



TUBERCULOUS MENINGITIS AND CRYPTOCOCCAL MENINGITIS CO-INFECTION IN HOSPITALISED PATIENTS IN DURBAN, KWA ZULU NATAL (KZN), SOUTH AFRICA

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Dedication

This thesis is dedicated to my husband Farhaad, my dearest son Abdullah, and my family

For their endless love, support, and encouragement

Acknowledgments

I am grateful to Dr. Mohammed Mitha, who encouraged me and assisted me with finding this topic and gave his unwavering support and guidance throughout the course.

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To the late Professor Coovadia for assistance accessing the data used in this project.

Abstract

Background: Cryptococcal meningitis (CCM) and Tuberculous meningitis (TBM) co-infection have been previously described, however, the diagnosis is rare. This may be due to the paucibacillary nature of TBM and the difficulty in diagnosing these conditions due to the overlap in symptoms and cerebrospinal fluid (CSF) findings. This study aimed to determine the frequency and outcome of TBM and CCM co-infection in hospitalised patients.

Methods: A retrospective review of routine laboratory and clinical records for TBM and CCM co-infection cases at four regional hospitals in a single district (Ethekwini) of South Africa between 01 January 2005 and 31 December 2009. CSF TB data at Inkosi Albert Luthuli Central Hospital TB Culture Laboratory, the only TB culture lab in Kwa Zulu Natal, was the starting point to identify CSF TB culture-positive cases. CSF microbiology laboratory data at each identified hospital study site was then reviewed for this retrospective analysis.

All adult patients with positive TB cultures in the cerebrospinal fluid were included. These positive MTB samples were then matched with CSF samples positive for cryptococcosis by CLAT, India ink, or culture. Variables analyzed included age, sex, HIV status, CD4 count, HIV viral load, and CSF chemistry and microbiology.

A chart review of dual-infected cases was then conducted. **Results:** A total of 418 patients were identified based on positive CSF TB culture extracted from laboratory data. A total of 15 patients had dual infection. The prevalence of dual infection was 3.5%. The mean age was 37 years. 12 of 15 patients were diagnosed with TBM posthumously. 6 patients were found to have multidrug-resistant TBM on CSF culture. The in-hospital mortality was 80% (12/15). Despite the age of the data set, the study remains valid and relevant due to the rarity of TBM and CCM co-infection.

Conclusion: In this study, we found that dual infection of the meninges with *mycobacterium tuberculosis* and *cryptococcus neoformans* in HIV-infected patients is rare, however the diagnosis may often be missed or not considered initially. The underdiagnosis and possible delay in diagnosis of dual infection may result in patients not being treated appropriately and adequately leading to increased morbidity and mortality.

This case series highlights the difficulty of diagnosing TBM-CCM co-infection. This study was undertaken prior to the introduction of the Xpert® MTB/RIF Ultra test as well as the lateral flow urine lipoarabinomannan (LF-LAM) assay, and we postulate that with the subsequent introduction of these tests, the diagnosis of TBM may improve earlier detection and yield. Co-infection reflects the advanced immunosuppression characteristic of patients with HIV-associated CCM and the complexities of diagnosis and management in patients at risk of intercurrent opportunistic infections. Our research demonstrates both the need to consider co-infection at baseline diagnosis and the need to remain vigilant for co-infection throughout the follow-up period.

Contribution: This study contributes to the limited data on TBM and CCM co-infection and emphasizes the ongoing need for early diagnosis and treatment.

Keywords: TB meningitis, Cryptococcal meningitis, co-infection, cerebrospinal fluid, TB culture, Cryptococcal Latex Agglutination Test (CLAT)

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Abbreviations:

ADC	Apparent Diffusion Coefficient
AFB	Acid-fast bacilli
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
BREC	Biomedical Research Ethics Committee
CCM	Cryptococcal Meningitis
CI	Confidence Interval
CLAT	Cryptococcal Latex Agglutination Test
CNS	Central Nervous system
CRS	Composite Reference Standard
CT scan	Computed Tomography Scan
CrAg	Cryptococcal Antigen
CSF	Cerebrospinal Fluid
DWI	Diffusion-Weighted Imaging
ELISA	Enzyme-Linked Immunosorbent Assay
FLAIR	Fluid-Attenuated Inversion Recovery
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IRIS	Immune Reconstitution Inflammatory Syndrome
LF-LAM	Lateral Flow Urine Lipoarabinomannan
LP	Lumbar Puncture
MDR TB	Multi Drug Resistant Tuberculosis
MRI	Magnetic Resonance Imaging
NHLS	National Health Laboratory Service
TB	Tuberculosis
TBM	Tuberculous Meningitis
SA	South Africa
WHO	World Health Organisation
XDR TBM	Extensively Drug-Resistant Tuberculous Meningitis
XPert [®] MTB/RIF	Gene eXpert Mycobacterium tuberculosis/resistance to rifampin (RIF)

Review of the literature

Tuberculous Meningitis (TBM) and Cryptococcal Meningitis (CCM) are two major causes of infectious meningitis in people living with HIV, presenting significant morbidity and mortality worldwide. While they are distinct entities, the co-existence of these infections poses diagnostic and therapeutic challenges.

Epidemiology

Findings from the Sixth South African National HIV Prevalence, Incidence, and Behaviour Survey (SABSSM VI) found that the percentage of all people living with Human Immunodeficiency Virus (HIV) in South Africa has decreased from 14.0% in 2017 to 12.7% in 2022. This translates to approximately 7.8 million people living with HIV in South Africa in 2022 compared to 7.9 million in 2017.¹ Meningitis remains a major cause of mortality in Africa and is the medical condition associated with the highest risk of inpatient death²

CCM is one of the top causes of meningitis in adults in Sub-Saharan Africa and other regions with a high prevalence of HIV, accounting for over 100,000 incident cases of meningitis per year in the region.³ Globally, about 15% of HIV-related deaths are attributable to CCM and 75% of these occur in Sub-Saharan Africa.³ It is further estimated that 10–20% of HIV-related deaths in Africa are due to CCM.³ Although the global incidence and deaths from HIV have continued to decline owing to increased access to antiretroviral therapy (ART), including pre-and post-exposure prophylaxis, the prevalence of the disease has not declined.³

In a recent South African study involving a large series of 11,891 cases of confirmed meningitis, the main causes were cryptococcal meningitis 62.3%, Tuberculous meningitis 24.6%, acute bacterial meningitis 10.1% and others 2.1%.⁴ The reported frequency of CCM in HIV varies within Africa from 3.25% in West Africa to 7% in Ethiopia, 40.4% in Uganda, and 1.7-9% in the Central African Republic with an overall estimated frequency there of 10.5%.⁴ Tuberculous meningitis (TBM) is the second most common cause of HIV-associated meningitis after CCM accounting for 10% of the TB/HIV cases and >20% (1-36%) of all cause meningitis.⁴ In TBM the mean age of onset in adults ranges from 26-37 years, and the duration of symptoms ranges from 9-42 days.⁴

Dual infection is rarely described in the literature with data on CCM-TBM co-infection being limited. The co-infection rate among various populations, as described by Wenjie Fang *et al.*, was 0.6% (23 co-infection cases/4053 total cases of patients with TBM) between 1993 and 2006 in China.⁵ Three other studies revealed that co-infection rates ranged from 2.7% to 3.8%.⁶

Pathophysiology

Tuberculous Meningitis

The initial stage of infection with *Mycobacterium tuberculosis* (MTB) occurs with aerosol transmission of MTB-containing droplets from an infected individual.⁷ The mycobacteria are then picked up by alveolar macrophages resulting in localised inflammation.⁷ The clearance of the infection is determined by the host's immune competence and response.⁷ Due to cell-mediated immunity, granulomas form, which restricts the spread and multiplication of the mycobacterium, however, this also facilitates disease latency. In the third and final stage, reactivation of the disease may occur.⁷

Dissemination of TB involves seeding of MTB to other sites including the central nervous system (CNS). Various mechanisms by which the bacilli migrate into the lymphatic system or bloodstream have been suggested.⁸ The meninges are seeded by MTB and form subependymal collections called Rich foci.⁸ These foci can rupture into the subarachnoid space and cause an intense inflammatory response that causes meningitis symptoms.⁸ The exudates caused by this response can encase cranial nerves and cause nerve palsies.⁸ They can entrap blood vessels causing vasculitis, and block cerebral spinal fluid (CSF) flow leading to hydrocephalus, which may be communicating or non-communicating.⁸ These immune responses can lead to complications associated with tuberculous meningitis.⁸

Influence of HIV infection on the pathogenesis of TBM

Infection with HIV is associated with an increased risk of activation of latent infection, as well as an increased risk of rapid progression of primary infection, without an intervening period of latency.⁹ Without HIV infection, individuals with latent infection have a lifetime risk of developing tuberculosis that ranges between 10% and 20%.⁹ In contrast, the HIV-infected individual will carry a 10% annual risk of progression to active infection, with

increasing risk as the CD4+ count declines.⁹ The use of antiretroviral therapy (ART) reduces this risk, but this reduction may be tempered by incomplete immune restoration of tuberculosis-specific lymphocytes, poor adherence, or treatment interruptions related to drug availability.⁹

Patients with HIV and active tuberculosis have an increased risk of extrapulmonary tuberculosis, and this risk will also increase with declining CD4+ count.⁹ Post-mortem examinations have commonly found disseminated tuberculosis among HIV-infected individuals from high-incidence countries.⁹ In an autopsy study from Kenya, disseminated tuberculosis was found in 41% of HIV-infected patients, compared with only 6% of HIV-uninfected patients.⁹ This increased risk of extrapulmonary disease leads to an increased risk of meningitis.⁹

Cryptococcal Meningitis

Cryptococcal disease is predominantly caused by *Cryptococcus neoformans* variant *grubii* (serotype A), and to a lesser extent by *Cryptococcus gatti* infections (formerly *Cryptococcus neoformans* serotypes B and C).^{4,10} *Cryptococcus neoformans* has a worldwide distribution.^{4,10} It is a saprophyte that is found in soil contaminated with pigeon droppings and has also been isolated from the heartwood of several tree species.^{4,10} After inhalation, there may be an initial pulmonary infection.^{4,10} Subsequent dissemination depends on the immune status of the host, and the number and virulence of the organisms inhaled.⁴ The initial infection may be cleared or contained within granulomata as a latent infection in the lungs in immunocompetent individuals whilst it can disseminate in immunocompromised individuals.^{4,10} Disseminated cryptococcal disease is associated with T-cell dysfunction (for example: malignancy, immunosuppressive medication, autoimmune disease, sarcoidosis, and HIV infection).⁴ Macrophages play a role in the dissemination of cryptococcus throughout the host, including dissemination across the blood-brain barrier.^{10,11} The central nervous system (CNS) is a predilected site for cryptococcus species.¹¹

Although the local host immune response is increasingly well described in each infection, a paucity of data exists regarding the immunology of co-infection.¹²

Both *Cryptococcus* and *Mycobacterium tuberculosis* are intracellular pathogens that induce a type 1 immune response marked by an interaction between cells of the adaptive immune

response innate immune cells like macrophages and dendritic cells.¹² HIV depletes both the number and functionality of all immune cells resulting in impaired immune responses and a predisposition toward disseminated tuberculosis and cryptococcosis.¹²

Influence of HIV infection on the pathogenesis of CCM

Cryptococcal immune reconstitution inflammatory syndrome (C-IRIS) may present as a clinical deterioration or new presentation of cryptococcal disease following initiation of antiretroviral therapy (ART) and is believed to be caused by recovery of cryptococcus-specific immune responses.¹³

Similar to the case definition of tuberculosis associated IRIS, two distinct modes of presentation of C-IRIS are recognized.¹³ First, in up to one-third of patients with cryptococcosis diagnosed pre-ART, “paradoxical” C-IRIS occurs following ART initiation.¹³ This presents as worsening or recurrence of treated cryptococcal disease in the same or new anatomical sites despite microbiologic treatment success.¹³ Second, new onset of cryptococcosis occurs in up to 1% of patients initiating ART in whom cryptococcosis was not recognized prior to ART.¹³

Infection with *Cryptococcus neoformans* can remain latent for years after initial exposure, and active disease may remain subclinical for some time in patients with advanced immunodeficiency, so it is not surprising that clinical cryptococcosis may emerge for the first time after ART initiation.¹³ The incidence of ART-associated cryptococcosis ranged from 0.2% to 1.6% in 6 studies including a total of over 2000 patients without evidence of cryptococcosis before initiating ART.¹³ However, the incidence may be as high as 33% in individuals with subclinical cryptococcal antigenemia without fluconazole pre-emptive therapy.¹³ Subclinical antigenemia is therefore the overwhelming risk factor for ART-associated cryptococcosis.¹³

Clinical Presentation

Cryptococcal meningitis usually presents as a subacute meningoencephalitis.¹⁴ The patient commonly presents with neurological symptoms such as a headache, altered mental status, and other signs and symptoms include lethargy, fever, stiff neck (both associated

with an aggressive inflammatory response), nausea, and vomiting. Some patients who are HIV positive may have minimal or nonspecific symptoms at presentation.¹⁴

The duration of symptoms from onset to presentation is usually 1 to 2 weeks in HIV cases and 6 to 12 weeks in non-HIV cases.¹⁴

Visual symptoms include diplopia, photophobia, and reduced acuity later in the disease (due to high CSF pressure or compression of the optic nerve and tracts).¹⁴ Other findings include hearing defects, ataxia, aphasia, seizures, and chorea.¹⁴

Although *C. neoformans* enters the body through the lungs, CNS is the main site of evident clinical infection.¹⁴

TBM also presents as a subacute meningitic illness and may present clinically as TBM alone or as disseminated TB.¹⁴ The clinical presentations of both CCM and TBM are very similar.¹⁴ Patients can present with headaches, neck stiffness, seizures, altered sensorium, and focal neurological signs.¹⁴

Three distinct phases of clinical presentation are usually found in TBM:¹⁵

1. The early prodromal phase is characterized by the insidious onset of low-grade fever, malaise, headache, and personality change. It usually lasts for one to three weeks.
2. It is followed by the meningitic phase, characterized by prominent neurologic features, such as protracted headache, vomiting, meningism, lethargy, confusion, and varying presentation of cranial nerve and long-tract signs.
3. Confusion gives way to stupor, seizures, coma, and often hemiparesis in the paralytic phase. Death frequently ensues within five to eight weeks of the onset of untreated illness.

