

DECLARATION

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DEDICATION

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57 To my dear husband, Ibrahim Sherif who remains willing to stand by my side until achieve my degree, A
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61 To all my family at Home Mother and Father, sisters and brothers, there are willing to help me with
62 anything that support my studies emotional and practical.

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OVERVIEW OF THE THESIS

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Cryptococcal meningitis (CM) is a common AIDS-defining illness and remains an important cause of morbidity and mortality among HIV-infected adults in South Africa (SA) despite antifungal therapy and anti-retroviral therapy (ART). Despite free access to treatment, ART experienced patients present with cryptococcal meningitis with low CD4 counts and have poor outcomes. The poor outcome is due to the disease itself, patient factors and poor adherence to treatment guidelines.

Over the last decade there was no significant reduction in the incidence of cryptococcal meningitis in South Africa. KwaZulu-Natal province has the highest prevalence of cryptococcal antigenemia (7.2%) vs. the national average (5.4%).

Mortality from CM also remains unacceptably high with a 3-month mortality of approximately 70% in Africa, compared to high income countries demonstrating mortality rates of 20-30 %. Underlying reasons for high CM associated mortality include: late presentation, sub optimal management from lack of access to drugs (flucytosine), use sub-therapeutic doses of fluconazole, sub-optimal monitoring of intra-cranial pressure (ICP) from lack of manometers. Multiple donor programmes, such as the Diflucan Access programme, the Global Fund for AIDFS TB, and Malaria, and the Presidents Emergency Fund for AIDS relief have contributed substantial resources aimed at improving patient outcomes and reducing morbidity and mortality. Despite free access to care, including in-patient services, to antiretroviral therapy and antifungal prophylaxis and treatment, morbidity and mortality from CM remains unacceptably high. We conducted a retrospective study in the tertiary teaching hospital to better understand factors that contribute to the ongoing high mortality among HIV infected patients presenting with cryptococcal meningitis. All HIV positive patients aged ≥ 13 years admitted to medical wards of this hospital, with microbiologically confirmed meningitis during the study period were included in the study.

Medical records of patients admitted to a tertiary teaching hospital from June to December 2016, who were diagnosed with meningitis were reviewed. We collected clinical and laboratory data from patient charts into pre-designed data collection forms. Data included clinical information upon presentation, subsequent clinical course and outcomes.

Patients with suspected meningitis had cerebrospinal fluid (CSF) evaluation either at acute admission, or after already being admitted to the adult medical wards. During the study period, 322 lumbar punctures (LP) were received from medical wards, including CSF samples from the 150/1680 patients presenting acutely with features suggestive of meningitis. A total of 44 CSF samples were deemed abnormal. Among

98 the abnormal LPs 61.3% (26/44) of patients had confirmed cryptococcal meningitis, with or without other
99 co-infections.

100 In the study of 27 patients with confirmed cryptococcal meningitis, 51.8% (14/27) were female and
101 48.2% (13/27) were male, mean age of 37.8 years (S.D 8.2 (Range: 21-62)). Headache was the most
102 common 91.3% (21/23) presenting feature, followed by vomiting 56.5% (13/23), with overall mean
103 duration of symptoms of 2 weeks (Range: 1 -3 weeks).

104 On admission 87% (20/23) were known HIV positive, while 13.0% (3/23) were offered testing during the
105 admission and confirmed HIV positive. Interestingly 43.5% (10/23) of patients were diagnosed with
106 cryptococcal meningitis within the same year of HIV diagnosis, among whom 50.0% (5/10) were
107 accessing ART at the time of admission with mean of duration from ART initiation of 3 months, whereas
108 17.0 % (4/23) of CM occurred one year after HIV diagnosis among whom 75.0% (3/4) were on ART. At
109 presentation with CM, thirteen percent (3/23) of patients were first diagnosed with HIV infection two
110 years previously, and all were ART naïve, with a CD4 count on admission in 23/23 patient of 89.4
111 cells/mm³ (range: 1-574). Mean duration of admission was 18 days (Range 1-15 day) and therapeutic
112 lumbar punctures (LP) were done in 31.8% (7/22). Complications secondary to CM treatment developed
113 in 39 % (9/23). New renal impairment occurred in 6/9, whereas worsening renal impairment occurred in
114 3/9 patients. Complications due to cryptococcal meningitis occurred: 2/23 (8.7%) patients had
115 hydrocephalus, 26.1% (6/23) of patients died, 30.4% (7/23) of patients required further care and referral
116 to a regional or quaternary hospital. Improved outcomes were noted in 43.4% (10/23) of patients whom
117 had either been discharged from the facility or had improved clinically and had been given a follow-up
118 clinic date for review.

119
120 This study helped understand the factors contributing to morbidity and mortality in HIV infected patients
121 presenting with cryptococcal meningitis. For the diagnosis of CM, a high index of suspicion and early
122 screening investigations are warranted. Improving medical management through ensuring that all patients
123 positive for serum cryptococcal antigen (CrAg) are triaged into effective treatment and prevention of
124 cryptococcal disease services, patients have the CSF opening pressure measured, the correct dose of
125 fluconazole is administered, therapeutic lumbar puncture regardless of initial opening pressure and close
126 observation and correction of commonly occurring drug related toxicities such as hypokalemia, and
127 anemia. Additional strategies such as use of flucytosine in combination with Amphotericin B to reduce
128 length of hospital stay, reduce risk of drug related toxicity, and improved patient outcomes is
129 recommended.

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133 **ABBREVIATIONS AND ACRONYMS**

134	AMA	Acute Medical Admission
135	AIDS	Acquired Immune Deficiency Syndrome
136	ADI	AIDS Defining Illness
137	ART	Antiretroviral Therapy
138	CrAg	Cryptococcal Antigen
139	CI	Confidence Interval
140	CNS	Central Nervous System
141	CM	Cryptococcal Meningitis
142	CSF	Cerebrospinal Fluid
143	ELISA	Enzyme Linked Immune Sorbent Assay
144	HIV	Human Immunodeficiency Virus
145	ICP	Intracranial Pressure
146	IQR	Interquartile Range
147	IRIS	Immune Reconstitution Inflammatory Syndrome
148	KEH VIII	King Edward Hospital VIII
149	LA	Latex agglutination
150	LP	Lumbar Puncture
151	NHLS	National Health Laboratory Service
152	OIs	Opportunistic Infections

153	SAS	Statistical Analysis Software
154	SSA	sub-Saharan Africa
155	TB	Tuberculosis
156	WHO	World Health Organisation
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CHAPTER 1

Background and Literature review

Human immunodeficiency virus (HIV) infection contributes to serious health challenges in resource limited settings. Since first reported, approximately 78 million people have been infected with the HIV virus and about 39 million people have died of acquired immune deficiency syndrome (AIDS) related disease, and another 35.3 million are currently living with HIV/AIDS, worldwide. Sub-Saharan Africa is still considered the most severely affected by HIV despite the introduction and scale-up of anti-retroviral therapy (ART) [1].

In 2017, an estimated 12, 5% of the total population in South Africa was HIV infected. The total number of persons living with HIV in South Africa increased from 4, 94 million in 2002 to 7.06 million by 2017[2]. The highest infection rates are among females in their early thirties and males in their late thirties. The province with the highest prevalence of HIV is KwaZulu-Natal accounting for 40% of HIV infections followed by 18% in Northern Cape and Western Cape [3,4]

Immunodeficiency results in increased susceptibility to a wide range of infections and diseases. Over the last few decades incidence of Cryptococcal meningitis has increased with HIV epidemic. Cryptococcal meningitis (CM) is a common AIDS defining illness that remains an important cause of morbidity and mortality among HIV-infected adults in South Africa (SA) despite free access to antifungal therapy and anti-retroviral therapy [5].

Globally an estimated 223 100 cases of cryptococcal meningitis resulted in 181 100 deaths among people living with HIV in 2014. Cryptococcal meningitis is the most common presentation of cryptococcal disease which is responsible for 15% of AIDS-related death, three quarters of which are in sub-Saharan Africa [6].

Cryptococcal meningitis affects immunocompromised individuals, either due to HIV infection or immune deficiency secondary to malignancies, solid-organ transplant, and steroid therapy, chronic diseases such as diabetes mellitus, renal failure and chronic liver disease. In the developed world the introduction of ART among HIV positive patients decreases incidence of cryptococcal infections, however the prevalence among other immunocompromised individuals has remained unchanged [7,8].

Cryptococcus yeasts exists everywhere in the environment and is acquired by inhalation. Primary infection is likely acquired in childhood by inhalation in a large proportion of individuals, although adult acquisition is also well documented. An effective cell-mediated immune response is required to contain the disease and resulting in clearance or establishment of a contained latent infection. Patients with AIDS

have a defective immune response due to low CD4 T cell count, therefore they are unable to naturally contain the cryptococcal infection [8].

Cryptococcal meningitis typically presents as an opportunistic infection among patients with advanced HIV with CD4 count less than a 100 cells/mm³. In 2016 the South African national government launched a reflex cryptococcal antigen (CrAg) testing program of all patients initiating ART. This study that attempted to understand the relationship between advanced HIV and serum CrAg positivity, was conducted in 49 NHLS CD4 laboratories in multiple districts across South Africa. Data showed that among reflex CrAg testing in patients with CD4 count less than 100 cells/mm³ the highest positivity was reported in Kwa-Zulu Natal with a total number of 3 545 (7.2% vs. national average 5.4%) [9]. In 2017 6294 patients presented to health care facilities in South Africa with Cryptococcal meningitis (cerebrospinal fluid positive for Cryptococcus species) with one third of cases from Kwa-Zulu Natal demonstrating the highest burden of cryptococcal meningitis in South Africa. Serum CrAg can be detected several weeks before patient becomes symptomatic especially in the first year of antiretroviral therapy initiation. The risk to develop cryptococcal meningitis is higher by 25% therefore serum cryptococcal antigen good, screening test for HIV infected patient with low CD4 cell count before initiation of ART to detect antigenemia and treat it with fluconazole before affecting central nervous system, prevent serious complications and significantly decrease mortality [7, 8].

