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Recurrent cryptococcal meningitis at Chris Hani Baragwanath academic hospital, Soweto, South Africa

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Recurrent Cryptococcal Meningitis at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.

Dineo Maphanga

A research report submitted to the University of the Witwatersrand, Johannesburg in partial fulfilment for the requirements of the degree of Master of Medicine 2017.

DECLARATION

I, Dineo Maphanga, declare that this research report is my own work, which is being submitted for the degree Master of Medicine in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature:

D.M. Maphanga

14th day of June 2017

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God, You constantly amaze me.

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Thank you to Dr. Nelesh Govender for the assistance particularly at the beginning of the project and for allowing me to use of the GERMS-SA database.

PUBLICATIONS AND PRESENTATIONS

None.

ABSTRACT

Background

Cryptococcal meningitis (CM) remains an ongoing and devastating disease with detrimental outcomes accounting for up to 44% of acquired immune deficiency syndrome (AIDS) related mortality. A significant portion of the cases have been attributed to recurrences, thought to be largely preventable. In the first world the recurrence rate is estimated at <5% but it is more than double that rate in developing countries. In the era of freely available antiretroviral therapy (ART) and secondary prophylaxis with fluconazole, we endeavoured to study the prevalence along with the clinical and laboratory features of patients with recurrence of CM in our setting.

Objectives

1. Determine the prevalence of recurrent CM at Chris Hani Baragwanath Academic Hospital (CHBAH) for patients with the incident presentation between the years 2012-2013.
2. Determine the use of ART and fluconazole in patients with recurrent CM.

The patients were divided into 4 groups reliant on whether they were on ART and fluconazole at the time of diagnosis of recurrent CM.

a. Patients on ART and fluconazole.
b. Patients on ART alone.
c. Patients on fluconazole alone.
d. Patients on no therapy.

3. Describe the clinical and laboratory features of patients with recurrent CM, as a cohort and by group. The groups were comparatively analysed. The variables studied included clinical presentation, mortality and cerebrospinal fluid (CSF) findings.

Methods

This was a retrospective review of adult patients presenting to Chris Hani Baragwanath Academic Hospital (CHBAH) with recurrent CM. The patients were identified using the Group for Enteric, Respiratory, and Meningeal disease Surveillance for South Africa (GERMS-SA) database. This is a nationwide network of clinical microbiology laboratories (both in the public and private sector) participating in an active laboratory-based surveillance programme for bacterial and fungal pathogens of public health importance. Specimen of patients identified to have CM are submitted to the National Institute of Communicable Diseases for confirmation and further characterisation. CHBAH is one of 25 enhanced surveillance sites where additional data including demographics, clinical findings and laboratory results were recorded. Hospital records and laboratory results were used to supplement the data. Patients with recurrent CM were identified between February 2012 and April 2014. The incident episode of CM had to have been from January 2012 to December 2013. The number of incident cases in that period was the denominator for the rate of recurrence.

Results

A total of 51 patients were identified from the database from an incident cohort of 658 patients with CM giving a prevalence rate for recurrent CM of 7.8%. These 51 patients had a total of 62 recurrent episodes of CM. Eight (15.7%) patients had multiple recurrences. There were 30 (58.8%) males and the median CD4 count was 85/mm^3 (IQR: 3-393/mm^3). The median time to recurrence was 143 days (IQR: 32-633 days).

Data on the use of ART and fluconazole was available for 56 (90.3%) of recurrent episodes in 45 patients. A total of 37 (66.1%) recurrent episodes were in patients on ART and 26 (46.4%) were on fluconazole. The 56 episodes of recurrent CM were grouped as follows:

1. A total of 20 (35.7%) recurrent episodes were in patients on both ART and fluconazole. Immune reconstitution syndrome (IRIS) contributed 14 cases.
2. A total of 17 (30.4%) of recurrent episodes were in patients only on ART.
3. A total of 6 (10.7%) of the recurrent episodes were in patients on fluconazole prophylaxis only.
4. A total of 13 (23.2%) of the recurrent episodes were in patients on no therapy.

The patients presented clinically with headaches (76.8%), meningism (57.1%), a Glasgow Coma Scale (GCS) <15 (30.4%) and seizures (14.3%). Twenty-seven (48.2%) cases died in hospital. Mortality was significantly higher in those with a GCS of less than 15 (82% vs. 33%, P=0.0008) and those with seizures (86% vs. 42%, P=0.0197).

No statistically significant differences were noted amongst the 4 groups with respect to the clinical presentation, cerebrospinal fluid profile, the time to recurrence and mortality.

Conclusion

The prevalence of recurrent CM was midway between that of the developed world and a pre-ART study in Gauteng. Recurrent CM had a high mortality. The finding that one-third of patients were not on ART and that more than half were not on fluconazole at the time of diagnosis of recurrent CM, together with the high rate of multiple recurrence requires further investigation. Explicit steps need to be taken to link patients with health care facilities to ensure reliable provision of fluconazole and the initiation of ART.

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ABREVIATIONS

AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
CD4	CD4+ T cell
CFU	Colony forming units
CM	Cryptococcal meningitis
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
FLUC	fluconazole
CRAG	Cryptococcal antigen
GERMS:SA	Group for Enteric, Respiratory and Meningeal disease Surveillance for South Africa
HIV	Human immunodeficiency virus
IRIS	Immune reconstitution inflammatory syndrome
II	India ink
L	Lymphocytes
MIC	Minimum inhibitory concentration
N	Neutrophils
TBM	Tuberculous meningitis
WHO	World Health Organization

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Cryptococcal meningitis (CM) is a common opportunistic infection in immune-compromised patients particularly those with human immunodeficiency virus infection/acquired immune deficiency syndrome [HIV/AIDS]. It remains amongst the leading causes of mortality in HIV particularly in Southern Africa despite the advent of antiretroviral therapy (ART). It accounts for up to 13%-44% of HIV associated mortalities [1]. The estimated current global burden of CM amongst individuals with HIV/AIDS ranges from 0.04%-12% per year, highest in developing countries [2]. There are about 500 000 deaths attributed to CM each year in sub-Saharan Africa with a case fatality of between 35%-65% as compared to 10%-20 % in developed countries [3].

1.2 Background

Cryptococcus neoformans is the causative organism, an encapsulated fungus, ubiquitous and largely benign in health. In immunocompromised states such as AIDS, inhalation leads to haematological spread and dissemination to multiple organs with particular predilection for the central nervous system. The initial primary pulmonary infection is often asymptomatic and manifestations of the disease depend on the host immune response. The organism is either eliminated or contained and in vulnerable individuals may present with asymptomatic cryptococcal antigenemia or invasive symptomatic mycoses manifesting as sub-acute or chronic meningitis, less commonly

as space-occupying intracranial lesions and at other foci including the liver, lungs and skin.

1.3 Diagnosis

The diagnosis of CM is on the basis of cerebrospinal fluid (CSF) analysis. The organism may be identified by india ink stain in 74%-88% of cases. Fungal culture is the gold standard with theoretically 100% cases of CM being culture positive. CSF cryptococcal antigen detection by latex agglutination is rapid and reliable in the diagnosis of CM, being positive in 99% of CM; it offers 93%-98% diagnostic sensitivity and specificity in the region of 93%-99% [4].

1.4 Treatment

The World Health Organization (WHO) recommends induction therapy with amphotericin B deoxycholate at 0.7-1mg per kilogram of body weight per day for 14 days in conjunction with flucytosine at 100mg/kg where available, followed by consolidation with fluconazole at doses of 400-800 mg for 8 weeks then subsequently at 200 mg daily as secondary prophylaxis until the CD4+ T-cell (CD4) count is maintained above 200 cells/mm^3 for a period of more than 6 months [3].