Atypical manifestations include rapidly progressive meningitic syndrome suggesting pyogenic meningitis, slowly progressive dementia over months, personality change, social withdrawal, memory deficits, and loss of libido.¹⁵ Patients may sometimes also present with an encephalitic course characterized by convulsions, stupor, and coma without overt signs of meningitis.¹

Diagnosis

The diagnosis of TBM is difficult for clinicians as it can clinically present similarly to other forms of meningitis.² The difficulty in diagnosis often leads to a delay in treatment and subsequent mortality.²

A study in Cape Town reported that TBM was present in 27 percent of hospitalised HIV infected patients.⁴ Diagnosis is based on a combination of clinical findings and fCSF examination. CSF findings are also very similar in TBM and CCM with a lymphocytic predominance, elevated protein, and low glucose often found in both conditions.¹⁶

The Cryptococcal Latex Agglutination Test (CLAT) is highly sensitive and specific, with very few false positives and negatives used in the diagnosis of CCM.¹⁷ The definitive diagnosis, however, remains on the culture of the organism from the CSF.¹⁷ In contrast, the rapid diagnosis of TBM remains a significant challenge for both clinicians and microbiologists due to the extremely poor yield of acid-fast bacilli microscopy on CSF samples and the lack of a reliable TB serological or molecular test.¹⁷ According to Palacios *et al.*, a 2014 WHO review of 18 Xpert® MTB/RIF studies for the diagnosis of extrapulmonary TB found a pooled sensitivity of 80.5% (95% confidence interval [CI], 59.0 to 92.2%) compared to traditional culture and 62.8% (95% CI, 47.7 to 75.8%) against a combined reference standard (CRS).¹⁷ For TBM specifically, Xpert® MTB/RIF had a sensitivity of 80.5% (95% CI 59.0–92.2%) and specificity of 97.8% (95% CI 95.2–99.0%) compared to culture results and a sensitivity of 62.8% (95% CI 47.7–75.8%) and specificity of 98.8 (95% CI 95.7–100%) compared to CRS.¹⁷

Another point of care test used in the diagnosis of TB is the use of the lipoarabinomannan (LAM) lateral flow assay (LFA) and LAM antigen detection enzyme-linked immunosorbent assay (ELISA), with sensitivities 29-70%, and specificity 93%, in advanced HIV positive patients.¹⁸

Clinicians in our setting base their treatment regimen on the results of CLAT or the India ink stain. If positive, patients are started on treatment for cryptococcal meningitis.¹⁸ However, it is well recognised that if a patient had previous CCM, the CLAT, and India ink may remain positive.¹⁸ Patients who present with new signs suggestive of meningitis may well have TBM.¹⁸ Due to the high TB burden, some patients may be co-infected with both organisms.¹⁸

Imaging

CT scan findings are often nonspecific for CNS cryptococcosis.⁴ In a retrospective study, CT scans of 35 patients with intracranial cryptococcal infection demonstrated unremarkable findings in 43% of the patients.⁴ However, positive findings included diffuse atrophy (34%), cryptococcomas (11%), hydrocephalus (9%), and diffuse cerebral oedema (3%).¹⁹

Cryptococcomas appear as round, hypoattenuating, or isoattenuating lesions that occur less commonly as gelatinous pseudocysts (rather than as granulomas or abscesses).¹⁹ In immunologically intact hosts, the cryptococci usually induce a chronic granulomatous reaction; on CT scans, these cerebral cryptococcal granulomas appear as hypoattenuating or isoattenuating lesions, with or without enhancement.²⁰

Intracranial mass lesions occur frequently in patients with AIDS.²⁰ The most common mass lesions detected by CT scanning or MRI are abscesses from toxoplasmosis, although lymphoma and less common infectious processes, such as cryptococcomas, must also be considered.²⁰

Besides basic structural information, neuroimaging of the brain has improved the understanding of the neuropathology of cryptococcal meningitis.²⁰ For example, diffusion-weighted imaging (DWI) with apparent diffusion coefficient maps (ADC) can identify foci of acute and subacute infarcts, while contrast-enhanced fluid-attenuated inversion recovery (FLAIR) imaging can demonstrate subtle foci of meningeal enhancement.²⁰

Commonly observed findings on MRI include infarcts, hydrocephalus, and enhancement of the leptomeninges in HIV-positive patients.²⁰

Most infarcts are categorized as lacunar, meaning less than 15 mm diameter, and typically multiple.²¹ Cryptococcosis also causes parenchymal lesions in the brain, often called cryptococcomas, which are masses of encapsulated cryptococci with little or no surrounding inflammation.²¹ Also seen are enlarged Virchow–Robin (VR) spaces, often referred to as pseudocysts when seen as clusters of punctate lesions, reflecting perivascular spaces filled with masses of cryptococci.²¹

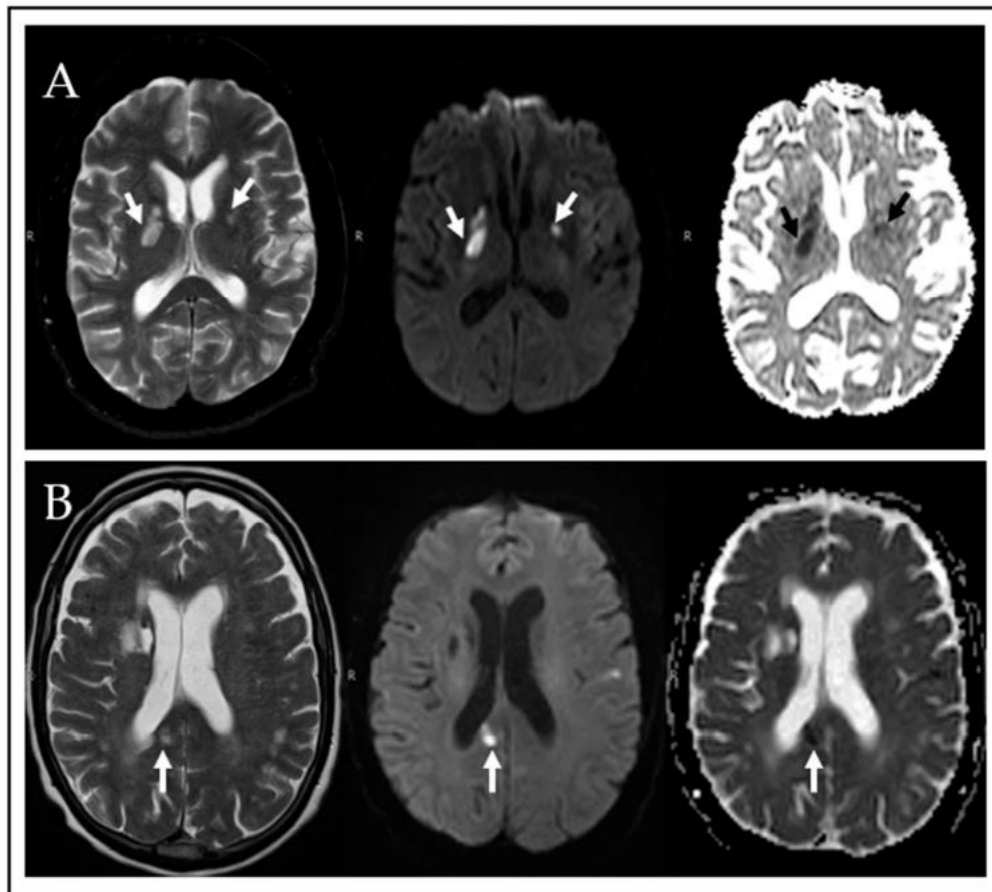


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Figure 1: MRI findings in CCM including ischemic infarcts or cryptococcal-space-occupying lesions. (A) Bilateral basal ganglia acute infarcts showing high signal on T2 weighted images (left image), restricted diffusion with high signal on DWI (mid image) and low signal on ADC maps (right image). (B) Image of likely cryptococcoma in the splenium of the corpus callosum (see arrows), showing high signal on T2 (left image), restricted diffusion with high signal on DWI (mid image) and low signal on ADC maps (right image). While acute infarcts show expected progression of ischemic lesions on follow-up, cryptococcomas are expected to show persistent restricted diffusion over weeks to months.

Neuroimaging plays a crucial role in the early and accurate diagnosis of tuberculous meningitis and its disabling complications.²² Magnetic resonance imaging is considered superior to computed tomography. Neuroimaging characteristics include leptomeningeal

and basal cisternal enhancement, hydrocephalus, periventricular infarcts, and tuberculoma. Partially treated pyogenic meningitis, cryptococcal meningitis, viral encephalitis, carcinomatous, and lymphomatous meningitis may have many similar neuroimaging characteristics, and differentiation from tuberculous meningitis at times based on neuroimaging characteristics becomes difficult.²²

Meningeal enhancement is defined as linear or nodular enhancement of meninges with contrast media at one or more locations: the basal meninges (e.g. basal cistern, ambient cistern, quadrigeminal cistern, prepontine cistern, cerebellopontine cistern, suprasellar cistern, pre medullary cistern), Sylvian fissure, cerebral or cerebellar convexity/sulci and ventricular system.²² Hydrocephalus may be present if one or more of the following are seen on MRI scan: dilated temporal horns of lateral ventricles, ballooning of frontal horns of lateral ventricle, ballooning of third ventricle, narrowed callosal angle, presence of flow void in T2W images at the Sylvian aqueducts.²² Evans' index of every patient with and without hydrocephalus is measured and compared.²² Communicating and non-communicating hydrocephalus is defined based on the absence or presence of an obstructing lesion along the intraventricular CSF pathways.²² Tuberculomas are defined as the presence of nodular or ring enhancement with contrast media and specified as millitary (<2mm) or non-millitary (>2mm) tuberculomas, or pseudo-abscesses.²² Abscesses are defined as the presence of ring enhancement with restricted diffusion appearance on DWI-ADC.²² Acute infarctions are defined as lesions with restricted diffusion on DWI-ADC, and increased T2-weighted and fluid-attenuated inversion recovery (FLAIR) signal intensity.²² Cranial nerve neuropathy is defined as enhancement with or without thickening of the oculomotor nerve, trigeminal nerve, abducens nerve, facial nerve, or vestibulocochlear nerve.²²

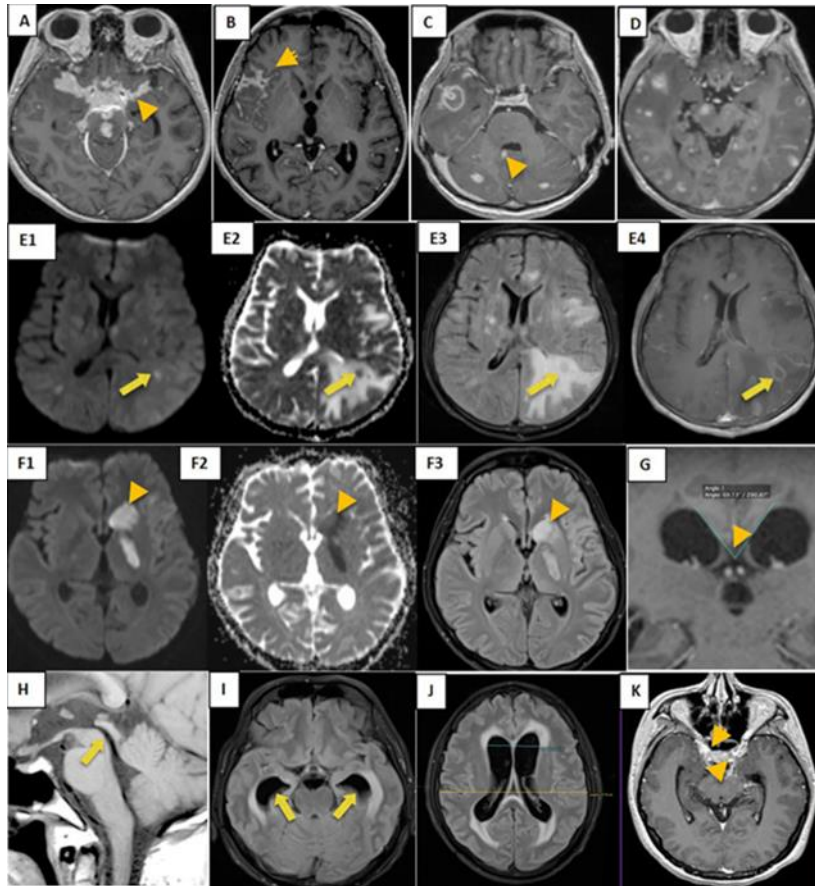


Image used with permission-Source: Dian S et al.: Brain MRI findings in relation to clinical characteristics and outcome of tuberculous meningitis

Figure 2: Common baseline MRI findings in adults with tuberculous meningitis.

Meningeal enhancement at basal meninges (A), right Sylvian fissure (B), and ventricular (C); non-miliary and miliary tuberculomas (D); pseudo abscess in axial DWI (E1), axial ADC (E2), axial T2 FLAIR (E3) and axial T1W1 post contrast (E4); multiple acute infarctions at the left basal ganglia in axial DWI (F1), axial ADC (F2), and axial T2 FLAIR (F3); Communicating hydrocephalus with narrowed Callosal angle (G), void signal in the aqueduct (H), and dilated temporal horn (I), broader of Evans' ratio (J); and oculomotor nerve enhancement (K).