Despite free access to care, including inpatient services to ART and antifungal therapy, outcomes remain poor in HIV infected patients presenting with cryptococcal meningitis namely; focal lesions, nerve palsies and blindness, thus requiring ongoing healthcare, family and community support. In Africa mortality due to cryptococcal meningitis remains unacceptably high with a 3-month mortality of approximately 70%, in comparison with mortality in high income countries of 20-30 %. Underlying reason for high CM associated mortality include: late presentation, and sub optimal management i.e. poor access to drugs (flucytosine), use of sub optimal therapeutic doses of fluconazole, lack of bedside manometers and lack of optimal clinical monitoring [11, 12].

All HIV infected patients with CD4 count less than 100 cells/mm³ should be screened for cryptococcal antigenemia [13]. If positive, patients must be evaluated for clinical evidence of cryptococcal meningitis. If the patient is symptomatic a lumbar puncture is indicated [13]. Contraindications for lumbar puncture include; significant coagulopathy or suspected space-occupying lesion based on focal nervous system signs (excluding cranial nerve VI palsy) or recurrent seizures or confirmed by brain computed tomography scan. Raised intracranial pressure (ICP) is not a contraindication for lumbar puncture in

patients with suspected cryptococcal meningitis. Other contraindications include major spinal deformity and patient refusal [14]. Serum CrAg can be detected several weeks before patient becomes symptomatic especially in the first year of ART initiation when the risk of development of cryptococcal meningitis is 25%, emphasizing the strength of the serum cryptococcal antigen assay, a good screening test for HIV infected patients [8, 15].

Diagnosis of cryptococcal meningitis can be made by clinical presentation and laboratory tests after lumbar puncture. Laboratory tests include microscopy with India ink stain that demonstrates a rounded cell with thick capsule. India Ink microscopy sensitivity is low, approximately 86%. Definitive diagnosis relies significantly on culture positivity. However, cultures can be negative if the patient is exposed to antifungal therapy or it requires longer incubation period. Detection of cryptococcal antigen (CrAg) polysaccharide of the fungal capsule in the serum and CSF is achieved by using either Latex agglutination (LA), Enzyme Linked Immune Sorbent Assay ELISA or Lateral Flow assay. Latex agglutination has higher sensitivity than India ink ranging from 83% to 97% and specificity of the LA on serum ranges from 93% to 100%. Lateral Flow assay has several advantages over the latex agglutination assay: including rapid (<10 minutes) turnaround time, requires little training for use and interpretation and can be performed with minimal laboratory infrastructure and without refrigerated storage. Enzyme Linked Immune Sorbent Assay ELISA is expensive, and needs laboratory infrastructure [8, 12, and 15].

The clinical presentation of cryptococcal disease is highly variable, it can result in asymptomatic disease, localized pulmonary disease or disseminated disease. Disseminated disease can occur in any organ with predilection for infection of the central nervous system (CNS). This results in meningoencephalitis and occasionally focal intracerebral granulomas known as Cryptococcomas. Patients with meningoencephalitis typically present with a severe headache, which may be present for several weeks to months, either isolated or associated with changes in the mental status or personality. Patients may also present with fever, lethargy, and coma [8, 16]. Cryptococcal meningitis can be a presentation of advanced HIV at the onset of diagnosis or within the first year of ART initiation as an immune reconstitution inflammatory syndrome IRIS.

Cryptococcal meningitis may be complicated by onset of hydrocephalus (communicating and non-communicating), papilledema that may lead to blindness, sudden onset of sensorineural deafness, cranial nerve palsies, motor and sensory deficits, cerebellar dysfunction, and seizures. Increased intracranial pressure due to occlusion of cerebrospinal fluid drainage by cryptococcal cells or shed polysaccharide, inflammation or combination of these factors [8].

The World Health Organization updated management guidelines of cryptococcal meningitis in March 2018 [12]. The new guidelines included an Induction phase: Short course (one week): amphotericin B 1

mg /Kg /Day, flucytosine 100 mg /Kg/day (divided in 4 doses). Studies have shown that 1 week of this combination has lower mortality rate at 10 weeks than 2-week duration also noted that there was a lower rate of developing amphotericin B related toxicity [15]. Depending on drug availability alternative options: 2 Weeks fluconazole 1200 mg daily and flucytosine or 2 weeks of amphotericin B and fluconazole 1200 mg daily followed by a Consolidation phase of 8 weeks of oral fluconazole 800 mg daily and lastly a Continuation phase with fluconazole 200 mg daily dose.

Since flucytosine is not available in South Africa, amphotericin B in combination with fluconazole is used instead. However, fluconazole doses are lower than recommended in the latest guidelines released by the WHO. Fluconazole is required to be continued until the CD4 count is more than 200 cells/mm³ on two occasions that are six months apart [13]. Direct antifungal approach together with supportive care which includes hydration, electrolyte monitoring, and regular therapeutic lumbar punctures is recommended. Raised intracranial pressure at the time of diagnosis of CM is common and frequently contributes to changes in mental status, headache, loss of vision and hearing or even death. Therefore, aggressive management of raised intracranial pressure is warranted. Each patient receiving a lumbar puncture should have a base line opening pressure measured and recorded, followed by therapeutic lumbar punctures to improve outcomes [16, 17]. Several studies have demonstrated that high intracranial pressure due to cryptococcal meningitis contributes to increased mortality if not managed aggressively [12].

Amphotericin B is an effective fungicidal but has many adverse effects including nephrotoxicity, electrolyte imbalance, chemical phlebitis and anaemia. Nephrotoxicity can be prevented by proactive intravenous fluid, electrolyte imbalances (hypokalaemia and hypomagnesemia) that can be prevented by close monitoring and prophylactic supplementation [18, 19].

Early clinical screening and detection of cryptococcal meningitis in an HIV endemic setting would enable early identification and triage of patients for closer clinical observation. This would facilitate early intervention for HIV infected patients at high risk for adverse clinical outcomes and mortality due to cryptococcal meningitis.

This study describes the prevalence, clinical presentation and outcomes on discharge of patients admitted with Cryptococcal meningitis, in the era of widespread access to ART and cryptococcal disease screening and prevention. Data from this study will allow better understanding of the clinical course of cryptococcal meningitis in HIV infected patients and help direct interventions to improve the clinical outcomes.

Hypothesis to be tested, or Research Question to be answered:

Cryptococcal meningitis occurs in patients on ART, with adverse outcomes

Aim

Describe the prevalence, clinical presentation and outcomes on discharge of patients admitted with Cryptococcus meningitis.

Objectives

1. Describe the prevalence of cryptococcal meningitis among all patients admitted to a tertiary facility over a six- month period

2. Describe the clinical presentation of CM by ART status with respect to:
 - a. Review clinical features of patients presenting with CM: Clinical signs and symptoms, opening pressure, ART status: (suppressed on ART, on ART - not suppressed, defaulted ART, ART naïve), CD4 count, Viral load, haematological parameters,
 - b. Review microbiologic features of CSF among patients presenting with CM: method of diagnosis (India Ink vs other), co-infections in CSF, cells, biochemistry

3. Describe clinical course and management:
 - a. Length of stay, number and type of invasive procedures (REPEAT LP) conducted, other.
 - b. drug therapy, ART management, other therapies administered
 - c. Co-morbidities: Drug toxicity, other infections, non-infective conditions
 - d. Nature of supportive care offered.

4. Patient outcomes on discharge, i.e.
 - a. Proportion alive with no complications
 - b. Proportion of patients alive with minor complications

- c. Proportion of patients alive with neurologic deficit
 - d. Proportion of patient that demised
5. Proportion of patients requiring step down in-patient care

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CHAPTER 2

Prevalence and outcome of cryptococcal meningitis among HIV infected patients admitted to a tertiary level facility in an HIV endemic setting in the ART era

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ABSTRACT

Background: Cryptococcal meningitis (CM) is a common AIDS (acquired immunodeficiency syndrome) - defining illness that contributes to morbidity and mortality among HIV-infected adults in South Africa (SA).

Methods: We conducted a retrospective study among HIV infected patients aged ≥ 13 years, admitted to medical wards to better understand factors that contribute to ongoing high mortality among patients presenting with cryptococcal meningitis.

Results: There were 322 lumbar punctures (LP) received from medical wards, from patients presenting with features suggestive of meningitis. A total of 44 CSF samples were deemed abnormal. 26 patients had confirmed cryptococcal meningitis. Among those patients, 51.8% (14/27) were female and 48.2% (13/27) were male. No further clinical data available for 3/27 patients due to missing charts, 1/27 was HIV uninfected therefore excluded from the study. Headache was the most common 91.3% (21/23) presenting feature, with overall mean duration of symptoms of 2 weeks (range: 1 -3 weeks). On admission 87% (20/23) were known HIV positive, with 13.0% (3/23) confirmed HIV positive during admission. Mean length of stay was 18 days IQR (1-15 day). Lumbar puncture (LP) was done to 95.6% (22/23) and therapeutic LPs were done only in 31.8% (7/22). Renal impairment developed in 39 % (9/23), 2/23 (8.7%) patients developed hydrocephalus, 26.1% (6/23) died, 30.4% (7/23) required further care, while 43.4% (10/23) were discharged.