1.5. Recurring crypotococcal meningitis

1.5.1 Disease burden in our environment

Cryptococcal meningitis still poses a major challenge especially in the developing world despite the availability of both subsidized fluconazole by Pfizer since 2000 and freely available ART [5]. Along with other variables that will be discussed, socio economic difficulties, modest access to equipped health facilities and difficulties with patients presenting late with advanced cryptococcal disease still pose a challenge in curbing the disease and its complications.

In the population of Soweto, a study conducted in 1996 examining the spectrum of meningitis encountered over a year showed CM to account for 13% of all cases, with the leading cause being tuberculous meningitis (TBM): 25.4% followed by acute bacterial meningitis: 22.5%, viral meningitis: 14.1% with the trend expected to increase particularly for TBM and CM given the increasing rate of HIV at the time (6)

In 2009, in sub-Saharan Africa there were an estimated 720000 cases of CM yearly with cryptococcosis contributing to 504 000 deaths compared to 347 871 due to tuberculosis [2].

Screening for serum cryptococcal antigen (CRAG) in patients prior to ART with a median CD4 of 97/mm^3 (IQR 46-157cells/ mm^3) has shed light into the prevalence of asymptomatic cryptococcal antigenemia, thus allowing for great strides to be taken in the decrease of CM and associated mortality.

The incidence of a positive serum CRAG which was 100% sensitive for predicting developing CM within the first year was found to be 7 percent [7].

Recurring CM still constitutes a sizeable quantity of the cases of CM to date, remaining fairly common even with more efforts directed at HIV/AIDS; in the developed world contributing approximately 5% of the cases seen [8]. In Gauteng, recurrences of CM were recognized to have an estimated incidence of 9.5% between 2002-2004 in a population-based surveillance in ART naïve patients [9].

Multiple variables have been described which may contribute to relapses. In our resource-limited environment where flucytosine is unavailable, amphotericin B is primarily used either alone or in combination with fluconazole in the induction phase, perhaps contributing to a number of relapses thought to be due to persistence of the infection from inadequate fungal CSF clearance. Multiple cohort studies have repeatedly shown that combination therapy with amphotericin B and flucytosine in the induction period increases CSF sterilization and significantly reduces relapses and mortality [10]. A recent randomized, three group open label trial comparing induction therapy with amphotericin B alone, in combination with flucytosine or with fluconazole in 299 patients showed significant reduction in mortality in the group who received flucytosine in addition to amphotericin B, (with 15 vs. 25 deaths at day 14 (P: 0.08) and 30 vs. 44 deaths at 70 days (P: 0.04).

There was no superiority in sterilizing the CSF, nor a survival benefit demonstrated in the group that received dual fluconazole and amphotericin B compared to the group on amphotericin B monotherapy [10].

Poor compliance or suboptimal drug dosing with fluconazole have also been shown to contribute significantly to relapses. A prospective study conducted at GF Jooste hospital in Cape Town (a public sector hospital which has been a site for numerous studies in CM) showed that as many as 43% of the 69 cases of CM relapses were due to inadequate secondary prophylaxis i.e. patients not taking fluconazole for various reasons [11].

There are concerns of emerging and growing resistance towards fluconazole, with 76 percent of the cases of culture positive CM being associated with isolates showing reduced susceptibility to fluconazole in a study from Cape Town [1]. This high rate of resistance has not been replicated in other studies.

Decreased efficacy of fluconazole with concurrent use of rifampicin has been proposed; It is thought that rifampicin may decrease the serological concentration of fluconazole and hence the efficacy when taken concurrently.

Immune reconstitution inflammatory syndrome (IRIS) is a well-recognised and perhaps a very common complication of ART initiation and accounts for a significant number of cases of CM recurrences.

1.5.2. Immune reconstitution inflammatory syndrome (IRIS)

IRIS has in recent years become a topic of much interest and research. The entity is grouped into unmasking [CM] and paradoxical IRIS [12]. The definitions used in this article are based on criteria published elsewhere. See Annexure C

Concisely, unmasking IRIS encompasses a new recognition of cryptococcal disease occurring after the introduction of effective ART. Paradoxical IRIS is worsening of a recognized disease, which previously showed symptomatic improvement. Evidence of immune reconstitution (most reliably a decline in viral load) is important in the diagnosis and equally so, the exclusion of other conditions and infections which may mimic the process. Refer to Annexure C for detailed proposed case definitions.

The CSF will mostly be culture negative if occurring after 3 months, with a decrease in the cryptococcal titer, the authors quoted have defined a significant decline to be 4 fold from the initial cryptococcal antigen titer [12].

Cryptococcal IRIS is fairly common and has been described in as many as 8-50 percent of individuals initiating ART [13]. The pathophysiology of IRIS continues to elude researchers, particularly due the heterogeneity of the patients with CM developing IRIS after commencing ART. IRIS is thought to occur from defective antigen clearance by the immune system from the preceding episode of CM, a high CRAG at initiation of ART as well as during the episode being both risk factors. When recovery of pathogen specific T cell immunity ensues, an aberrant inflammatory response occurs, demonstrated by high-designated T helper 1 type cytokines.

Other theories such as a dysregulation in immunity between the effector and suppressor cells have been disproven [14].

In an American study conducted in Texas examining CM IRIS in an HIV seropositive population, the incidence was 30% in the cohort of 84 patients. The patients at risk for IRIS were ART naïve with a high CSF CRAG supporting the hypothesis mentioned earlier [15].

Work conducted in Uganda examining the mortality of CM before ART and after the ART era found the figures to be comparable primarily due to the development of IRIS in the latter group. In the study IRIS was reported in 7 of the 24 patients after commencing ART, with 5 patients presenting with aseptic meningitis [16].

Another prospective study conducted in South Africa with 65 patients initiating ART revealed 17% of the individuals developed CM IRIS at a median time of 29 days [17]. The reported time of onset of IRIS varies widely and occurs from as early as 4 days up to 3 years, the median time is 1 to 10 months with individuals bearing a CD4 of $50/mm^3$ or less being at a particularly increased risk. Other recognized risk factors include a high viral load prior to ART initiation and early initiation of ART i.e. commencement of ART earlier than 5 weeks after the episode of CM [12]. The risk of IRIS seems to increase with profound immune deficiency and appears to be lower if switched from a failing ART regimen.

A high fungal burden as evidenced by an elevated CRAG is also associated with IRIS. Other factors include a paucity of initial CSF inflammation, which also seems to have a strong association with IRIS [14].

The diagnosis of IRIS can be challenging. Shelburne et al. reported patients with IRIS to show higher mean opening pressures and white blood cell counts as opposed to individuals with HIV associated CM; of the 84 patients followed up over 1.48 years, 17 patients developed culture negative meningitis (3 unmasking and 14 paradoxical IRIS). [15] They also found the risk factors for developing IRIS to be a higher viral load pre-ART initiation along with a higher CSF CRAG (1:4096 vs 1:1024 p=0.020) and early ART commencement at less than 30 days (RR 1.73; CI 1.03-2.29; P=0.031). Patients who developed IRIS presented earlier as opposed to those presenting with relapse of CM (59 days vs 165 days; P=0.29) with higher intracranial pressures documented in the IRIS group (45 vs 31cm H20 P=0.38). [15]

There are no conclusive tests for a definitive diagnosis of IRIS, and the diagnosis is generally made once relapses due to therapy failure and other causes are excluded. The factors that favour the diagnosis include evidence of CSF inflammation with a higher CSF white cell count, decreasing CSF CRAG and a decrease in the HIV viral load. The test shown to be more sensitive is a negative CSF culture; this may delay the diagnosis as the turnaround time for the result can be weeks with a low CSF fungal load [18].

