability of manometers. Venepuncture was also done to obtain a blood specimen. Specimens were processed the same day in the Microbiology Laboratory of the University of Zimbabwe, College of Health Sciences. Blood was allowed to clot for 15 minutes and serum was aspirated into a separate tube. CSF and serum specimens were centrifuged for 15 minutes and the supernatant was used for CRAG test while the sediment was used for India ink and culture for cryptococcus. For CRAG tests, the Cryptococcal Antigen Latex Agglutination System (CALAS) testing kits were used. The kit is a product of the Meridian Bioscience Incorporated based in the United States. It is a qualitative and semi-quantitative latex test which detects capsular polysaccharide antigens of cryptococcus neoformans in serum and cerebrospinal fluid with a reported sensitivity and specificity of 100%.

**CRAG testing:** Controls were run daily to ensure reliable results. After centrifuge, 25µL of CSF supernatant were placed in each of two labeled rings and a drop of detection latex (latex particles coated with anti-cryptococcal globulin) was added to
each ring. A drop of control latex (latex particles coated with normal globulin) was also added to each ring and the contents of the rings mixed. Results were read immediately and strengths of reactions graded as indicated below. For serum, after centrifuge, 200µL of serum were mixed with 200µL of pronase and incubated for 15 minutes at 56 degree Celsius. The mixture was then boiled for a further 5 minutes and allowed to cool to room temperature. Treatment with pronase is recommended by the manufacturer and allows reduction of false positive results. Then 25µL of pronase-treated serum was aspirated and added to 2 rings according to the procedure outlined above for CSF. After addition of detection latex the strength of reactions were read as follows:

- Negative (-) a homogenous suspension of particles with no visible clumping
- One plus (+) fine granulation against milky background
- Two plus (2+) small but definite clumps against slightly cloudy background
- Three plus (3+) large and small clumps against a clear background
- Four plus (4+) large clumps against a very clear background

Negative and 1+ are normally considered as negative CRAG test. However 1+ may be suggestive of cryptococcal infection. Patient specimens showing a 2+ or greater agglutination were considered presumptive evidence of cryptococcal infection (CRAG positive). Reaction of 2+ and greater is supposed to be followed up with serial dilutions to determine titer but this was not done because of financial limitations.

*CSF India ink and culture procedure:* The sediment (after centrifuge) was examined under a microscope with India ink stain to identify the presence or absence of yeasts cells of cryptococcus species. An amount of 0.5 – 1.0 ml of the sediment was
inoculated onto Sabauroud’s dextrose agar plate for aerobic culture. Culture results were read after 72 hours and after 7 days and recorded as positive culture (growth) or negative culture (no growth) for cryptococcus species.

Patient’s CSF India ink, CSF and serum CRAG and CSF culture results were recorded. In addition the following laboratory test results (which were done as part of routine care by the treating doctors) were also recorded i.e. Full Blood Count (FBC), serum electrolytes, current or most recent CD4 count, CSF protein, CSF glucose and CSF gram stain.

When cryptococcal test results became available, the treating doctors were informed and treatment was adjusted according to local guidelines. Consent was sought for the storage of left-over specimens which were refrigerated in the University of Zimbabwe Bacteriology Laboratory for the purpose of future meningitis-related studies.

Patients in whom a diagnosis of cryptococcal meningitis was made were followed up for a determination of short-term in-hospital outcome - either survival to discharge or death.

4.8 Statistical analysis plan

Patients’ socio-demographic and clinical characteristics and measurement results for the main variables were evaluated using descriptive statistics i.e. frequency tables. Proportions were used to compare differences in various categories and the standard method for comparing proportions, chi-squared test ($\chi^2$ test) or Fishers exact test were used. Descriptive statistics were used to identify factors associated with a diagnosis of CM and death among CM patients. Analysis was performed using Stata version 12.0 (Stata Corporation, College Station, Texas, USA). All statistical
evaluations were carried out using p=0.05 as the level of significance. Means (standard deviations) or medians (inter-quartile ranges) were used where necessary.

4.9 Permission to carry out the study and ethical considerations

Permission to carry out the study and ethical clearance was granted by the institutional Joint Research and Ethics Committee (JREC) and the Medical Research Council of Zimbabwe (MRCZ). The results of cryptococcal testing for enrolled patients were made available to the treating doctors as soon as they were available because they were necessary for treatment decisions. CRAG latex agglutination is currently the most routinely used test for diagnosis of CM because of its quick turn-around time and reliability. However the test is expensive and not routinely available even at central hospitals. In many patients the diagnosis of CM is delayed. The study helped the treating physicians get quick and reliable cryptococcal test results for their patients; a situation which is not universal in routine practice. Therefore decisions about treatment were made early. Only the results of cryptococcal testing were made available to the treating doctors. All other information collected as part of the study was kept confidential unless if it had direct bearing on the patient’s care. Beyond the initial diagnosis of cryptococcal infection, the study was not responsible for any subsequent cryptococcal tests that may have been required for monitoring or other purposes of patient care.