Treatment

The antifungal treatment of HIV-associated CCM is divided into three phases: induction, consolidation, and maintenance phase.²³ The 2022 WHO guidelines recommend that the first choice for induction phase treatment is a single high dose (10 mg/kg) of liposomal amphotericin B with 14 days of flucytosine (100 mg/kg per day divided into four doses per day) and fluconazole (1200 mg/daily for adults; 12 mg/kg per day for children and

adolescents up to a maximum of 800 mg daily).²³ If liposomal amphotericin is not available: A seven-day course of amphotericin B deoxycholate (1 mg/kg per day) and flucytosine (100 mg/kg per day, divided into four doses per day) followed by seven days of fluconazole (1200 mg daily for adults and 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).²³ If no amphotericin formulation is available: 14 days of fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents) and flucytosine (100 mg/kg per day, divided into four doses per day).²³ Fluconazole and flucytosine are the only recommended oral combination regimens and have been associated with lower mortality compared with amphotericin B deoxycholate and fluconazole.²³ If flucytosine is not available: 14 days of liposomal amphotericin B (3–4 mg/kg per day) and fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).²³ If liposomal amphotericin B and flucytosine are not available: 14 days of amphotericin B deoxycholate (1 mg/kg per day) and fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).²³ The consolidation phase follows with fluconazole 800mg/day for 8 weeks or until CSF is sterile followed by a secondary suppressive phase of maintenance therapy with fluconazole 200mg/day until CD4 counts are $>200/\text{mm}^3$ and virologic suppression has been established.²³ If the CSF pressure at diagnosis is elevated (>20 cm H₂O) then serial daily/ alternate day lumbar punctures are indicated to decrease intracranial pressure which has been shown to reduce mortality.²³

Current South African and World Health Organization tuberculosis guidelines recommend 9 months of treatment for TBM, consisting of intensive-phase therapy for 2 months with a combination of rifampicin, isoniazid, ethambutol, and pyrazinamide, followed by 7 months of rifampicin and isoniazid as first-line treatment.²⁴ High-dose corticosteroid treatment with prednisone 1-2mg/kg daily for 4 weeks and then taper off gradually over 2 weeks is recommended in the treatment of TB meningitis.²⁴

Timely treatment dramatically improves the outcome of TBM and CCM, however, both these conditions require prolonged courses of drug treatment which exposes patients to a wide array of drug toxicities which further complicates the morbidity and mortality of patients with dual infections.²⁴

Complications

Mortality and serious long-term sequelae still occur in about 50% of patients with tuberculous meningitis despite anti-tuberculosis treatment.²⁴ Complications include hydrocephalus due to obstruction of CSF outflow causing raised intracranial pressure.²⁵ Hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion may occur in 40 to 50% of patients with TBM.²⁵ Tuberculomas can occur independently of TBM or in conjunction with TBM.²⁵ Vasculitis and stroke occur in 15 to 57% of patients with TBM depending on which diagnostic modalities are used in diagnosis, with MRI being diagnostically superior in diagnosis to CT.²⁵ Seizures, generally focal, result from hyponatremia, infarction, and meningeal irritation.²⁶ Loss of vision that could be permanent due to compression of the optic chiasma by the dilated third ventricle as the optic chiasma and optic nerve are encased by thick tuberculous exudates.²⁷ Transverse myelitis manifests as paraparesis or quadriparesis, sensory symptoms, and urinary retention.²⁷

Complications of CCM include persistent infection, whereby individuals are treated for at least 4 weeks with anti-fungal therapy at an effective dose but the CSF cultures remain persistently positive.²⁷ Elevated CSF pressure requiring repeated therapeutic lumbar punctures can complicate the clinical course of CCM. Immune Reconstitution Inflammatory Syndrome (IRIS) may also occur.²⁸ Unmasking IRIS, in which cryptococcal symptoms appear after the initiation of antiretroviral therapy, and paradoxical IRIS during the treatment of cryptococcosis and administration of antiretroviral therapy.²⁸

Tuberculosis and Cryptococcal meningitis co-infection:

Literature on co-infection of TBM in cryptococcal meningitis amongst HIV-infected patients is limited.

In a case report of 5 HIV-infected Ugandan adults with cryptococcal and tuberculous (TB) meningitis co-infection, all unmasked meningitis occurred within 5 weeks of starting HIV therapy. Xpert® MTB/RIF Ultra facilitated prompt diagnosis; however, 60% in-hospital mortality occurred. TB meningitis coinfection prevalence was 0.8% (5/586) among cryptococcal meningitis, 2 of which occurred during second cryptococcal episodes.²⁹

Another case series from Mali described 3 cases of neuromeningeal cryptococcosis and lung tuberculosis coinfection collected prospectively over a year at the Department of Infectious Diseases of the Point G Teaching Hospital in Bamako.³⁰ Two patients had HIV disease, and the third patient had no underlying immunosuppressive illnesses.³⁰

CCM and TBM co-infection has been described in other case reports in patients with a range of underlying immune deficiencies, including HIV (n = 3), systemic lupus erythematosus (n =1), Waldenstrom's macroglobulinemia (n =1), and reticulum cell sarcoma (n =1) and in patients with no underlying immunodeficiency (n = 2).^{31,32}

Silber *et al.*, describe seven HIV-1 seropositive patients with cryptococcal meningitis, three of whom had a proven nervous system infection with a second organism: concurrent tuberculous meningitis, a tuberculoma, and the first documented case of cryptococcal meningitis and neurosyphilis.³³ They concluded that co-infection of the nervous system by two distinct nonviral organisms is uncommon and often undiagnosed.³³

To the best of our knowledge, the presence of dual infection with TBM and CCM has yet to be described in Durban. This study aimed to determine the frequency, outcomes, and if patients were appropriately diagnosed and treated in the regional public hospitals in Durban.

References

1. South African National HIV Prevalence, Incidence and Behavior and Communication Survey 2017. Human Sciences Research Council. Available: <http://www.hsrbpress.ac.za>
2. 2022 Guidelines for Diagnosing, Preventing and Managing Cryptococcal Disease among adults, adolescents and children living with HIV. Available: <https://www.nicd.ac.za/wp-content/uploads/2023/01/2022-WHO-Guideline-Crypto-Final-9789240052178-eng.pdf>
3. Muzazu SGY, Assefa DG, Phiri C, Getinet T, Solomon S, Yismaw G, Manyazewal T. Prevalence of cryptococcal meningitis among people living with human immunodeficiency virus and predictors of mortality in adults on induction therapy in Africa: A systematic review and meta-analysis. *Front Med (Lausanne)*. 2022 Sep 8;9:989265. doi: 10.3389/fmed.2022.989265

4. Jarvis JN, Meintjies G, William A et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infectious Diseases* 2010;10:67 doi:10.1186/1471-2334-10-67
5. Fang W, Zhang L, Liu J, Denning DW, Hagen F, Jiang W, Hong N, Deng S, Lei X, Deng D, Liao W, Xu J, Boekhout T, Chen M, Pan W. Tuberculosis/cryptococcosis co-infection in China between 1965 and 2016. *Emerg Microbes Infect.* 2017 Aug 23;6(8):e73. doi: 10.1038/emi.2017.61
6. Marais S, Wilkinson R J. The diagnosis and medical management of tuberculous meningitis in adults. *SAMJ, S. Afr. med. j.* [Internet]. 2014 Dec [cited 2023 Aug 13] Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0256-95742014001200023&lng=en. <http://dx.doi.org/10.7196/SAMJ.9060>
7. Sasindran, S. & Torrelles, J. Mycobacterium Tuberculosis Infection and Inflammation: what is Beneficial for the Host and for the Bacterium? *Frontiers in Microbiology* 2(2011).
8. Thwaites G, Chau TT, Mai NT, Drobniewski F, McAdam K, Farrar J. Tuberculous meningitis. *J Neurol Neurosurg Psychiatry.* 2000 Mar;68(3):289-99.
9. Vinnard C, Macgregor RR. Tuberculous meningitis in HIV-infected individuals. *Curr HIV/AIDS Rep.* 2009 Aug;6(3):139-45. doi: 10.1007/s11904-009-0019-7.
10. Antinori S. New Insights into HIV/AIDS-Associated Cryptococcosis. *Isrn aids.* 2013;2013:471363.
11. Williamson PR, Jarvis JN, Panackal AA, Fisher MC, Molloy SF, Loyse A, et al. Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. *Nat Rev Neurol.* 2017;13(1):13-24
12. Rutakingirwa MK, Cresswell FV, Kwizera R, Ssebambulidde K, Kagimu E, Nuwagira E, Tugume L, Mpoza E, Dobbin J, Williams DA, Muzoora C, Meya DB, Boulware DR, Hullsiek KH, Rhein J. Tuberculosis in HIV-Associated Cryptococcal Meningitis is Associated with an Increased Risk of Death. *J Clin Med.* 2020 Mar 13;9(3):781. doi: 10.3390/jcm9030781.
13. Haddow LJ, Colebunders R, Meintjies G, Lawn SD, Elliott JH, Manabe YC, Bohjanen PR, Sungkanuparph S, Easterbrook PJ, French MA, Boulware DR; International Network for the Study of HIV-associated IRIS (INSHI). Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *Lancet Infect Dis.* 2010 Nov;10(11):791-802. doi: 10.1016/S1473-3099(10)70170-5.

14. Williamson PR, Jarvis JN, Panackal AA, Fisher MC, Molloy SF, Loyse A, Harrison TS. Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. *Nat Rev Neurol*. 2017 Jan;13(1):13-24.
15. Udani PM, Dastur DK. Tuberculous encephalopathy with and without meningitis. Clinical features and pathological correlations. *J Neurol Sci*. 1970 Jun;10(6):541-61.
16. Bhigjee AI, Padayachee R, Paruk H et al. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. *International Journal of Infectious Diseases* 2007 11, 348-354
17. Carlo Foppiano Palacios, Paul G. Saleeb, Challenges in the diagnosis of tuberculous meningitis, *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, Volume20,2020,100164,ISSN2405-5794, <https://doi.org/10.1016/j.jctube.2020.100164>.
18. Howlett WP. Neurological disorders in HIV in Africa: a review. *Afri Health Sci*.2019;19(2): 1953-1977. <https://dx.doi.org/10.4314/ahs.v19i2.19>
19. Popovich MJ, Arthur RH, Helmer E. CT of intracranial cryptococcosis. *AJR Am J Roentgenol*. 1990 Mar. 154(3):603-6.
20. Harris DE, Enterline DS. Neuroimaging of AIDS. I. Fungal infections of the central nervous system. *Neuroimaging Clin N Am*. 1997 May. 7(2):187-98
21. Anjum, S.H.; Bennett, J.E.; Dean, O.; Marr, K.A.; Hammoud, D.A.; Williamson, P.R. Neuroimaging of Cryptococcal Meningitis in Patients without Human Immunodeficiency Virus: Data from a Multi-Center Cohort Study. *J. Fungi* 2023, 9, 594. <https://doi.org/10.3390/jof9050594>
22. Dian S, Hermawan R, van Laarhoven A, Immaculata S, Achmad TH, Ruslami R, Anwary F, Soetikno RD, Ganiem AR, van Crevel R. Brain MRI findings in relation to clinical characteristics and outcome of tuberculous meningitis. *PLoS One*. 2020 Nov 13;15(11):e0241974. doi: 10.1371/journal.pone.0241974.
23. Guidelines for diagnosing, preventing and managing cryptococcal disease among adults, adolescents and children living with HIV. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO
24. Moodley S, Dlwati M, Bhigjee A. Treatment of tuberculous meningitis in adults: Is the duration of intensive-phase therapy adequate? *S Afr Med J*. 2021 Apr;111(5):412-415.

25. Misra UK, Kalita J, Bhoi SK, Singh RK. A study of hyponatremia in tuberculous meningitis. *J Neurol Sci.* 2016 Aug 15;367:152-7.
26. Misra UK, Kumar M, Kalita J. Seizures in tuberculous meningitis. *Epilepsy Res.* 2018 Dec;148:90-95.
27. Garg RK, Malhotra HS, Kumar N, Uniyal R. Vision loss in tuberculous meningitis. *J Neurol Sci.* 2017 Apr 15;375:27-34.
28. Wong EB, Omar T, Setlhako GJ, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. *PLoS ONE* 2012;7:e47542.
29. Ellis J, Cresswell FV, Rhein J, Ssebambulidde K, Boulware DR. Cryptococcal Meningitis and Tuberculous Meningitis Co-infection in HIV-Infected Ugandan Adults. *Open Forum Infect Dis.* 2018 Aug 29;5(8):ofy193. doi: 10.1093/ofid/ofy193. Erratum in: *Open Forum Infect Dis.* 2018 Oct 16;6(3):ofy252.
30. Ouedraogo D, Cissoko Y, Soumare M, Alle Akakpo AE, Loua OO, Konate I, Niare Doumbo S, Dao S. Neuromeningeal Cryptococcosis and Tuberculosis Coinfection in Bamako: A One-Year Case Series. *Open Forum Infect Dis.* 2023 Aug 18;10(8):ofad438. doi: 10.1093/ofid/ofad438.
31. Chen M, Al-Hatmi AM, Chen Y, et al. Cryptococcosis and tuberculosis co-infection in mainland China. *Emerg. Microbes Infect.* 2016;5:e98.
32. Mete B, Saltoglu N, Vanli E, et al. Simultaneous cryptococcal and tuberculous meningitis in a patient with systemic lupus erythematosus. *J Microbiol Immunol Infect* 2016; 49:289–94.
33. Silber E, Sonnenberg P, Koornhof HJ, Morris L, Saffer D. Dual infective pathology in patients with cryptococcal meningitis. *Neurology.* 1998 Oct;51(4):1213-5. doi: 10.1212/wnl.51.4.1213

Part 2: A submission ready manuscript

Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa

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Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa

Abstract

Background: Cryptococcal meningitis (CCM) and Tuberculous meningitis (TBM) co-infection have been previously described, however, the diagnosis is rare. This may be due to the paucibacillary nature of TBM and the difficulty in diagnosing these conditions due to the overlap in symptoms and cerebrospinal fluid (CSF) findings. This study aimed to determine the frequency and outcome of Tuberculous meningitis (TBM) and Cryptococcal meningitis (CCM) co-infection in hospitalised patients.

Methods: A retrospective review of routine laboratory and clinical records for TBM and CCM co-infection cases at four regional hospitals in a single district (Ethekeini) of South Africa between 01 January 2005 and 31 December 2009.

CSF TB data at Inkosi Albert Luthuli Central Hospital TB Culture Laboratory, the only TB culture lab in Kwa Zulu Natal, was the starting point to identify CSF TB culture positive cases. CSF microbiology laboratory data at each identified hospital study site was then reviewed for this retrospective analysis.

All adult patients with positive TB cultures in the cerebrospinal fluid were included. These positive MTB samples were then matched with CSF samples positive for cryptococcosis by CLAT, India ink, or culture. Variables analyzed included age, sex, HIV status, CD4 count, HIV viral load, and CSF chemistry and microbiology.

