Dual infection with tuberculous meningitis and cryptococcal meningitis in HIV-positive patients

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Declaration

I, Comfort Shaba, declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

_____ (Signature of candidate)

4th day of May 2018

Abstract

Introduction: HIV-positive individuals may have multiple CNS opportunistic infections. CM and TBM have been described to occur as dual infection in HIV-positive patients. However, the literature in this regard is scant.

Aim: To determine the prevalence of dual infection with tuberculous and cryptococcal meningitis in HIV-positive patients at CMJAH

Method: Records of 123 HIV-positive patients admitted at CMJAH with a diagnosis of cryptococcal meningitis between 1 May 2015 and 30 April 2017 were reviewed. Tests that would determine or suggest the presence of co-occurring TBM were also reviewed. Co-occurrence of TBM was diagnosed by isolating TB on CSF (auramine stain, genexpert MTB/RIF or TB culture) as well as on the basis of CSF profile and imaging findings. The diagnosis was also supported by TB findings in non-CNS sites such as chest radiography and abdominal ultrasound.

Results: A total of 24 patients of the 123 patients with CM (19,5%) were diagnosed with TBM dual infection. The median CD4 count of the patients also diagnosed with TBM was 61 cells/mm^3 (IQR 16-102) compared to those without dual infection (25 cells/mm^3, IQR 8-54). Of the 24 patients, 14 (58,3%) were on HAART. Factors significantly associated with TBM were raised CSF protein >1g/L, low glucose <2.2 and high CSF lymphocytes > 15. The absence of photophobia was found to be significantly associated with the diagnosis of dual infection. Patients on HAART were found to be more likely to be diagnosed with TBM compared to those not on HAART.

Conclusion: TBM and CM may occur concomitantly. CM patients with raised CSF protein >1g/L, low glucose <2.2mmol/L and lymphocytes >15 cells×10^6/L warrant further investigation and treatment for TB meningitis.

Keywords: cryptococcal meningitis, tuberculous meningitis, HIV

For Wezi

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Abbreviations

ADA	adenosine deaminase
AIDS	acquired immunodeficiency syndrome
CM	cryptococcal meningitis
CNS	central nervous system
CrAg	cryptococcal antigen
CSF	cerebrospinal fluid
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DNA	deoxyribonucleic acid
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
LP	lumbar puncture
PCR	polymerase chain reaction
SA	South Africa
TB	tuberculosis
TBM	tuberculous meningitis
WHO	World Health Organisation

CHAPTER 1: BACKGROUND AND EXTENDED LITERATURE REVIEW

1.1 Background to the study

Infectious meningitis is a substantial cause of morbidity and mortality in the HIV-infected population. (Békondi et al., 2006; Marais et al., 2011; (Veltman, Bristow and Klausner, 2014) Many studies have reported the increasing prevalence of *Mycobacterium tuberculosis* and *Cryptococcus neoformans* particularly in the context of the HIV epidemic both globally and specifically in Southern Africa. (Adeyemi & Ross, 2014; Jarvis et al., 2010; Thwaites et al., 2005) Of interest is the co-occurrence of these infections.

1.2 The burden of HIV in South Africa (SA)

Approximately seven million people were estimated to be living with HIV in 2016. This is equivalent to around 12,7% of the total South African population. Adults aged 15 - 49 years, with an estimated 18,9% of this age group being HIV positive, carry the highest burden of HIV. (Mid year population estimates, 2016) The impact of this growing epidemic on the population statistics is evident in the decrease in life expectancy and in the number of children who are orphaned. This creates a critical public health issue that requires prioritisation.

1.3 Opportunistic infections

The central nervous system is frequently a site of infection in HIV-positive individuals. (Collazos, 2003) Opportunistic infections are defined as infections that occur as a result of a severely depleted immune system. They are caused by pathogens that typically do not cause infections in an immunocompetent host.

The immune system may be impaired due to underlying conditions such as: malignancies, diabetes, autoimmune disease, solid organ transplants and drugs for example chemotherapy, immunosuppressants and steroids.

(Bicanic, 2005) In sub-Saharan Africa, HIV is the most common cause of immunosuppression. HIV infection results in progressive depletion of the CD4 subset T-lymphocytes causing an immunodeficiency state. Extra-pulmonary tuberculosis infection, such as TBM occurs within a CD4 count between 250-500 cells/mm^3 whereas cryptococcus infection is said to occur at a CD4 count range between 75 and 125/mm^3. (Crowe et al., 1991) Both of these conditions are AIDS-defining illnesses. (WHO case definitions of HIV, 2007)

1.4 Cryptococcal meningitis

Cryptococcal meningitis (CM) is an example of an opportunistic infection that is encountered frequently at CMJAH. In fact, *Cryptococcus neoformans* is the most commonly found meningitis-causing pathogen in SA according to a Cape Town study by Jarvis et al (2010) with incidence reported as high as 63% of patients presenting with meningitis. Similar results have been demonstrated in other local (Bhagwan and Naidoo, 2011) as well as international studies. (Ghate et al., 2011).

1.4.1 Pathogenesis

Two types of fungus namely, *Cryptococcus neoformans* and *Cryptococcus gattii*, can cause meningitis and as mentioned above, the most common cause is *neoformans*. It is an encapsulated yeast that is ubiquitous to many parts of the world and is associated with pigeon droppings usually found in soil. (Tenforde et al., 2017)

1.4.2 Clinical presentation

Patients infected with CM may present variably from minor ailments to severe illness. The most common symptom is headache that may or may not be associated with fever. They may also present with symptoms and signs related to inflamed meninges including neck stiffness. However in HIV-positive patients it is important to

note that there may be a distinct lack of fever or meningism. (Karstaedt, 1998)

Raised intracranial pressure symptoms such as headache and confusion; as well as signs such as altered level of consciousness; sixth cranial nerve palsies with diplopia, visual impairment and papilloedema may also feature. There may also be an overlap with encephalitis in that some patients present with cognitive decline, memory loss, personality changes, bizarre behaviour or other new-onset psychiatric symptoms. (Baradkar et al., 2009)

1.4.3 Diagnosis

1.4.3.1 Laboratory diagnosis

CM is diagnosed by demonstrating the encapsulated yeasts on cerebrospinal fluid (CSF) via India ink staining or by identifying a positive cryptococcal latex agglutination test (CLAT). India ink stains have a sensitivity of approximately 90%. (Bicanic, 2005) A fungal culture may also be used to demonstrate *Cryptococcus neoformans* with results available usually within 72 hours.