Conclusion: Improving medical management through more effective treatment and prevention services for cryptococcal disease is required.

Keywords: Cryptococcal meningitis, HIV, cerebrospinal fluid, ART, CLAT, fluconazole, flucytosine, amphotericin

INTRODUCTION

Cryptococcal meningitis (CM) is a common AIDS (acquired immunodeficiency syndrome) - defining illness that remains an important cause of morbidity and mortality among HIV-infected adults in South Africa (SA) despite free access to care including inpatient services to ART and antifungal therapy [1].

Cryptococcal meningitis typically presents as an opportunistic infection among patients with advanced HIV with CD4 cell count less than 100 cells/mm^3 , since 2016 the national government launched a reflex CrAg testing program of all patients initiating ART. The study that was conducted in 49 NHLS CD4 laboratories in different districts across South Africa attempted to understand the relationship between advanced HIV disease and serum CrAg positivity. Data showed that positive reflex CrAg testing in patients with CD4 cell count less than 100 cells/mm^3 , the highest positivity was reported in KwaZulu-Natal with a total number of 3,545 (7.2% vs. national average 5.4%) [3]. In 2017, 6294 patients that presented to health care facilities were diagnosed with Cryptococcal meningitis (cerebrospinal fluid positive for *Cryptococcus* species) with one third of these cases in KwaZulu-Natal province which shows the disproportionate burden of cryptococcal meningitis in this region. Over the last decade there was no significant reduction of the incidence of cryptococcal meningitis in South Africa and in hospital fatality ratio remains static [4].

Serum CrAg can be detected several weeks before patient becomes symptomatic especially in the first year of antiretroviral therapy initiation. The risk to develop cryptococcal meningitis is higher by 25% therefore serum cryptococcal antigen good, screening test for HIV infected patient with low CD4 cell count before initiation of ART to detect antigenemia and treat it with fluconazole

before affecting central nervous system, prevent serious complications and significantly decrease mortality [7, 8].

Increased intracranial pressure occurs due to occlusion of cerebrospinal fluid drainage by cryptococcal cells or shed polysaccharide, inflammation or combination of these factors [8]. World Health Organization updated guidelines of management cryptococcal meningitis March 2018 [10, 11]. Recommended Induction phase: Short course (one week): amphotericin B 1mg/Kg/Day, flucytosine 100 mg /Kg/day (divided in 4 doses). Studies have shown that 1 week of this combination has lower mortality rate at 10 weeks than 2-week duration. Depending on drug availability, alternative options: 2 Weeks of fluconazole 1200 mg daily and flucytosine or 2 weeks of amphotericin B and fluconazole 1200 mg daily followed by consolidation phase of 8 weeks of oral fluconazole 800 mg daily then continuation phase with fluconazole 200 mg daily dose.

Since flucytosine is not available South Africa, amphotericin B in combination with fluconazole used instead [12]. However, in practice, fluconazole dose is lower than recommended in the last guidelines released by WHO. Direct antifungal approach together with supportive care which include hydration, electrolyte monitoring and proper replacement, regular therapeutic lumbar punctures are recommended [13]. Raised intracranial pressure at the time of diagnoses of CM is common and frequently leads to changes in mental status, headache, loss of vision and hearing or even death. On that account, aggressive management of raised intracranial pressure is recommended for each patient. Lumbar puncture should have base line opening pressure measured and subsequent therapeutic lumbar puncture regardless of base line opening pressure which has been noted to improve the outcomes. In a study was conducted in Uganda and Washington, USA showed that therapeutic lumbar puncture improve survival [7, 9]. Several studies were conducted to understand benefits of therapeutic lumbar puncture to decrease high ICP all supported the fact that high intracranial pressure resulting from cryptococcal meningitis carry a high mortality if not managed aggressively [11].

Amphotericin B is an effective fungicidal, but a number of side effects have been reported which include nephrotoxicity which can be prevented by proactive intra-venous fluid, electrolyte imbalance namely hypokalaemia and hypomagnesemia which can be prevented by close monitoring and prophylactic supplement and treatment. Chemical phlebitis and anaemia are also reported as side effects of amphotericin B [14, 15, 16].

Even though antiretroviral access is rapidly expanding in Africa, management of opportunistic infections (OIs) remains a major challenge of HIV/AIDS care.

METHODS

Study Setting

We conducted a retrospective, cross sectional study. patient chart records were reviewed for adult patients admitted to medical wards with symptoms suggestive of meningitis at a tertiary teaching hospital (King Edward Hospital VIII (KEH VIII) over a period of 6 months (from June to December 2016).

King Edward Hospital is a referral hospital in the eThekweni district of KwaZulu-Natal where HIV prevalence of 17% and approximately 4 million patients access ART within the district. The hospital has 852 beds with 22000 outpatients monthly.

Study Population

All HIV positive patients aged ≥ 13 years admitted to KEH VIII medical wards with confirmed meningitis during the study period were included in the study. Patients who were HIV negative and presented with cryptococcal meningitis as well as HIV infected patients admitted with diagnoses other than meningitis were not included in this study.

Important definitions:

Patient with Suggestive meningitis: patient who suspected of having meningitis (presented with one or more of following symptoms such as headache, meningism, confusion, fever, etc.)

Confirmed meningitis: based on

1. Clinical presentation (headache, meningism, confusion, fever) and CSF microscopic examination and serum CrAg positivity
2. Clinical presentation and serum CrAg positivity and radiological features suggestive of meningitis.

Data extraction

Identification of patients occurred in 2 steps:

Step 1: review of a register of all patients admitted to Acute Medical Admission (AMA) ward with suspected meningitis.

Step 2: review of data base of central laboratory of the specimens received from AMA and all adult medical wards during the study period after obtaining the Institutional Ethical Clearance. Data was extracted from the charts of patients with confirmed microbiological evidence of cryptococcal meningitis on CSF. The extracted information was documented on pre-designed data collection forms and uploaded on a data management system then to statistical analysis software using Statistical Package for the Social Sciences software. Data variables included: HIV status on presentation, year of diagnosis, ART status, presenting symptoms, duration of illness, hematologic and biochemistry parameters, base line CD4 count, current CD4 count, viral load, lumbar puncture result, number of therapeutic LPs done, length of stay, clinical course, outcome.

Patient outcomes were studied:

- a. Proportion alive with no complications
- b. Proportion of patients alive with minor complications
- c. Proportion of patients alive with neurologic deficit
- d. Proportion of patient that demised
- e. Proportion of patients requiring step down in-patient care

Descriptive data were tabulated and mean, standard deviation of the data was calculated. The association of clinical and laboratory features with the outcome was analysed.

Protocol was submitted to UKZN research biomedical ethics committee for regulating oversight, Ref Number: BE 434/16 and the protocol approved for higher degree on the 26/04/2017

Amendment was submitted on the 26/March/2018 to authorize reviewing the National Health Laboratory Service (NHLS) result records of the lumbar puncture done in the medical wards during the study period, which approved on the 24/April/2018

RESULTS

During the study period a total of 1680 patient were admitted to the medical wards, of these, 150 patients presented acutely with clinical features suggestive of meningitis. NHLS laboratory

received CSF samples from 322 lumbar punctures (LP) performed at medical wards during the study period. A total of 44 CSF samples were deemed abnormal. Among the abnormal LPs 59% (26/44) of patients had confirmed cryptococcal meningitis, with or without other co-infections. Other infective causes of meningitis comprised of a total of 49.9% (18/44) of which 77.7% (14/18) had isolated Tuberculosis (TB) meningitis, 11.1% (2/18) had viral meningitis and 11.1% (2/18) had isolated bacterial meningitis (Figure 1).

Baseline demographic and clinical presentation

In this study, 27 patients had confirmed cryptococcal meningitis either on CSF examination in 96%(26/27) or based on clinical, radiological finding suggestive of meningitis with elevated intracranial pressure and positive serum CrAg in 3.7%(1/27), Additional clinical information was not available for 11.1% (3/27) of patients due to missing clinical charts and 3.7% (1/27) patients was confirmed HIV negative, and therefore excluded from the analysis. 51.8% (14/27) were female and 48.2% (13/27) were male, mean age of 37.8 years (SD 8.2 (range: 21-62)). Headache was the most common 91.3% (21/23) presenting feature, followed by vomiting 56.5% (13/23), with overall mean duration of symptoms of 2 weeks (range: 1 -3 weeks) (Table 1) (Figure 2).

On admission 87% (20/23) were known HIV positive, while 13.0% (3/23) were offered testing during the admission and confirmed HIV positive. Interestingly 43.5% (10/23) of patients were diagnosed with cryptococcal meningitis within the same year of HIV diagnosis, among whom 50.0% (5/10) were accessing ART at the time of admission with mean duration of initiation of 3 months, whereas 17.0 % (4/23) of CM occurred one year after HIV diagnosis among whom 75.0% (3/4) were on ART. Thirteen percent (3/23) of patients presented with CM 2 years after HIV diagnosis and all were not on ART at the time of presentation, 4.3% (6/23) presented 4 years or more after HIV had been diagnosed, among whom 66.0% (4/6) were on ART at the time of presentation with CM (Table I).

Baseline CD4 count was recorded in only 91.3 %, (20/23) of patients with mean of 94.86 cells/mm³ (range 1-504). While 100 % (23/23) of patients had CD4 counts recorded during this presentation with mean of 89.39 cells/mm³ (range 1-574) (Table 1).