A chart review of dual infected cases was then conducted.

Results: A total of 418 patients were identified based on positive CSF TB culture extracted from laboratory data at each study site. A total of 15 patients had dual infection. The prevalence of dual infection was 3.5%. The mean age was 37 years. 12 of 15 patients were diagnosed with TBM posthumously. 6 patients were found to have multidrug-resistant TBM

on CSF culture. The in-hospital mortality was 80% (12/15). Despite the age of the data set, the study remains valid and relevant due to the rarity of TBM and CCM co-infection.

Conclusion: In this study, we found that dual infection of the meninges with *Mycobacterium tuberculosis* and *Cryptococcus neoformans* in HIV-infected patients is rare, however the diagnosis may often be missed or not considered initially. The underdiagnosis and possible delay in diagnosis of dual infection may result in patients not being treated appropriately and adequately leading to increased morbidity and mortality.

This case series highlights the difficulty of diagnosing TBM-CCM co-infection. This study was undertaken prior to the introduction of the Xpert® MTB/RIF Ultra test as well as the lateral flow urine lipoarabinomannan (LF-LAM) assay, and we postulate that with the subsequent introduction of these tests, the diagnosis of TBM may improve earlier detection and yield.

Contribution: This study contributes to the limited data on TBM and CCM co-infection and emphasizes the ongoing need for early diagnosis and treatment.

Keywords: TB meningitis, Cryptococcal meningitis, co-infection, cerebrospinal fluid, TB culture, Cryptococcal Latex Agglutination Test (CLAT)

Introduction:

According to the South African National HIV Prevalence, Incidence, Behavior, and Communication survey in 2017, the prevalence of HIV in South Africa was estimated at 14.0%, translating to 7.9 million people living with HIV.¹ Among the medical conditions associated with the highest mortality risk in hospitals in Africa, meningitis is the leading cause of death.² Cryptococcal meningitis (CCM) is the most common causative pathogen in advanced HIV; especially in those with low CD4 counts.² Approximately 250,000 cases of cryptococcosis were recorded in 2014, which accounted for 15% of deaths associated with HIV.³ Tuberculous meningitis (TBM) is the second most common cause of HIV-associated meningitis.¹ Due to its similar clinical presentation to other forms of meningitis, TBM is difficult to diagnose.² Diagnostic challenges often lead to treatment delays and subsequent mortality.

A study in Cape Town reported that TBM was present in 27 percent of hospitalised HIV-infected patients admitted with meningitis.⁴ TBM presents as a subacute meningitic illness and may present clinically as TBM alone or as disseminated TB.⁵ The clinical presentations of both CCM and TBM are very similar.⁴ Patients can present with headaches, neck stiffness, seizures, altered sensorium, and focal neurological signs. CSF findings are also very similar in TBM and CCM with a lymphocytic predominance, elevated protein, and low glucose, often found in both conditions.⁶

Although the Cryptococcal Latex Agglutination Test (CLAT) is highly sensitive and specific, with very few false positives and negatives, the definitive diagnosis remains on culture of the organism from the CSF.⁷ The disadvantage of the CLAT however, is in the evaluation of patients who have had CCM and then re-present with meningitis, as the CLAT may remain positive in the serum and the CSF for an extended period.⁷ In contrast, the rapid diagnosis of TBM remains a significant challenge for both clinicians and microbiologists due to the extremely poor yield of acid-fast bacilli microscopy on CSF samples and the lack of a reliable TB serological or molecular test.⁷ An analysis of 18 Xpert[®] MTB/RIF studies for the diagnosis of extrapulmonary tuberculosis by Palacios *et al.* in 2014 found that the pooled sensitivity for extrapulmonary tuberculosis was 80.5% (95% confidence interval [CI], (59.0 to 92.2%)) when compared to traditional culture and 62.8% when compared with the composite reference standard (CRS).⁷ The reference standards were mycobacterial culture or a composite reference standard (CRS) defined by the study authors of the individual studies.⁷

Clinicians in our setting base their treatment regimen on the results of CLAT or the India ink stain. If positive, patients are started on treatment for cryptococcal meningitis. However, it is well recognised that if a patient had previous CCM, the CLAT, and India ink may remain positive for an extended period. Patients who present with new signs suggestive of meningitis may well have TBM. Due to the high TB burden, some patients may be co-infected with both organisms. Dual infection with both TBM and CCM have been documented. A study in Taiwan demonstrated that co-infection in the brain is the second most common site after the lung.⁸ A small study published in 1998, conducted on

mineworkers in rural South Africa revealed that 2 of 7 (<30%) patients with HIV and suspected meningitis had co-infection with tuberculosis and cryptococcus.⁹

Wong et al. found that 62% of HIV-infected patients with Mycobacterium infection had multiple infectious causes of death at autopsy, including cryptococcosis.¹⁰ However, most of the available literature on the co-infection of TB in cryptococcal meningitis amongst HIV-infected patients is limited to case series and reports. A retrospective study by Britz *et al* described the epidemiology of meningitis in CSF among adults in Gauteng province in South Africa. They noted cryptococcal meningitis, tuberculous meningitis, and pneumococcal meningitis accounted for 62.3% (n = 7,406), 24.6% (n = 2,928) and 10.1% (n = 1,197) of cases over a four-year period.⁸ A total of 112 cases (0.9%) of mixed aetiology occurred, of which 95 cases were cryptococcal and tuberculous meningitis.⁸

To the best of our knowledge, the presence of dual infection with TBM and CCM has not been well described in Durban. This study aimed to describe the frequency and outcome of TBM and CCM co-infection in hospitalised patients and to review the diagnostic and therapeutic processes during hospitalisation in the regional, public hospitals in Durban, South Africa.

Materials and methods:

Retrospective data from the laboratory and clinical records of patients were reviewed for CCM in those with culture-proven TBM on cerebrospinal fluid. The patients were admitted from 1 January 2005 to 31 December 2009 at regional hospitals in the Durban Metropolitan Complex, South Africa. All adult patients who had positive TB culture in the CSF and positive for CCM either by India ink, CLAT, or culture were included in the study. Patients with incomplete medical records i.e.: where CSF results or other data were not available, were excluded.

CSF TB data at Inkosi Albert Luthuli Central Hospital TB Culture Laboratory, the only TB culture lab in Kwa Zulu Natal, was the starting point to identify CSF TB culture positive cases. CSF microbiology laboratory data at each identified hospital study site was then reviewed for this retrospective analysis.

All adult patients with positive TB cultures in the cerebrospinal fluid were included. These positive MTB samples were then matched with CSF samples positive for cryptococcosis by CLAT, India ink, or culture. Variables analyzed included age, sex, HIV status, CD4 count, HIV viral load, and CSF chemistry and microbiology.

A chart review of dual infected cases was then conducted.

TBM and CCM infection were concurrent to the best of our knowledge.

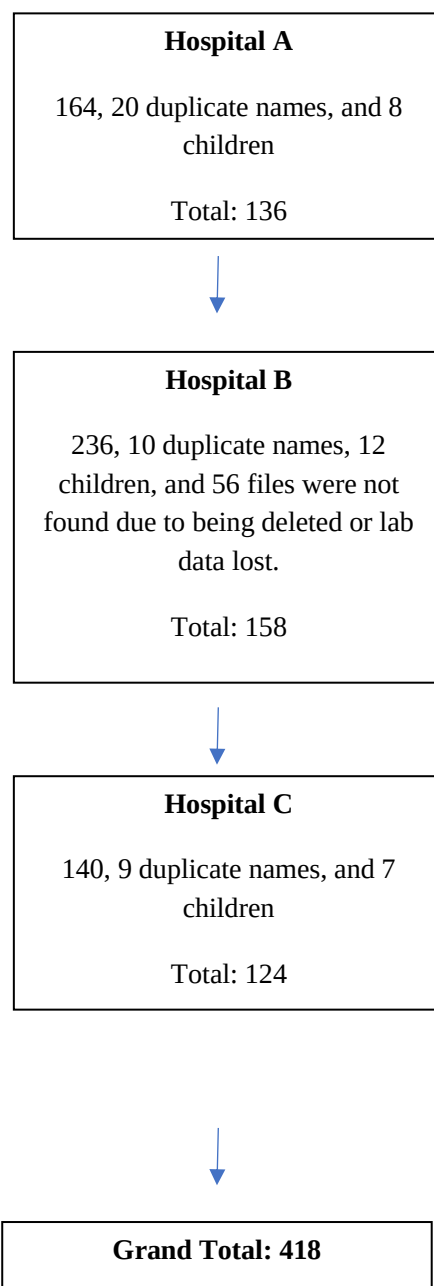


Table 1: Number of patients per hospital

Ethics: The proposal was submitted to the Biomedical Research Ethics Committee (BREC) of the University of Kwa Zulu Natal and the Kwa Zulu Natal Department of Health for evaluation and approval. The ethics approval number for this study is BREC/00005669/2023. The data was analysed anonymously to ensure the privacy and confidentiality of the participants' information.

Statistical Analysis:

Data was collated on a Microsoft® Excel spreadsheet and was then analysed using SPSS software version 24.

Results:

There was a total of 418 patients identified with confirmed TB meningitis between 01 January 2005 to 31 December 2009, with 15 patients having dual infection (3.5%). Amongst the patients with dual infection, females were comparatively more than males (6 males vs 9 females). The age range of the study subjects was between 14 and 56 years with the mean age being 37 years.

The most common symptoms described were headaches (86.6%), fever (33.3%), photophobia (33.3%), and disturbances in the level of consciousness (26.6%). Several patients also reported a productive cough (40%), loss of appetite (33.3%), and loss of weight (33.3%).

The median CSF neutrophil count/uL was 0/uL, and lymphocyte count/uL was 44/uL, whilst the median CSF glucose was 0.8mmo/l and protein of 0.83g/L respectively in patients with TBM and CCM co-infection.

Ten of the fifteen patients with dual infection were known to be HIV positive at the time of admission. Six of the ten patients had CD4 counts of < 250 cells/ul and four patients with an unknown CD4 count. TBM was diagnosed posthumously in 12 patients. 6 patients with co-infection were found to have multidrug-resistant TBM on culture, with one of those patients

having extensively drug-resistant TBM (XDR TBM). The in-hospital mortality was 80% (12/15), with only three patients who survived to discharge.

The detailed histories, mode of diagnosis, and treatment with dual infection are outlined below:

1. 27-year-old female, HIV positive with an unknown CD4 count and no background medical history. He presented with a 3-week history of cough, loss of appetite, weight loss, and night sweats. He also had a 1-week history of headaches not associated with photophobia. Examination revealed a clinically immunocompromised patient with lymphadenopathy, oral thrush, and a 5cm hepatomegaly. The central nervous examination revealed a confused patient with neck stiffness and no neurological deficits. Chest radiography had bilateral infiltrates and left lower zone consolidation. Ultrasound of the abdomen indicated intra-abdominal lymph nodes. The patient's sputum was positive for TB on smear microscopy, CSF CLAT was positive and the CSF TB culture was also positive. The patient was commenced on anti-TB treatment and Amphotericin B. The patient demised 11 days after admission.
2. 23-year-old female with a background history of epilepsy and HIV. Her CD4 count was unknown. She presented with a 1-month history of cough, loss of appetite, loss of weight, night sweats, nausea, and vomiting. On examination, she appeared clinically immunocompromised with generalized wasting, pallor, and oral thrush. A respiratory examination revealed bilateral crackles. She had terminal neck stiffness on neurological examination with no other neurological deficits. The rest of the examination was normal. Chest radiography was suggestive of miliary TB with bilateral nodular infiltrates. She was commenced on TB treatment and prednisone. She was not initiated on CCM treatment as her low CSF glucose and lymphocyte pleocytosis were attributed to TBM. Her CSF CLAT result was unavailable until after her death. She demised two days after admission.

3. 31-year-old female with no background medical history and HIV status unknown. She presented with a 3-month history of headaches with no photophobia and diarrhoea. She had a 3-week history of coughing, night sweats, fever, loss of appetite, loss of weight, and generalized weakness. Examination revealed a clinically immunocompromised patient with oral candidiasis, herpes labialis, and pallor. A respiratory examination revealed bronchial breathing in the right upper zone. She had a 4 cm hepatomegaly on abdominal examination, and on neurological examination, she had terminal neck stiffness only with a normal mental state. Chest radiograph revealed nodular shadowing in keeping with TB on our chart review. She was commenced on IV antibiotics. TBM nor CCM was not considered. The CLAT as well as the TB culture on CSF were positive but the result was received posthumously by the attending team. The patient demised three days later.
4. 38-year-old female with no previous medical history and unknown HIV status presented with a 2-week history of headache, photophobia, loss of weight, loss of appetite, neck pain, and vomiting. She was clinically immunocompromised on examination, and the only positive finding was neck stiffness. Her chest radiograph was normal. She had yeast cells on the gram stain, and her serum and CSF CLAT were positive. She was commenced on intravenous ceftriaxone and had a test dose of amphotericin B. She subsequently demised the next day. TBM was not considered. The patient's CSF had cultured MDR TB after her death.
5. 37-year-old male, HIV positive with unknown CD4 count. He had CCM two years ago. He was previously on fluconazole prophylaxis but had defaulted treatment. He presented to the hospital with headaches, drowsiness, and an inability to speak. On examination, he had a depressed level of consciousness with neck stiffness. All other systems were normal. A CT scan of his brain revealed hydrocephalus. There was no record of a chest radiograph. CSF India Ink stain showed yeast cells and was CLAT

positive. *Cryptococcus neoformans* was also cultured. The patient received Amphotericin B and Fluconazole. TBM was not considered. The patient demised ten days later. The patient also had concurrent drug-sensitive TBM, which was unknown then.