The Cryptococcal Antigen (CrAg) Lateral Flow assay of serum is a relatively new, low-cost, highly sensitive screening test. A screen-and-treat strategy, whereby all HIV-positive patients with a CD4 count below 100cells/mm^3 are tested for cryptococcal antigen is now being recommended and implemented. A positive serum CrAg in the correct setting should direct the physician to perform a lumbar puncture. The serum CrAg test has become more widely used and is a useful screening tool as it is positive in >99% of subjects with cryptococcal meningitis (van der Horst et al., 1997)

As part of diagnosis and treatment, patients with CM should have recordings of opening pressures done in the lateral recumbent position. Normal values are generally agreed to be below 20cm H_2O. (Lascelles, 1992)

1.4.3.1 Imaging studies

Due to the high fungal load and high sensitivity of India ink staining, confirming CM infection microbiologically is usually not problematic in clinical practice. (Bicanic, 2005) Imaging is rarely required. There are no typical brain findings on imaging in CM. Computed tomography (CT) scans are often normal or may show cryptococcomas. Other rare findings include cerebral oedema or hydrocephalus. Magnetic resonance imaging (MRI) scans are more sensitive for these findings but may also demonstrate other features of advanced immunodeficiency and underlying HIV-associated dementia such as cortical atrophy. These findings are often non-specific. (Garg, Malhotra and Jain, 2016; Kastrup, Wanke and Maschke, 2005)

1.4.4 Treatment

The treatment of CM in HIV-positive patients consists of three phases: induction, consolidation and maintenance. The current South African guidelines are as follows:

i) Induction phase: amphotericin B (0.7–1 mg/kg/d) plus fluconazole (800mg/d) for 2 weeks. The WHO-recommended flucytosine is not readily available in South Africa.

ii) Consolidation phase: fluconazole (400 mg/d) for 8 weeks

iii) Maintenance phase: fluconazole 200 mg/d, depending on the patient's clinical status and CD4 count. In some cases fluconazole should be continued for life. (Bicanic, 2005; Saag et al., 2000)

Raised intracranial pressures contribute to the morbidity and mortality of CM. Repeated lumbar punctures to lower this pressure are an integral part of the treatment plan.

1.5 Tuberculous meningitis

The second most common cause of meningitis in HIV-positive patients is *Mycobacterium tuberculosis.* (Veltman, Bristow and Klausner, 2014) Jarvis et al (2010) also demonstrated that the proportion of meningitis due to tuberculosis (TB) is increasing. The study found TB confirmed microbiologically in 28% of the patients studied.

1.5.1 Pathogenesis

Tuberculous meningitis is caused by the organism *Mycobacterium tuberculosis.* The organism spreads haematogenously from the primary site, usually the lungs, to the brain. Once there, rich foci of granulomas form below the arachnoid and pia meninges. The breakdown of their purulent material into the cerebrospinal fluid results in TBM. "The most characteristic pathologic feature of TBM is meningeal inflammation and formation of thick gelatinous exudates in the basal parts of the brain". (Garg, Malhotra and Jain, 2016; Jha, 2017)

1.5.2 Diagnosis

1.5.2.1 Laboratory diagnosis

TBM is the most serious complication of infection with *Mycobacterium tuberculosis* and if left untreated causes severe neurological sequelae ranging from epilepsy, cognitive impairment, moderate to severe physical disability and persistent vegetative states. "Approximately a third of patients die soon after presenting to hospital. In patients with HIV co-infection, mortality exceeds 60%. Early diagnosis and treatment for TBM have been shown in

numerous studies to be the best prognosticator of survival". (Nhu et al., 2013)

However, the diagnosis of TBM has also proven to be a clinical challenge as tests available have varying sensitivities. "The gold standard for microbiological confirmation is the demonstration of *Mycobacterium tuberculosis* in the CSF". (Garg, Malhotra and Jain, 2016) This can be done by identifying the organisms on smear analysis with Ziehl–Neelsen or Auramine O staining. The identification of acid-fast bacilli (AFB) on smear results is largely based on the experience and expertise of the laboratory technician. It also depends on the availability of a larger volume of CSF analysed (>6ml) as well as length of meticulous microscopic examination (>30 minutes). (Nhu et al., 2013; Vidal et al., 2017)

Mycobacterium tuberculosis can also be visualised by culture: either conventionally on Lowenstein-Jensen medium or in a mycobacterial growth indicator tube. The sensitivity is once again dependant on the volume of CSF but is reported to be approximately 45% to 90%. The disadvantage of culture is the length of time before a result is available. Culture may be positive in two weeks but usually takes up to six weeks of incubation. (Brancusi, Farrar and Heemskerk, 2012)

GeneXpert MTB/RIF (Cepheid) is a relatively new test that uses immediate polymerase chain reaction (PCR) to detect the presence of *Mycobacterium tuberculosis* DNA. Its advantages of nearly 100% sensitivity of ease of use and rapid turnover time are outweighed by its lack of sensitivity. The sensitivity varies between 30% and 60% thus limiting its value. (Baldwin and Zunt, 2014; Bhigjee et al., 2007; Nhu et al., 2013) Repeat analysis of serial CSF samples may however improve the likelihood of a positive result.

Due to the high specificity but low sensitivity of the microbiological tests for TB, they are fantastic "rule-in" tests but cannot exclude the diagnosis of TB. Therefore in the clinical setting, diagnosis is often based on characteristic CSF abnormalities such as mononuclear cell pleocytosis, low glucose and elevated protein concentration.

A Peruvian study published in 2013, in addition to the above, found that CSF ADA (adenosine deaminase) more than 6 U/l in patients with clinical features of meningitis is a strong indicator for TBM and warrants the initiation of anti-tuberculous treatment. (Solari et al., 2013) Other authors recommend a cut-off value of 8 - 9.5 U/l to differentiate between TBM and other types of meningitis. (Sun et al., 2012; Tuon et al., 2009) However, as there is no standardisation to this test, ADA cannot be used to differentiate between TBM and other types of meningitis.

- 1.5.2.2 Imaging studies

  Radiological studies are a valuable part of the diagnostic process due to the challenging clinical diagnosis. Although MRI is said to be superior, in our experience CT scan is more readily available.