1.5.3. Inadequate secondary prophylaxis

There is a rising regularity of poor adherence to secondary prophylaxis. Some of the encountered challenges are poor patient education, socioeconomic difficulties leading to patients being lost to follow up and failure to be retained within facilities that will ensure continued adherence. Sub-therapeutic doses of fluconazole may contribute to relapse of disease especially important in the maintenance/suppressive phase of therapy.

A prospective observational study referenced previously at GF Jooste Hospital in Cape Town indicated the commonest cause for relapses to be patients not taking their fluconazole prophylaxis, this number constituting 43% of the total number in the study. The in-hospital mortality was also high in this group (33%). They found that 47% of the individuals did not have fluconazole prescribed by health care providers [11].

1.5.4. Antifungal resistance

There are fears regarding the emergence of resistance to current antifungal therapy Bicanic et al. suggested that there may be an emergence of resistant isolates of *cryptococcus neoformans* [1]. This is plausible concern especially given the free use of fluconazole for CM as well as other fungal infections.

Pfaller et al. at the University of Iowa conducted a surveillance of cryptococcal isolates from 5 different global sites including Africa/ Europe/Latin America /Pacific and North America, in the surveillance conducted between the years 1990-2004, a total of 1811 clinical isolates of *cryptococcus neoformans* were tested against commonly used antifungals including amphotericin B, fluconazole and flucytosine. 98-100% of isolates were susceptible to amphotericin B irrespective of geographic region with minimal inhibitory concentrations (MIC) less or equal to 1mcg/ml. Isolates from North America were 75% susceptible to fluconazole (MIC< or equal to 8mg/ml) as compared to the rest of the regions where 94-100 % showed susceptibility. Susceptibility to flucytosine ranged from 35% to 68% in North America and Latin America respectively. Five sites from Africa were involved in the study, 395 isolates contributed to the total number mentioned. There was 99% susceptibility to amphotericin B at an MIC of 1mcg/ml. 97 % of isolates were susceptible at an MIC of 8mcg/ml for both fluconazole and flucytosine [19].

1.5.5 Drug interactions

Concomitant therapy that the patient may be on may have significant drug-drug / pharmacokinetic interactions, which could considerably lower drug concentrations for fluconazole particularly in the CSF. This may have significant implications for further recurrences. Rifampicin is a potent inducer of the hepatic cytochrome p450 oxidative enzyme, and a number of drugs including fluconazole are affected. A Thai study revealed that co-administration of rifampicin with fluconazole caused significant changes in the pharmacokinetic parameters of fluconazole. They demonstrated a 39% increase in the elimination rate constant, a 28% shorter elimination half time, 17 % decrease in the maximum concentration and 30% increase in clearance of fluconazole ($p<0.05$). [20]

This is in line with a paper by Coker et al (1990) who reported clinical relapse in 3 case studies in patients on concurrent fluconazole and rifampicin, [21] with another study revealing fluconazole to be less effective in decreasing candida colony counts in 4 individual on anti-tuberculosis therapy [22]. This raises questions about the possible need to adjust the fluconazole dose for patients receiving tuberculosis therapy.

Jarutanasirikul et al. conversely reported no clinically significant effect of fluconazole on rifampicin; they postulated that this might be due to a low inhibitory affinity of fluconazole to mammalian cytochrome p450 systems and the metabolic pathways of rifampicin not being primarily independent on the activity of cytochrome p450 [23].

1.5.6. Suboptimal therapy at initial episode of cryptococcal meningitis

The gold standard for the treatment of CM involves an intensive phase of high dose amphotericin B at 0.7mg/kg-1mg/kg daily along with flucytosine at 100mg/kg for a period of 2 weeks. This regimen has repeatedly been shown to be superior in sterilizing the CSF [3].

In a randomized 3 group trial, combination therapy with flucytosine and amphotericin B showed better organism clearance from the CSF (-0.42 log10 colony forming units (CFU) per milliliter per day vs 0.31 and 0.32 log10 CFU per milliliter per day in the groups receiving monotherapy with amphotericin B and amphotericin B with fluconazole respectively($P<0.001$). [10]Further support from work by Van Der Horst et al. showed that combination therapy with flucytosine and amphotericin B was associated with marked CSF sterility [24], along with a study by Saag et al. demonstrating that the best predictor of relapse was the lack of flucytosine in the initial 2 weeks of therapy [25]. There appears to be synergy between amphotericin B and flucytosine therapy supporting the data that it is the most fungicidal regimen available [26].

In a study comparing the fungicidal activity of various antifungal drug combinations, 64 patients presenting with an index episode of CM were randomly assigned to groups. They were randomized to receive the following treatment: amphotericin B; amphotericin B with fluconazole; amphotericin B with flucytosine and amphotericin

B/fluconazole/ flucytosine triple therapy, with the endpoint being the reduction in CFU at day 3,7,14 of treatment. Clearance was faster in the amphotericin B and flucytosine group than in the amphotericin B (p:0.0006) , amphotericin B plus fluconazole (p: 0.02) or triple therapy group (p:0.02). [27]

In 1993, Spitzer et al. proposed inadequate clearance of the initial CM to be the major factor in recurrent disease. Pulsed field electrophoresis of intact chromosomes and southern blot hybridization with two genomic DNA probes were utilized to investigate the genetic relation between the initial case of CM and the subsequent episode. Four patients were studied and the strains were differentiated using electrophoretic karyotyping using three independent methods. Results showed the initial and recurrent isolates of *cryptococcus neoformans* to be clonally related and thus corroborated the notion of recurrence of the initial strain in recurrent disease, This data suggests that inadequate *cryptococcus neoformans* clearance may be a contributor to relapses [28].

Other contributions may be the use of fluconazole monotherapy. Amphotericin B deoxycholate is a drug with a significant side effect profile; It is not uncommon due to drug related toxicities for therapy to be interrupted and at times completely stopped leaving only fluconazole, a fungistatic agent as the backbone for therapy.

The benefit of a repeat lumbar puncture at the end of therapy to ensure CSF sterility is uncertain and is not standard practice in most institutions. Whether ensuring CSF sterility at discharge may decrease relapses in the future is unclear.

1.5.7 Immunodeficiency

Ongoing profound immunodeficiency translating to impaired CSF immunity plays a major role in disease eradication and recurrences. It is not unusual for patients to be lost to follow up, often presenting with persistent immunodeficient states with recurring disease. In a study conducted in Cape Town at GF Jooste hospital looking at relapses of CM, 8% of the 69 patients studied relapsed pre-ART while on fluconazole [1].

Increased fungal burden at the time of presentation associated with delayed presentations to health care facilities is an important consideration, and may predispose to future relapses from suboptimal fungal clearance. There are studies that also suggest a link between the initial high CRAG titers and immunodeficiency to the subsequent development of IRIS [11].

Ample work has taken place in trying to determine the optimal time for ART initiation after an incident of CM, most studies seem to concur with delayed ART initiation in improving survival and the incidence of IRIS.

Commencement of ART at 5 weeks or later from the date of the initial CM episode is recommended as earlier initiation is associated with significant mortality. There is a mortality risk of 45% vs 30% in the group where ART was commenced before 2 weeks. The same study showed no statistically significant benefits in the secondary outcome (i.e. IRIS and relapses) occurring in similar numbers in both groups: 8/88 in the group with ART at 5 weeks and 2/89 in the group with ART at 1-2 weeks (p: 0.06). [29]

2.0. Study objectives

1. Determine the prevalence of recurrent CM for the period under analysis at Chris Hani Baragwanath Academic Hospital [CHBAH].

2. Determine the profile of the patients who presented with recurrences of CM.

To aid in this, the patients are divided into 4 groups. The groups were based on the presence of ART and /or fluconazole therapy at the time of diagnosis.

The groups were as follows:

2.1. Patients on ART and fluconazole

This group included patients with IRIS.