Haematological and biochemical parameters

Baseline blood investigations comprised of the following; 47.8% (11/23) of patients had abnormal haemoglobin level with mean haemoglobin of 10.6g/dl (SD 1.73), 17.3% had leukopenia, and 8.3% had leucocytosis (2/23). Mean white cell count was of $5 \times 10^9/L$ (SD 5.5) and mean platelet count 246.17 (SD 99.7). Renal function tests on admission elevated urea and creatinine in 5/23, (21.7%) with mean urea of 5 mmol/l (range 7.3 - 12.5 mmol/l) mean creatinine of 83.5 $\mu\text{mol/l}$ (range 97-256 $\mu\text{mol/l}$), mean potassium level of 4.25 mmol/l (SD 0.9 (range 3.2-5.7 mmol/l)) and mean total albumin of 32.65g/l (SD 6.9 (range 20-45g/l)). On discharge, 39.1 % (9/23), developed new (6/9) or worsening (3/9) renal impairment, 6/23 (26%) patients developed hypokalaemia with mean potassium level on discharge 4.25 mmol/l (range 2.6- 5.3 mmol/l). 13/23, (56%) patients had elevated total protein level with mean total protein level 83.5 (SD 12.2 (range 79-120 mmol)) (Figure 3).

Microscopic features of CSF

On admission 95.6% (22/23) of patients had a lumbar puncture done, with opening pressures recorded in only 22.7% (5/22) of patients, all were elevated, mean of 31 cmH₂O (range 50-15). Microbiologically confirmed cryptococcal meningitis diagnosis was as follows: India ink positive in 63.6% (14/22) of patients, CSF CLAT in 100% (22/22) of patients and CSF culture positive for *Cryptococcus Neoformans* in 77.2% (17/22) of patients. Combined CLAT and Culture positive in 31.8% (7/22), Combined CLAT and India ink in 18.1% (4/22), Combined India ink, CLAT, Culture positive in 44.4% (10/22). Nine percent (2/22) of patients were diagnosed with cryptococcal meningitis with TB co-infection and 4.5% (1/22) of patients were diagnosed with cryptococcal infection with bacterial co-infection. Chemistry documented in all 22 patients was a mean CSF glucose of 3mmol/l (range 0.7- 3.5 mmol/l) and mean CSF protein of 1.5 g/l (range 0.23 – 5.6 g/l). White cell count (WCC) was recorded in CSF of 95.4 % (21/22) patients. The mean CSF WCC was 26.67 cell/ μl (range: 0-262) (Table II).

Clinical course and outcome

Mean duration of admission was 18 days (range 1-15 days). Therapeutic LPs were done in 31.8% (7/22). Complications secondary to treatment developed in 39.1 % (9/23) (patients developed

renal impairment, 66.6% (6/9) of which had normal renal function on admission while 33.3 % (3/9) patients with known abnormal renal function on presentation, had experienced worsening. Hypokalaemia was reported in 23% (6/23) patients, requiring prolonged duration of hospital stay. Complications due to cryptococcal meningitis also has been recorded, 8.7% (2/23) of patients had CT scan performed which revealed hydrocephalus. Death occurred in 26.1% (6/23) of patients, with a mean length of stay of up-to 9 days, range (1-24). Although 30.4% (7/23) of patients experienced complications that required further care and referral to a regional or quaternary hospital, improved outcomes were noted in 43.4% (10/23) of patients who had either been discharged from the facility or had improved clinically and given a follow-up clinic date for review. (Table III) (Figure 3).

DISCUSSION

Cryptococcal meningitis is a common cause of high morbidity and mortality in HIV infected patient in South Africa despite free access to health care for screening, and pre-emptive treatment. In this study, 61.3% of patients with suspected meningitis was due to cryptococcal meningitis. Over the last decade there was no significant reduction of the incidence of cryptococcal meningitis in South Africa.[4]

While there is a good reflex screening program in South Africa, the success of it depends on the results being communicated to the frontline clinician timeously for prompt communication to patients before advanced and disseminated cryptococcal disease develops. The ongoing high prevalence of cryptococcal meningitis, indicates that the benefit of reflex testing can only be realized with commensurate strengthening of the health system across all levels. Almost all patients in this study have very low CD4 cell counts and unsuppressed HIV viral load. Furthermore, almost 10% of patients were already diagnosed with HIV but not initiated on ART, suggesting that neither guidelines for HIV test and treat, nor guidelines for serum cryptococcal antigen screening and linkage to preventive and treatment services were followed. Reflex screening programs are helpful when efficient and timeous communication of results to patients is possible. Hence, while the idea is excellent implementation fails due to lack of proper systems to cascade positive results and appropriate treatment to eligible patients.

Deviation from guidelines has a significant impact on the clinical outcome of patients presenting with cryptococcal meningitis. In a systemic review and meta-analysis published in 2018 to assess the prevalence of blood CrAg positivity (31 studies) and asymptomatic CM in CrAg-positive participants, this review also looked at the incidence of cryptococcal meningitis and mortality rate in all screened patient. It found that the incidence of cryptococcal meningitis was 21.4% without pre-emptive fluconazole and 5.7% with pre-emptive fluconazole therapy initiated at 800 mg daily. Furthermore, conducting lumbar punctures and initiating pre-emptive fluconazole in CrAg positive participants who are still asymptomatic, reduced the incidence of cryptococcal meningitis to zero with improved survival benefits [16].

We confirmed our hypothesis that cryptococcal meningitis had poor outcomes irrespective of ART status. In our study we found 56.5% of patients experienced negative outcomes and improvement was noted in 43.4% of patients despite the reflex CrAg testing and investments in widespread ART

access. The high proportion of patients that still develop life-threatening AIDS defining illness (ADI) and high mortality that indicate a failure of the reflex CrAg testing and the ART programmes. We report 26.1% mortality with HIV-associated CM. This mortality is much higher than the mortality reported in North American studies 5.5%–15%, but comparable to that reported elsewhere in sub-Saharan Africa [17, 18, 19].

Multiple factors contributed to this high mortality such as advanced disease and late presentation. Our study highlights the substantial contribution of ART experienced patients to ongoing AIDS related morbidity and mortality [20]. Most of our patients who presented with cryptococcal meningitis had a very low CD4 cell count and unsuppressed HIV viral load. Among the ART experienced patients, 43.4% had defaulted treatment and experienced poor outcome. On the other hand, patients who are newly diagnosed HIV or chronic HIV on treatment had better outcomes.

Mean age of patients was 37.8 years, similar to another local study done but lower than the mean age of 39.05 years, reported in a study in India [1, 21]. In this study, the overall mean duration of symptoms was 2 weeks. The commonest presenting symptom was headache reported in 91.3% followed by vomiting 56.5%, findings that are similar to other studies conducted in Africa and India. The difficulty is that headache is non-specific, and it is easy to overlook and in the vast majority of patients who have died in this study, headache was the presenting symptom highlighting the need to pay close attention to headache as a presenting clinical feature. A significant proportion had altered mental status (34.8%), similar to the experience in Uganda (38%) and France (33%), but much higher than the 10–12% in U.S. reports [22].

Failure to monitor and manage high intracranial pressure (ICP) has also contributed to the high morbidity and mortality seen. In this study, opening pressure was measured only in five patients (22.7%), far lower rates of opening pressure measurement than studies from the United States - 50%, and Uganda 92% in. In Western Cape, South Africa, opening pressure measurement was performed more consistently in the regional hospital, than in the district hospitals in 2010 [22, 23]. Furthermore, this study highlights a major deviation in following South African and World Health Organization guidelines for managing raised intracranial pressure. In this study therapeutic lumbar puncture was done in only seven patients (31.8%). While this is slightly higher than reports from a study done in KwaZulu-Natal 2012, twenty-nine patients had therapeutic lumbar puncture (23.4% 29/114), contrastingly, in an observational study in Tanzania 100 % of patients had

opening pressure measured and therapeutic lumbar puncture performed and show a reduction in the mortality rate [1, 7].

Another factor that contributed to the poor outcome in our study was the poor recognition and management of drug toxicity. Amphotericin B nephrotoxicity was observed in 39.1 % of patients. Hypokalaemia, reported in 23% of patients, resulted in prolonged duration of hospital stay. Interestingly, this shows improvement from a study conducted in South Africa in 2012, where nephrotoxicity and hypokalaemia were documented in 55% and 71% of patients respectively [1]. However, with careful observation and clinical management, a 1% incidence of nephrotoxicity was reported in Uganda and in 8.8 % of patient in the multicentre ACTA study conducted in South Africa in patients treated with Amphotericin B [11, 22]. There was no proper haemoglobin monitoring in our study, unlike other published studies. According to the guidelines monitoring twice weekly renal function and electrolyte and weekly haemoglobin monitoring with supplementation of electrolytes are cost-effective measures that will improve the outcome and decrease length of stay in health facilities.

Among patients who died in our study, most were chronically infected with HIV, all presented with advanced disease, very low CD4 cell count and very high viral load, and most were ART experienced who had defaulted treatment. Two patients were already on second line ART indicating chronicity of care and failure of the health care system to prevent this AIDS related mortality by failing to re-engage these patients to the program, and provision of frequent CD4 count testing and early screening and prevention of opportunistic infections (OIs). While headache was the most common presenting complaint among these patients, only one patient had CSF opening pressure measured, and therapeutic lumbar puncture performed. Furthermore 50% of these patients developed drug toxicity, further contributing to overall morbidity and poor outcomes. The risk factors for high mortality associated with cryptococcal meningitis found in our study includes: advanced HIV disease, failure of the screening program to prevent the disease, late presentation of patients, failure to follow guidelines in treating cryptococcal meningitis especially measuring of opening pressure and conducting therapeutic lumbar punctures, and failure in preventing drug related toxicity through proper clinical monitoring.