  An important finding in patients with TBM is meningeal enhancement, which is defined as "the enhancement of pia and arachnoid meninges after administration of a contrast material". (Garg, Malhotra and Jain, 2016) Enhancement around the basal cisterns and the sylvian fissure characterises the thick basilar exudate associated with TBM. Some authors even suggest that basal meningeal enhancement amounts to a definite diagnosis of TBM. (Modi, Mochan and Modi, 2004)

Three other characteristic features of TB on brain imaging are hydrocephalus, periventricular infarcts and tuberculomas. "Tuberculomas are hypodense or isodense lesions with regular walls of varying thickness, associated with oedema and mass effect. They are cortical in location and demonstrate ring or nodular enhancement". (Modi, Mochan and Modi, 2004). A combination of the above radiological features increases the specificity of TB diagnosis.

The presence of tuberculosis elsewhere can also contribute to the positive diagnosis of TBM. Chest radiography may demonstrate abnormal findings in up to 43% of cases. Typical findings include hilar adenopathy, miliary pattern, and bronchopneumonic infiltrates. (Yaramis et al., 2007)

1.6 Differentiating between CM and TBM

CM and TBM present very similarly and distinguishing the two on clinical diagnosis alone may be difficult. (Veltman, Bristow and Klausner, 2014) Both cryptococcus and mycobacterium separately have been found to cause chronic meningitis. "Chronic meningitis is defined as an inflammatory CSF profile that persists for at least one month". (Baldwin and Zunt, 2014) The features of chronic meningitis include elevated protein, predominantly lymphocytic pleocytosis and decreased glucose. In CM, lumbar puncture is often characterised by an increased opening pressure. (Baldwin and Zunt, 2014) Tuberculous meningitis (TBM), which presents similarly, is also more common in patients co-infected with HIV.

Compared to CM however, the distinguishing feature of TBM is the presence of a thick basilar meningeal exudate as described above. This results in multiple cranial neuropathies and hydrocephalus as it blocks the flow of CSF; a finding that is very rare in CM.

Other features in support of TB include chest radiography findings as discussed above as well as low serum sodium suggestive of the inappropriate secretion of anti-diuretic hormone.

1.7 Dual infections

Infections occurring concomitantly have been described in literature. In 1998 Silber et al from the University of Witwatersrand conducted a study looking at the presence of dual infections affecting the nervous system sequentially or simultaneously. Researchers concluded that dual infection of the nervous system by two distinct non-viral organisms is uncommon but often undiagnosed. (Silber et al., 1998) They found CNS TB co-infection in two out of seven patients with CM. One patient had a ring-enhancing mass in the parietal lobe that was proven to be a tuberculoma after biopsy. The second patient who was known with pulmonary TB presented with a decreased level of consciousness. His CSF sample was positive for cryptococcal antigen and culture and also cultured *Mycobacterium tuberculosis*. Both patients with co-infection had elevated protein and cell counts. Their recommendations were that "immunocompromised patients, who present with high levels of CSF protein and a significant lymphocytosis, should be investigated thoroughly for other pathology and that co-infection with tuberculosis should always be considered in patients from communities with high rates of pulmonary TB".

Following the above Rawat et al (2008) described four HIV-positive patients with concomitant tuberculosis and cryptococcosis infection. A recent study published in the Indian Journal of Tuberculosis (Singh et al., 2013) echoes the above sentiments. They described two case reports of patients with dual TB and CM at a hospital in Patiala, India. Both patients were taking treatment for tuberculosis: one being pulmonary and the other abdominal. Due to deterioration in their condition and mental state they

were suspected of having meningitis, which proved to be cryptococcal. The authors suggest that all patients diagnosed as suffering from TB must be screened for HIV and further work up should be done to diagnose and treat multiple opportunistic infections.

Although various researchers have shown acknowledgement of co-infection, literature demonstrating dual infection of both central nervous system (CNS) TB and CM is scant.

A Turkish case report from Mete et al (2016) describes a patient, with systemic lupus erythematosus on immunosuppressive agents, who was diagnosed with simultaneous cryptococcal and TB meningitis. The patient presented with headache, fever, nausea, vomiting, incontinence, somnolence, and convulsions. Clinically she had neck stiffness and dysarthria. Acid-fast bacilli were detected in a CSF sample and PCR confirmed *Mycobacterium tuberculosis*. The patient however deteriorated despite appropriate treatment with anti-TB drugs. Repeat CSF examination revealed capsulated yeasts using Indian ink staining, and *Cryptococcus neoformans* was isolated on culture. The same authors described seven other cases of dual CM and TBM. Two of the patients had underlying HIV infection and only one was from Africa. (Mete et al., 2016)

In conclusion, many researchers highlight the burden of disease with respect to TB and CM occurring separately in patients with HIV. There is however a lack of data within the literature regarding the rate of CM and CNS TB dual infection. The above case reports show that although the presence of TBM and CM dual infection is rare, it does occur. Considering the high prevalence of HIV and TB in our population one can only postulate that many of the cases remain undiagnosed.

The aim of our study was to determine the prevalence of this dual infection in our setting, and to assist physicians who treat patients with HIV in determining which patients with CM need further investigation for TB.

CHAPTER 2: OBJECTIVES AND METHODS

2.1 Study objectives

To determine the rate of concomitant infection with TBM and CM in patients with HIV

To describe the demographic profile of patients presenting with CM

To determine predictors of concomitant infection with CM and TBM

To develop guidelines for the investigation of patients with possible dual infection

To compare the rate of CM and TBM concomitant infection by sociodemographic and biological factors such as sex, CD4 count, viral load and HAART

2.2 Methods

2.2.1 Study site

CMJAH is a tertiary / quaternary level care hospital that caters for patients in and around Parktown as well as those referred from surrounding community health centres and peripheral hospitals within Gauteng province. At CMJAH the vast majority of patients with CM are admitted to the neurology wards in 586 or 587 and each patient has a discharge summary available.

2.2.2 Study design

A retrospective descriptive record review was conducted in the neurology wards of Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). Records between 1 May 2015 and 30 April 2017 were reviewed. HIV-positive patients admitted to the neurology wards with a diagnosis of CM were included in the study. The diagnosis was based on a positive India ink,

cryptococcal latex agglutination test or culture of *Cryptococcus neoformans* on CSF. Patient records were identified using the neurology ward admission books. Further review of clinical records and laboratory data was done in order to obtain information on the tuberculosis testing on CSF. The data sources are linked with the patient's hospital file number. Information was collected on a data collection sheet and the data was captured onto a Microsoft® Excel spread sheet.

2.2.3 Study population

According to the admission and discharge registers in ward 586 and 587, a total of 176 were admitted to the wards during the study period.