6. 43-year-old male, HIV positive with a CD4 of 74 cells/uL. He previously had pulmonary TB in 2004 but did not complete treatment. In 2006 he developed reactivation pulmonary TB based on positive sputum as well as CCM on CSF. He continued TB treatment but interrupted his fluconazole treatment after 4 months. Whilst in the continuation phase of TB treatment, he presented with a 3-day history of headaches, photophobia, seizures, and diarrhoea. Examination revealed oral candidiasis, confusion, and neck stiffness. All other systems were normal. Chest radiograph indicated right upper lobe fibrosis. CT brain showed age-inappropriate atrophy. The CSF result showed that the CLAT was positive however the CSF culture was negative for cryptococcal species. This may have been due to his previous episode of CCM. The patient refused amphotericin B but received fluconazole, sodium valproate, pyridoxine, and drug-sensitive TB treatment. The CSF later cultured MDR TB; however, the patient had already demised 14 days prior.
7. 24-year-old female, HIV positive with an unknown CD4 count, presented with a 2-week history of headache, neck pain, and vomiting. Examination revealed a wasted patient with pallor and oral thrush. Her cardiovascular, respiratory, and abdominal examination was normal. Neurologically she had neck stiffness with no other focal neurological signs. Her CSF showed cryptococcus species and yeast cells on gram stain and India Ink stain. She was commenced on amphotericin B. She became confused in the ward and developed focal neurological deficits. She died ten days later. TBM was not considered in the diagnosis. She was found to have MDR TB subsequently after death.

8. 29-year-old female, HIV status was not recorded in patient's file, PTB on 4th month of continuation phase of treatment, presented with a 3-day history of progressively worsening headache. Examination revealed a globally wasted patient with no other signs on general and systemic examinations. The CSF result showed *cryptococcal* species on India ink staining and cultured the organism. The patient was maintained on a rifampicin/isoniazid combination as well as receiving 14 days of amphotericin B. She was discharged on fluconazole. The patient presented two months later, complaining of severe headaches for an uncertain duration. She had a CT Brain, which showed basal enhancement. The repeat CSF (shown in table 2 below) revealed a negative India Ink stain; however, the CLAT was positive. The patient was continued on the continuation phase of TB treatment, corticosteroids, amphotericin B, and fluconazole. The patient unfortunately demised. The patient was subsequently found to have XDR TB on culture.
9. 38-year-old male with no previous medical history and unknown HIV status presented with a 2-week history of headaches and a 2-day history of confusion. On examination, the patient had generalized lymphadenopathy and was confused. CNS examination revealed only neck stiffness. The rest of the examination was normal. CSF revealed *cryptococcus* on India Ink staining and a positive CSF CLAT. The patient was commenced on amphotericin B; however, he demised one week later. It was subsequently found that the patient also had drug-sensitive TBM. The diagnosis was not considered as the patient had a positive India Ink stain for CCM.
10. 46-year-old male, HIV positive but not on ARVs as he had interrupted treatment due to incarceration. He was previously treated ten years ago for PTB. The patient was once again diagnosed with PTB six months earlier and was on continuation phase treatment. He then developed signs and symptoms of meningitis and was diagnosed with TBM based on positive CSF culture. He continued to have headaches and diarrhoea and was found to have disseminated *cryptococcus neoformans* in the CSF and serum. He was commenced on amphotericin B and fluconazole and continued

continuation phase TB treatment for nine months. The patient was discharged well and continued to be managed on a routine basis including re-initiation of ARVs.

11. A 14-year-old male with unknown HIV status and no previous medical history. He presented with a 1-month history of fever, night sweats, and cough. He had diarrhoea, vomiting, and a skin rash for two days. Examination revealed a pyrexial patient, hypotensive with pallor and oral candidiasis. A respiratory exam revealed bibasal crackles, and he had a 4cm hepatomegaly. Chest radiograph showed nodular shadowing in the left lower lung field. Sputum was positive for Acid Fast Bacilli, and the patient was commenced on anti-TB treatment. Six days after admission (3 days of TB treatment), the patient develops neck stiffness. The CSF revealed a positive CLAT, and yeast cells on staining. The patient was commenced on amphotericin B; however, he demised two weeks later. He was subsequently found to have MDR TB on CSF as well.
12. 56-year-old female, HIV positive with a CD4 count of 218 cells/ul, not on HAART as per HIV treatment guidelines at that time (2005). She had previous CCM four years prior and was on fluconazole 200mg daily and previous PTB, for which she was treated for six months. She presented with a 2-month history of headache, vomiting, and confusion and a 2-day history of diarrhoea. There was no cough, loss of appetite, or loss of weight. The only significant finding on examination was neck stiffness. Chest radiograph revealed reticulonodular shadowing. A computed tomography (CT) scan of the brain indicated a prominent ventricular system and hypodensities in the basal ganglia. The lumbar puncture was CLAT positive, and she also cultured TB and *cryptococcus* on CSF. The patient commenced TB and CCM treatment. The patient was treated appropriately and discharged well.
13. 24-year-old male, HIV positive with a CD4 count of 53 cells/ul, not on ARVs as he had interrupted his treatment after three months. The patient also had sputum-positive

PTB 4 months prior to presentation and had also interrupted treatment. He presented with a 10-day history of headache, photophobia, and blurred vision. He also had a loss of appetite, loss of weight, night sweats, productive cough, and pleuritic chest pain. On examination, he was emaciated with melanonychia and axillary lymphadenopathy. Respiratory, cardiovascular, and abdominal examinations were normal. The only positive finding on CNS examination was neck stiffness. The chest radiograph and CTB were normal. The CSF was CLAT positive, and *cryptococcus neoformans* was cultured on CSF. The patient was treated with amphotericin B and fluconazole. The patient subsequently demised. TB was cultured posthumously on his CSF.

14. 33-year-old female, HIV positive on ARV's, CD4 count was seven cells/ul. She was initially diagnosed with CCM based on positive CLAT. The patient was treated with amphotericin B for two weeks and was discharged on fluconazole. She presented two weeks later with headache, nausea and vomiting, and neck stiffness. The only significant positive finding on examination was terminal neck stiffness. The CT brain was normal. The CSF cultured cryptococcus and yeast cells were observed on gram stain. The patient was re-commenced on amphotericin B; however, she developed acute kidney injury and was changed to fluconazole. The patient had reported improvement and was discharged but lost to follow-up. It was subsequently found on this chart review that the patient cultured drug-sensitive TB on CSF.

15. 26-year-old female, HIV positive with a CD4 count of 218 cells/ul. She was not on HAART. She initially was diagnosed with pulmonary TB on sputum. One month later she presented with a 3-week history of paraparesis with an L3 sensory level, a normal magnetic resonance imaging (MRI) scan of the spine, and a normal LP. She had hypodensities of the right parietal lobe with scattered calcifications on the CT scan of her brain. Chest radiograph at this time revealed a bronchopneumonia with hilar adenopathy, and an ultrasound abdomen highlighted an enlarged liver with abdominal lymph nodes. TB treatment was continued. Six months later, she

remained with paraparesis and was now seen with complaints of pleuritic chest pain and generalized lymph nodes while still on her 8th month of TB treatment. Chest radiography at this stage showed hilar adenopathy with nodular infiltrates. She was admitted a month later with a 2-day history of headache and neck pain. Examination revealed generalized lymphadenopathy and oral thrush. She had scattered crackles on the respiratory exam, and her central nervous system examination revealed an orientated and lucid patient with neck stiffness. CSF revealed *cryptococcus neoformans* both on gram stain and culture and the CSF CLAT was positive. The patient had remained on TB treatment before admission and was treated for CCM. She was discharged but subsequently lost to follow-up. The CSF culture results were never followed up. The patient was later found to have MDR TB cultured on CSF during our chart review.

Table:2 Summary of patients with dual infection:

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age	27	23	31	38	37	43	24
Sex	F	F	F	F	M	M	F
HIV status	Positive	Positive	Unknown	Unknown	Positive	Positive	Positive
CD4 T Cells/ul	Unknown	Unknown	Unknown	Unknown	Unknown	74	Unknown
Glasgow coma scale	14	15	15	15	12	14	13
CSF:							
Neutrophils/uL	22	0	0	0	0	70	0
Lymphocytes//uL	10	4	4	94	0	170	250
Glucose mmol/L	1.1	0.7	0.7	2.3	0.3	0.6	0.7
(serum glucose mmol/L)	(4.4)	(5.5)	(3.5)	(5.7)	(6.0)	(5.8)	(5.0)
Protein g/L	0.37	0.62	0.62	0.83	1.07	3.9	4.59
CLAT	Positive	Positive	Positive	Positive	Positive	Not done	Positive
CSF TB culture	Positive	Positive	Positive	Positive MDR TB	Positive	Positive MDR TB	Positive MDR TB
Other TB diagnosis	Bilateral infiltrates on chest radiography Sputum: Positive for TB (AFB microscopy)	Infiltrates in keeping with miliary TB on chest radiography	Nodular shadowing in keeping with TB Sputum: Positive for TB (AFB microscopy)	Normal chest radiograph	Chest radiograph not available	Chest radiograph showed right upper lobe fibrosis	Chest radiograph not available
Patient outcome	Demised	Demised	Demised	Demised	Demised	Demised	Demised

Abbreviations: AFB: Acid Fast Bacilli/MDR TB: Multi-Drug Resistant Tuberculosis/XDR TB: Extreme Drug

Resistant Tuberculosis, CLAT: Cryptococcal Latex Agglutination Test

Table:3 Summary of patients with dual infection:

	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Age	29	38	46	14	56	24	33	26
Sex	F	M	M	M	F	M	F	F
HIV status	Unknown	Unknown	Positive	Unknown	Positive	Positive	Positive	Positive
CD4 T Cell mm ³	Unknown	Unknown	68	Unknown	218	53	7	218
Glasgow coma scale	15	14	15	15	14	15	15	15
CSF:								
Neutrophils/uL	8	40	0	28	0	0	0	40
Lymphocytes/uL	214	984	44	12	240	0	0	690
Glucose mmol/L	0.9	0.5	3.1	1.2	0.8	2.7	0.3	1.3
(serum glucose mmol/L)	4.8	4.6	4.8	5.8	7.3	6.1	4.4	4.6
Protein g/L	2.24	1.94	1.6	0.55	3.83	0.41	0.34	No result
CLAT	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
CSF TB culture	Positive (XDR TB)	Positive	Positive	Positive (MDR TB)	Positive	Positive	Positive	Positive (MDR TB)
Other Tuberculosis Diagnosis	Chest radiograph not available	Chest radiograph not available	Chest radiograph not available	Chest radiograph showed consolidation in the right upper zone and reticular nodular shadowing in the left lower zone. Sputum: Positive AFBs	Chest radiograph showed reticular nodular infiltrates and hilar adenopathy .	Chest radiograph normal Sputum positive AFBs	Chest radiograph not available	Chest radiograph showed hilar adenopathy with nodular infiltrates Sputum: negative AFBs

Patient outcome	Demised	Demised	Discharged well	Demised	Discharged well	Demised	Demised	Discharged well
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Abbreviations: AFB: Acid Fast Bacilli/MDR TB: Multi-Drug Resistant Tuberculosis/XDR TB: Extreme Drug Resistant Tuberculosis, CLAT: Cryptococcal Latex Agglutination Test

Discussion:

Tuberculous meningitis (TBM) and cryptococcal meningitis (CCM) co-infection have been infrequently documented in the literature, with limited available data on its occurrence.^{11,12,13} In our study cohort comprising severely immunocompromised HIV-infected adults with TBM, 3.5% (5/15) were diagnosed with co-infection with TBM/CCM, a rate consistent with existing literature.^{11,12} Notably, Wenjie Fang *et al.* reported a co-infection rate of 0.6% (23 cases of co-infection out of 4053 total TBM cases) in China between 1993 and 2006.¹¹ Additionally, other studies have documented co-infection rates ranging from 2.7% to 3.8%.¹² The higher prevalence of co-infection observed in our population compared to more developed regions may be attributed to the heightened incidence of tuberculosis (TB) in South Africa.⁴ Furthermore, a study conducted in Ethiopia by Jemal *et al.* examining the prevalence of cryptococcal antigenemia among HIV/AIDS patients found a prevalence of 11.43%, with a subset of TB-positive patients demonstrating rates of 43.8% and 31.2% for pulmonary and extrapulmonary TB, respectively.¹⁴ Although this study provides insights into the potential incidence of co-infection, it focused on extrapulmonary TB rather than specifically on TBM.

Analysis of the demographic characteristics of our study participants revealed an age range from 14 to 56 years, with a mean age of 37 years. These findings mirror those reported by Wenjie Fang *et al.*, whose investigation into TBM and CCM co-infection in Chinese patients revealed a mean age at co-infection diagnosis of 35.0 ± 2.8 years (95% confidence intervals=29.3–40.7).¹¹

In our cohort, the proportion of females among patients with dual infection exceeded that of males (9 females vs. 6 males), in contrast to previous findings on co-infection with CCM/TBM, which demonstrated a male predominance.^{11,15} This observed gender distribution may be attributed to the higher prevalence of HIV among females (20%)

compared to males (12%) in South Africa, a significant factor considering the majority of our patients were HIV positive.¹

Notably, our study revealed a similarity in the clinical presentation of CCM and TBM co-infection, characterized by symptoms such as headaches, fever, photophobia, and altered consciousness levels, potentially contributing to diagnostic delays or missed diagnoses.^{9,11,15} Further complicating the diagnostic process, our investigation identified similarities in cerebrospinal fluid (CSF) profiles between TBM and CCM, although co-infected individuals tended to exhibit higher CSF protein and cell counts.⁸ Specifically, among patients with TBM and CCM co-infection in our cohort, the median CSF neutrophil count was 0/uL, lymphocyte count was 44/uL, glucose was 0.8mmol/L, and protein was 0.83g/L. These findings align with those reported by Ellis *et al.* in their case series from Uganda, where the baseline median CSF white cell count was 60 cells/ μ L (<5–2200 cells/ μ L) and median protein was 1g/L(0.2–3.56g/L).¹⁵ Multivariate analysis conducted by Vidal *et al.* in patients with TBM infection alone showed CSF neutrophil predominance, pleocytosis, and protein levels exceeding 1.0g/L.¹⁶ CSF microscopy, including Ziehl-Neelsen staining for acid-fast bacilli (AFB) in TBM cases, demonstrated limited diagnostic utility with acid-fast bacilli (AFB) only present in 5.5% of cases.¹⁶ Moreover, while a high opening CSF pressure, low CSF white blood cell count, and positive cryptococcal antigen latex agglutination test were indicative of CCM, they were not universally present.¹⁶ India ink staining of CSF showed yeast compatible with *cryptococcus* spp. in 84% of cases.¹⁶ A cryptococcal antigen latex agglutination test of CSF was positive in only 82% of cases.¹⁶ Collectively, our results underscore the challenge of relying solely on CSF studies to differentiate between CCM and TBM. Integration of clinical, radiological, and additional laboratory assessments is essential for accurate diagnosis and appropriate management of these dual infections.¹⁶

The diagnosis of co-infection poses significant challenges, particularly in cases of tuberculous meningitis (TBM), given its paucibacillary nature.¹² This study predates the introduction of advanced diagnostic modalities such as the Xpert® MTB/RIF Ultra and the lateral flow urine lipoarabinomannan (LF-LAM) assay, which have since augmented tuberculosis (TB) detection.¹⁷ The Xpert® MTB/RIF assay, capable of real-time PCR detection of *Mycobacterium tuberculosis* and rifampicin resistance, has demonstrated potential in expediting TBM diagnosis.¹² Recognizing its value, the World Health Organization (WHO)

advocates for its primary use in TBM diagnosis, boasting a sensitivity of approximately 60-70% and a specificity nearing 100%, thereby enhancing diagnostic timeliness.¹⁸ Additionally, the LF-LAM assay, with a sensitivity of 45% and specificity of 92% in severely immunocompromised individuals, holds promise as a point-of-care TB diagnostic tool, particularly endorsed for HIV-positive populations by the WHO.¹⁷ Anticipating the introduction of superior diagnostic methodologies, such as Xpert® MTB/RIF, to bolster microbiological confirmation of TBM, it is conceivable that the prevalence of TBM/CCM co-infection may rise.