LIMITATION

This study was a retrospective chart review that relied on the accuracy and completeness of written medical records from routine care services. While missing data and incomplete information may have undermined the quality of the data we provide, the interpretation and generalizability of our finding remains unaffected. The prevalence estimates are based on testing patients that accessed care at a single central facility where only the sickest patients are seen. Furthermore, only patients present with overt symptoms of CM are triaged for CSF testing, and those with subtle features may have been missed, and patients with advanced HIV often present with multiple co- morbidities.

Conclusion

Our study highlights the major contributing factors to ongoing morbidity and mortality in patients with cryptococcal meningitis in South Africa despite the clear guidelines to how to prevent and treat cryptococcal meningitis as well as guidelines for ART initiation to test and treat to prevent AIDS related mortality.

From a public health perspective, the scaling up of routine HIV counselling and testing, offer the potential to intervene with ART before AIDS-related OIs occur. Our study demonstrates a high cryptococcal meningitis mortality despite amphotericin therapy and the availability of ART. Although use of ART and early detection of HIV may eventually lead to a reduction in OIs, including CM, management of OIs remains a key aspect of AIDS care in Africa.

Other public health implication in setting with endemic disease sub-Saharan Africa, is that we should have a manometer in every medical ward.

Recommendations:

1. Increase level of awareness of the individuals of the importance of early HIV testing and initiation of ART its lifesaving and prevent morbidity and mortality.
2. Primary health care facility to repeat CD4 cell count every 6 months but not only HIV viral load and those patients with low CD4 cell count should be screened and tested for serum cryptococcal antigenemia to start antifungal therapy and refer for lumbar puncture even before patients become symptomatic to diagnose subclinical cryptococcal meningitis and initiate treatment.

4. Flucytosine and amphotericin B regimen for 1 week has been recommended by WHO in the last guidelines released in 2018 to decrease duration of exposure to the nephrotoxic drug and decrease other side effects such as anaemia, also it will decrease hospital stay for patient treated for cryptococcal meningitis.

5. Since flucytosine is expensive and not available in South Africa, fluconazole 1200 mg daily with amphotericin B for duration of 2 weeks with prophylaxis measure to prevent any drug related toxicity is recommended.

6. Post exposure prophylaxis: to continue 200 mg fluconazole for HIV infected patients with CD4 cell count of less than 100 cells/mm³ for at least 1 year then to discontinue if patient adherent to ART and CD4 cell count improved to greater than 100 cells/mm³

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Figures & Tables

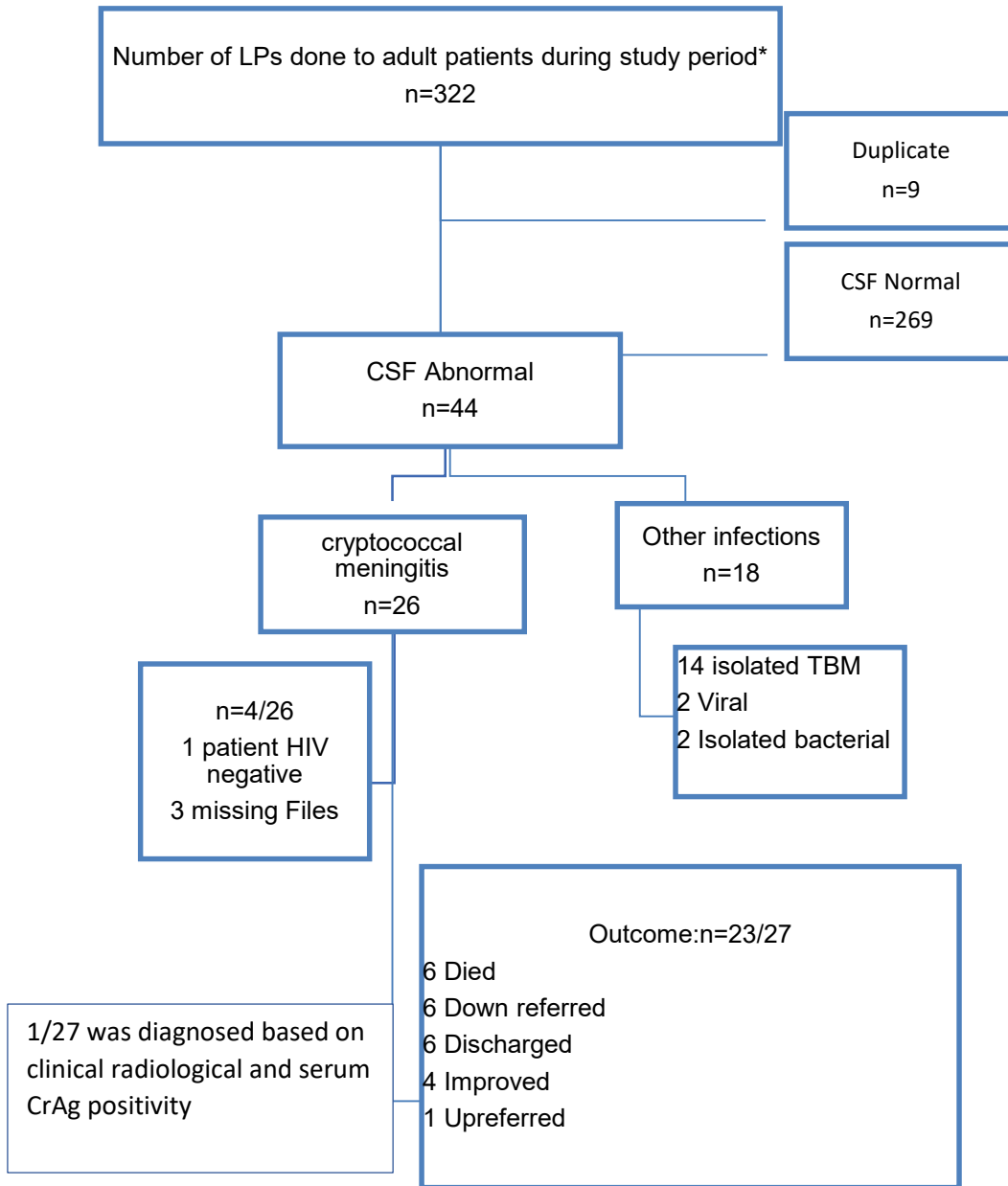


Figure 1: Results from lumbar puncture of suspected meningitis patients from adult medical wards over a 6 Months period, diagnosis and outcome. (*from regional laboratory)

LP lumbar puncture, TBM Tuberculosis meningitis, CrAg cryptococcal antigen

Table I: Baseline characteristics and clinical features at presentation among patients with cryptococcal meningitis

Characteristics	N
Gender, n (%)	
Male	13/27 (48.2)
Female	14/27 (51.8)
Age (years), mean (SD)	37.8 (8.2)
Presenting symptom and signs, n (%)	
Headache	21/23 (91.3)
Vomiting	13/23 (56.5)
Confusion	8/23 (34.8)
Loss of weight	8/23 (34.8)
Loss of appetite	8/23 (34.8)
Photophobia	7/23 (30.4)
Duration of symptoms	2 weeks (1-4)
Length of stay, in days (range)	18 (1-15).
Current CD4, cells/mm ³ (range)	89.9 (1-574)
Viral load, copies/ml (range)	646310.4 (40 - 6650000)
Newly diagnosed 2016 n (%)	10/23 (43.4)
Known chronic HIV n (%)	13/23 (56.5)
On ART n (%)	11/23 (47.8)
Haemoglobin (SD)	10.6 (1.73)
Serum albumin (SD)	32.6 (6.9)
LP done n (%)	22/23 (95.6)
Opening pressure measured	5/22 (22.7)
Therapeutic LP	7/22 (31.8)
CSF protein (range)	1.5 (0.23-5.6)
Renal impairment n (%)	3/23 (21.7)
Anaemia n (%)	11/23(47.8)
Low GCS n (%)	8/21 (38.1)
TB (at any site) n (%)	3/23 (13)
Other infection n (%)	1/23 (8.6)
Co morbidities n (%)	3/23 (13)
Died n (%)	6/23 (26)
Patient needed further medical care n (%)	7/23 (30.3)

ART antiretroviral therapy, CSF cerebrospinal fluid, LP lumbar puncture, TB tuberculosis, GCS Glasgow comma scale SD standard deviation