Patient records were reviewed to determine if there was evidence of CNS TB based on CSF microscopy, TB culture or geneXpert MTB/RIF. TBM diagnosis was classified as 'definite TBM' if any of the above criteria were positive. Classification of 'probable TBM' and "possible TBM" was based on other clinical, laboratory and radiological features. This was based on criteria published by Marais et al. (2010) (Table 2.1) Patients were also classified as 'not TBM' if none of the above criteria were present. (Bhigjee et al., 2007)

Definite TBM	Probable TBM	Possible TBM
1) AFB were seen in CSF or 2) AFB or M. tuberculosis was cultured from CSF or 3) M. tuberculosis was detected by PCR from CSF	1) Suggestive CSF findings of TBM (total white cell count >5 cells×10^6/L, protein >0.45 g/L and glucose <2.2 mmol/L) plus 2) One or more of the following i) chest radiograph findings consistent with pulmonary TB, ii) an extra-meningeal specimen positive for AFB, iii) other evidence of extra-meningeal TB (e.g. abdominal ultrasound features) or iv) brain computed tomography (CT) evidence of TBM including one or more of the following: basal meningeal enhancement, hydrocephalus or infarctions.	1. Either four or more of the following were present i) a history of TB ii) a predominance of CSF lymphocytes (>50%) iii) illness duration of more than five days iv) CSF glucose <2.2 mmol/L v) altered consciousness vi) clear or yellow CSF with protein>1 g/L vii) focal neurological signs, or 2. markedly abnormal' CSF (excluding isolated hypoglycaemia) with evidence of TB elsewhere.

Table 2.1: TBM case definition criteria reproduced and slightly modified from Marais et al (2010)

2.2.4 Exclusion criteria:

Patients who had not had TB testing on CSF

2.2.5 Sample size

Statistical software used for sample size calculation: StatCalc/ Epi Info 7.1 and Mobile version 2.0.1

Number of CM patients admitted over the 24 months = 176

Estimated frequency of TBM among HIV-positive patients with CM = 50% as no data currently available

Acceptable margin of error 5%, 95% confidence interval

Design effect and clusters = 1

Sample size (minimum) = 121

The sample was selected by consecutive sampling looking at most recent records first until at least the minimum sample size required was reached.

2.2.6 Data collection

The investigator collected the data on a data collection sheet. (Appendix C) Information obtained includes demographic profile, CSF findings in relationship to cryptococcal and tuberculous infection, presenting symptoms such as headache, fever and photophobia as well as presenting signs such as neck stiffness, confusion or coma.

2.2.7 Data analysis

Methods used were frequencies and percentages to summarise categorical data. Mean ± standard deviation was used to describe variables that are normally distributed. If not normally distributed then they were represented as median (interquartile range).

To compare the relationship or association between the prevalence of concomitant CM and TBM with gender or HAART or other categorical group, Pearson's chi squared test was used.

To compare the association between dual infection and continuous variables such as CD4 count or viral load, the Mann Whitney U test was used.

Univariate logistical regression and subsequent multivariate logistical regression was used to determine predictors of dual infection.

The p value level of statistical significance was accepted as <0.05.

All analysis was done with Stata Data Analysis and Statistical Software Version 14.

2.3 Ethical considerations

The Human Research Ethics Committee (HREC) of the University of the Witwatersrand approved the study. Ethics clearance certificate number M170486 (Appendix A) Permission was also obtained from the Chief Executive Officer of CMJAH. (Appendix B)

To maintain confidentiality, patient data such as names were kept confidential by allocating each patient a research number. The data sheet only contained research numbers and the data was only used for the purpose of the study. No consent was required due to the retrospective design of the study.

2.4. Funding

There was no funding required for this study. The investigator assumed the cost of stationery, printing and photocopying.

CHAPTER 3: RESULTS

There were 176 patients admitted with CM over the study period. 53 patients were excluded from the study, as they had no TB testing done on their CSF samples. Therefore ultimately 123 patients were included.

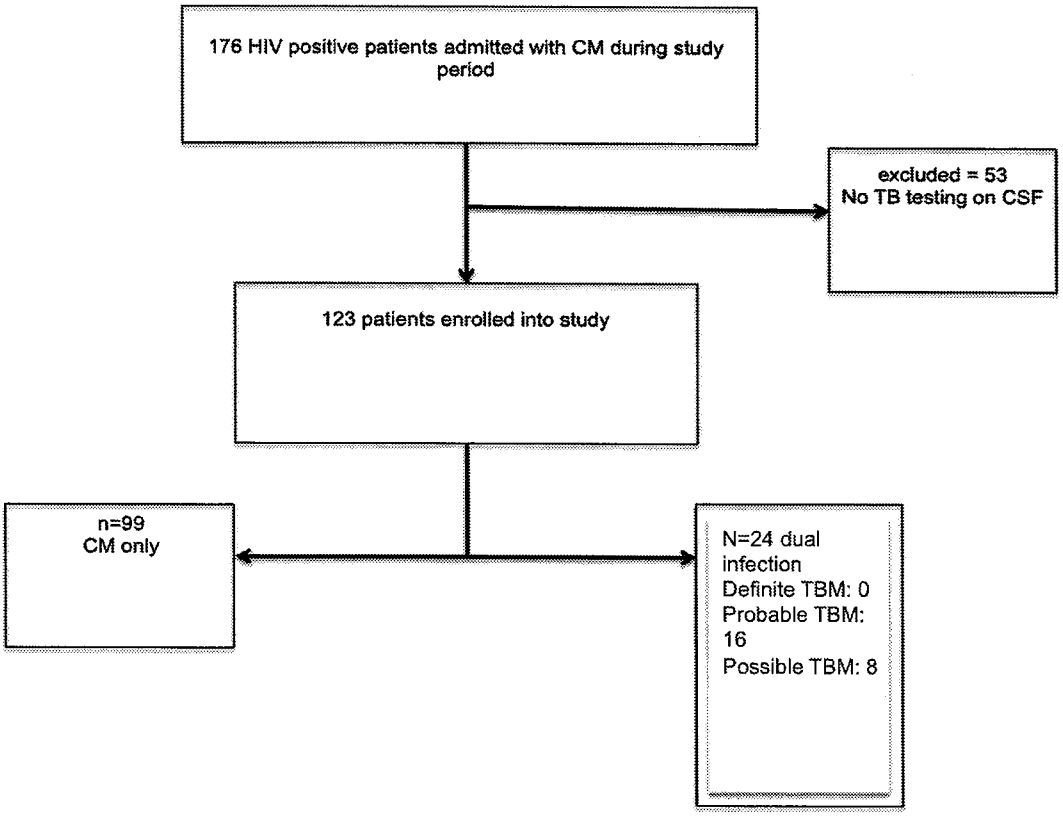

Figure 3.1: Flow chart of diagnosis for patients included in the study

Sociodemographic and clinical profile of patients with CM at CMJAH

Of the total patients included in the study, 69 (56,6%) were male. CD4 counts ranged from 1 to 315 cells/mm^3. The median CD4 count was 48 (IQR= 48 (8 - 72) cells/mm^3.