2.2. Patients on ART alone

2.3. Patients on fluconazole alone

2.4. Patients on no ART or fluconazole/no therapy

3. To describe the clinical and laboratory features and outcome of patients with recurrent CM, as a cohort and by group. The variables compared included the time to relapse, the clinical presentation, CSF profiles and mortality.

Chapter 2: Materials and Methods

2.1. Methodology

This was a retrospective descriptive study of patients with recurrent CM.

Patients were identified using the Group for Enteric, Respiratory, and Meningeal disease surveillance for South Africa (GERMS-SA) database. A nationwide network of clinical microbiology laboratories (both in the public and private sector) participating in an active laboratory-based surveillance programme for bacterial and fungal pathogens of public health importance. Approximately 200 South African clinical laboratories participate in the surveillance programme. Clinical isolates and specimen are submitted to the National Institute for Communicable Diseases (NICD) for confirmation and further characterisation. CHBAH is one of 25 enhanced surveillance sites were additional data is recorded. The additional information is recorded on a case report form and entered onto the database. Hospital and laboratory records were used to supplement the data.

Patients with recurrent CM between February 2012 and April 2014 were identified. The incident episode of CM had to have been from January 2012 to December 2013. The number of incident cases in that period was the denominator for the rate of recurrence.

2.2. Site of study:

CHBAH, Soweto, South Africa is a 2700-bed public sector university hospital serving the population of Soweto.

2.3. Inclusion criteria

1. All adult patients (≥18 years of age) admitted to CHBAH medical wards with recurrent CM between February 2012 and April 2014 where another cause of the clinical presentation was excluded.

2. Patients needed to have documented evidence of previous (CSF positive) CM >30 days preceding the admission, the first episode occurring during the period of 01/01/2012-31/12/2013.

3. The denominator in calculating the prevalence was the total number of cases of incident CM at CHBAH between 01/01/2012-31/12/2013.

The patients were grouped as follows:

A. Patients on ART and fluconazole: this included patients with IRIS and relapse.

B. Patients on ART alone and not on fluconazole: these patients were considered to have a relapse.

C. Patients on fluconazole

D. Patients on no treatment

Please refer to annexure A: flow diagram on study objectives

2.4. Data Analysis

STATA 12.0 software program was used for data analysis with the aid of a statistician. Baseline characteristics for each group were summarized as means and standard deviations or medians with interquartile ranges for continuous data depending on normality of the data. For categorical data, proportions or percentages were used. Box plots and other graphical measures were utilized to describe the data, and tests for normality were performed. For categorical data, proportions between the groups were compared using the Chi squared test if the expected value was above 5, and Fisher exact if less than 5. Means and medians for the continuous variables between the groups were compared using the student T test and Wilcoxon sum rank respectively depending on normality. A P value of less than 0.05 was considered as significant for the analysis.

2.5. Ethics

Approval from GERMS-SA to use the database was obtained. Since there was patient anonymity, no consent from the patients was required. For the denominator, only the number of patients was obtained. For patients with recurrent CM, patients were assigned a study number and the patient names and folder numbers were kept separately on a master sheet to protect confidentiality. A letter from CHBAH for permission to perform the study was obtained. The proposed study was approved by the University of Witwatersrand Human Research Ethics Committee. See Annexure D

Chapter 3: Results

3.1. The prevalence of cryptococcal meningitis recurrence

There were 658 incident cases of CM between the 1st of January 2012 and the 31st of December 2013. There were 51 patients identified with recurrences of CM who had a total of 62 episodes of recurrence. The prevalence of CM recurrence in our setting was thus 51/658, calculated at 7.8%. Amongst the 51 patients, there were 43 patients with 1 recurrence (84.3%), and 8 patients with multiple recurrences (15.7%). (Amongst the 8 patients with multiple recurrences; 1 patient had 4 recurrences, 1 patient had 3 recurrences, and 6 patients had 2 recurrences).

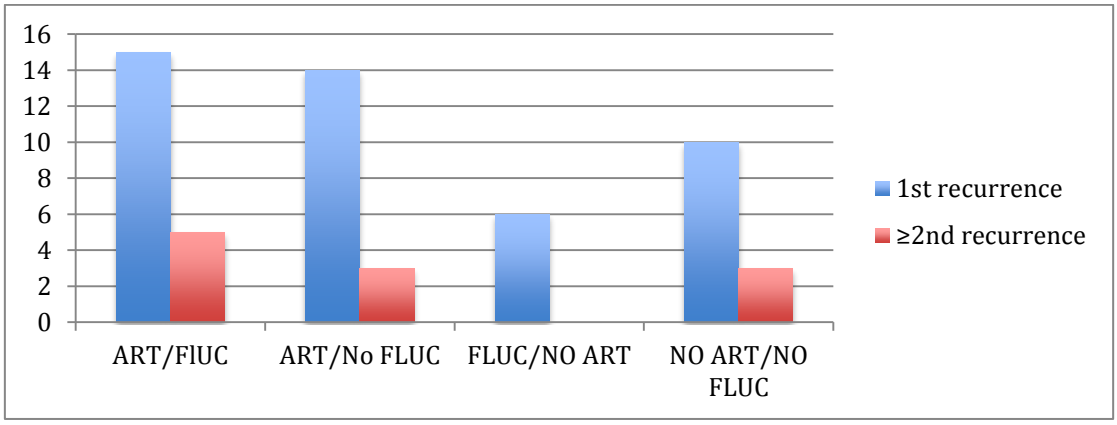
3.2. Profile of patients

All patients were HIV seropositive with AIDS by definition. Within the 51 patients, there were 30 (58.8%) males and 21 (41.2%) females. The mean age was 37.5 years (IQR: 24-54 years).

Taking into account that a number of patients had multiple recurrences, I have elected to describe the various episodes of recurrences and cluster these into the groups dependent on the use of ART and fluconazole.

In 51 patients with 62 episodes of recurrences, 51(82.3%) episodes was the first recurrence of CM and 11 (17.7%) episodes of recurrence were in patients who had had at least 1 previous recurrence of CM and are therefore placed in a category of ≥2nd recurrence of CM.

Figure 1: Profile of episodes of recurrent CM



FLUC: fluconazole

ART: Antiretroviral therapy

Data on the use of ART and fluconazole was available for 56 out of the 62 episodes of recurrent CM in 45 patients. These 56 episodes of recurrent CM are further studied in the sections that follow, and are referred to as episodes of recurrence

3.3. Profile of recurrent episodes of CM

Episodes of recurrent CM in patients on ART contributed a total of 66.1% and 33.9% of the episodes were in patients not on ART.

The Group distributions of the 56 recurrent episodes of CM.

A. Twenty (35.7%) of the recurrent episodes were in patients on ART and fluconazole.
B. Seventeen (30.4%) of the recurrent episode were in patients on ART alone.
C. Six (10.7%) of the recurrent episodes were in patients on fluconazole alone.
D. Thirteen (23.2%) episodes of recurrences were in patients on no therapy.

The biggest group of 20 episodes of recurrent CM was in patients on both ART and fluconazole. IRIS was diagnosed in 14 of these patients, which was 25% of the whole cohort and 70% of this group. These 14 cases of IRIS had recurrent symptomatic CM with negative CSF fungal cultures without evidence of ART failure, that is a recovering CD4 count and a decreasing viral load based on the proposed criteria. See Annexure C. Those patients who were still had a positive CSF fungal culture beyond 3 months from the initial episode of CM were not considered to have IRIS. It must be noted that some cases called IRIS may still have been from relapses from treatment failure. There were 5 deaths amongst the 14 patients with IRIS.