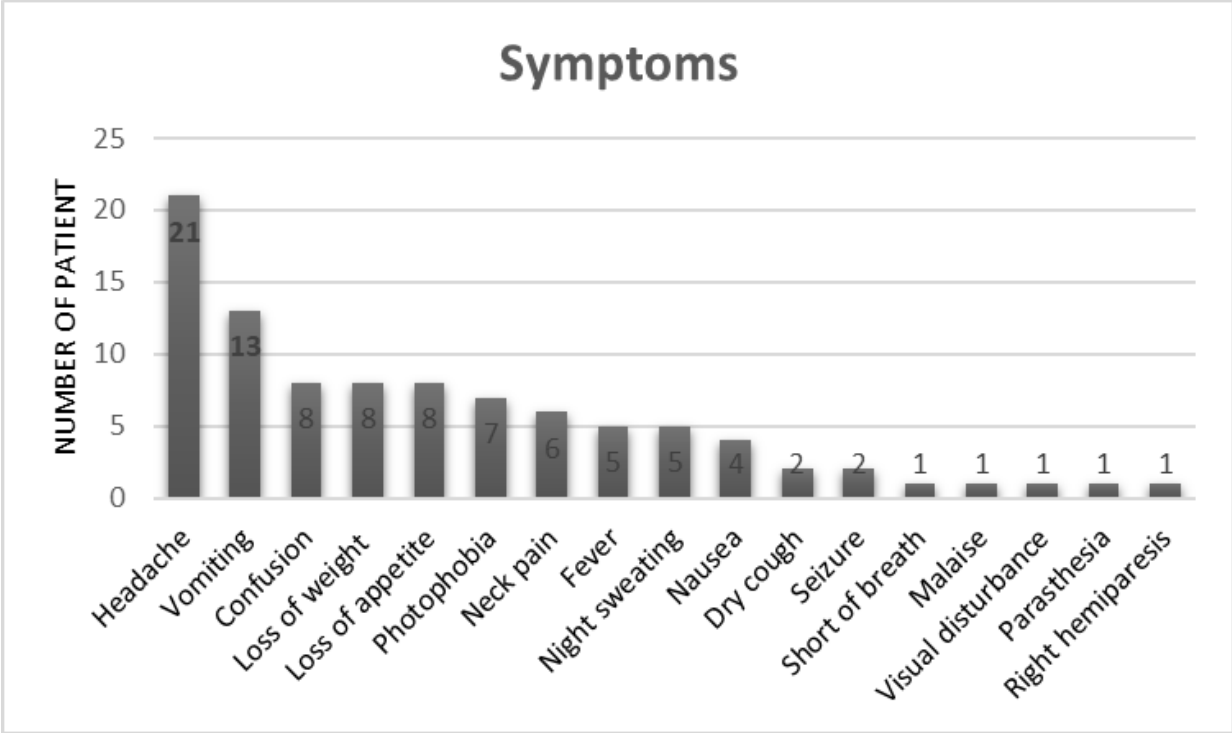


Figure 2: Symptoms of 23 patients presenting with cryptococcal meningitis

Table II: Comparison between HIV diagnosis year, ART duration and CM treatment outcome

	Year HIV Diagnosis	Current CD4 count	Current Viral load	Date HIV dx	Out come
Patient Not on ART at CM Presentation					
1	2016	1	399000	0/12	discharged
2	2016	40	15300	2/12	Follow up clinic date
3	2016	136	984000	0/12	Follow up clinic date
4	2016	117	842000	0/12	Follow up clinic date
5	2016	183	507000	3/12	Died
Patients on ART at CM presentation (recently initiated)					
	Year HIV diagnosis	Current CD4 count	Viral load	ART duration	Outcome
1	2016	40	78000	2/12	Down referred
2	2016	4	927000	6/12	Died
3	2016	4	208	2/12	Discharged
4	2016	182	818	1/12	Down referred
5	2016	61	415000	4/12	Discharged
Patient Not on ART at the time of CM presentation (defaulted ART treatment)					
1	2015	70	173800		Discharged
2	2014	2	11042		Follow up clinic date
3	2014	64	3420000		Died
4	2014	68	19900		Died
5	2011	1	257		Died
6	2011	104	2200000		Discharged
7	2010	7	279000		Down referred
Patient on ART at the time of CM presentation (non-adherent)					
1	2012	4	18800		Up referred
2	2011	2	261000		Died
3	2005	93	6650000		Down referred

Patient on ART (supressed)					
1	2015	164	71	11/12	Down referred
2	2015	574	Not detectable	23/12	Discharged
3	2015	135	392		Down referred

Table III: Clinical and laboratory data at diagnosis in 22 HIV infected individuals with CM and outcome

CSF obtained	N=22	On ART	Proportion Alive
Opening pressure	5/22 (22.9%)	5/5 (100%)	4/5 (80%)
CSF			
Combined CLAT & culture	7/22 (31.8%)	3/7 (42.8%)	5/7 (71.2%)
CLAT & culture & India ink	10/22 (44.4%)	6/10 (60%)	7/10 (70%)
Combined CLAT & India ink	4/22 (18.1%)	2/4 (50%)	2/4 (50%)
Blood Investigation			
Blood culture done	11/23 (47.8%)	5/11 (45%)	8/11 (72.7%)
-Blood culture positive	4/11 (17.3%)	2/4 (50%)	2/4 (50%)
Leukopenia	4/23 (17.3%)	3/5 (60%)	1/4 (25%)
Leucocytosis	2/23 (8.6%)	2/2 (100%)	1/2 (50%)

CSF cerebrospinal fluid, ART antiretroviral therapy, CLAT cryptococcal latex antigen test

Table IV: Clinical AND laboratory Characteristic in CM patients who demised

Patients						
Characteristics	1	2	3	4	5	6
Gender	F	M	F	M	M	M
Age	38	44	46	42	48	31
Year HIV diagnosis	2016	2011	2014	2011	2014	2016
CD4 count	183	2	68	1	64	4
Viral load	507000	2601000	19900	257	3420000	927000
ART status	Not on ART	On ART 2 nd line	Not on ART	On ART2 nd line	On ART	On ART
Presentation	Headache, Confusion, vomiting	Headache	Confusion	Headache, Photophobia	Headache, Confusion	Headache, photophobia, vomiting
Haemoglobin	9.7	10.9	7	9.5	14.9	12.2
Albumin	27	26	21	25	45	39
CSF protein	1.05	5.6	0.23	0.36	0.46	0.31
CSF glucose	2.8	3.3	2.6	1.3	1.8	3.5
Open/pressure	No	No	No	No	50	No
Therapeutic lumbar puncture	No	No	No	Yes	No	No
Comorbidities	None	None	Pancytopenia and Sepsis	Renal impairment and Pancytopenia	None	None
Complication	Hypokalaemia	Hypokalaemia	Renal impairment	None	None	None
Days to death from admission	8	1	24	16	3	5

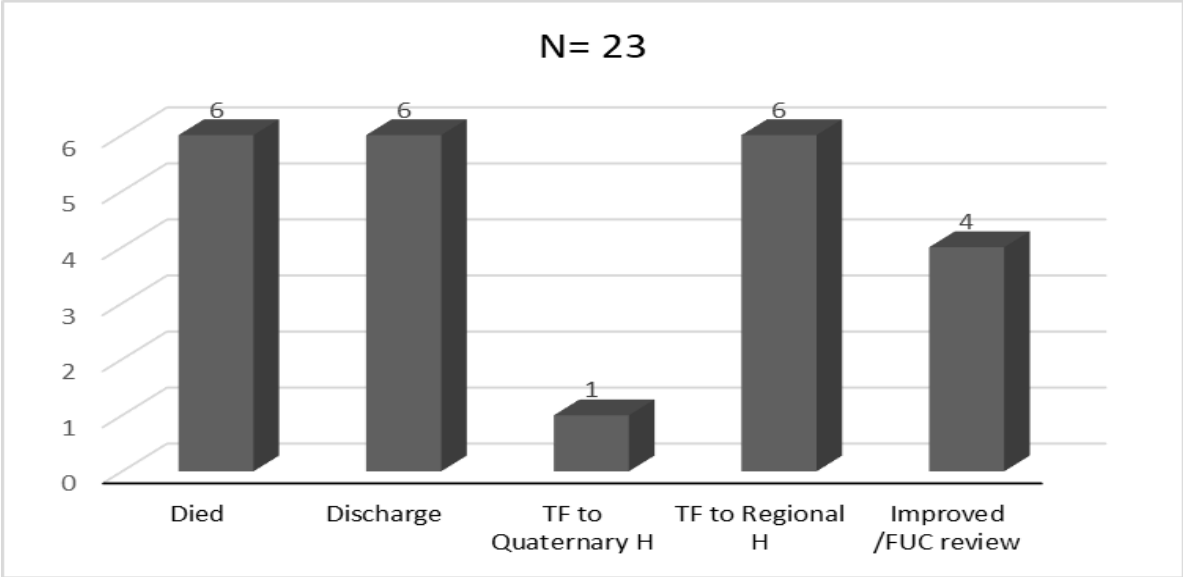


Figure 3: Outcome upon discharge
TF transferred, H Hospital, FUC follow up clinic

Declarations

Ethics approval

This study was approved by the Biomedical Research Ethics Committee Ref Number: BE 434/16 and the protocol approved for higher degree on the 26/04/2017

University of KwaZulu-Natal.

Conflict of interest

Funding

Authors Contributions

NGA.NM conceived the study. NGA, KN. and NM. designed and conducted the study. NGA prepared, extracted, and analyzed the data. KN, provided ongoing support, and study co-ordination and oversight of data analysis, interpretation and write-up. NGA, and KN wrote the article. NGA, and KN interpreted the data. All authors approved submission of this article

APPENDICES

APPENDIX 1: THE FINAL PROTOCOL OF THE STUDY APPROVED BY BREC

**Prevalence of cryptococcal meningitis among HIV infected patients
Admitted to a tertiary level facility in an HIV endemic setting in the
ART era**

MMed Internal medicine

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**Version 1.1
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Aim

Describe the prevalence, clinical presentation and outcomes on discharge of patients admitted with Cryptococcus meningitis.

Objectives

1. Describe the prevalence of cryptococcal meningitis among all patients admitted to a tertiary facility over a six- month period
2. Describe the clinical presentation of CM by ART status with respect to:
 - a. Review clinical features of patients presenting with CM: Clinical signs and symptoms, opening pressure, ART status: (suppressed on ART, on ART - not suppressed, defaulted ART, ART naïve), CD4 count, Viral load, haematological parameters,
 - b. Review microbiologic features of CSF among patients presenting with CM: method of diagnosis (India Ink vs other), co-infections in CSF, cells, biochemistry
3. Describe clinical course and management:
 - a. Length of stay, number and type of invasive procedures (REPEAT LP) conducted, other.
 - b. drug therapy, ART management, other therapies administered
 - c. Co-morbidities: Drug toxicity, other infections, non-infective conditions
 - d. Nature of supportive care offered.
4. Patient outcomes on discharge, i.e.