The majority of the patients overall (87%) had a CD4 count of less than 100 cells/mm^3 while 10% had a CD4 count between 100 and 200 cells/mm^3 and the other 3% had a CD4 count above 200 cells/mm^3. (Figure 3.2)

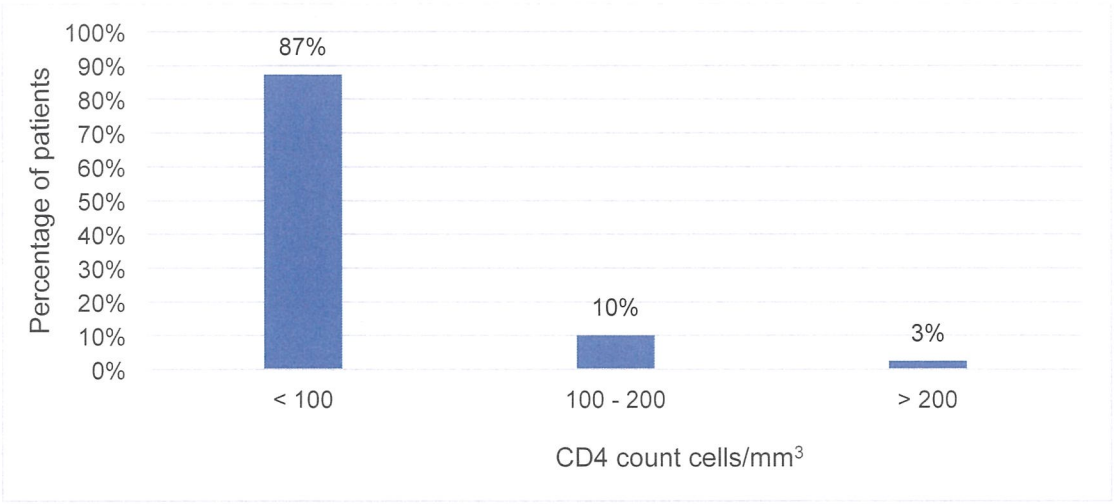

Figure 3.2 distribution of CD4 count in patients with CM

HIV viral loads were obtained in 83 out of 123 patients. Three patients were found to have a viral load that was lower than detectable. The majority of the patients (60%) were not yet on HAART or presented with a diagnosis of CM as the first presentation of HIV. Patients commonly presented with headache (found in 91% of the total patient sample) neck stiffness (71%) and confusion. The opening pressures varied with some patients (19%) having normal opening pressures below 20 cmH_2O.

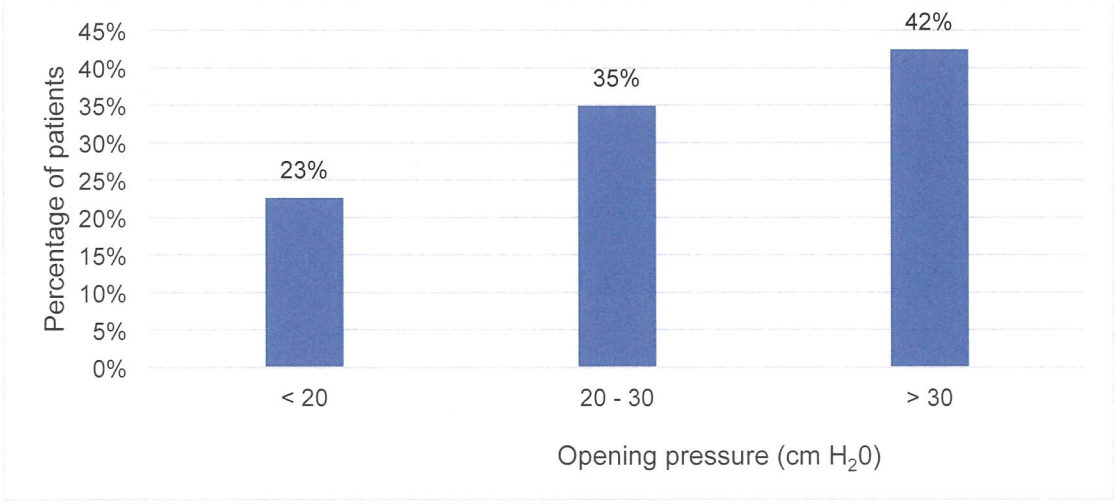

Figure 3.3: distribution of opening pressure

The CD4 count and selected CSF characteristics of patients with CM are represented in table 3.1 below.

Variable	Median (IQR)
Protein g/L	0.72 (0.405 - 1.175)
Glucose mmol/L	2.1 (1.4 - 2.9)
Lymphocytes cells×10^6/L	4 (0 - 19)
ADA U/l	1.6 (0.9 - 4.5)
CSF opening pressure cmH20	28.5 (20 - 40)
CD4 count cells/mm^3	48.08 (8 - 72)

Table 3.1: CSF characteristics in patients diagnosed with CM

Dual infection

Sociodemographic and clinical profile

Twenty-four patients out of the 123 patients (19.51% [95% CI 13.4-27.6]) in the study were diagnosed with dual infection. Their mean age was 37.9 years +- SD 9.8 with range of 24-59 years. The median CD4 count was 61 cells/mm^3 (IQR = 16-102 cells/mm^3) compared to those without dual infection (25 cells/mm^3, IQR 8-54 cells/mm^3). Nine of the patients with dual infection (37,5%) were not on HAART compared to 15 (62,5%) who were on HAART.

Presentation

The most frequent symptoms in patients with dual infection were headache, neck stiffness and confusion. The symptoms are summarised in the graph below.