5.0 Results

From June 2013 to November 2013, 144 patients with suspected meningitis were consecutively enrolled into the study. The criteria for cryptococcal meningitis were satisfied in 59 participants giving a proportion of 41%. The following diagram shows the study procedure.
Figure 1: Flow diagram showing study procedure

Enrolled (n=144)

CSF culture
CSF CRAG
CSF india ink
Serum CRAG

125
Negativ
e

45 CSF culture pos

80 Perpos

14 India ink pos

5 India ink neg

42 CSF CRAG pos, 3 CSF CRAG neg
34 CSF india ink pos, 11 CSF india ink neg
42 serum CRAG pos, 3 serum CRAG neg

19

= cryptococcal meningitis cases

Pos = positive

Neg = negative
Table 1 below shows baseline features among participants with CM.

**Table 1: Baseline clinical and demographic features among participants with CM**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Present (%)</th>
<th>Absent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache (n=58)</td>
<td>55 (94.8)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>Vomiting (n=55)</td>
<td>33 (60)</td>
<td>22 (40)</td>
</tr>
<tr>
<td>*Disorientation (n=54)</td>
<td>30 (55.6)</td>
<td>24 (44.4)</td>
</tr>
<tr>
<td>Cough (n=56)</td>
<td>16 (28.6)</td>
<td>40 (71.4)</td>
</tr>
<tr>
<td>Female (n=59)</td>
<td>27 (45.8)</td>
<td>32 (54.2)</td>
</tr>
<tr>
<td>ART (n=58)</td>
<td>32 (55.2)</td>
<td>26 (44.8)</td>
</tr>
<tr>
<td>GCS 13 – 15 (n=58)</td>
<td>54 (93.1)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Neck stiffness (n=57)</td>
<td>32 (56.1)</td>
<td>25 (43.9)</td>
</tr>
</tbody>
</table>

*disorientation reported at any point during the illness. GCS Glasgow coma scale. *some data were missing

Notably, slightly more than half of participants with CM (55.2%) were taking ART at the time of diagnosis of CM; ranging from those who had commenced ART within the week prior to CM diagnosis to those who had been on ART for many years. The majority of participants with CM complained of headache and most participants with CM had GCS 13-15.

Table 2 compares some baseline clinical features between CM patients and non-CM patients.
Table 2: Comparison of clinical features between CM and non-CM patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CM (%)</th>
<th>Non-CM (%)</th>
<th>$\chi^2$ test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male (n=76)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n=68)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32(42.1)</td>
<td>44(57.9)</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27(39.7)</td>
<td>41(60.3)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Age(years) (n=139):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36.8(±11.0)</td>
<td>38.6(±10.3)</td>
<td>0.315</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache (n=115)</td>
<td>55(47.8)</td>
<td>60(52.2)</td>
<td>0.510</td>
<td></td>
</tr>
<tr>
<td>Vomiting (n=62)</td>
<td>33(53.2)</td>
<td>29(46.8)</td>
<td>0.473</td>
<td></td>
</tr>
<tr>
<td>Disorientation (n=83)</td>
<td>30(36.1)</td>
<td>53(63.9)</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>Cough (n=43)</td>
<td>16(37.2)</td>
<td>27(62.8)</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Sputum (n=21)</td>
<td>9(42.9)</td>
<td>12(57.1)</td>
<td>0.355</td>
<td></td>
</tr>
</tbody>
</table>

The variables that were significantly different between the two groups were disorientation and cough where non-CM patients were more likely to have reported disorientation and cough. There were some differences with gender where among females, the greater percentage had non-CM (p=0.016).
Table 3 shows more variables.

### Table 3: Comparison of clinical features between CM and non-CM patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CM (%)</th>
<th>Non-CM (%)</th>
<th>χ² test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consciousness level (GCS):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 – 12 (n=15)</td>
<td>4(26.7)</td>
<td>11(73.3)</td>
<td>0.011</td>
</tr>
<tr>
<td>13 – 15 (n=119)</td>
<td>54(45.4)</td>
<td>65(54.6)</td>
<td>0.154</td>
</tr>
<tr>
<td><strong>Tuberculosis (past history) (n=35)</strong></td>
<td>19(54.3)</td>
<td>16(45.4)</td>
<td>0.473</td>
</tr>
<tr>
<td><strong>Past history of non-CM (n=13)</strong></td>
<td>5(38.5)</td>
<td>8(61.5)</td>
<td>0.239</td>
</tr>
<tr>
<td><strong>Neck stiffness (n=71)</strong></td>
<td>32(45.1)</td>
<td>39(54.9)</td>
<td>0.240</td>
</tr>
<tr>
<td><strong>Focal neurological deficits (n=13)</strong></td>
<td>7(53.9)</td>
<td>6(46.2)</td>
<td>0.695</td>
</tr>
<tr>
<td><strong>ART (current) (n=77)</strong></td>
<td>32(41.6)</td>
<td>45(58.4)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Fluconazole (current) (n=7)</strong></td>
<td>3(42.9)</td>
<td>4(57.1)</td>
<td>0.593</td>
</tr>
<tr>
<td><strong>Anti-TB drugs (current) (n=17)</strong></td>
<td>7(41.2)</td>
<td>10(58.8)</td>
<td>0.304</td>
</tr>
<tr>
<td><strong>Abnormal CXR (n=25)</strong></td>
<td>9(36.0)</td>
<td>16(64.0)</td>
<td>0.048</td>
</tr>
</tbody>
</table>

*GCS – Glasgow coma scale

Participants with GCS 9 – 12 were more likely to have non-CM (p=0.011) and more patients on ART ended up with non-CM (p=0.036). Abnormal chest x-rays were noted more often among non-CM patients (p=0.048).