- a. Proportion alive with no complications
 - b. Proportion of patients alive with minor complications
 - c. Proportion of patients alive with neurologic deficit
 - d. Proportion of patient that demised
5. Proportion of patients requiring step down in-patient care

Background and Literature review

Approximately 78 million people have been infected with the HIV virus and about 39 million people have died of HIV since the epidemic started. Worldwide, 36.7 million [34.0 million–39.8 million] people living with HIV in 2015. Sub-Saharan Africa is considered the most severely affected with HIV. Virtually 1 in every 20 adults living with HIV and globally represent approximately 71% of the people living with HIV. (1,2)

The proportion of South Africans infected with HIV has increased from 10.6% in 2008 to 12.2% in 2012. For 2015, an estimated 11,2% of the total population is HIV positive. The total number of persons living with HIV in South Africa increased from an estimated 4,02 million in 2002 to 6,19 million by 2015. (3,4)

The highest infection rates noted among females in early thirties and males in late thirties. Provincially, HIV prevalence is almost 40% KwaZulu-Natal compared with 18% in Northern Cape and Western Cape. As a result of the introduction of antiretroviral treatment, this has played a significant role in increasing life expectancy for patient living with HIV. (3,8)

The Human Immunodeficiency Virus (HIV) targets the immune system and weakens people's defence systems against infections and some types of cancer. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient. Immune function is typically measured by CD4 cell count.

Immunodeficiency results in increased susceptibility to a wide range of infections and diseases that people with healthy immune systems can fight off.

HIV symptoms vary depending on the stage of infection. Though people living with HIV tend to be most infectious in the first few months, many are unaware of their status until later stages. *Within 2 to 4 weeks after initial infection*, patients may be asymptomatic or an influenza-like illness. As the infection progressively weakens the immune system, other signs and symptoms may occur, such lymphadenopathy, weight loss, fever, diarrhoea and cough. Without treatment, they could also develop severe illnesses such as tuberculosis, cryptococcal meningitis, and cancers such as lymphomas and Kaposi's sarcoma.

The most advanced stage of HIV infection is Acquired Immunodeficiency Syndrome (AIDS), which can take from 2 to 15 years to develop depending on the individual. (8)

The neurological disease in HIV infection it might be related to primary effect of HIV infection on brain tissue or secondary to opportunistic infection or malignancies (5)

Opportunistic infections include: Cryptococcosis, Toxoplasmosis Progressive multifocal leukoencephalopathy, Cytomegalovirus, syphilis, Mycobacterium tuberculosis, HTLV-I infection, Amoebiasis (5)

Neoplasms are Primary CNS lymphoma, Kaposi's sarcoma, Aseptic meningitis, HIV-associated neurocognitive disorders - including HIV encephalopathy/AIDS dementia complex (5)

Cryptococcal meningitis (CM) is a common AIDS-defining illness and remains an important cause of morbidity and mortality among HIV-infected adults in South Africa (SA) (6,7).

Cryptococcus was isolated from the tibia of a 31-year-old woman with disseminated disease in 1894. The first described case of meningo-encephalitis was in 1905 by von Hansemann (7).

Cryptococcus meningitis affect immunocompromised individuals, patients who are HIV positive as well as patient how are HIV negative with malignancies, diabetes, steroid therapy, solid-organ transplant, and chronic medical diseases such as renal and liver failure are also at risk of developing Cryptococcus meningitis. After introduction of antiretroviral therapy there is decline in incident cryptococcal infections in the developed world, though the prevalence among other immunocompromised individuals has remained stable. (7)

Cryptococcus yeasts are everywhere in the environment and acquired by inhalation. Primary infection is likely acquired in childhood by inhalation in a large proportion of individuals, although adult acquisition is also well documented. An effective cell-mediated immune response is required to contain the disease and resulting in clearance or establishment of a contained latent infection.

In patients with AIDS, there is defect in their immune response to contain cryptococcal infection due to low CD4 T cell count (7).

The clinical presentation of cryptococcal disease is highly variable and can result in asymptomatic disease, localized pulmonary disease or disseminated disease.

Disseminated disease can occur in any organ there is a predilection for infection of the central nervous system (CNS), resulting in meningoencephalitis and occasionally causing focal intracerebral granulomas known as Cryptococcomas. Patients with meningoencephalitis typically present with a severe headache, which may be present for several weeks to months, and it might be associated with changes in the mental status or changes in the personality also it may present with fever, lethargy, and coma. Cryptococcus meningitis may be complicated by onset of hydrocephalus (communicating and non-communicating), papilledema that may lead to blindness, sudden onset of sensorineural deafness, cranial nerve palsies, motor and sensory deficits, cerebellar dysfunction, and seizures (7).

To reduce disability and deaths associated with HIV infection, screening and pre-emptive antifungal treatment of cryptococcal disease has been suggested for routine implementation as a part of the South African National Strategic Plan for HIV, STIs and TB, 2012 - 2016.

According to the guidelines patient who are HIV positive and CD4 Count less than 100, not on ARVs, and no previous CM should be screened for Cryptococcal antigenemia. If cryptococcal antigen is positive to contact the patient for urgent follow up and screen for symptoms and signs of meningitis. If symptomatic start daily 1200 mg fluconazole and refer immediately for lumbar puncture .If LP positive treat as CM amphotericin B and fluconazole 800 mg daily dose . Close monitor of renal function and electrolyte is important, then continue with 400 mg fluconazole daily dose for 2 months, followed by 200 mg daily for minimum 1 year. Daily fluconazole is discontinued, when patient has 2 CD4 counts of >200 cells/mm³ taken at least 6 months apart. In patient who are asymptomatic start fluconazole 800 mg daily for 2 weeks as out-patient and continue 200 mg fluconazole daily for minimum 1 year and discontinue when patient had 2 CD4 counts of >200 cells/ mm³ taken at least 6 months apart. LP may be considered if available. (4)

Despite amphotericin B-based therapy, in hospital case-fatality ratio (CFR) remain high. Advanced HIV disease at diagnosis (CD4 count <200 cells/ mm³) high cerebrospinal fluid (CSF) fungal burden, sub-optimal management of cryptococcal meningitis (CM), delayed initiation of antiretroviral treatment (ART) and the presence of co-morbid conditions such as tuberculosis all contribute to the high case-fatality ratio (CFR) (11).

In South Africa 2015, number of 6174 of cases (KwaZulu-Natal 1745 case) presented with symptomatic disease were diagnosed with Cryptococcus meningitis (cerebrospinal fluid positive for Cryptococcus species); 4% were diagnosed with fungaemia (12). A total of 4,295 cases of cryptococcal antigenaemia (with no concurrent clinical or laboratory evidence of cryptococcal meningitis or fungaemia) were detected. (11)

Because neurological disease affecting patient with HIV has significant mortality and morbidity, treatment and prophylaxis against this conditions is mainly by control of HIV infection by using ART (8) , and it is important to advise patients regarding the adherence to antiretroviral therapy and explain clearly the risk of defaulting treatment and its implication on the effectiveness of treatment, and risk of intercurrent AIDS related opportunistic infections and drug resistance.

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- 1.Global Health Observatory (GHO) data ,2016 update.
- 2.WHO/HIV –AIDS ,November 2015 update
- 3.SA has highest number of new HIV infections worldwide –survey/01APR2014
- 4.Guideline for the prevention, diagnosis and management of cryptococcal meningitis among HIV-infected persons: 2013 update by the Southern African HIV Clinicians Society
- 5.Harrison's Principles of Internal Medicine, 19e
- 6.Management of cryptococcal meningitis in a district hospital in KwaZulu-Natal: a clinical audit, Afr J Prim Health Care Fam Med,2014
7. New approaches to the diagnosis and treatment of cryptococcal meningitis, Makadzange, A. T.McHugh, G. ,Semin Neurol,2014
- 8.Progressive multifocal leukoencephalopathy in an AIDS patient, J Formos Med Assoc, 2007(PMC)
9. Mid-year population estimates 23, July,2015.
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12. GERMS-SA Annual Report 2015

keywords used in this research and MeSh terms

Cryptococcus meningitis

Human immunodeficiency virus

AIDS

NEURO

Manifestation

Hypothesis to be tested, or Research Question to be answered:

Cryptococcal meningitis occurs in patients on ART, with adverse outcomes

Study design

Cross sectional study, retrospective chart review

Study location

Adult medical wards, tertiary facility hospital (King Edward Hospital VIII), Durban, South Africa

Study duration

Period of 6 months June 2016-to December 2016

Study strategy

Medical records of all patients who were admitted to medical wards in King Edward Hospital VIII during period June 2016 to the end of December 2016 will be assessed at AMA, and records of admissions to individual medical wards. Charts of all patients admitted for cryptococcal meningitis will be drawn for archives and undergo a thorough chart review of medical records.

Review NHL data base for all LP results done to the patients admitted to all medical wards and AMA in King Edward Hospital VIII during study period (First of June 2016 to the end of December 2016).

The inclusion criteria

Adult patient presented with cryptococcal meningitis during the study period

The exclusion criteria

Patient age <13

HIV patient presented with diagnosis other than meningitis.