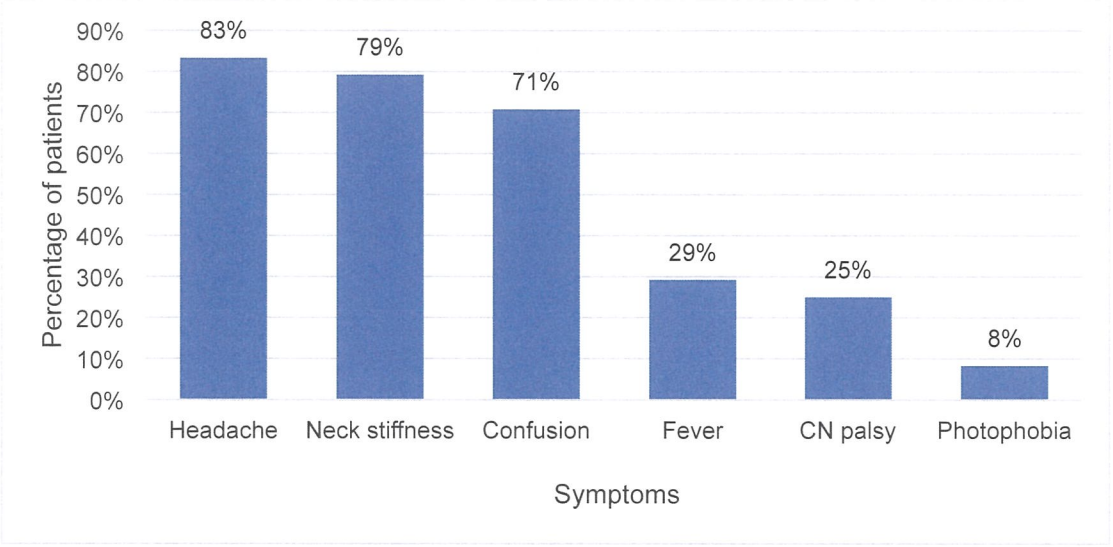

Figure 3.4: frequency of symptoms in patients with dual infection

Below is a comparison of the symptoms among the 24 patients with dual infection compared to the 99 without concomitant TBM.

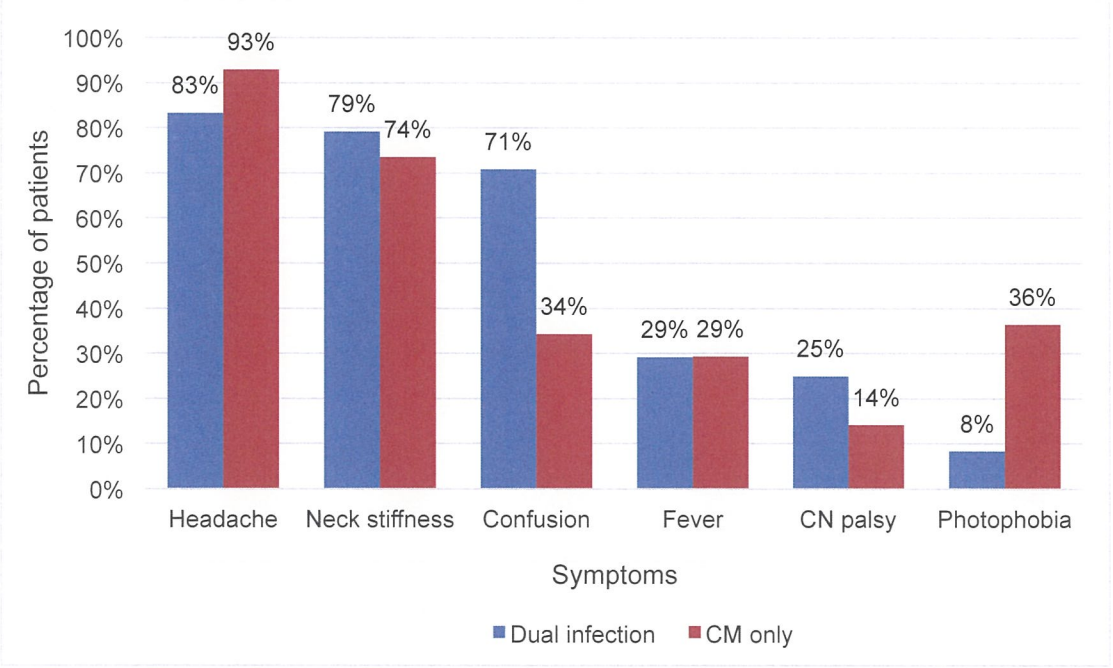

Figure 3.5: frequency of symptoms in patients with dual infection compared to those without dual infection

TBM diagnosis

The diagnosis of TB meningitis was classified as probable in 16 patients and possible in 8 patients. No patients had definite TBM as none of the patients had a positive identification of *Mycobacterium tuberculosis* on smear, culture or genexpert. Diagnosis was mostly based on typical CSF profile (54%) followed by typical brain imaging findings (29%) and abdominal ultrasound. (29%)

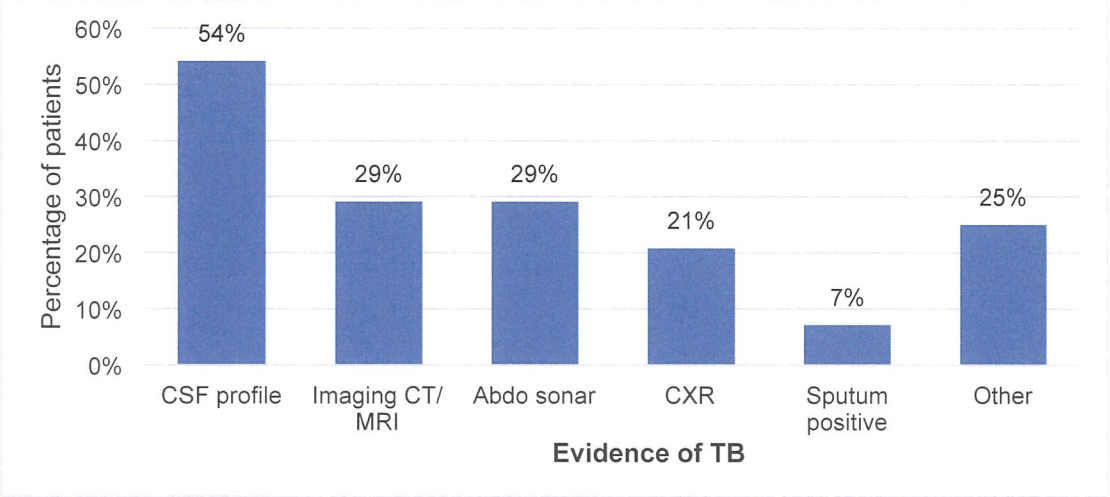

Figure 3.6: frequency of evidence of TB (other includes: cholestatic picture on liver function tests and temperature spikes)

The association between TBM and a number of categorical variables was assessed using Chi-square and the results are shown in Table 3.2 below.