The following table compares laboratory features between CM and non-CM patients.
Table 4: Baseline laboratory values and comparison between CM and non-CM patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>CM</th>
<th>Non-CM</th>
<th>(x^2) - p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF protein [median(IQR)]</td>
<td>0.9(0.6-1.63)</td>
<td>0.9(0.6-1.7)</td>
<td>0.8(0.5-1.5)</td>
<td>0.333</td>
</tr>
<tr>
<td>(n=129)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF glucose [median(IQR)]</td>
<td>2.8(1.8-3.5)</td>
<td>2.5(1.6-3.0)</td>
<td>3.10(1.9-3.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(n=132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current CD4 [median(IQR)]</td>
<td>62.0(19.0-197.0)</td>
<td>32.0(10.5-64.0)</td>
<td>158.0(47.0-324.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(n=85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCC [median(IQR)]</td>
<td>5.1(3.6-6.8)</td>
<td>4.8(3.5-6.0)</td>
<td>5.7(3.9-7.0)</td>
<td>0.300</td>
</tr>
<tr>
<td>(n=119)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin [median(IQR)]</td>
<td>10.9(8.9-13.1)</td>
<td>11.0(9.5-13.0)</td>
<td>10.6(8.9-13.3)</td>
<td>0.653</td>
</tr>
<tr>
<td>(n=119)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV [median(IQR)]</td>
<td>90.0(83.5-95.4)</td>
<td>91.3(85.5-96.7)</td>
<td>88.0(83.0-93.0)</td>
<td>0.036</td>
</tr>
<tr>
<td>(n=119)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets [median(IQR)]</td>
<td>262.0(167.0-328.0)</td>
<td>290.0(199.0-353.0)</td>
<td>227.0(142.5-300.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>(n=118)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium [median(IQR)]</td>
<td>132.0(127.0-136.0)</td>
<td>132.0(126.0-136.0)</td>
<td>133.0(128.0-137.0)</td>
<td>0.543</td>
</tr>
<tr>
<td>(n=118)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium [median(IQR)]</td>
<td>4.2(3.6-4.7)</td>
<td>4.2(3.6-4.8)</td>
<td>4.2(3.7-4.7)</td>
<td>0.832</td>
</tr>
<tr>
<td>(n=119)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea [median(IQR)]</td>
<td>4.6(3.1-6.4)</td>
<td>3.9(2.8-5.4)</td>
<td>5.2(3.2-7.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>(n=118)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine [median(IQR)]</td>
<td>77.5(60.5-105.5)</td>
<td>69.0(57.0-96.0)</td>
<td>81.0(64.0-117.0)</td>
<td>0.061</td>
</tr>
<tr>
<td>(n=116)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant differences were that patients with CM had lower median CSF glucose concentration (p<0.001) and lower median current CD4 count (p<0.001) compared to non-CM patients. In the FBC, patients with CM had higher median MCV.
(p=0.036), higher median platelet counts (p=0.002) and on serum electrolyte tests, CM patients had lower median urea (p=0.009) and lower median creatinine (p=0.061) concentration compared to non-CM patients.

In other results, among patients with CM and taking ART, 53% (17/32) had initiated ART within one year prior to the diagnosis of CM; and 47% (15/32) had initiated ART more than one year before the diagnosis of CM. Latex agglutination CRAG (CSF and serum) and CSF India ink were compared to CSF culture for the diagnosis of CM.

Table 5: Comparison of CSF India ink to CSF culture in diagnosis of CM

<table>
<thead>
<tr>
<th>CSF Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF India ink</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>80</td>
<td>91</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>80</td>
<td>125</td>
</tr>
</tbody>
</table>

*India ink sensitivity = 75.6%. India ink specificity = 100%. India ink PPV = 100%

Table 6: Comparison of CSF CRAG to CSF culture in diagnosis of CM

<table>
<thead>
<tr>
<th>CSF Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF CRAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>80</td>
<td>125</td>
</tr>
</tbody>
</table>

*CSF CRAG sensitivity =93.3%. CSF CRAG specificity = 96.3%

Table 7: Comparison of serum CRAG to CSF culture in diagnosis of CM

<table>
<thead>
<tr>
<th>CSF Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>80</td>
<td>125</td>
</tr>
</tbody>
</table>

*PPV of serum CRAG = 93%. NPP of serum CRAG = 96%
Outcomes among participants with CM

Outcome data were ascertained for 51 of the 59 participants with CM. The following table outlines the data.