Data collection methods and tools

Data will be collected by using existing records (medical record review) patients chart who admitted to adult medical wards at King Edward Hospital VIII during period between first of June 2016 till the end of December 2016. Review of medical records that already exist, and by using data collection sheet that contain the demographic profile of the patient, race, symptoms and signs that patient presented with, provisional diagnosis on admission, blood investigation, lumbar puncture results, opening pressure if was measured, and if not measured Why?, CD4 count, viral load, radiological studies, final diagnosis, and the outcome upon completing treatment and discharge.

Review NHL data base for all LP results done during study period (during period between first of June 2016 till the end of December 2016) to help understand the prevalence of meningitis.

Statistical analysis methods

Descriptive statistics will be used to determine means, medians (interquartile range) for continuous variable and frequency counts and percentages for categorical variables. Univariate and bivariate analysis will be performed to describe the prevalence of patients presented with Cryptococcal infection in ART era.

Limitation of the study

Relies on accuracy of written record or recall of individuals Important data may not be available.

Ethical consideration

This study involves a review of the existing records there is no patient participation involved in the process

This is a minimum risk, non-invasive study. therefore, there are minimal ethical issues involved in the research process in this case.

APPENDIX 2: INSTRUCTIONS TO THE AUTHOR FOR THE JOURNAL SELECTED FOR SUBMISSION

Manuscript preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this are Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

- An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.
- Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.
- Mask self-citations by referring to your own work in third person.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, **full** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and °C), e.g. '40 kg' and '20 cm' but '50%' and '19°C'.
- Please be sure to insert proper symbols e.g. μ not u for micro, α not a for alpha, β not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.

- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

****NB:** Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. *J Genet Counsel* 2008; 17:424-433: standard human pedigree nomenclature.

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:

- **Background:** why the study is being done and how it relates to other published work.
- **Objectives:** what the study intends to find out
- **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
- **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
- **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc) that may have an impact on the study results. Clearly define how participants were enrolled and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the \pm symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

CME (by invite only)

CME is intended to provide readers with practical, up-to-date information on medical and related matters. It is aimed at those who are not specialists in the field.

From January 2016, all CME articles will be printed in full in the *SAMJ*. Please try to adhere strictly to the guidelines on word count as we have a page limit for the print issue of the *SAMJ*. We reserve the right to place some tables and reference lists online if this is necessary for space.

In practice, this means that each CME topic usually covers two issues of the print issue of the *SAMJ*.

The guest editor, in consultation with the editor, is responsible for convening a team of authors, deciding on the subjects to be covered and for reviewing the manuscripts submitted. The suggestion is for 4 - 5 articles, although there is some room for flexibility contingent on discussions with the editor.

For queries about these guidelines please feel free to contact the CME editor, Dr Bridget Farham, by email (ugqirha@iafrica.com) or telephone (+27 (0)21 789 2331).

Review process

The guest editor reviews the articles and returns them to the CME editor for review and final approval.

Guest editorials

Guideline word limit: 1 000 words

- Include the guest editor's personal details (qualifications, positions, affiliation, e-mail address, and a short personal profile (50words)).
- If possible, include a photograph of the author(s) at high enough resolution for print. It is preferable to provide two guest editorials, one for each issue, so that the content of the articles in each issue is covered.

Articles

Guideline word limit: 2 000 - 3 000 words

- Each article requires an abstract of ± 200 words.
- The editor reserves the right to shorten articles but will send a substantially shortened article back for author approval.

Personal details

Please supply: Your qualifications, position and affiliations and MP number (used for CPD points); Address, telephone number and fax number, and your e-mail address; and a short personal profile (50words) and a few words about your current fields of interest.

In Practice

Guideline word limit: 2 000 - 3 000words

This section includes articles that would previously have been accepted into the Forum section, and case reports.

In practice articles are those that draw attention to specific issues of clinical, economic or political interest regarding medicine and healthcare in southern Africa. They are assigned to a topic:

- Case report
- Clinical practice
- Clinical alert
- Issues in medicine
- Issues in public health
- Healthcare delivery
- Consensus/Position statement
- Medicine and the environment
- Medicine and the law

- Cochrane corner

An In Practice article should follow the following format – sub-headings are not necessary, but may be used for clarity:

- Author affiliations and qualifications: to be the same as for Research. Provide all authors' names and initials, qualifications and full affiliations, and corresponding author.
- Short abstract: does not need to be structured, but should capture the essential features of the article
- Introduction: the reason for the article and the issue being addressed
- Recent research, discussion, local policy around the issue – include your own research where appropriate
- All statements should be referenced and, if opinion only, this should be stated
- Discussion: how this article adds to the discussion around a particular topic
- If a clinical practice or policy point is at issue, this needs to be emphasised, using a box with highlights if appropriate.

Essentially In practice is an opportunity for a more discursive approach to topics of clinical, economic or political importance in southern African health systems. It is not an opportunity to put forward unsubstantiated opinions!

Case reports

The *SAMJ* has recently started to accept case reports. The cases must come from Africa, preferably southern Africa unless the condition is common to all African countries and must be either a completely new description of a clinical condition or result (use Google!) or a case that highlights important practice or management issues.

Please use the following format for case reports:

- Title of case: do not include the words 'a case report' in the title
- Summary/abstract: up to 150 words summarising the case presentation and outcome
- Background: why is this case important and why did you write it up?
- Case presentation: presenting features, medical, social, family history as appropriate
- Case management: should be according to best practice, and if not, please explain why
- Investigations, if relevant: save space by simply saying 'normal' if, for example, renal function was completely normal, rather than listing normal results, highlight the abnormal – or indeed the normal if this is clinically significant
- Differential diagnosis, if relevant
- Treatment, if relevant
- Outcome and follow-up
- Discussion – a VERY BRIEF review of similar published cases
- Teaching points: 3 - 5 bullet points
- References: as per the *SAMJ* house style
- Tables and figures: keep to a minimum. Use clinical images where relevant – we need hi-res versions for print, and identifiable persons must have a consent form
- Patient consent: please include a statement about patient consent to a written case report. This should be uploaded as a supplementary file.

Clinical trials

Guideline word limit: 4000 words

As per the recommendations published by the International Committee of Medical Journal Editors (ICMJE), clinical trial research is any research that assigns individuals to an intervention, with or without a concurrent comparison/control group to study the cause-and-effect relationship between the intervention and health outcomes. All clinical trials should be registered with the appropriate national clinical trial registry (or any international primary register, if relevant), and the trial registration number should be cited at the end of the abstract. Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the [South African National Clinical Trials Register](#). The SAMJ therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Please refer to the general guidelines for all papers at the top of this article for additional requirements with respect to ethics approval, funding, author contributions, etc. The format of original research articles should be followed for reporting of clinical trial results.

Review articles

Guideline word limit: 4 000 words

These are welcome but should be either commissioned or discussed with the Editor before submission. A review article should provide a clear, up-to-date account of the topic and be aimed at non-specialist hospital doctors and general practitioners.

Please ensure that your article includes:

- Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
- Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
- When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you have to, say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations and provide advice specific to southern Africa.
- Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Correspondence (Letters to the Editor)

Guideline word limit: 500 words

Letters to the editor should relate either to a paper or article published by the SAMJ or to a topical issue of particular relevance to the journal's readership

- May include only one illustration or table
- Must include a correspondence address.

Book reviews

Guideline word limit: 400 words

Should be about 400 words and must be accompanied by the publication details of the book. Provide a hi-res image of the cover if possible (with permission from the copyright holder).

Obituaries

Guideline word limit: 400 words

Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Guidelines

Guidelines should always be discussed with the Editor prior to submission.

Because of the intensive review process required to ensure Guidelines are independent, evidence-based and free from commercial bias, they are usually published as a supplement to the *SAMJ*, the costs of which must be covered by sponsorship, advertising or payment by the guideline authors/association. We will provide a quote based on the expected length of the guideline and whether it is to appear online only, or in print, which must be accepted by the body putting the guidelines together before submitting the work to the *SAMJ*.

The Editor reserves the right to determine the scheduling of supplements. Understandably, a delay in publication must be anticipated dependent upon editorial workflow.

All guidelines should include a clear, transparent statement about all sources of funding and an explicit, clear statement of conflicts of interest of any of the participants in the guidelines about industry funding for lectures, research, conference participation etc.

All guidelines should be structured according to [Agree II](#).

Please access this website before putting the guidelines together, download the Agree 11 instrument and use this to put the guidelines together.

All submitted guidelines will be sent to the local Agree II appraisal committee for review and must be endorsed by an appropriate body prior to consideration and all conflicts of interest expressed.

A structured abstract not exceeding 400 words (recommended sub-headings: *Background, Recommendations, Conclusion*) is required. Sections and sub-sections must be numbered consecutively (e.g. 1. Introduction; 1.1 Definitions; 2.etc.) and summarised in a Table of Contents.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'. Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain)*. –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for n and %:

Rather:

Combine into one column, n (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must **not** be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,^[2] and others.^[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the [List of Journals in Index Medicus](#).
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.

- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI link). Authors are encouraged to use the DOI lookup service offered by [CrossRef](#):
 - On the Crossref homepage, paste the article title into the 'Metadata search' box.
 - Look for the correct, matching article in the list of results.
 - Click Actions > Cite
 - Alongside 'url =' copy the URL between { }.
 - Provide as follows, e.g.: <https://doi.org/10.7196/07294.937.98x>

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. *Stat Med* 1998;289(1):350-355. <http://dx.doi.org/10.1000/hgjr.182>
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. <http://www.who.int/whr/2002> (accessed 16 January 2010).
- Legal references

- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) would indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format: Author(s). Title. Publisher place: Publisher name, year; pages.*
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

APPENDIX 3: ETHICAL APPROVAL LETTERS