Variable	**Category**	**TBM**			**P-value**
		Yes (n= 24)	**No (n= 99)**	**Total (n= 123)**	
Sex	Male	54.2%	56.6%	56.1%	1.000
	Female	45.8%	43.4%	43.9%	
HAART	Yes	62.5%	34.7%	40.2%	0.019*
	No	37.5%	65.3%	59.8%	
Headache	Yes	83.3%	92.9%	91.1%	0.223

	No	16.7%	7.1%	8.9%	
Fever	Yes	29.2%	29.3%	29.3%	1.000
	No	70.8%	70.7%	70.7%	
Photophobia	Yes	8.3%	36.4%	30.9%	0.007*
	No	91.7%	63.6%	69.1%	
Neck stiffness	Yes	79.2%	73.5%	74.6%	0.794
	No	20.8%	26.5%	25.4%	
Confusion	Yes	70.8%	34.3%	41.5%	0.002*
	No	29.2%	65.7%	58.5%	
Cranial nerve palsy	Yes	25.0%	14.1%	16.3%	0.221
	No	75.0%	85.9%	83.7%	

Table 3.2 Association between TBM and selected categorical variables
**statistically significant p value <0.05*

There was a significant association between HAART and TBM (p-value = 0.019) meaning that patients on HAART were more likely to be diagnosed with TBM. It can be noted from Table 3.2 that 62.5% of the patients diagnosed with TBM were on HAART compared to only 34.7% on HAART not diagnosed with TBM. A significant association was also noted between TBM and the absence of photophobia (p-value = 0.007) and presence of confusion (p-value = 0.002)

The results show that there is no association between sex, presence of headache (p-value = 0.223), fever (p-value = 1.000), neck stiffness and cranial palsy (p-value = 0.221) as the p-values were greater than 0.05.

The Mann-Whitney U was conducted to assess whether there was a significant association between TBM and a number of continuous variables. Mann-Whitney U test, a non-parametric test, was used because the variables were not normally distributed. The results are shown below.

Variable	TBM			P-value
	Yes (n= 24)	No (n= 99)	Total (n= 123)	
Duration of HAART months	0.25 (0 - 4)	0 (0 - 2)	0 (0 - 3)	0.054
Viral load cps/ml	160500 (52100 - 487500)	115000 (13600 - 537000)	115000 (14711 - 510000)	0.940
Protein g/L	1.39 (0.705 - 3.02)	0.68 (0.395 - 0.925)	0.72 (0.405 - 1.175)	0.001*
Glucose mmol/L	1.25 (0.9 - 2.25)	2.3 (1.6 - 3)	2.1 (1.4 - 2.9)	0.003*
Lymphocytes cells$\times 10^6$/L	15 (0 - 157.5)	2 (0 - 14)	4 (0 - 19)	0.039*
Erythrocytes cells$\times 10^6$/L	0 (0 - 0.75)	0 (0 - 0)	0 (0 - 0)	0.499
ADA U/l	0.9 (0.7 - 2.8)	1.6 (1.1 - 4.7)	1.6 (0.9 - 4.5)	0.159
Opening pressure cm H_2O	28 (15.75 - 33.5)	29.5 (21.5 - 40.5)	28.5 (20 - 40)	0.137
CD4 count cells/mm^3	61 (16 - 102)	25 (8 - 54)	48.08 (8 - 72)	0.013*

*Table 3.3: Multivariate analysis of continuous variables associated with TBM (the figure in brackets reflects the interquartile range) *statistically significant p value <0.05*

The results show that patients with dual infection had significantly higher CSF protein (median = 1.39 g/L) compared to a median of 0.68 g/L for patients without TBM (p-value = 0.01). A significantly higher CSF lymphocyte count (median = 15 cells$\times 10^6$/L) compared to a median of 2 cells$\times 10^6$/L for patients without TBM (p-value = 0.039). A significantly higher CD4 count (median = 61 cells/mm^3) compared to a median of 25 cells/mm^3 for patients without TBM (p-

value = 0.013). They also had a significantly lower CSF glucose (median = 1.25 mmol/L) compared to a median of 2.3 mmol/L for patients without TBM (p-value = 0.003).

There was no significant relationship between duration of HAART (p-value = 0.054), HIV viral load (p-value = 0.940), erythrocyte count (p-value = 0.499), ADA (0.159), and opening pressure (p-value = 0.137) given that the p-values were greater than 0.05.

CHAPTER 4: DISCUSSION & CONCLUSION

3.1 Demographic profile of all patients

The aim of this study was to describe the sociodemographic profile of patients presenting with CM and to look at the prevalence and predictive factors of dual infection with tuberculous meningitis.

The majority of patients (87%) demonstrated severe immunodeficiency as evidenced by a low CD4 count below 100 cells/mm^3. This is in keeping with the study by Crowe et al who described CM as occurring between 75-125 cells/mm^3. A low CD4 count in relation to CM is well documented in various other studies. (Heyderman et al., 1998; Kisenge et al., 2007) This was also demonstrated by Tanzanian researchers who found 93% of their CM patients presented with a CD4 count below 100 cells/mm^3. (Kisenge et al., 2007)

In our study, CM was found to be the first presentation of HIV in 60% of patients. This figure is lower than the 80% and 79% reported by Heyderman et al (1998) and Patel et al (2010) respectively. This could be as a result of the decreasing incidence of CM due to the implementation of the CrAg screen and test programme.

Typical presentations of headache, neck stiffness and confusion correlate well with other studies. (Aslam & Chandrasekhara, 2009; Heyderman et al., 1998; Moosa and Coovadia, 1997)

3.2 Dual infection

The main objective of this study was to determine the prevalence of dual CM and TBM infection. Dual infection was found in 19,5% of patients. This figure is lower than that found in an Indian study by Aslam & Chandrasekhara (2009). They found dual TBM and CM in 30% of HIV-

positive patients diagnosed with CM. Aslam & Chandrasekhara do not mention how the diagnosis of TBM was derived in their study.