### Table 8: Mortality rates among CM Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM cases</td>
<td>51</td>
<td>23 (45)</td>
</tr>
<tr>
<td>CM on ART</td>
<td>32</td>
<td>12 (37.5)</td>
</tr>
<tr>
<td>CM not on ART</td>
<td>27</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td>CM within 1 yr of starting ART</td>
<td>17</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>CM after 1 yr of starting ART</td>
<td>15</td>
<td>9 (60.0)</td>
</tr>
</tbody>
</table>

The overall mortality rate was 45%. Patients with CM while on ART had slightly lower mortality rate compared to those with CM without ART. Mortality was much lower among CM patients who had started ART within one year prior to meningitis diagnosis compared to those who had commenced ART more than one year before meningitis diagnosis. There was a trend towards higher overall mortality with lower CD4 counts as shown below.

### Table 9: Mortality rate according to CD4 count category

<table>
<thead>
<tr>
<th>CD4 range</th>
<th>Frequency (n=35)</th>
<th>Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 49</td>
<td>21</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>50 – 99</td>
<td>10</td>
<td>3 (30.0)</td>
</tr>
<tr>
<td>100 – 150</td>
<td>4</td>
<td>1 (25.0)</td>
</tr>
</tbody>
</table>
The median time to discharge was 24 days (range 11 – 40) and the median time to death was 14.5 days (range 7 – 22).

**6.0 Discussion**

Meningitis is one of the most serious infections occurring in HIV/AIDS patients and may be associated with greater mortality compared to respiratory or gastro-intestinal illnesses. In this study the proportion of CM cases was high. Despite that the sample size was relatively small; there are reasonable grounds to suggest that the burden of CM is still substantial despite widespread access to ART in Zimbabwe. This probably holds true for other sub-Saharan African countries. Current use of ART was associated with a protective effect on CM because among participants on ART a significantly greater percentage had non-CM. This would be expected as long as patients are on effective ART with high CD4 counts (typically greater than 200cells/µL). However among participants with CM, approximately half of them were on ART suggesting that patients diagnosed with CM were equally likely to be taking or not taking ART. It is likely the most important factor in this case, which was common to all CM patients regardless of ART status, was severe immune-suppression (median CD4 count <100cells/µL).

Fifty-three percent of participants with ‘CM on ART’ were diagnosed with meningitis within one year after starting ART. This group probably included those that had meningitis symptoms at the time of ART initiation (but diagnosis was missed), those that had subclinical cryptococcal infection and those that acquired the infection later (in the face of persisting low CD4). Therefore there is potential for preventing cryptococcal disease by screening for overt and subclinical cryptococcal infection among patients enrolling for ART. After one year of ART use, the risk of ART
treatment failure (either due to drug resistance or poor drug adherence) begins to increase. It can be assumed that the majority of participants with ‘CM on ART’ who had started ART more than one year before meningitis diagnosis, were failing ART. When the CD4 count falls below 200 cells/ µL, the risk for CM increases. Therefore early diagnosis and management of HIV drug resistance and ART treatment failure can also reduce the number of hospital cases of CM. In addition patients with ART treatment failure and low CD4 count (less than 200) are also targets for cryptococcal infection screening and pre-emptive fluconazole treatment.

Compared to non-CM participants, CM participants were less likely to report disorientation or cough in the history, less likely to have more severe impairment of consciousness (GCS 9 -12) and less likely to have an abnormal chest x-ray. However chest x-rays were usually done only when a participant had chest symptoms and/or signs in addition to meningitis. Possibly fewer participants with CM obtained chest x-rays because they tended to report cough less than non-CM participants. Therefore the possibility of asymptomatic chest x-ray abnormalities cannot be excluded among participants with CM.

In this study, there was a significant difference in median CD4 counts between CM and non-CM participants. This was in contrast to the study by Hakim et al. where CD4 counts could not discriminate between the different types of meningitis in the study (i.e. CM, pyogenic meningitis and tuberculosis meningitis) possibly because median CD4 counts were generally very low in that study. ART was not yet widely available. HIV infected patients with suspected meningitis now comprise a heterogeneous group in terms of degree of immune-suppression (CD4 count). The CD4 count is therefore a very important discriminator because higher CD4 counts (greater than 100 – 200) had negative predictive value for CM.
The finding of lower median CSF glucose concentration among participants with CM is interpreted with caution. Firstly plasma glucose was not routinely available for comparison with CSF glucose and the quality of the CSF biochemistry results cannot be ascertained as these tests were not handled by the investigators. However if there were IRIS cases among the ‘CM on ART’ participants, this may have contributed to these results because IRIS is associated with more inflammation and hence lower CSF glucose and higher CSF protein values (the median CSF protein was slightly higher among CM participants but this did not reach statistical significance).

It is uncertain at this point whether a true relationship is suggested by the significant difference in median platelet counts and median MCV between CM and non-CM participants. This requires further exploration. The significance of such a relationship is also not known. The lower median serum urea and creatinine concentrations in patients with CM may simply reflect lower body-mass index, lower muscle bulk and debilitation associated with severe immune-suppression.