3.4.The clinical presentation

Table 1: The clinical presentation of the groups

Group	N	Headache	Meningism	GCS <15	Seizures	Died
ART/FLUC	20	15 (75.0%)	7 (35.0%)	5 (25.0%)	3 (15.0%)	9 (45%)
ART/NO FLUC	17	14 (82.4%)	12 (70.6%)	4 (23.5%)	2 (11.8%)	9 (52.9%)
FLUC/NO ART	6	4 (66.67%)	3 (50%)	4 (66.7%)	0	4 (66.7%)
NO ART/NO FLUC	13	10 (76.9%)	10 (76.92%)	4 (30.8%)	3 (23.1%)	5 (38.5%)
TOTAL	56	43 (76.8%)	32 (57.1%)	17 (30.4%)	8 (14.3%)	27 (48.2%)

GCS: Glasgow Coma Scale

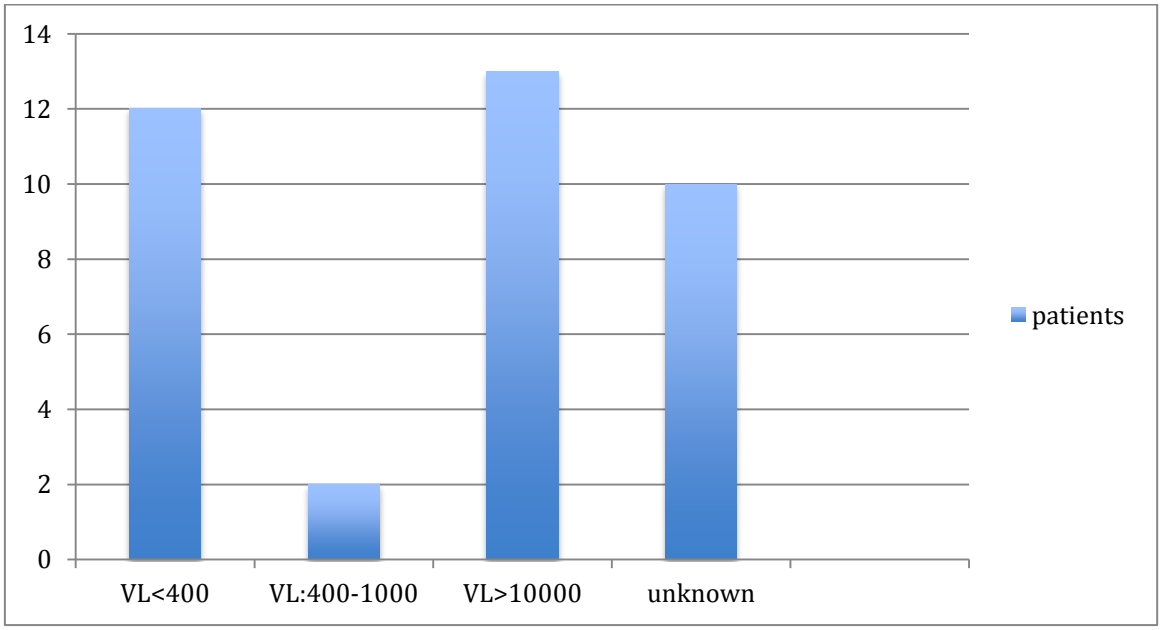
ART: Antiretroviral therapy

FLUC: fluconazole

Table 2: CSF findings, CD4 count and time to recurrence

Characteristic	Total(N)	Median(interquartile range)
CD4 count: index episode($mm^{3)}$	24	52 (0-182)
CD4 count: recurrent episode mm^3	52	85 (3-393)
	CSF	
Lymphocytes/mm^3	54	42 (0-385)
Neutrophils/mm^3	54	8 (0-121)
Erythrocytes/mm^3	54	14 (0-360)
Protein g/l	51	1.19 (0.29-8.3)
Glucose mmol/l	51	1.7 (0.2-3.9)
Time to recurrence (days)	55	142 (32-633)

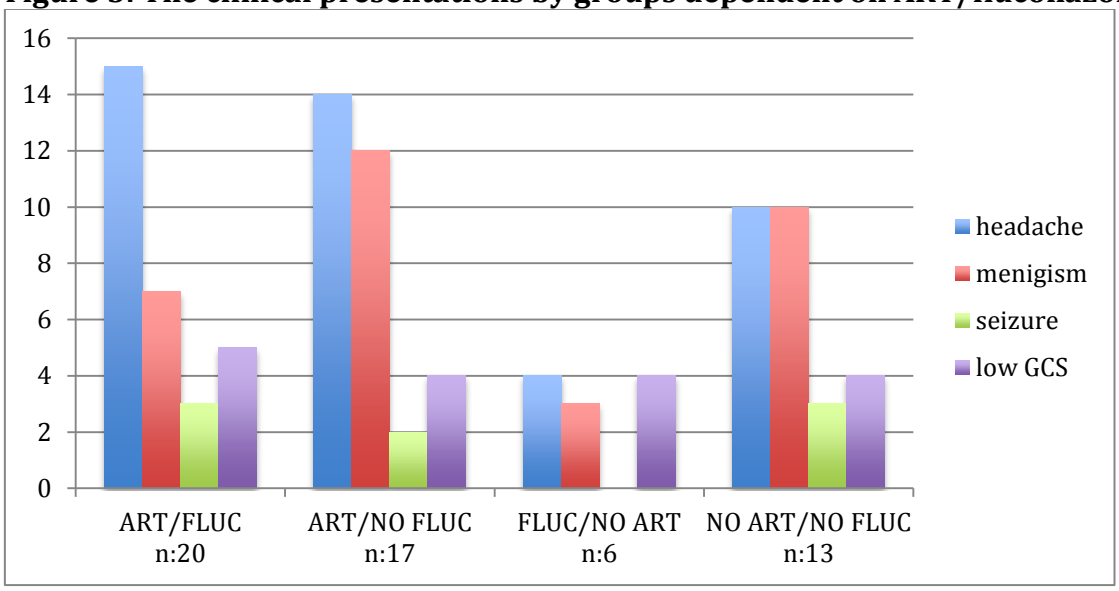
Figure 2: Patients on ART: viral loads



Viral loads expressed as (copies/ml)

37 patients were on ART, 27 had viral loads results and only 12 were virally suppressed.

Figure 3: The clinical presentations by groups dependent on ART/fluconazole



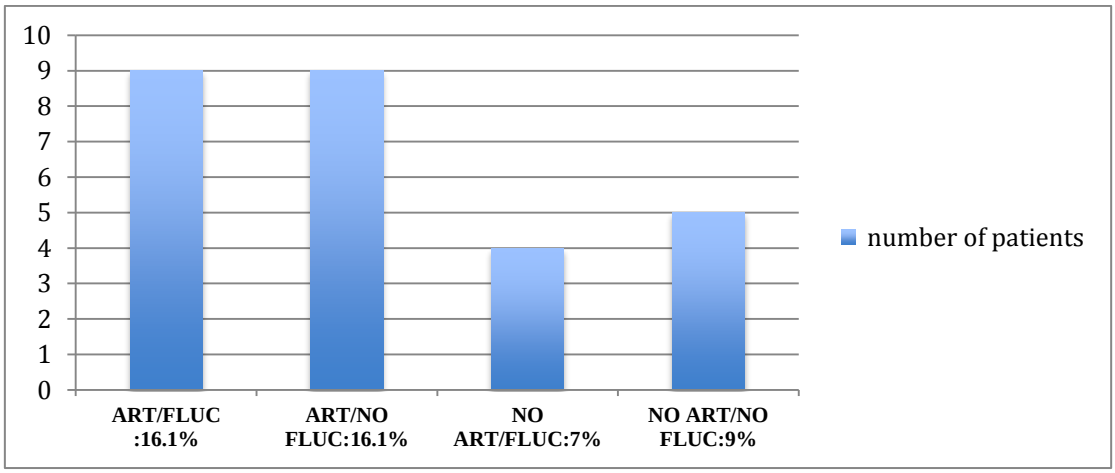
Headache and meningism were the commonest presenting features occurring in 76.8 % and 57.1% of the cases respectively. A decline in GCS occurred in less than a third of the cases. The occurrence of seizures seemed to be evenly distributed amongst the groups, occurring in all groups apart from the group on fluconazole only.