Our diagnosis of TBM echoed many previous studies in that microbiologically confirmed diagnosis remains elusive. A definite diagnosis of TBM was not made in any of the patients studied. Although dissatisfying to not have confirmed any cases microbiologically, this finding is in keeping with previous studies on TBM. (Luma et al., 2013) Luma et al found acid-fast bacilli in one CSF sample (1,9%). The reasons for such a low yield can be attributed to the low sensitivities of smear microscopy, culture and GeneXpert MTB/RIF. Furthermore a positive yield is dependent on adequate analysis of CSF as well as sufficient volume of CSF sent to the laboratory. (Vidal et al., 2017; Thwaites et al., 2002) We could not control how much CSF was sent for laboratory analysis in this retrospective record review. Repeat CSF examinations are recommended and this is also not often done in our setting. Moreover TB culture requests from CSF are not routinely done in our setting.

Of the patients diagnosed with TBM (8/24) 33% were based on a probable diagnosis with the remainder having possible TBM. Consistent with Marais et al. (2011), in our setting the diagnosis relied on clinical evidence combining laboratory and radiological evidence. This lack of microbiological evidence has been well documented in various studies and a rapid, cheap sensitive tool to diagnose TBM on CSF is urgently required. (Karstaedt, 1998; Thwaites and Hien, 2005) As in our study, treatment for TBM is often initiated empirically at the discretion of the attending physician. Other clinical features that have been described to be associated with TBM such as duration of illness and optic atrophy on fundal examination were not investigated in this study. (Thwaites et al., 2002)

The diagnosis of dual infection was associated with a raised CSF protein > 1g/L, elevated lymphocyte and low glucose. The criterion of elevated protein once again correlates well with Aslam et al (2009) who found this to be significant in their study as well.

Similar to other studies such as that by Vidal et al (2017) our study found an association between confusion or altered mental state and a diagnosis of TBM.

The finding of the absence of photophobia associated with TBM needs to be interpreted with caution as the symptom itself is quite subjective.

Limitations

This study has several limitations. As it was a retrospective record review, invariably missing data was a component of the data collection. Some investigations such as CSF TB cultures are not done routinely. We, for example, did not have the CD4 counts, viral loads and opening pressures for all the patients in the study. Furthermore including only CM patients who had TB testing on CSF may introduce selection bias. Standardisation of the measurement of opening pressure was impossible due to the retrospective nature of the study.

CM and CNS TB are defined microbiologically. Although the gold standard in the diagnosis of TB meningitis remains the identification of acid-fast bacilli either by Ziehl-Nielsen smear microscopy or culture and now geneXpert, these may be negative even in those patients with TBM.

Despite these limitations, this study provides evidence of the presence of concomitant CM and TBM. It also highlights the association between dual infection and raised CSF protein, elevated lymphocyte and low glucose findings on CSF.

Future studies

Future research should look at investigating the outcome of patients diagnosed with dual infections. Prospective studies in this area would be beneficial.

Conclusion

Patients who present with signs and symptoms of meningitis, specifically headache and neck stiffness, should have CSF testing for CM. This study is the first in Africa investigating the prevalence of dual infection with CM and TBM and we have shown that both CNS opportunistic infections do occur concomitantly in our population. We have emphasised the need to maintain a high index of suspicion for dual infection especially in immunocompromised patients. Furthermore patients with raised CSF protein >1g/L, low glucose 2.2mmol/L and raised CSF lymphocyte >15 cells$\times 10^6$/L warrant further investigated for TB.

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APPENDIX A: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Comfort Shaba

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170486

NAME: (Principal Investigator)	Dr Comfort Shaba
DEPARTMENT:	Neurosciences/Neurology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Dual Cerebrospinal Fluid Infection with Tuberculosis in HIV Positive Patients Diagnosed with Cryptococcal Meningitis
DATE CONSIDERED:	05/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Modi
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	10/05/2017

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

APPENDIX B: CMJAH CHIEF EXECUTIVE OFFICER PERMISSION

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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

Dear Dr. Comfort Shaba

STUDY TITLE: Dual Cerebrospinal Fluid Infection with Tuberculosis in HIV Positive Patients Diagnosed with Cryptococcul Meningitis.

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not anyway incur or inherit costs as result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 6/6/2017

Approved/not approved

Ms C. Bogoshi
Chief Executive Officer
Date: 07.06.2017

APPENDIX C: DATA COLLECTION SHEET

CODED DATA COLLECTION SHEET

Research number:

Age

Sex

Male	Female
1	2

CD4 count (cells/mm³)

Viral Load (cps/ml)

HAART

No	Yes
1	2

Duration in months

Headache

No	Yes
1	2

Fever

No	Yes
1	2

Photophobia

No	Yes
1	2

Neck stiffness

No	Yes
1	2

Confusion

No	Yes
1	2

Cranial nerve palsy

No	Yes
1	2

Initial CSF findings

Opening pressure		
Glucose		
Protein		
Polymorphs		
Lymphocytes		
Erythrocytres		
India Ink	Neg 1	Pos 2
CLAT	Neg 1	Pos 2
ADA		

TBM diagnosed

No	Yes
1	2

Evidence of TB

No	AFBs on microscopy	CSF genexpert	CSF culture	CXR	Abdo sonar	Imaging CT/MRI	CSF profile	Other (specify)
1	2	3	3	4	5	6	7	

APPENDIX D: TURNITIN REPORT

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ORIGINALITY REPORT

13%	11%	9%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	spiral.imperial.ac.uk Internet Source	1%
2	Suzaan Marais, Dominique J. Pepper, Charlotte Schutz, Robert J. Wilkinson, Graeme Meintjes. "Presentation and Outcome of Tuberculous Meningitis in a High HIV Prevalence Setting", PLoS ONE, 2011 Publication	1%
3	Submitted to University of South Africa Student Paper	1%
4	jiasociety.org Internet Source	<1%
5	Gonzalez-Duarte, Alejandra, Maria del Mar Saniger-Alba, and Jesús Higuera-Calleja. "Cryptococcal meningitis in HIV-negative patients with systemic connective tissue diseases", Neurological Research, 2015. Publication	<1%
6	Adeyemi, Benjamin O., and Andrew Ross. "Management of cryptococcal meningitis in a	<1%

Corrections

Page 13 line 2 “opportunistic infections” replaced with “immunosuppression”

Page 14 paragraph 2 rewritten with the addition of “as well as signs such as “

Page 16 section 1.5.1 line 13 “or” replaced with “of”

Page 17 paragraph 2 issue regarding sensitivity of identifying *Mycobacterium tuberculosis* is addressed further on in same paragraph

Page 18 section 1.5.2.2 line 23 “cistern” replaced with “cisterns”

Page 21 line 23 “of data” is added