In this study, the in-hospital mortality rate was noted to be 80% (12/15), with only three patients who survived to discharge. Ellis *et al.* reported a 60% in-hospital mortality rate in their cohort of patients with CCM/TBM co-infection.¹³ Wong *et al.* found 62% of HIV-infected patients with *Mycobacterium* infection had multiple infectious causes of death at autopsy, including cryptococcosis.¹⁰ Mortality from cryptococcal meningitis alone remains unacceptably high in sub-Saharan Africa.¹⁹ The in-hospital mortality rate of CCM reported from a district hospital in the Eastern Cape, South Africa was around 30% whilst a study in a rural area in KwaZulu Natal reported around 40.5% and in comparison, assumed 3 months mortality rates of high-income regions, such as Eastern and Central Europe and Oceania, of only 9%.¹⁹ The high early mortality rate associated with CCM may also lead to an underdiagnosis of co-infection, as CCM patients may die before diagnosing TBM.

Another disconcerting finding was the number of multi-drug resistant TBM cases observed in our study. Six patients with co-infection were found to have multidrug-resistant TBM on culture, with one of those patients having extensively drug-resistant TBM (XDR TBM). Heemskerk *et al.* described the clinical outcome of patients with drug-resistant TBM which had a high mortality rate, with eleven of 16 (68.8%) of those with MDR TB dying by nine months.²⁰ The majority of patients with MDR TBM demised by day 27 despite being initiated timeously on treatment.²⁰ Hence it can be postulated that dual infection would lead to much poorer patient outcomes with higher mortality, as was evident in this case series. The expeditious initiation of treatment significantly ameliorates the prognosis of both TBM and CCM; however, prolonged drug regimens expose patients to diverse drug toxicities, further complicating morbidity and mortality in cases of dual infection.²⁰

Despite a growing understanding of the local host immune response in each individual infection, there is a lack of information regarding the immunology of co-infection.⁸ Both *cryptococcus* and *mycobacterium tuberculosis*, being intracellular pathogens, can activate a type 1 immune response that is characterised by the interplay between adaptive immune response cells and innate immune cells e.g., macrophages and dendritic cells.⁸ HIV reduces the quantity and capability of all immune cells, causing weakened immune responses and an increased likelihood of developing disseminated tuberculosis and cryptococcosis.⁸

Although the epidemiology, immune responses, and major impact on HIV-related mortality of TB and cryptococcosis overlap, the co-infection of the two is not well documented.^{21,22} The low sensitivity and specificity of the available TB diagnostic tests for those with advanced HIV may be the source of missed cases of TBM.²¹ Using sensitive and specific TB diagnostics for systematic screening of patients who are severely immunosuppressed with HIV will likely increase the number of microbiologically confirmed cases of TB co-infection and could result in an increase in the number of hospitalized HIV patients with confirmed TB diagnosis.

Limitations:

The findings of our study necessitate cautious interpretation, recognizing the inherent challenges in diagnosing extrapulmonary or disseminated tuberculosis (TB). The absence of specific TB diagnostics for such cases likely introduced a bias towards underdiagnosis. Our investigation solely focused on patients meeting the criteria for “definite” tuberculous meningitis (TBM) as outlined by the consensus clinical case definition proposed by Marais *et al.*²³ Patients categorized as “probable” or “possible” TBM were excluded from our study and this could have resulted in some patients with TBM being missed.²³ Given the retrospective nature of this case series, incomplete medical records and data gaps may have impacted our analysis. The limited number of patients included in this series reflects the inherent difficulty in diagnosing co-infection of TBM and CCM. Moreover, not all specimens may have been sent for TB culture, potentially leading to missed diagnoses.

Conclusion:

In this study, we found that dual infection of the meninges with *mycobacterium tuberculosis* and *cryptococcus neoformans* in HIV-infected patients is rare, however the diagnosis may often be missed and therefore under-reported. The underdiagnosis of dual infection may result in patients not being treated appropriately and adequately.

This case series highlights the difficulty of diagnosing TBM-CCM co-infection. This study was undertaken prior to the introduction of the Xpert® MTB/RIF Ultra test as well as the lateral flow urine lipoarabinomannan (LF-LAM) assay, and we postulate that with the subsequent introduction of these tests, the diagnosis of TBM may improve earlier detection and yield. Co-infection reflects the advanced immunosuppression characteristic of patients with HIV-associated CCM and the complexities of diagnosis and management in patients at risk of intercurrent opportunistic infections. Our research demonstrates both the need to consider co-infection at baseline diagnosis and the need to remain vigilant for co-infection throughout the follow-up period.

Data availability:

The data that support the findings of this study are available from the corresponding author, F.M., upon reasonable request.

Conflicts of interest:

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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Disclaimer:

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy of the affiliated institutions of the authors.

References:

1. South African National HIV Prevalence, Incidence and Behavior and Communication Survey 2022. Human Sciences Research Council. Available: <http://www.hsrcpress.ac.za>
2. 2022 Guidelines for Diagnosing, Preventing and Managing Cryptococcal Disease among adults, adolescents and children living with HIV. Available: <https://www.nicd.ac.za/wp-content/uploads/2023/01/2022-WHO-Guideline-Crypto-Final-9789240052178-eng.pdf>
3. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017 Aug;17(8):873-881. doi: 10.1016/S1473-3099(17)30243-8. Epub 2017 May 5
4. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis*. 2010;10:67. doi: 10.1186/1471-2334-10-67. PubMed.
5. Lan SH, Chang WN, Lu CH, Lui CC, Chang HW. Cerebral infarction in chronic meningitis: a comparison of tuberculous meningitis and cryptococcal meningitis. *QJM*. 2001 May;94(5):247-53. doi: 10.1093/qjmed/94.5.247
6. Bhigjee AI, Padayachee R, Paruk H, Hallwirth-Pillay KD, Marais S, Connolly C. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. *Int J Infect Dis*. 2007 Jul;11(4):348-54. doi: 10.1016/j.ijid.2006.07.007. Epub 2007 Feb 23.
7. Carlo Foppiano Palacios, Paul G. Saleeb, Challenges in the diagnosis of tuberculous meningitis, *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*,

8. Huang CT, Tsai YJ, Fan JY, Ku SC, Yu CJ. Cryptococcosis and tuberculosis co-infection at a university hospital in Taiwan, 1993-2006. *Infection*. 2010 Oct;38(5):373-9. doi: 10.1007/s15010-010-0045-9. Epub 2010 Jul 27.
9. Silber E, Sonnenberg P, Koornhof HJ, Morris L, Saffer D. Dual infective pathology in patients with cryptococcal meningitis. *Neurology*. 1998 Oct;51(4):1213-5. doi: 10.1212/wnl.51.4.1213.
10. Wong EB, Omar T, Setlhako GJ, Osih R, Feldman C, Murdoch DM, Martinson NA, Bangsberg DR, Venter WD. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. *PLoS One*. 2012;7(10):e47542. doi: 10.1371/journal.pone.0047542. Epub 2012 Oct 16.
11. Fang W, Zhang L, Liu J, Denning DW, Hagen F, Jiang W, Hong N, Deng S, Lei X, Deng D, Liao W, Xu J, Boekhout T, Chen M, Pan W. Tuberculosis/cryptococcosis co-infection in China between 1965 and 2016. *Emerg Microbes Infect*. 2017 Aug 23;6(8):e73. doi: 10.1038/emi.2017.61.
12. Marais S, Wilkinson R J. The diagnosis and medical management of tuberculous meningitis in adults. *SAMJ, S. Afr. med. j.* [Internet]. 2014 Dec [cited 2023 Aug 13] Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0256-95742014001200023&lng=en. <http://dx.doi.org/10.7196/SAMJ.9060>
13. Ellis J, Cresswell FV, Rhein J, Ssebambulidde K, Boulware DR. Cryptococcal Meningitis and Tuberculous Meningitis Co-infection in HIV-Infected Ugandan Adults. *Open Forum Infect Dis*. 2018 Aug 29;5(8):ofy193. doi: 10.1093/ofid/ofy193. Erratum in: *Open Forum Infect Dis*. 2018 Oct 16;6(3):ofy252.
14. Mohabaw Jemal, Teshiwal Deress, Teshome Belachew, Yesuf Adem, "Prevalence of Cryptococcal Antigenemia and Associated Factors among HIV/AIDS Patients at Felege-Hiwot Referral Hospital, Bahir Dar, Northwest Ethiopia", *International Journal of Microbiology*, vol. 2021, Article ID 8839238, 6 pages, 2021. <https://doi.org/10.1155/2021/8839238>
15. Ouedraogo D, Cissoko Y, Soumare M, Alle Akakpo AE, Loua OO, Konate I, Niare Doumbo S, Dao S. Neuromeningeal Cryptococcosis and Tuberculosis Coinfection in Bamako: A One-Year Case Series. *Open Forum Infect Dis*. 2023 Aug 18;10(8):ofad438. doi: 10.1093/ofid/ofad438. PMID: 37663093; PMCID: PMC10469546.

16. Vidal J E, Peixoto de Miranda E J F, Gerhardt J, Croda M, Boulware D R. Is it possible to differentiate tuberculous and cryptococcal meningitis in HIV-infected patients using only clinical and basic cerebrospinal fluid characteristics?. *SAMJ, S. Afr. med. j.* [Internet]. 2017 Feb [cited 2023 July 20] ; 107(2): 156-159. Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0256-95742017000200021&lng=en. <http://dx.doi.org/10.7196/samj.2017.v107i2.11162>
17. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO.
18. Mitha M, Pillay M, Moodley JY, Balakrishna Y, Abbai N, Bhagwan S, Dangor Z, Bhigjee AI. Laboratory diagnosis of tuberculous meningitis in human immunodeficiency virus-seropositive patients: Correlation with the uniform case definition. *S Afr J Infect Dis.* 2020 Aug 26;35(1):135. doi: 10.4102/sajid.v35i1.135
19. Hiesgen J, Schutte C, Olorunju S, Retief J. Cryptococcal meningitis in a tertiary hospital in Pretoria, mortality and risk factors - A retrospective cohort study. *Int J STD AIDS.* 2017 Apr;28(5):480-485. doi: 10.1177/0956462416653559. Epub 2016 Jul 10.
20. Heemskerk AD, Nguyen MTH, Dang HTM, Vinh Nguyen CV, Nguyen LH, Do TDA, Nguyen TTT, Wolbers M, Day J, Le TTP, Nguyen BD, Caws M, Thwaites GE. Clinical Outcomes of Patients With Drug-Resistant Tuberculous Meningitis Treated With an Intensified Antituberculosis Regimen. *Clin Infect Dis.* 2017 Jul 1;65(1):20-28.doi: 10.1093/cid/cix230.
21. Chen M, Al-Hatmi AM, Chen Y, Ying Y, Fang W, Xu J, Hagen F, Hong N, Boekhout T, Liao W, Pan W. Cryptococcosis and tuberculosis co-infection in mainland China. *Emerg Microbes Infect.* 2016 Sep 7;5(9):e98. doi: 10.1038/emi.2016.95.
22. Britz E, Perovic O, von Mollendorf C, von Gottberg A, Iyaloo S, Quan V, Chetty V, Sriruttan C, Ismail NA, Nanoo A, Musekiwa A, Reddy C, Viljoen K, Cohen C, Govender NP. The Epidemiology of Meningitis among Adults in a South African Province with a High HIV Prevalence, 2009-2012. *PLoS One.* 2016 Sep 26;11(9):e0163036. doi: 10.1371/journal.pone.0163036.
23. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, Donald PR, Wilkinson RJ, Marais BJ. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis.* 2010 Nov;10(11):803-12. doi: 10.1016/S1473-3099(10)70138-9. Epub 2010 Sep 6.

Appendix 1: The Final Study Protocol

Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa

Hypothesis

1. Dual infection of the meninges with *Mycobacterium tuberculosis* (MTB) and *Cryptococcus neoformans* (CN) is not uncommon in HIV-infected patients.
2. The underdiagnosis of dual infection results in patients not being treated appropriately and adequately.

Aim of Study

1. To determine the frequency of dual infection of the meninges with *mycobacterium tuberculosis* and *cryptococcus neoformans* in hospitalised patients with TB meningitis in Durban, Kwa Zulu Natal.
2. To determine whether patients with dual infections have been appropriately and adequately treated.