Meningism was less reported in patients on fluconazole with or without ART, 10/26 (38.5%) compared to those not on fluconazole, 22/30 (73.3%), p=0.018. There were no other significant differences in the manner of presentation among the various groups.

3.5. Mortality rates

The total in-hospital mortality rate for CM recurrences was 48.2% (27/56 died). The patients with seizures had a significantly increased in-hospital mortality rate of 85.7 % (7/8died) as compared to 41.7% (20/48) without seizures; p=0.0197. Patients with decline in level of consciousness as measured by the Glasgow Coma Scale (GCS) also demonstrated an increased in-hospital mortality rate of 82.4 % (14/17 died) as compared to the mortality in individuals with a normal GCS of 33% (13/39died); p=0.0008. There were 5 (35.7%) deaths in the 14 IRIS patients.

FIGURE 4 Mortality rates



The mortality of 48.2%: group distribution

3.6. A comparison between the incident and recurrent CM episodes.

45 (80.4%) of the 56 cases with recurrent CM had CSF results from both the index episode and the recurrent episode, which enabled comparison.

The results are shown on the table that follows:

Table 3: CSF comparison of incident and recurrent episode of CM.

		ART/FLUCONAZOLE	ART, NO FLUCONAZOLE	NO ART,ON FLUCONAZOLE	NO ART AND NO FLUCONAZOLE	TOTAL	P-value
	n=	15	13	7	10	45	
GENDER	Male n (%)	8 (53.3)	10 (76.9)	3 (42.9)	9 (90.0)	30 (66.7)	0.109
	Female n (%)	7 (46.7)	3 (23.1)	4 (57.1)	1 (10.0)	15 (33.3)	
AGE (years)		37±7	37±7	45±7	38±5	38±7	0.047
CLINICAL PRESENTATION- No (%)							
	n=	**13**	**13**	**7**	**10**	**43**	
HEADACHES	Yes n (%)	12 (92.3)	10(76.9)	6 (85.7)	9 (90.0)	37 (86.0)	0.692
	No n (%)	1 (7.7)	3 (23.1)	1 (14.3)	1 (10.0)	6 (14.0)	
SEIZURES	Yes n (%)	3(20)	2(15.4)	-	3(30)	8(17.78)	
GCS	Normal	10 (66.7)	9 (69.2)	3 (42.9)	7 (77.8)	29 (65.9)	0.515
	Abnormal <15	5 (33.3)	4 (30.8)	4 (57.1)	2 (22.2)	15 (34.1)	
CSF findings							
	n=	15	13	7	10	45	
CSF ON FIRST PRESENTATION	Lymphocytes $/mm^3$	60±94	11±18.	143±159	159±241	102±192	0.317
	Neutrophils $/mm^3$	0±1.5	9±20	3.8±8	28±61	10±31	0.192
	Erythrocytes $/mm^3$	3±10	1±3	60±159	39±82	20±75	0.257
	Protein g/l	1.11±0.66	0.93±0.46	1.22±0.61	1.23±0.42	1.1±0.52	0.544
	Glucose mmol/l	1.91±0.78	2.06±0.62	1.63±0.68	1.11±0.7	1.73±0.78	0.020
	Culture (Positive)	12 (92.3)	11 (100.0)	7 (100.0)	10 (100.0)	43 (97.7)	0.577
	Culture (Negative)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	
	CRAG titre	1888±938	2234±618	1765±750	1869±567	1963±707	0.513

		ART/FLUCONAZOLE	ART, NO FLUCONAZOLE	NO ART,ON FLUCONAZOLE	NO ART AND NO FLUCONAZOLE	TOTAL	P-value
	n=	15	13	7	10	45	
CSF ON SECOND PRESENTATION	Lymphocytes $/mm^3$	40±40	54±110	28±31	63±101	65±148	0.761
	Neutrophils $/mm^3$	8±23	19±36	2±4	19±57	12±34	0.634
	Erythrocytes $/mm^3$	0±2	5±14	8±14	49±102	15±51	0.129
	Protein g/l	1.09±0.69	1.03±0.57	0.74±0.23	0.91±0.2	0.99±0.49	0.374
	Glucose mmol/l	1.71±1.17	1.99±0.85	2.31±0.98	1.11±0.72	1.71±0.99	0.081
	Culture (Positive)	8 (66.7)	9 (81.8)	2 (28.6)	7 (87.5)	27 (65.9)	0.091
	Culture(Negative)	4 (33.3)	2 (18.1)	3 (42.8)	1 (12.5)	14 (34.1)	
	CRAG titre	1274	1834	658	1754	1380	0.587
CD4 count $/mm^3$		134±103	69±69	92±48	42±21	89 ± 78	
OUTCOME n (%)	ALIVE	5 (38.5)	7 (63.6)	2 (28.6)	8 (80.0)	24 (54.5)	0.175
	DIED	8 (61.5)	4 (36.4)	5 (71.4)	2 (20.0)	20 (45.5)	
TIME TO RELAPSE days ± SD		127±57	122±67	141±161	115±50	132±93	0.701

The variables were compared to elicit any differences between the first and second CSF. No statistically significant difference was found in any parameter.

The clinical parameters in the 45 cases used here were similar to those in the total group and therefore suggest that they were representative of the total group.

Chapter 4: DISCUSSION

The study was descriptive aimed at defining the prevalence of recurrent CM and describing the clinical, laboratory profiles and outcomes of recurrent CM in the age of freely available ART and secondary prophylaxis in the population of Soweto, Gauteng, South Africa. The prevalence of cryptococcal meningitis recurrences in our population was 7.8%. The figure was lower than the 9.5% reported in a surveillance study in patients pre-ART conducted in Gauteng in 2002-2004, but was higher than the 5% reported in the developed world [8,9]. The prevalence of recurrent CM remains high, however it must be kept in mind that the CM IRIS contributed 25% to the cohort. IRIS/ART related recurrences of CM would not have contributed to the pre-ART sample in 2002-2004.

Among the 56 recurrent episodes of CM scrutinized, a total of 66% were on ART and only 46% were on fluconazole. This was an indirect measure of the efficacy of the health system and reflects suboptimal rates of ART initiation and fluconazole treatment and secondary prophylaxis in patients who had experienced a serious opportunistic infection.

The biggest group of patients (35.7%) were documented to be on both ART and fluconazole. IRIS accounted for recurrent CM in a majority (70%) of this group and for 25 % of all CM recurrences. It is challenging to accurately define the incidence of IRIS in most clinical settings. It is largely a diagnosis of exclusion as there are no definitive markers for a diagnosis and mostly becomes a presumptive inference based on patterns. These include a negative CSF fungal culture, a fall in CRAG titre, and a

decrease in HIV viral load [12,17]. Not all, if any, of these laboratory tests are available in many areas of sub-Saharan Africa where the majority of patients with CM are situated. An additional challenge is the criteria for IRIS in the various studies are generally not uniform, and largely dependent on resources [12].

The 25% of IRIS cases in this study is midway between the incidences reported both locally and internationally. Data out of Cape Town by Bicanic et al. showed IRIS to occur in 17% of their sample population. (11 of the 65 patients in this 2009 prospective study). Interestingly another earlier study conducted in the same centre in 2007/2008 by Jarvis et al. showed IRIS to contribute to 45% of the relapses [11, 17]. It must be noted that in the two studies the patients commenced ART at different times, which may have accounted for the higher rates in the one study. A Thai study by Sungkanuparph et al. showed an incidence of 13%, 13 patients with IRIS were identified out of a cohort of 101 patients [12]. A higher prevalence in Uganda was reported as seen in a prospective cohort by Kambugu et al, with 42 cases in this study of 85 demonstrating features of probable IRIS. The incidence was calculated at 42 [12,16]. The lowest incidence was documented in a french study with an incidence of 8% reported [12].