Comparison of different tests in the diagnosis of CM revealed results that are consistent with what has been reported in literature. India ink had excellent positive predictive value when used for previously un-diagnosed patients. Serum CRAG also had very good positive and negative predictive value and when used for patients with suspected meningitis, serum CRAG may be very useful for establishing diagnosis or excluding CM where CSF testing cannot be done. This result indicates that bloodstream fungaemia is a pre-requisite for CNS or disseminated infection.

The observed in-patient mortality was higher in this study (45%) compared to the previous Zimbabwean study (39%). Comparing these 2 figures is difficult for the
following reasons. Firstly there were 8 participants in the current study whose outcomes could not be ascertained because patients were regularly shifted between wards (due to pressure for hospital beds) with poor transfer record keeping. This ‘lost to follow-up’ figure could significantly affect the current mortality rate either positively or negatively; giving a mortality rate ranging from 39\% - 52.5\%. Secondly no antifungal treatment was available in the older study and the investigators concluded that the ‘relatively low in-hospital mortality was due to early discharge from hospital’.³

In our study, all patients with CM were treated with amphotericin B (AmB) deoxycholate (0.7-1mg/kg/day) and/or fluconazole 800-1200mg/day according to local guidelines. Two studies done in South Africa showed a 14 day mortality rate of 32\% and 28\% among CM patients treated with AmB and/or fluconazole.⁸,⁵⁶

Compared to these figures, the mortality rate in our study was much higher.

Potentially modifiable factors contributing to mortality in this study include erratic supplies of AmB which often results in intermittent AmB dosing and suboptimal monotherapy with fluconazole. Interruption of treatment due to toxicity of AmB is also very common. AmB is known to cause azotaemia and loss of electrolytes due to tubular kidney damage in many patients during the course of treatment.⁴¹,⁴² These adverse effects are largely reversible but whether they are independently associated with mortality is not known. In addition, adequate management of raised intracranial pressure remains challenging and poor adherence to this has been noted in other studies. ⁴³

The risk of developing hospital-acquired infections increases with the longer duration of stay in hospital and this may have contributed to poor outcomes among participants with CM. This risk was studied in a retrospective cohort study in Uganda and South Africa which identified bacteraemia (associated with fever) due to drug
resistant staphylococcus, klebsiella, pseudomonas and other bacteria in hospitalised CM patients.53

The current use of ART was not associated with a significant difference in overall mortality among participants with CM possibly because both those on ART and those not on ART had severe immune-suppression (median CD4 < 100). CD4 count is often a strong and independent determinant of mortality and table 9 bears some evidence to this.

7.0 Conclusions and recommendations

Despite improvements in the ART coverage and the availability of anti-fungal medications, the morbidity and mortality of HIV-associated CM among hospitalised patients is still unacceptably high. Efforts should be directed at strengthening methods of preventing overt cryptococcal disease. As highlighted, a cryptococcal infection screening programme for patients enrolling for ART has the potential to prevent many CM cases. The WHO recommends a cryptococcal screening program and outlines the management of patients who are found positive at screening.37 Zimbabwean guidelines for HIV and ART care have recently been updated (December 2013) and now include a recommendation for cryptococcal screening.55 However screening in Zimbabwe is severely hampered by the cost of CRAG latex agglutination testing. It is therefore imperative that the new Lateral Flow Assay (LFA) test kits are acquired and made widely available for use. This new cryptococcal antigen test kit is cheap and can be used in remote settings to diagnose symptomatic and asymptomatic cryptococcal infection. The LFA has been endorsed by the WHO.
Improved detection and diagnosis of HIV drug resistance and ART treatment failure is another important arm for prevention of cryptococcal disease. This poses a significant challenge in RLS because it is best achieved with regular measurement of HIV viral load. In the absence of such, health workers require training to promptly recognise ART treatment failure either due to falling CD4 counts or development of an opportunistic illness. ART clinics also require systems that allow minimal delays in changing patient treatment where necessary.

The recent adjustment of ART initiation thresholds by WHO in 2013 to CD4 500 is a move in the right direction in terms of preventing cases of CM. More individuals will qualify for ART before developing severe immune suppression (CD4 < 200) and this may reduce further the number of patients developing CM without ART.

Where diagnostic facilities are limited, priority for CM testing may be given to a suspected meningitis patient who, in addition to symptoms and signs of meningitis, has a low CD4 count (typically less than 100 cells/µL), has normal or mildly impaired consciousness, lacks a cough and has a normal chest x-ray. A negative serum CRAG is very useful for excluding CM and may be done where the clinical probability for CM is low e.g. where CD4 count is high (typically greater than 200 cells/µL).

The treatment of CM is challenging, complicated and the medications are potentially toxic. Monitoring of infusions is difficult in resource-limited areas. There is extended hospital stay which is risky for already immune-suppressed individuals and is costly for institutions. Due to the intensity of treatment for CM in the acute setting, a CM unit/ward at Parirenyatwa Hospital may afford the care that would improve short-term mortality rates. It is hypothesized that such a unit would improve treatment outcomes because it would be easier to apply recommended treatment strategies for all
patients (such as for elevated intracranial pressure) and help standardise care for every patient according to the best available current evidence. It is also an ideal place for obtaining data that is required for research into CM prevention and treatment.

This study has some limitations. The relatively small sample size (largely driven by financial limitation) could have resulted in an over-estimation of the proportion of CM cases and affected the ability to detect true associations in various analyses. Therefore a larger study of this nature may give a more precise view of CM in this modern-day period of ART. Due to limitations in diagnostic testing, the study did not determine the causes of meningitis in the non-CM group. The value of the comparison ‘CM to non-CM’ may be compromised since it was not known exactly what ‘non-CM’ comprised of. Enhancing the diagnosis of other causes of meningitis (i.e. TB, bacterial and viral) is crucial to enable full characterization of HIV associated meningitis.
8.0 References


9.0 Appendices

9.1 Appendix 1: Data Collection Form

Date of Completion - Patient Number - Ward -

Gender  M  F  Age -  Weight -  Height -

Symptoms

Headache  Y  N  Duration (record Duration) -

Vomiting  Y  N  Duration (record Duration) -

Disorientation  Y  N

Cough  Y  N  Duration (record duration) –

Sputum  Y  N

Night sweats  Y  N

Past Medical History

Tuberculosis  Y  N  Pulmonary  Extra-Pulmonary (specify site)

Meningitis  Y  N  Specify Type -

Drugs (Current)

ARVs  Y  N  Year and Month of commencement -

Fluconazole  Y  N  Dose-  Duration of Treatment –

Anti TB drugs  Y  N  Duration of Treatment-

Other:
**Examination**

<table>
<thead>
<tr>
<th>Examination</th>
<th>Y</th>
<th>N</th>
<th>Specify site and size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Consciousness (GCS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (admission)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck Stiffness</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Focal Neurologic Deficits</td>
<td>Y</td>
<td>N</td>
<td>Specify</td>
</tr>
<tr>
<td><strong>Chest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crackles/Effusion</td>
<td>Y</td>
<td>N</td>
<td>Specify</td>
</tr>
<tr>
<td><strong>Abdomen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>Y</td>
<td>N</td>
<td>Specify</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Y</td>
<td>N</td>
<td>specify</td>
</tr>
</tbody>
</table>

**Other Findings** *(Eg Kaposi sarcoma, cutaneous lesions etc)*
Laboratory Tests

Most recent CD4 count - Date/month of CD4 measurement –

FBC – Date/Month of test –

WCC - HB - MCV - Platelets –

Electrolytes –Date/Month of Test –

Sodium - Potassium- Urea- Creatinine-

Serum CrAg Result Negative Positive Titre –

CSF CrAg result Negative Positive Titre –

Other CSF Results Cell count – Differential count –

Protein –

Glucose –

Gram Stain –

India Ink –

Other
9.2 Appendix 2: English Consent Forms

P. O. Box A 178
Avondale
HARARE, Zimbabwe

DEPARTMENT OF MEDICINE

Telephone: 263-4-791631
Fax: 263-4-251017
Telegrams: UNIVERSITY

Chairperson: Prof M.Z Borok, FRCP (UK)

Email:medicine@medsch.uz.ac.zw

====================================================================

COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF ZIMBABWE

English Informed consent

Title of Research: Cryptococcal Infection among HIV infected Patients admitted with Meningitis at Parirenyatwa Hospital

Principal Investigator

Dr Tendai R. Machiridza
Department of Medicine
University of Zimbabwe College of Health Sciences
Mazowe Street
P O Box A 178 Avondale, Harare
Zimbabwe
Telephone 077 2 755 135

Introduction

You are being asked to participate in the research study named above because you are suspected of having a disease known as meningitis. Meningitis is a serious infection in the brain which commonly presents itself with headache. With the increase in HIV infection cases the occurrence of meningitis has increased as well as meningitis due to previously uncommon organisms such as Cryptococcus neoformans, which is a micro-organism known as a fungus.

This document gives you information about the study and what you will be expected to do if you accept to participate. If you understand the study and you agree to take part, you will be asked to sign this document or make your mark in front of someone. You will be given a copy to keep.

Your participation in this study is entirely voluntary and you may decide not to take part or to withdraw from the study at any time without losing any of the benefits of your standard medical care.
Purpose of the study

The purpose of the study is to determine the proportion or number of patients who are infected with Cryptococcus neoformans from among patients admitted with meningitis. Cryptococcus neoformans is a small organism (fungus) causing disease in humans. It mainly causes meningitis. The occurrence of cryptococcal meningitis (meningitis due to cryptococcus neoformans) has increased markedly in patients with HIV infection.

The following individuals will be included in the research (inclusion criteria):

- Have HIV infection
- May or may not be taking Antiretroviral Drugs
- Be 18 years or older, either male or female (pregnant or non-pregnant)
- Be admitted with a suspected diagnosis of meningitis before any tests are done

The following individuals will be excluded from the study (exclusion criteria):

- Those with a past history of infection with Cryptococcus (meningitis or other illness)

What will be done (study procedures)

If you agree to participate in the study, the following samples will be required to test for cryptococcus - blood (4ml) and cerebrospinal fluid (4ml). Cerebrospinal fluid (abbreviated CSF) is a clear watery fluid that circulates around the brain and spinal cord (which runs down the back). Testing CSF is the definite way of determining the presence of meningitis.

Some information will be collected from you such as age, gender, the symptoms you have, whether or not you are taking antiretroviral drugs and other. You will be given a code number which is recorded on your information sheet and also on your samples. Therefore your name will not appear anywhere and the information collected or results obtained cannot be traced back to you by any person except by the individual who has collected the information from you, who happens to be the Principal Investigator.

Risks and discomfort

The risks in this study are minimal. The procedures that are intended (drawing blood and CSF) are routinely performed for patients with suspected meningitis. Drawing of blood from the vein is safe and is associated with minimal discomfort from needle prick. Other side effects include bleeding and feeling faint but are exceedingly rare. CSF is obtained by a procedure known as lumbar puncture where a needle prick in the lower back after a local anaesthetic is given (to minimise pain) obtains the fluid. A lumbar puncture performed by a skilled physician has a low risk of complications which may include headache. Severe complications are extremely rare and include bleeding and direct injury to spinal cord. Because very few patients experience these side effects or complications, a lumbar puncture is recommended for every patient with suspected meningitis (unless there are certain specific reasons against it) because it is the only definite way of diagnosing meningitis and the micro-organism causing it. This is essential for treatment. The principal investigator will be responsible for performing the lumbar puncture and drawing of blood. This will provide specimen both for the study and for the routine tests required by your doctor. Therefore you won’t have to undergo the procedures again.

The specimens that you provide for the study (bearing only a number) may be kept for use in future during other meningitis related studies. If you agree with this you will be asked to sign another form. It is not possible to say exactly what tests may be carried out on the
specimens due to the advances and the changes that occur in research. However the specimens may be used for meningitis-related research only after approval by appropriate committees. Because your name will have been removed, it would not be possible to inform you of results of any further tests. If you wish to withdraw your specimen from future studies at any time, you may contact Dr Tendai R. Machiridza on 077 2 755 135.

**Benefits**

You will directly benefit from this study in that the study will bear the cost of doing the cryptococcal test for you. The cryptococcal test is usually requested by your doctor on the ward because it is a good test to say whether or not you have cryptococcal meningitis. The laboratory usually requires cash upfront (no less than USD $10) before the test is performed. The study will pay for your test. The results of this test will be made available to you and your doctor so that they can make decisions about your treatment. Only the principal investigator (who will be collecting your information) can link the coded specimen result to your name. It is only the test results that will be made available to your doctors because they need these results for your treatment.

The study hopes to establish ways of quickly making the diagnosis of cryptococcal meningitis to improve the survival of patients with the disease.

You will be told of any new information during the course of the study that may influence your decision to stay in the study. If any important information is discovered during the study that might affect your health, you will be informed right away.

**Costs of the study**

There is no cost to you for participating in the study. Instead the study will benefit you by paying for one of the tests that is usually required for your treatment.

**Confidentiality**

The information that is collected from you and your test results will be confidential. You will only be identified by a code number. You will not be personally identified in any publication of this study. No persons other than research staff and health workers overseeing your care will have right of entry to any information that identifies you individually. Only the principal investigator can connect the information and samples to your name.

Study records that identify you by name may be reviewed by representatives from the Medical Research Council of Zimbabwe. These representatives look at study records to make sure the procedures are done correctly, that records are accurate and complete and that your rights are being protected.

**Treatment**

The results of your blood and CSF (cryptococcal) tests will be made available to your treating doctors and all decisions regarding your treatment will be made by them. Therefore the study will help your doctors make quick decisions by assisting with laboratory tests without delay.

**Research-Related Injury**

If you are injured as a result of participation in this study, you will receive immediate necessary treatment for your injuries. No payment will be made directly to you for research related injuries. You are not giving up any of your liability rights for personal injury by signing this informed consent document.
Refusal to participate or withdrawal from the study

You may decline to participate in this study or you may choose to withdraw at any time during the study. In such a case there is no fine or loss of benefits to which you were otherwise entitled. You do not have to explain your reasons for declining to participate. The samples that you may have provided will be destroyed. You are encouraged to ask any questions before, during or after the study. You may contact the Medical Research Council of Zimbabwe (04 791 792 or 791 193) or the principal investigator, Dr Tendai R. Machiridza (0772 755135) if you have any questions.
Signature Page

Title of Research: Cryptococcal Infection among HIV infected Patients admitted with Meningitis at Parirenyatwa Hospital

Statement of agreement to participate in the study

I have read and understood this consent form or it has been read to me in a language that I understand and its contents explained to me. All my questions have been answered. I understand that my rights and privacy will be maintained. I freely and voluntarily choose to participate in the study.

By signing your name or making your mark in the space below you voluntarily agree to join this study.

______________________________  _______________________________  ___________
Participant’s Name                  Participant’s Signature               Date

______________________________  _______________________________  ___________
Witness Name                      Witness Signature                     Date

______________________________  _______________________________  ___________
Investigator’s Name                Investigator’s signature             Date
INTRODUCTION:
You have decided to take part in the investigational research study named above. While you are in this study, blood and cerebrospinal fluid will be collected from you. You are kindly being asked to agree to the storage of these samples for use during the study and after the study has ended. This consent form gives you information about the collection, storage, and use of these samples. These samples may be useful for future research. The principal investigator will talk to you about this information. Please ask if you have any questions. You will be asked to sign or make your mark on this form to indicate whether you agree to have your samples stored and tested. You will be offered a copy of this form to keep.
YOUR PARTICIPATION IS VOLUNTARY:

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study. Even if you decide now that your samples can be stored for future research, you may change your mind at any time. If this happens, you must tell the study staff that you have changed your mind. If you decide not to have your samples stored or used for future research, they will be destroyed at the end of the study.

PURPOSE:

Your samples will only be used to look for cryptococcal infection, damage caused by infection, or how your body reacts to the infection. For example, the tests may look at cells, proteins, and other chemicals in your body. The study researchers do not plan to contact you or your regular doctor with any results from tests done on your stored samples. This is because research tests are often done using experimental procedures, so the results may not help for making decisions on managing your health. In the very rare case that a specific test result gives important information about your health, the researchers will tell the study staff and the study staff will try to contact you. If you wish to be contacted with this type of test result, you must give the study staff any change to your contact information. If you have a regular doctor and you want the study staff to tell this doctor your test results, you must give the study staff your doctor’s contact information.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by a special committee at the Medical Research Council of Zimbabwe.

PROCEDURES:

Each time your blood or cerebrospinal fluid is drawn, up to 4 mL of the sample may be stored. For each sample given, part of the sample will tested immediately and the rest will be stored. Your samples will be stored safely and securely in a storage facility at the University of Zimbabwe Department of Medical Microbiology laboratory. Only the people who work at the facility and approved researchers will have access to your samples. The people who work at the facility will not have any information that identifies you. The approved researchers may be given information about you such as your age and sex, but they will not be given your name or any other information that identifies you. There is no time limit on how long your samples will be stored.

RISKS and/or DISCOMFORTS:

There are few risks related to storing your samples. When tests are done on the stored samples there is a rare but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests it could cause you problems with your family (having a family member learn about a disease that may be passed on in families) or problems getting a job or insurance.

POTENTIAL BENEFITS:

There are no direct benefits to you from having your samples stored. You and others could benefit in the future from research done on your blood.
CONFIDENTIALITY:
To keep your information private, your samples will be labelled with a code that can only be traced back to your study clinic. Your name, where you live, and other personal information will be protected by the study clinic. When researchers are given your stored samples, they will not be given your personal information. The results of future tests will not be included in your health records. Every effort will be made to keep your personal information confidential, but we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Efforts will be made to keep your study records and test results confidential to the extent permitted by law. However, we cannot guarantee absolute confidentiality. You will be identified by a code, and personal information from your records will not be released without your written permission. Any publication of this study will not use your name or identify you personally. However, your records may be reviewed by the Joint Ethics and Research Committee, the Medical Research Council of Zimbabwe, study staff and study monitors.
In addition to the efforts made by the study staff to keep your personal information confidential, an Oath of Confidentiality was signed by all our staff working in this study. This Oath requires study staff not to tell people who are not connected with this study, information about you or other study participants or any other information related to the study.

PROBLEMS OR QUESTIONS:
For questions about the storage of your samples, contact:

- Dr Tendai R. Machiridza
  Department of Medicine
  University of Zimbabwe College of Health Sciences
  Mazowe Street
  P O Box A 178 Avondale, Harare
  Zimbabwe
  Telephone 077 2 755 135

- For questions about your rights as a research subject, contact:

  - The National Coordinator
    Medical Research Council of Zimbabwe
    National Institute of Health Research
    CnrMazoe Street/ Josiah Tongogara Avenue
    Harare
    Ph : +263 4 791792, 791193
    Cell : +263  912 433 166
SIGNATURE PAGE

CONSENT FOR SPECIMEN STORAGE AND SHIPMENT

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide it will not affect whether you can be in the research study, or your routine health care.

________ I agree to have samples of my blood and cerebrospinal fluid stored and used for future testing related to cryptococcal infection.

________ I do not agree to have samples of my blood and cerebrospinal fluid stored and used for future testing related to cryptococcal infection.

____________________________________  Participant Name (print)

____________________________________  Participant Signature or Mark and Date

____________________________________  Study Staff Conducting Consent Discussion (print)

____________________________________  Study Staff Signature and Date

____________________________________  Witness Name (print)
(As appropriate)

____________________________________  Witness Signature and Date