Background and Literature Review

According to the South African National HIV Prevalence, Incidence, Behavior, and Communication survey in 2017, the prevalence of HIV in South Africa was estimated at 14.0%, translating to 7.9 million people living with HIV¹

Cryptococcal meningitis (CCM) is a common opportunistic infection in HIV-positive individuals, especially those with a low CD4 count. It is the commonest cause of meningitis in HIV-infected patients in South Africa and is believed to be the second commonest cause of mortality amongst HIV infected individuals.^{2, 3}

Tuberculous meningitis (TBM) is the second commonest cause of meningitis in HIV positive patients. A recent study in Cape Town reported that TBM was present in 27 percent of hospitalised HIV infected patients.² TBM may present clinically as TBM alone or as disseminated TB.

The clinical presentations of both CM and TBM are very similar.⁴ Patients can present with headache, neck stiffness, seizures, altered sensorium as well as focal neurological signs.

CSF findings are also very similar in TBM and CM in that a lymphocytic predominance, elevated protein, and low sugar is often found in both conditions.⁵

Although the Cryptococcal Latex Agglutination Test [CLAT] is highly sensitive and specific, false positives and negatives have been rarely reported. The definitive diagnosis remains on culture of the organism from the CSF.

In contrast, the rapid diagnosis of TBM remains a major challenge for both clinicians and microbiologists because of the extremely poor yield of AFB microscopy on CSF samples and the lack of a reliable TB serological or molecular test.

Clinicians in our setting base their treatment regimen on the results of CLAT or the India ink/gram stain. If positive, patients are started on treatment for cryptococcal meningitis.

However, it is well recognised that if a patient had previous CM, the CLAT and India ink/gram stain may remain positive. Patients who present with new signs suggestive of meningitis may well have TBM. Due to the high TB burden, and the delay in obtaining TB cultures, some patients may be co-infected with both organisms.

Dual infection with both TBM and CM has been documented. A recent study in Taiwan demonstrated that co-infection in the brain is the second most common site after the lung.⁶ A limited study, published in 1998, conducted on mineworkers in rural South Africa revealed that of 2 of 7 (< 30%) patients had co-infection with tuberculosis and cryptococcus.⁷

To the best of our knowledge, dual infection with TBM and CM has not been adequately studied or reported in this province. We therefore embarked on this retrospective study to document the prevalence of dual infection in hospitalized patients in Durban, KZN. We believe that the findings of this study will impact the management practices of physicians treating these patients in KZN.

References:

1. South African National HIV Prevalence, Incidence and Behaviour and Communication Survey 2008. Human Sciences Research Council. Available: <http://www.hsrbpress.ac.za>
2. Jarvis JN, Meintjies G, William A et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC Infectious Diseases 2010, 10:67 doi:10.1186/1471-2334-10-67
3. Department of Health, Republic of South Africa: The burden of cryptococcosis in South Africa. Statistical Notes February 2008. Available: <http://www.doh.gov.za/docs/stats/2008/cryptococcosis>
4. Lan SH, Chang WN, Lu CH et al. Cerebral infarction in chronic meningitis: A

- comparison of tuberculous meningitis and cryptococcal meningitis. Q J Med 2001
5. Bhigjee AI, Padayachee R, Paruk H et al. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. International Journal of Infectious Diseases 2007 11, 348:354
6. Huang CT, Tsai YJ, Fan JY et al. Cryptococcosis and tuberculosis co-infection at a university hospital in Taiwan, 1993-2006. Infection 2010 38:373-379
7. Silber E, Sonnenberg P, Koornhof HJ et al. Dual infective pathology in patients with cryptococcal meningitis. Neurology 1998, 51:1213-1215

Study Methodology

Study Design

Retrospective laboratory analysis of CM co-infection in patients identified with culture proven TBM.

Study Period

1 January 2005- 31 December 2009

Study Population

Laboratory data analysis of CSF specimens received from patients with suspected meningitis admitted to hospitals within the Durban metro complex: King Edward VIII, R K Khan, Addington, and Mahatma Gandhi Memorial Hospitals. (PMMH was left out because of difficulties in accessing stored laboratory data)

Data Collection and Methods

1. Laboratory data: CSF microbiology laboratory data at each of the identified

hospitals will be the starting point for this retrospective analysis. In addition, CSF TB data at IALCH TB Culture Lab, which is the only TB culture lab in KZN will also be reviewed to identify any cases of TBM that were not included in the data obtained from the individual hospital laboratories. As TB is endemic in South Africa, it will be used as the index infection to determine the temporal sequence of co-infection with Cryptococcus. All adult patients with positive TB culture in the cerebrospinal fluid will be included. These positive MTB samples will then be matched with CSF samples positive for cryptococcosis either by CLAT, India ink/ Gram stain or culture.

2. Clinical data: The clinical records of all dual infected patients will be analysed to try and ascertain:

- a. Whether TBM or CM was the most likely initial infection,
- b. Was there any evidence of previous tuberculosis (any form) or cryptococcal meningitis.

Statistical planning

Variables that will be analysed:

1. Age
2. Gender
3. HIV status
4. CD4 count
5. Viral load
6. CSF chemistry and microbiology

Case Definitions

1. Cryptococcal Meningitis: Any patient who has a positive CSF on India ink/Gram stain, CLAT, or culture for *cryptococcus* species.
2. TB Meningitis: Any patient who has a positive culture in the CSF for *mycobacterium tuberculosis*
3. Co-infection: Due to the endemic nature of TB in South Africa, it will be used as the index infection to determine the temporal sequence of co-infection with *cryptococcus*.

Inclusion/ Exclusion Criteria

Inclusion: All adult patients who had positive TB culture in the CSF and positive for CCM either by India ink/Gram stain, CLAT or culture.

Data analysis techniques

Statistical analysis

The assistance of a biostatistician will be obtained if required

Data management:

The anonymity of patients included in the study will be maintained. Patient hospital numbers will be used instead of names to protect patient identity.

All clinical information will be retrieved from the patients' clinical notes.

There will be no specific mention of any personal patient information in the study.

The master copy of patients' names and information will be available to the primary investigator only after the data is captured. Collected de-identified data encrypted by a number system will be entered into a password-protected database. The data will only be handled by the research team and kept with the main investigator until submission for data analysis. Only de-identified data will be submitted for analysis.

Original data files will be kept in a locked office and will be destroyed in keeping with

the university policy once 5 years have elapsed.

Limitations

1. Not all specimens are sent for TB culture, therefore cases may be missed.
2. Only 40% of TB meningitis cases are culture-positive and therefore it is likely that 60% of TB meningitis will be missed
3. Rare occurrence of false positive and negative CLAT results

Funding

All costs will be incurred by Dr. Faaizah Mohamed (principal investigator)

Ethical considerations

The anonymity of patients included in the study will be maintained. There will be no specific mention of any personal patient information in the study. We anticipate an expedited ethical review bearing in mind that the study is a retrospective audit which is confidential and non-interventional.

Appendix 2: The Guidelines for Authorship for the Journal selected for submission of the manuscript

Journal of the Colleges of Medicine South Africa

The *Journal of the Colleges of Medicine of South Africa* (JCMSA) is the first official scientific journal of the Colleges of Medicine of South Africa. As the national examining body for medical and dental professions in South Africa, the CMSA is unique in that it embraces 29 constituent Colleges representing all disciplines of medicine and dentistry. The JCMSA leverages this unique structure to foster aspects of medical and dental education and professionalism common to all specialities while showcasing the diversity of medical research and scholarship across different fields.

The JCMSA is a peer-reviewed and open-access publication promoting the highest skill, efficiency, and ethical medical and dental practice standards. The scope of the JCMSA encompasses original research articles, reviews, case reports, and other scholarly contributions that advance basic medical sciences, medical and dental knowledge, education, and practice.

Author submission guidelines:

Overview

The author guidelines include information about the types of articles received for publication and preparing a manuscript for submission. Other relevant information about the journal's policies and the reviewing process can be found under the about section. The compulsory cover letter forms part of a submission and must be submitted together with all the required forms. All forms need to be completed in English.

Original Research Articles

Report of original scientific research conducted. Ethical approval is essential. See the full structure of the original research articles below.

Submission status	open
Word limit	7000 words (<u>excluding</u> the abstract, tables, figures, graphs, and references)
Abstract	maximum: 250 words requires structural headings: Background, Methods, Results, Conclusion and Contribution
Main text	requires structural headings, refer to the full structure 'Ethical considerations' is a sub-section in the manuscript and must include: • Name of the ethical review committee • Study approval number • Manner of consent (written, oral) for human participants • Description of measures taken to maintain the confidentiality of data • If the study was not human or animal research or the study was determined to be non-human subjects research or exempt, the authors must provide a statement with those details in this section.

References	40 or less, adhere to the Vancouver referencing style
Tables, figures and graphs	7 or less, adhere to the Illustrations requirements found in the AOSIS House style guide
Formatting requirements	apply the guidelines located on the Formatting requirements page and the AOSIS house style guide
Compulsory supplementary file(s)	the Authorship, disclosure statements, copyright, and license agreement form , Ethical Clearance/Waiver Documentation and any other relevant form applicable to your submission
Ethical clearance/waiver documentation	evidence of ethical clearance for the study, such as the study approval letter or certificate from the Institutional Review Board (IRB), a waiver from the IRB et cetera

Style and format

File format

- Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected.
- LaTeX documents (.tex) should be converted into Microsoft Word (.doc) before submission online.
- Rich Text Format (RTF): Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available that will make this process easier.

Length

Manuscripts should adhere to the author guidelines of the journal. There are restrictions on word count, number of figures, or amount of supporting information.

Font

Use a standard font size and any standard font family.

Special characters

Do not use the font named 'Symbol'. To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character. Refer to our AOSIS house style guide on mathematical and Unicode font guidelines.

Headings

Ensure that formatting for headings is consistent in the manuscript. Limit manuscript sections and sub-sections to four heading levels. To avoid confusion during the review and production process, ensure that the different heading levels used in your work are visually distinct from one another. The simplest way to achieve this is to use different font sizes and/or a combination of bold/italics for different heading levels.

Keywords

Identify eight keywords that represent the content of your manuscript and are specific to your field or sub-field. Test your keywords: when you enter your keywords into various journal and academic databases like Google Scholar, do the results include papers similar to your topic? If not, revise the terms until they do.

Layout and spacing

Manuscript text should have a 1.5 line spacing.

Page and line numbers

Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).

Footnotes

Footnotes are not ideal. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.

Language

Manuscripts must be written in British English, according to the Oxford English Dictionary (avoid Americanisms [e.g. use 's' and not 'z' spellings], and set your version of Microsoft Word default language to UK English). Refer to the AOSIS house style guide for more information.

Abbreviations

Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.

Illustrations

Illustrations fall into two categories:

- Figures: Photographs, drawings, diagrams, graphs, flowcharts, maps, etc.
- Tables and/or Boxes: Text and/or numbers arranged in orderly columns and rows.

Every time a Figure, Table and/or Box is presented in your manuscript, it should be referred to three times:

- In a legend, which includes a number, a title, and its source. The legend is placed below a Figure and above a Table and/or Box. The source section should consist of the in-text citation, creator or owner and its year of creation, and any other attribution required as stipulated by the permission received (person and place) to reproduce.
- In the body of your written manuscript. You should include an in-text citation and a sentence or two about the image explaining what it illustrates and why it is there.
- As a reference entry within your reference list.

References

Referencing style guide

The manuscript must adhere to the [Vancouver referencing style](#).

Permission to use copyright material

The following information will assist you in understanding your responsibilities and in requesting permission to reproduce copyrighted material in your work. All permissions granted must be submitted to the journal together with your manuscript, and you must ensure that a clearly written source accompanies the work.

Your responsibilities

As the author, you are responsible for obtaining permission and paying any fees to use the third-party copyrighted material that your manuscript contains.

Material that will need clearance

Content not in the public domain or freely available to use under a license such as a creative commons license will require clearance. It includes the use of photographs, figures, maps, tables, cartoons, advertisements, epigraphs and quotations that are over the limits referred to under 'Modification/adaptation of figures and tables'.

Web material

Image or text material on the Web may not be the intellectual property of the site hosting it. You must always identify the original copyright source and obtain explicit permission. Take particular care with photographs obtained from websites, blogs, Google image searches, YouTube, Wikimedia, etc.

Material previously published by you or your colleague

Check the contract with the other publisher to see whether, and under what conditions, the material can be reused in this AOSIS publication. If in any doubt, permission must be obtained.

Images of, or information about, identifiable individuals

It is your responsibility to obtain consent from patients and other individuals for the use of information, images, audio files, and video clips from which they may be identified. Bear in mind the following points:

- Masking a person's eyes is not an adequate or acceptable means of rendering an image anonymous.
- People may still be recognizable to individuals or their families, even if head/shoulders are not included.
- People may recognize themselves from clinical descriptions or case reports.

Modification/adaptation of figures and tables

Use the original figure as first published where appropriate. However:

- No clearance is required if you create figures or tables using factual data from copyrighted material.
- No clearance is required if, after you have created a single figure or table using data from two or more figures or tables, no single source comprises more than 75% of the new figure or table.
- No clearance is required if, after you have created a new figure or table by adding your own data to an existing figure or table, your data comprises more than 25% of the new figure or table.
- Clearance is required if you create a figure or table using parts from two or more third-party sources, and each part contains more than 75% of the content of the original figure/table part.

Quotations

For prose, permission is required for single quotations of over 400 words or multiple quotations from the same source that cumulatively total more than 800 words. But note that, even if below these limits, permissions must be cleared for quotations that represent the 'heart of the work' or a substantial portion of the overall original source material.

Clear before you submit your final manuscript

Permissions must be cleared before the final version of your manuscript is submitted for publication. If permission cannot be obtained, you should find an alternative or remove the material. Provide electronic copies of all consent forms obtained when you submit your final manuscript, numbered and named accordingly.