The second commonest group accounting for 30% of the relapses was fluconazole interruption in those taking ART. Cases not on fluconazole irrespective of ART contributed 54% of the cohort. This supports other studies showing failure to continue secondary prophylaxis contributed significantly to cases of relapse.

In a prospective Cape Town study, there were 69 relapse episodes accounting for 23% of all cases of cryptococcal meningitis. 43% (n: 30) of these relapse episodes were in patients not taking fluconazole prophylaxis and of the 30 patients not taking fluconazole 47% (n: 14) had not been prescribed secondary prophylaxis by their healthcare providers [11]. In our study the reasons for fluconazole interruption were not available. In many cases, it likely reflected failures in the health system. In some cases, fluconazole could have been stopped by a doctor or nurse if a patient on ART with a suppressed viral load had a CD4 count >200/mm^3.

The smallest group of 10.7% was in cases on fluconazole without ART. Fluconazole used as secondary prophylaxis has been shown to prevent relapse of CM [3]. The group on no therapy (23.2% of cases) may have represented a group with no follow-up or loss-to-follow up following the incident episode of CM.

The overall median time to relapse was 142±115 days in the 56 episodes of recurrence, but with a wide range of one month up to almost 2 years. The longest median time to relapse was in the group on fluconazole at 141 days. The shortest time was in the group on no therapy (115 days). The groups on ART and fluconazole relapsed at 127 days, this may be explained by the cases of probable IRIS occurring earlier than relapses, commonly within 1- 10 months as described in the literature [13].There was a male predominance with a total of 58.8% patients (male-to-female ratio of 1.4:1). This was higher than the 49% described in a previous surveillance study in South Africa [9]. Whether this points to males being at an increased risk of

relapses is uncertain, we did not have the gender distribution of the incident patients. The clinical presentation did not differ amongst the groups. The most common signs and symptoms were headache, meningism, a decreased level of consciousness and seizures in order of decreasing frequency. It is noteworthy that headache and meningism did not occur in all patients, which can make diagnosis of CM difficult. The signs and symptoms of cases with recurrent CM were no different to those seen in other cases of CM. A local study reported headache to be the commonest symptom (79%) followed by meningism (67%), fever, altered GCS (32%) with seizures only contributing 4% [30].

There was no significant difference in the CSF profiles between the groups. The CSF parameters of recurrent CM changed slightly relative to the initial episode. There was a higher CSF protein, lower glucose level, and a higher CSF neutrophil cell count as compared to the initial episode in patients on ART and fluconazole; but the difference was not statistically significant. A high CSF white blood cell count has been reported as a common occurrence in IRIS cases [17,18].

The findings of lower CSF culture rates and CRAG titers may be explained by the cases of IRIS. Interestingly there were CSF culture negative recurrences in the group on fluconazole and no ART. The reason may have been a low fungal burden in the presence of fluconazole. The laboratory may have discarded cultures before they could become positive, as culture may be delayed up to a month before becoming positive [31].

The overall mortality for recurrent CM was 48.2%. In the sub-group of patients taking fluconazole without ART, 4 out of the 6 demised. Due to the small numbers the significance of this high mortality is uncertain. There was significantly increased mortality in patients with seizures and a low GCS, these features occurred evenly amongst the groups. The mortality was 85, 7% and 82, 5% respectively in patients with seizures and a low GCS. The mortality rate of CM described in the literature ranges between 13-44%, largely influenced by the availability of resources. [3]. The in-hospital mortality rate found in this study of recurrent CM is at the higher end. This is higher than the mortality rate in sub-Saharan Africa of 30%. Higher rates were reported in Uganda in the pre- ART era, with 42 % in-hospital mortality improving in the HAART era to 20% at 14 days [16]. In a prospective IRIS study in Cape Town, the reported mortality in IRIS was 36% (4 patients out of 11), which is similar to that in the current study [17]. The mortality rate for IRIS in this study was 35.7%. The reason for such the increase in the mortality rate in this study is likely multifactorial. The mortality rate is largely influenced by the availability of resources in institutions, along with timeous presentation to healthcare facilities. The mortality rates are much lower in developed resource rich environments, especially with the use of flucytosine together with amphotericin B for induction therapy [3].

Chapter 5: Limitations

This was a retrospective study and as such some information may have been missing or not documented correctly. This lack of accurate data may have been exacerbated by the fact that almost a third of patients had a documented reduced GCS.

The prevalence rate may have been affected by a number of possible factors. For the numerator, some patients with recurrent CM may have died in the community or sought medical attention at other hospitals; so the numerator for prevalence may have been falsely reduced. Similarly some of those discharged after the incident episode of CM may have died without recurrence and the denominator for prevalence may have been falsely elevated. We were not able to obtain adequate records for 11 (20%) episodes of recurrent CM. Because some patients had more than one episode of recurrence, this led to confusion between patients and episodes, so we have used the terms cases or episodes for these recurrences.

Since resistance testing is not routine and repeat lumbar punctures to determine sterility of CSF were not usually performed, we were not able to comment on resistance or possible organism persistence from CSF on the initial presentation

We accepted GERMS-SA data and diagnosis as CM when rehospitalised. There was no detailed information on fluconazole prescription or its use prior to hospitalization in the database and some of the bed letters, thus it was difficult to be certain of the compliance on fluconazole prior to hospitalization using GERMS-SA records and bed letters. IRIS still carries challenges with regards to a confident diagnosis.

There may be differences in clinical and laboratory features of patients with recurrent CM but the numbers were too small to show these. Bigger numbers of patients may be required to further elucidate the significance of a number of the variables.

Chapter 6: Conclusion

The prevalence of CM recurrence was 7.8%, which was midway between the developed world rate and the pre-ART Gauteng study rate [8,9]. More than 1 in 6 episodes of recurrence were in patients with a history of a previous recurrence of CM.

Two-thirds of the cases were documented to be on ART, while almost half were on fluconazole. This means that one-third of the cases had not yet started ART and more than half were not receiving fluconazole. The reason for this together with the high rate of multiple recurrences requires further investigation. The largest group in the cohort was on both ART and fluconazole but this was still merely 35.7%, while 11% were on no therapy. IRIS was common and contributed 25 % to the recurrent cases.

There was no significant difference in the clinical features or CSF findings between groups. The in-hospital mortality of cases was 48.2%, and was significantly more common in those who had a low GCS or who had seizures.

The management of patients with CM remains a challenge. This includes management of the hospitalized patient as well as education of the patient and family members as to the nature of his or her illness and the need for lifelong treatment. It also requires explicit steps to link the patient with a clinic or hospital to ensure the reliable provision of fluconazole and the initiation of ART.

Recommendations for future work

The possible impact of TB and its treatment on recurrences of CM needs to be examined. This would involve measuring fluconazole levels in patients receiving rifampicin to investigate whether fluconazole levels become subtherapeutic.