Ethical considerations

This section should include the statements of compliance with standards of research involving either animal or human involvement. If your study involves human subjects and/or animals, and if your manuscript includes case reports/case series, you need to provide the following:

- Authors must provide the name of the ethical approval committee/Institutional Review Board they have obtained consent from and the approval number/ID.
- Authors should specifically mention if a waiver was obtained for the study and the reason for the waiver. They should confirm that the study was conducted in accordance with Helsinki Declaration as revised in 2013.
- Authors must state that written informed consent was obtained from the participants of the study (and the relevant document(s) must be provided when requested by the journal). If verbal informed consent was obtained, the reason(s) for the absence of written consent must be provided.
- For case reports/case series involving minor subjects/children/infants, authors should confirm that the statements of written informed consent from legally authorized representatives/parents/guardians are available; if verbal informed consent was obtained, reasons for this must be mentioned.
- Since patients have a right to privacy, identifying information (including patients' images, names, initials, or hospital numbers) should not be included in recordings, written descriptions, or photographs unless the information is essential for scientific purposes. In any case, written informed consent from the patient must be obtained for publication of these graphics in print and electronic form. If such consent has not been obtained, personal details of patients in any part of the paper and in any supplementary materials (including illustrations) must be removed before submission.

Subject	Scenario	Suggested statement of compliance

Animals: Include the statement of compliance with standards of research involving animals.	If your work includes animals, it is necessary to have a statement of compliance with research standards involving animals.	All procedures performed in studies involving animals followed all international, national, and/or institutional guidelines for the care and use of animals.
	If you publish an article and there are reasons to <u>believe</u> that you could have used animals but did not.	This article does not contain any studies involving animals performed by any of the authors.
Humans: Include the statement of compliance with standards of research involving humans.	If your work does not include humans as subjects, you may finish the ethical statement to include.	This article does not contain any studies involving human participants performed by any of the authors.
	If your work involves humans as a subject, you had to receive ethics approval.	An application for full ethical approval was made to the [committee/Institutional Review Board] and ethics consent was received on [date]. The ethics approval

		number is [full ethics approval number].
	If your work involves humans as a subject, but you received an ethics waiver.	An application for full ethical approval was made to the [committee/Institutional Review Board] and ethics consent was received on [date]. The ethics waiver number is [full number]. The [committee/Institutional Review Board] issued an ethics waiver for the study because [reason for the ethics waiver].
	<i>Additional statements:</i> If your work involves humans, it is necessary to <u>include one of the above and the following two additional statements.</u>	All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments

		or comparable ethical standards.^a Informed Consent: [Written/Verbal] informed consent was obtained from all individual participants involved in the study. [If verbal informed consent was obtained, the reason(s) for the absence of written consent must be provided.]
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^aDo keep in mind: The necessary and sufficient condition is to conduct research in accordance with the [1964 Helsinki Declaration](#). If your institution has no department that deals with ethics, then you should not mention institutional standards. If your country has no committee that deals with ethics, then you should not mention national standards.

Acknowledgement's structure

Acknowledgements

The acknowledgement section follows the conclusions section and addresses formal, required statements of gratitude and required disclosures. It includes listing those who contributed to the work but did not meet authorship criteria, with the corresponding description of the contribution. Acknowledge anyone who provided intellectual assistance, technical help (including with writing and editing), or special equipment and/or materials.

Authors are responsible for ensuring that anyone named in the Acknowledgements agrees to be named.

Also provide the following, each under their own subheading:

- Competing interests
- Author contributions
- Funding information
- Data availability statement
- Disclaimer

Competing interests

This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect. Read our [policy on competing interests](#).

The following are examples of competing interest statements. If you use one of the examples, you should modify it to fit your specific relationship.

Scenario	Suggested competing interest statements
----------	---

Example 1	The author(s) declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.
Example 2	The author reported that they [have a financial and/or business interests in] [are a consultant to] [received funding from] a company that may be affected by the research reported in the enclosed publication. They have disclosed those interests fully and have in place an approved plan for managing any potential conflicts arising from [that involvement].
Example 3	A.B. developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both A.B and B.C. contributed to the final version of the manuscript. B.C. supervised the project.
Example 4	A.B., B.C., C.D., D.E., E.F., F.G., and G.H. conceived and planned the experiments. A.B., B.C., C.D. and D.E. carried out the experiments. A.B., F.G. and E.F. planned and carried out the simulations. J.K., K.L., A.B., B.C., D.E., C.D., F.J., and F.G. contributed to sample preparation. A.B., B.C., C.D., D.E., FJ, E.F., F.G. and G.H. contributed to the interpretation of the results. A.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.
Example 5	A.B. and B.C. designed the model and the computational framework and analysed the data. A.B. and C.D. carried out the implementation. A.B. performed the calculations. A.B. and B.C. wrote the manuscript with input from all authors. D.E. and E.F.

	conceived the study and were in charge of overall direction and planning.
Example 6	A.B. designed and performed the experiments, derived the models and analysed the data. B.C. assisted with XYZ measurements and C.D. helped carry out the XYZ simulations. A.B. and D.E. wrote the manuscript in consultation with C.D., B.C. and E.F..
Example 7	A.B. devised the project, the main conceptual ideas and proof outline. B.C. worked out almost all of the technical details, and performed the numerical calculations for the suggested experiment. C.D. worked out the bound for quantum mechanics, with help from D.E.. E.F. verified the numerical results of the XYZ by an independent implementation. F.G. and G.H. proposed the XYZ experiment in discussions with A.B.. B.C., C.D., G.H. and A.B. wrote the manuscript.
Example 8	A.B., B.C. and C.D. designed the study. A.B., D.E. and E.F. performed the XYZ experiments. F.G. and G.H. performed XYZ simulations. I.H. and M.C. expressed and purified all proteins. A.B., H.J., B.C. and C.D. analysed the data. A.B., B.C. and C.D. wrote the paper with input from all authors.
Example 9	A.B. and B.C. designed and directed the project; C.D., D.E., A.B. and B.C. performed the experiments; C.D. and B.C. analysed spectra; A.B. and E.F. made the simulations; B.C. developed the theoretical framework; C.D., A.B. and B.C. wrote the article.
Example 10	The author of this publication receives research funding from [Entity], which is developing products related to the research

	described in this publication. In addition, the author serves as a consultant to [Entity] and receives compensation for these services. The terms of this arrangement have been reviewed and approved by the [University name; Institution name] in accordance with its policy on objectivity in research.
Example 11	The author of this publication receives research support from [name of research sponsor] with an equipment loan from [Entity]. The author also [has an equity interest in, serves as a consultant to, serves on an advisory board or board of directors for] [Entity]. The terms of this arrangement have been reviewed and approved by the [University name; Institution name] in accordance with its policy on objectivity in research.

Author contributions

All authors must meet the criteria for authorship as outlined in the [authorship](#) policy and [author contribution](#) statement policies.

The following are examples of an author contribution statement. If you use one of the examples, you should modify it to fit your specific relationship.

Scenario	Suggested author contribution statements
Example 1	A.B. and B.C. conceived of the presented idea. A.B. developed the theory and performed the computations. C.D. and D.E. verified the analytical methods. B.C. encouraged A.B. to investigate [a specific aspect] and supervised the findings of this

	work. All authors discussed the results and contributed to the final manuscript.
Example 2	A.B. and B.C. carried out the experiment. A.B. wrote the manuscript with support from C.D.. D.E. and E.F. fabricated the XYZ sample. F.G. and G.H. helped supervise the project. G.H. and H.I. conceived the original idea. H.I. supervised the project.
Example 3	A.B. developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both A.B and B.C. authors contributed to the final version of the manuscript. B.C. supervised the project.
Example 4	A.B., B.C., C.D., D.E., E.F., F.G., and G.H. conceived and planned the experiments. A.B., B.C., C.D. and D.E. carried out the experiments. A.B., F.G. and E.F. planned and carried out the simulations. J.K., K.L., A.B., B.C., D.E., C.D., F.J., and F.G. contributed to sample preparation. A.B., B.C., C.D., D.E., FJ, E.F., F.G. and G.H. contributed to the interpretation of the results. A.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.
Example 5	A.B. and B.C. designed the model and the computational framework and analysed the data. A.B. and C.D. carried out the implementation. A.B. performed the calculations. A.B. and B.C. wrote the manuscript with input from all authors. D.E. and E.F. conceived the study and were in charge of overall direction and planning.

Example 6	A.B. designed and performed the experiments, derived the models and analysed the data. B.C. assisted with XYZ measurements and C.D. helped carry out the XYZ simulations. A.B. and D.E. wrote the manuscript in consultation with C.D., B.C. and E.F..
Example 7	A.B. devised the project, the main conceptual ideas and proof outline. B.C. worked out almost all of the technical details, and performed the numerical calculations for the suggested experiment. C.D. worked out the bound for quantum mechanics, with help from D.E.. E.F. verified the numerical results of the xyz by an independent implementation. F.G. and G.H. proposed the xyz experiment in discussions with A.B.. B.C., C.D., G.H. and A.B. wrote the manuscript.
Example 8	A.B., B.C. and C.D. designed the study. A.B., D.E. and E.F. performed the xyz experiments. F.G. and G.H. performed XYZ simulations. I.H. and M.C. expressed and purified all proteins. A.B., H.J., B.C. and C.D. analysed the data. A.B., B.C. and C.D. wrote the paper with input from all authors.
Example 9	A.B. and B.C. designed and directed the project; C.D., D.E., A.B. and B.C. performed the experiments; C.D. and B.C. analysed spectra; A.B. and E.F. made the simulations; B.C. developed the theoretical framework; C.D., A.B. and B.C. wrote the article.
Example 10	A.B., B.C. and C.D. performed the measurements, D.E. and E.F. were involved in planning and supervised the work, A.B. and B.C. processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. F.G., and G.H. performed the xyz calculations. H.I., and I.J. manufactured the

	samples and characterized them with xyz spectroscopy, J.K. performed the xyz characterization. K.L. aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.
Example 11	A.B., B.C., C.D. and D.E. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Funding information

All research articles should have a funding acknowledgement statement included in the manuscript in the form of a sentence under a separate heading entitled 'Funding information'. The funding agency should be written out in full, followed by the grant number in square brackets.

The following are examples of a funding statement. If you use one of the examples, you should modify it to fit your specific relationship.

Scenario	Suggested funding statements
Example 1	The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Medical Research Council [grant number xxx].
Example 2	This work was supported by the Trust [grant numbers xxxx, yyyy]; the Natural Environment Research Council [grant number

	zzzz]; and the Economic and Social Research Council [grant number aaaa].
Example 3	The author(s) received no financial support for the research, authorship, and/or publication of this article.

Data availability statement

All research articles should have a data availability statement included in the manuscript in the form of a sentence under a separate heading entitled ‘Data availability statement’.

The following are examples of a data availability statement. If you use one of the examples, you should modify it to fit your specific relationship.

Availability of data	Suggested data availability statements
Data openly available in a public repository that issues datasets with DOIs	The data that support the findings of this study are openly available in [repository name e.g ‘figshare’] at http://doi.org/[doi] , reference number [reference number].
Data openly available in a public repository that	The data that support the findings of this study are openly available in [repository name] at [URL], reference number [reference number].

does not issue DOIs	
Data derived from public domain resources	The data that support the findings of this study are available in [repository name] at [URL/DOI], reference number [reference number]. These data were derived from the following resources available in the public domain: [list resources and URLs]
Data available within the article or its supplementary materials	The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.
Data generated at a central, large-scale facility, available upon request	Raw data were generated at [facility name]. Derived data supporting the findings of this study are available from the corresponding author [initials] on request.
Embargo on data due to commercial restrictions	The data that support the findings will be available in [repository name] at [URL / DOI link] following a [6 month] embargo from the date of publication to allow for the commercialisation of research findings.
Data available on request due to	The data that support the findings of this study are available on request from the corresponding author, [initials]. The data are not publicly available due to [restrictions, e.g. their

privacy/ethical restrictions	containing information that could compromise the privacy of research participants].
Data subject to third party restrictions	The data that support the findings of this study are available [from] [third party]. Restrictions apply to the availability of these data, which were used under licence for this study. Data are available [from the authors / at URL] with the permission of [third party].
Data available on request from the authors	The data that support the findings of this study are not openly available due to [reasons of sensitivity, e.g. human data] and are available from the corresponding author, [author initials], upon reasonable request [include information on the data's location, e.g. in a controlled access repository where relevant].
Data sharing not applicable – no new data generated	Data sharing is not applicable to this article, as no new data were created or analysed in this study.

Disclaimer

A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

Appendix 3: Ethics Approval Letter



24 August 2023

Dr Faaizah Mohamed (206500057)
School of Clinical Medicine
Medical School

Dear Dr Mohamed,

Protocol reference number: BREC/00005669/2023

Project title: Prevalence of Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa

Degree: MMed

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 24 August 2023. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 24 August 2023. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 September 2023.

Yours sincerely,

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

Appendix 4: District Office Approval Letter



health

Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE: DISTRICT

Physical address: 83 King Cetshwayo Highway, Highway House; Mayville 4091

Postal Address: Private Bag X54318, Durban 4000

eThekweni District Office, Tel: 031 240 5309 Fax: 031 240 5550

Email: Thabisile.Sakye@kznhealth.gov.za

Enquiries: Miss O Magwaza
Date: 21/07/2023

Dear Dr Faaizah Mohamed
University of KwaZulu Natal
School of Clinical Medicine, Department of internal medicine

RE: "Prevalence of Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa."

I am pleased to inform you that the District is granting you support to conduct the research study titled "Prevalence of Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa."

Please note the following:

1. Please ensure you adhere to all the policies, procedures, protocols, and guidelines of the Department of Health about this research.
2. This research will only commence once this office has received confirmation from the provincial health research committee in the KZN health department.
3. Please ensure this office is informed before you commence your research.
4. The District office/facility will not provide any resources for this research.
5. You will be expected to provide feedback on your findings to the district office/facility

Thanking you,

Thanking you,



P.P (ANM: M&E)

(District director) EThekweni Health District

Date: 21/07/2023

Appendix 5: Department of Health Approval Letter



KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2005/3189/3123 Fax: 033 394 3782
Email: hrkm@kznhealth.gov.za

Health Research & Knowledge
Management

NHRD Ref: KZ_202306_005

Dear Dr F Mohamed
(UKZN)

Approval of research

1. The research proposal titled 'The prevalence of Tuberculous Meningitis and Cryptococcal Meningitis co-infection in hospitalised patients in Durban, Kwa Zulu Natal (KZN), South Africa' was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

The proposal is hereby **approved** for research to be undertaken at Mahatma Gandhi Memorial, RK Khan and King Edward VIII hospitals.

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

For any additional information please contact Dr. G Shezi on 033-395 3189.

Yours Sincerely


Dr E Lutao