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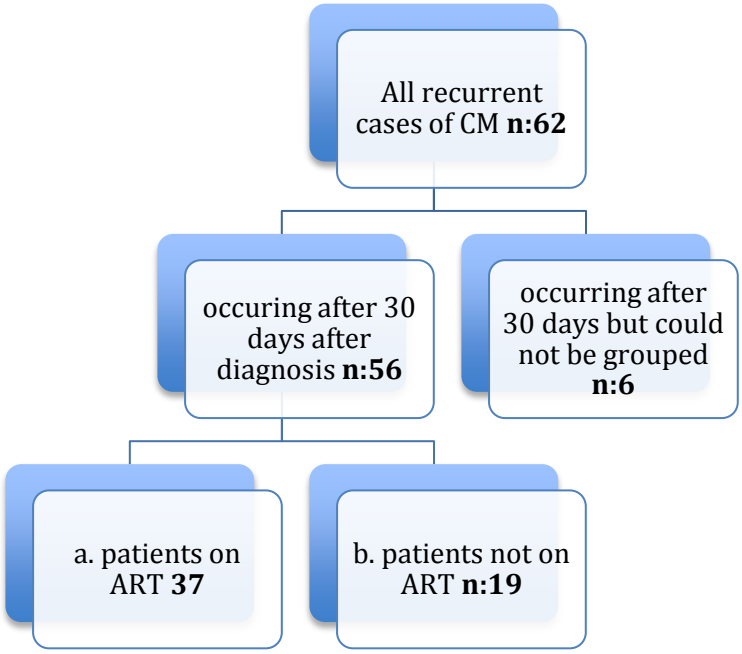
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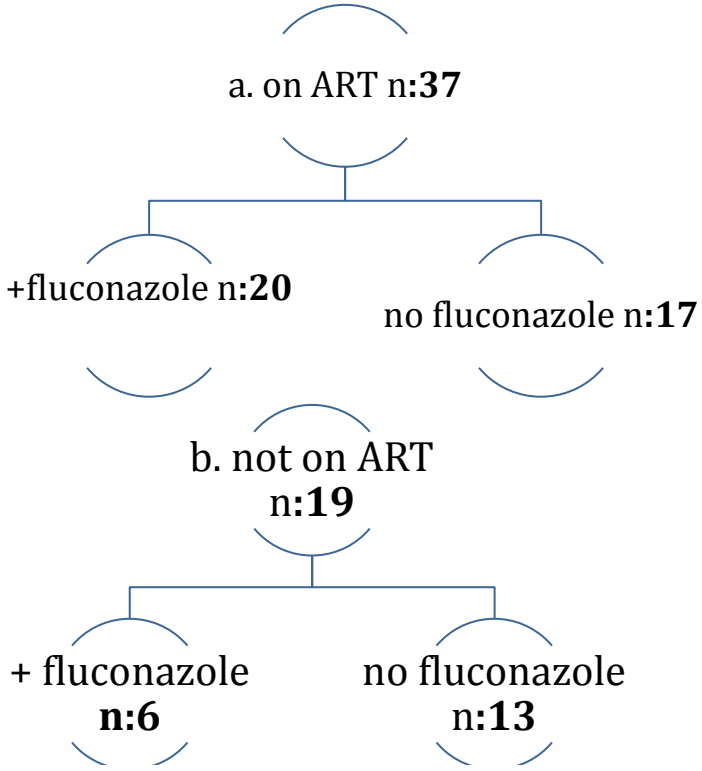
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ANNEXURE A


All recurrent cases of CM **n:62**
occuring after 30 days after diagnosis **n:56**
occurring after 30 days but could not be grouped **n:6**
a. patients on ART **37**
b. patients not on ART **n:19**


a. on ART n:37
+fluconazole n:20
no fluconazole n:17
b. not on ART n:19
+ fluconazole n:6
no fluconazole n:13

ANEXURE B

DATA SHEET

Study Number

Age:

Gender:

HIV: Y/N
Fluconazole: Y/N
DOSE:
DURATION:
BACTRIM: Y/N

ART: Y / N

IF Yes: Duration
: Regimen

CD4 AT ADMISSION:
Baseline:

VL: At admission
At Baseline
OTHER COMRBIDITIES:

PREV EPISODES OF CM: 1-6M
6-12M
>1 YEAR

SYMPTOMS AND SIGNS

Headache: Y/ N

Decline in GCS: Y/N
FOCAL SIGNS Y/N
If yes list:

	L	N	E	PROT	GLUC	CL	I. INK	CRAG	CULTURE
EPISODE1									
EPISODE2									
EPISODE3									
EPISODE4									

	WCC	HB	MCV	PLT	NA	K	CL	UR	CR
ADMISSION 1									
2									
3									
4									

Discharged: Y/N

Died: Y/N

ANEXURE C

IRIS DEFENITIONS BASED ON THE INTERNATIONAL NETWORK FOR THE STUDY OF HIV ASSOCIATED IRIS(INSHI)

Panel 1: Case definition for paradoxical cryptococcal immune reconstitution inflammatory syndrome in patients HIV-1

Antecedent requirements

• Taking antiretroviral therapy

• Cryptococcal disease diagnosed before ART by positive culture or typical clinical features plus positive India ink staining or antigen detection

• Initial clinical response to antifungal therapy with partial or complete resolution of symptoms or signs, fever, or other lesions, or reduction in CSF cryptococcal antigen concentration or quantitative culture

Clinical criteria

• Event occurs within 12 months of ART initiation, reintroduction, or regimen switching after previous failure

• Clinical disease worsening with one of the following inflammatory manifestations of cryptococcosis (see text for possible rarer manifestations):

• Meningitis

• Lymphadenopathy

• Intracranial space-occupying lesion or lesions

• Multifocal disease

• Cutaneous or soft-tissue lesions

• Pneumonitis or pulmonary nodules

Other explanations for clinical deterioration to be excluded

• Non-adherence or suboptimum antifungal therapy, indicated by an increase in quantitative culture or antigen titre, or any positive cryptococcal culture after 3 months of antifungal therapy

• Alternative infection or malignant disease in the affected site

• Failure of ART excluded if possible (eg, failure to achieve ≥1 log10 copies/mL decrease in viral load by 8 weeks of ART)

ART=antiretroviral therapy. CSF=cerebrospinal fluid

Panel 2: Proposed case definitions for antiretroviral-therapy-associated cryptococcosis and unmasking cryptococcal immune reconstitution inflammatory syndrome

ART-associated cryptococcosis

• Patient taking ART
• No recognised cryptococcal disease at ART initiation
• Clinical disease worsening caused by cryptococcosis occurs after initiation, re-introduction, or regimen switch after previous failure (supported by microbiological, histological, or serological evidence)
• Cryptococcal infection characterised by meningitis, CNS complications, skin or soft-tissue lesions, lymphadenopathy, lung disease, or disseminated disease

Unmasking cryptococcal IRIS (provisional)

• Criteria for ART-associated cryptococcosis are met
• Unusual, exaggerated, or heightened inflammatory manifestations, such as the following:
• Meningitis with CSF WBC >50Å~10^6 /L or CSF opening pressure >20 cm that is refractory to therapy
• Painful or suppurating lymphadenopathy
• Rapidly expanding CNS lesions, cryptococcomas
• Unusual focal site (ie, not within the CNS, lung, skin, or lymph nodes)
• Granulomatous inflammation on histology
• Pneumonitis, particularly if cavitating or necrotic
• Event occurs early after ART initiation*
• Failure of ART excluded if possible (eg, ≥1.0 log10 copies/mL decrease in HIV-1 viral load by 8 weeks treatment)

ART=antiretroviral therapy. IRIS=immune reconstitution infl ammatory syndrome. CSF=cerebrospinal fluid. WBC=white blood cell count. *No specific time limit is proposed for unmasking cryptococcal IRIS, pending further research. Typically, onset within 3 months of starting ART could be assumed to support a diagnosis of IRIS owing to early and rapid changes in immune function.

However, late presentations of cryptococcal IRIS have been reported in patients with good responses to therapy assessed by CD4 cell counts.

ANEXURE D

R14/49 Dr Dineo Maphanga

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140819

NAME: (Principal Investigator)	Dr Dineo Maphanga
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Recurrent Cryptococcal Meningitis at Chris Hani Baragwaneth Hospital
DATE CONSIDERED:	29/08/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Alan Karstaedt
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	22/10/2014

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES