PREVALENCE AND FACTORS ASSOCIATED WITH CRYPTOCCOCAL ANTIGENEMIA AMONG PATIENTS WITH ADVANCED HIV IN MBALE REGIONAL REFFERRAL HOSPITAL

BY

DR. EBONU ERIC,
MBCHB (MUST).
BU/GS16/MMM/2

SUPERVISORS:

1. **BWAYO DENIS;** MBChB (MUK), MMED Internal Med (MUK), MPH (University of Queensland, Australia).
   Lecturer Department of Internal Medicine; Faculty of Health Sciences, Busitema University.

2. **DAVID B. MEYA;** MBChB (MUK), MMED Internal Med (MUK), PHD (MUK).

   Associate Professor, Department of Internal Medicine, Makerere College of Health Sciences, Makerere University.

A RESEARCH DISSERTATION SUBMITTED TO BUSITEMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE.

2021

DECLARATION

I EBONU ERIC declare that this dissertation is my original work, except where due acknowledgement has been made. I declare that this work has not been submitted to this University or to any other institution for funding or for any academic award.

Signature  Date;21/05/2021

Name: Ebonu Eric

Registration Number: BU/GS16/MMM/2

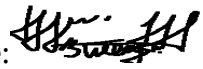
APPROVAL

This dissertation has been submitted as a partial fulfillment for the award of Master of Medicine in Internal Medicine of Busitema University, with our approval as the academic supervisors.

Name: **Dr. BWAYO DENIS**

Academic Qualifications: MBChB (MUK), MMed Internal Med (MUK), MPH (University of Queensland, Australia).

Lecturer Department of Internal Medicine; Faculty of Health Sciences, Busitema University

Signature: 

Date: 21/05/2021.

Name: **Dr. DAVID B. MEYA**

Academic Qualifications: MBChB (Mak), MMed Internal Med (Mak), PHD (Mak).

Associate Professor, Department of Internal Medicine, Makerere College of Health Sciences, Makerere University.

Signature…… 

Date;21/05/2021

DEDICATION

I dedicate this book to **God** and my **family** for being there for me at all times during this endeavor.

ACKNOWLEDGMENT

I with utmost gratitude wish to acknowledge the following people and institutions for their priceless contribution towards the success of this dissertation:

Busitema University, Faculty of Health Sciences but most especially the Department of Internal Medicine. The Head of the Department of Internal Medicine, **Dr. JPM Masaba**, for his persistence and fatherly guidance towards this cause. My supervisors; **Dr. Denis Bwayo**, **Associate Professor D.B. Meya** for their academic guidance, patience and thought-provoking discussions. They contributed significantly to completion of this research. I would also want to acknowledge **Dr. Richard Katuramu** for his input to the completion of this paper. To Professor **Peter Olupot-Olupot**, for his wisdom and amazing attention to fine detail, thank you.

I would also like to acknowledge SIDA Makerere and IDI Mulago for facilitating this research.

The administration, staff, and my colleagues at Mbale Regional Referral Hospital for their unconditional support and encouragement; Soroti Regional Referral Hospital for allowing me study and for the financial support. My father, **Dr. Engoru Charles** who has always shown unending interest in this research. Mr. **Okello Francis** the Biostatistician, the patients, manager and staff of TASO Mbale for their hospitality while collecting data.

In a special way, I would like to express intense gratitude to my **family** who were there for me emotionally, economically, through their earnest prayer and endurance for the extremely long days and sleepless nights, thank you.

Most importantly all praise be to **God** my Everlasting father who granted me the chance, wisdom and prudence to accomplish this task with spiritual blessing through **Christ**.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AHD	Advanced HIV Disease
ART	Anti-Retroviral Therapy
BMI	Body Mass Index
BUFHS	Busitema University Faculty of Health Sciences
CBC	Complete Blood Count
CM	Cryptococcal Meningitis
CD4	Cluster of Differentiation 4
CrAg	Cryptococcal Antigen
CSF	Cerebral Spinal Fluid
ENT	Ear, Nose and Throat
FR	Fluconazole Resistance
HDRC	Higher Degrees Research Committee
HIV	Human Immunodeficiency Virus
HW	Health Worker
LAB	Laboratory
LFTS	Liver Function Tests
LP	Lumbar Puncture
MIC	Mean Inhibitory Concentration
MBALE RRH	Mbale Regional Referral Hospital
OR	Odds Ratio
PI	Principle Investigator
PLHIV	People Living with HIV
PNFP	Private Not for Profit
PR	Prevalence Ratio
RCT	Routine Counseling and Testing
REC	Research and Ethics Committee
RFTS	Renal Function Tests
RPM	Rotations Per Minute
SD	Standard Deviation
TASO	The AIDS Support Organization
TB	Tuberculosis
WBC	White Blood Cells
WHO	World Health Organization.

DEFINITIONS

Term	Definition
Prevalence	Is the proportion of the patients with Advanced HIV disease who have positive CrAg test in this study.
Advanced HIV disease	Is a disease condition in which an HIV infected person has a CD4 cell count of less than 200cells/mm^3 or first clinical presentation with clinical stage 3 and 4 or all children 5 years of age and younger.
Cryptococcal antigen positivity or antigenemia	The presence of cryptococcal antigen as demonstrated by a positive cryptococcal antigen test using serum, plasma, or cerebrospinal fluid.
Cryptococcal disease	Infection of a person with *Cryptococcus* species resulting in relevant clinical symptoms and signs depending on the affected organ/tissue.
Cryptococcal meningitis	Infection of the tissues covering the brain and spinal cord with *Cryptococcus neoformans*, these tissues are called meninges.
Kernig's sign	Is positive when the thigh is flexed at the hip and knee at 90-degree angles, and subsequent extension in the knee is painful (leading to resistance).

ABSTRACT

Background

Cryptococcal infection is an opportunistic infection caused by a fungal infection with *Cryptococcus neoformans*. It is found majorly among severely immunocompromised people, especially those living with advanced HIV. It is associated with a high rate of morbidity and mortality. A positive serum test for cryptococcal antigen (CrAg) is the current clinical practice gold standard for diagnosis of cryptococcal infection. This antigen-based test is the basis for categorizing patient's antigenemia status. Cryptococcal infection is an independent predictor for cryptococcal meningitis which is the most severe form of Cryptococcal infection and the second commonest cause of morbidity and death among People Living with HIV (PLHIV) with Advanced HIV disease (AHD). AHD is defined as having a CD4 cell count of less than 200cells/mm^3 or World Health Organization (WHO) clinical stage 3 or 4 event at presentation in adults.

In Uganda, Cryptococcal antigenemia and AHD have been described specially in central Uganda, but there are no formal studies describing the condition in Eastern Uganda. This study, therefore, assessed the prevalence and the factors associated with cryptococcal antigenemia among PLHIV with advanced HIV disease in Mbale Regional Referral Hospital (Mbale RRH).

Aims/Rationale

The main objective of this study was to determine the prevalence and factors associated with cryptococcal antigenemia among PLHIV with advanced HIV disease.

Materials and Methods

In this cross-sectional study, we recruited participants of 18 years of age and above who were HIV positive with advanced disease in Mbale RRH medical ward. Eligible participants were; all patients with AHD aged 18 years and above, voluntarily willing to participate in the study and able to give informed consent. They were recruited consecutively until a sample size of 228 was achieved. We collected data on social, demographic and clinical characteristics using a pre-tested customized data collection tool administered to study participants. Cryptococcal antigenemia was determined using Lateral flow assay (Immuno-Mycologics IMMY^{R}) test. Besides the consent to participate in this study, additional mandatory clinical care consent was obtained as part of the hospital protocol from all CrAg positive patients to do a lumbar puncture (LP) and obtained Cerebral spinal fluid (CSF). The CSF was tested using the Lateral flow assay and if found positive was cultured using Sabouraud Dextrose agar culture media. All the positive cultures were subjected with a drug sensitivity testing (DST) for fluconazole and Amphotericin B sensitivity. The collected data were entered into an excel based databased, exported and were analyzed using STATA version 14.0. proportions of socio demographic factors were reported and prevalence of cryptococcal antigenemia was reported as proportion of those patients with positive serum CrAg compared to the total number of patients enrolled in the study. Factors associated with cryptococcal antigenemia were reported first as proportion and further analyses to determine the associations were conducted using multiple logistic regression models.

Results

Between May to June 2019, we enrolled 228 participants, 152 (66.7%) females, mean age (SD) 42 (12.4) years and median CD4 count of 194 cells/mm^3 (IQR 129-370). Of the total 228 patients, only 10/228 (4.4%; 95% CI, 0.024 - 0.080) had cryptococcal antigenemia. Although the factors associated with cryptococcal antigenemia were significant at bivariate analysis, they were found to lack significance at multivariate analysis, these factors included; CD4 cell count of less than 100cells/mm^3 (AOR=3.70), keeping poultry at home, taking ART was protective (AOR=0.240). Clinical features associated with a positive serum CrAg; headaches (AOR=1), neck and back pains (AOR=8.817), altered vision (18.061), recent confusion (AOR=6.323) and neck stiffness (AOR=676.217). 30% of those with positive serum CrAg had a suppressed viral load.

Conclusions

Cryptococcal antigenemia is common among the people living with AHD with a CD4 of <100cells/mm^3, independent predictors associated with were CD4cell count <100cells/mm^3, clinical symptoms & signs of meningeal irritation and poultry keeping. We recommend regular screening for CD4 counts, prophylaxis for those found with CD4 counts <100cells/mm^3, prophylaxis for those who have AHD and also keep poultry at home, early & effective ART initiation and education of people living with HIV/AIDS (PLWHA) as a means of early detection of patients at risk, prevention and reduction of risks respectively.

CHAPTER 1:

INTRODUCTION

1.1 Background of the study

Cryptococcosis is an infection with *Cryptococcus neoformans* a fungus that is ubiquitous in the environment worldwide and is associated with severe health consequences (Hakim *et al*., 2000; Park *et al*., 2009a). It is an opportunistic infection that occurs among people with severe immune suppression. Globally the prevalence of cryptococcosis is estimated to be 6.0%, a majority of whom are PLWHA with AHD; typically, with either CD4 200cells/mm3 and below or stage 3 or 4 WHO clinical stage event at presentation irrespective of CD4 cell count among adults (Rajasingham *et al.*, 2017; WHO, 2018). The disease spectrum includes Cryptococcal meningitis (CM), which is the commonest and severest accounting for about 80% all the cryptococcal infections globally (Rajasingham *et al*, 2017). A 2017 estimation of burden of disease estimated a global cryptococcal infection prevalence of 6.0% with about 278,000 people annually estimated to develop cryptococcal disease resulting in 181,100 deaths every year (Rajasingham *et al*., 2017). This is about 15% of all HIV related deaths every year (WHO, 2018). Prevalence of Cryptococcosis seems to vary significantly across different geographical locations, for instance in 2007 in Cambodia and in 2009 in South Africa the prevalence of cryptococcal antigenemia was found to be 18% and 13% respectively among those with advanced HIV disease with CD4 cell count of less than 100 (Jarvis *et al*, 2009; Micol *et al*., 2007b). It is estimated that between 73% to 75% of Cryptococcal disease cases occur in sub-Saharan Africa (Park *et al*., 2009a; Radha Rajasingham *et al*., 2017). Early HIV diagnosis and early initiation of antiretroviral therapy (ART) has resulted in a significant decrease in number of deaths from cryptococcal disease since the previous 2009 estimate (Park *et al*., 2009b; R. Rajasingham *et al*., 2017). Cryptococcal disease

remains the second most common cause of AIDS-related mortality after tuberculosis among people living with HIV (Castelnuovo *et al*., 2009). Cryptococcal antigenemia represents both the mild or severe forms of infection.

A Meta-analysis in 2015 of Ugandan data showed that cryptococcal prevalence was estimated to be 7.1% among the estimated 56,000 PLHIV with CD4 cell count below 100cells/mm^3, 2.8% of the estimated 147,000 PLHIV with CD4 cell counts between 100-200cells/mm^3 and the total number of cryptococcal infections was estimated to be 4,050 with 2,412 (60%) estimated to die annually from the disease (R.Parkes Rantanshi, 2015). Even with introduction of ART and administration of recommended treatment, Cryptococcal meningitis among PLHIV is still associated with high 14-day mortality rates of 20%- 42% in Ugandan cohorts (Butler *et al*,, 2012; Kambugu *et al*., 2008; Park *et al*., 2009a)**.** A major reason for the high mortality from cryptococcosis in low-income countries is delay in diagnosis because of ineffective screening programs (Meya *et al*., 2010).

Early screening for cryptococcal antigenemia does not only provide an opportunity for early diagnosis and opportunity for early treatment but also minimizes the risk of immune reconstitution inflammatory syndrome (IRIS) when the patients are initiated on ART often complicating treatment (Lortholary *et al*., 2005; Sungkanuparph *et al*., 2009). Early screening has also been proven to be cost effective (Ramachandran *et al*, 2017). WHO 2018 guidelines on screening and treatment of Cryptococcal infection suggests screening of PLHIV with a CD4 cell count of 100cells/mm^3 and less while the Ugandan national HIV guidelines recommend screening for cryptococcosis for all those with AHD (WHO, 2018.; MoH, 2020). Most studies on effectiveness

of cryptococcal screening among PLHIV have used a cut off of CD4 count <100 cells/mm^3 (A. O. Andama *et al*., 2013; Liechty *et al*, 2007; Oyella, 2012).

Studies on cryptococcal antigen screening and outcomes in Uganda have been limited mostly to central Uganda. They largely focused on ART naïve patients and those with CD4 cell counts of 100cells/mm^3 and below. The prevalence of Cryptococcal antigenemia and factors associated with infection among PLHIV in this community in eastern Uganda is unknown. This study aimed at determining the prevalence and factors associated with cryptococcal antigenemia among people with advanced HIV disease (either CD4 200cells/mm^3 and below or stage 3 or 4 WHO clinical stage event at presentation irrespective of CD4 cell count) in Mbale Regional Referral hospital (Mbale RRH).

1.2 Problem statement

Despite improved HIV treatment options, Cryptococcal infection is still a major health problem in Uganda with high morbidity and mortality among people living with advanced HIV. The proportion of cryptococcal infection in Uganda among PLHIV with AHD is estimated to be 4,050 (7.1%) with mortality at 2,412 (60%). Despite these data, very few studies it at all any have been carried out in eastern Uganda to describe Cryptococcal infections, especially among people with AHD.

As an initial formal description, understanding of this condition among people with AHD is paramount, especially, describing its prevalence, associated factors and outcomes against this background, the description of these social demographic, clinical and laboratory factors may be useful for improvement of care of, especially people with AHD. For instance, in resource-limited

settings these findings may contribute the screening protocols for cryptococcal infection in patients with AHD.

1.3 Justification of the study

This being the first formal description of cryptococcal antigenemia it provides a baseline on which local understanding of the condition will be referenced. This study provides information specific for the population that receives services at Mbale RRH. Previous studies have been carried out elsewhere in Uganda and thus their findings may not be generalizable because of different local and regional factors. The target population around Mbale RRH comprises of mixed tribal and religious affiliation, there is a small urban population with majority being semi-urban and rural. The greater majority mainly comprise of the Bamasaba (Gishu) tribe who have unique cultural characteristics. The target population mostly reside in hilly/mountainous areas which have been known to interfere with health seeking behavior, health service delivery, and treatment interruptions among patients on chronic care.

In addition, in the era of advanced combination highly effective antiviral therapy for the care of HIV, it provides a contribution towards advancing of understanding which patients are at risk of the condition in Eastern Uganda. Moreover, at hospital level, this study advances knowledge on the current prevalence of disease and factors associated, therefore, informing resource allocation for optical care and outcomes.

At the national level, it is hoped that this study will provide additional information that may be useful for policy makers, health care providers and program managers about the prevalence and factors associated. It will usefully inform screening guidelines, prevention practices and appropriate treatment interventions.

1.4 Research Objectives

General objective:

The overall objective of this study was to determine prevalence and factors associated with cryptococcal antigenemia among PLHIV with advanced disease in Mbale Regional Referral Hospital.

Specific Objectives;

1. To determine the proportion of patients with Cryptococcal antigenemia among PLHIV with advanced HIV disease in Mbale Regional Referral Hospital.
2. To determine factors associated with Cryptococcal antigenemia among PLHIV with advanced HIV disease in Mbale Regional Referral Hospital.
3. To determine the drug sensitivity patterns of *Cryptococcus neoformans* in CSF isolates to fluconazole and amphotericin B among Cryptococcal antigen positive PLHIV with advanced HIV disease in Mbale Regional Referral Hospital.

1.5 Research questions

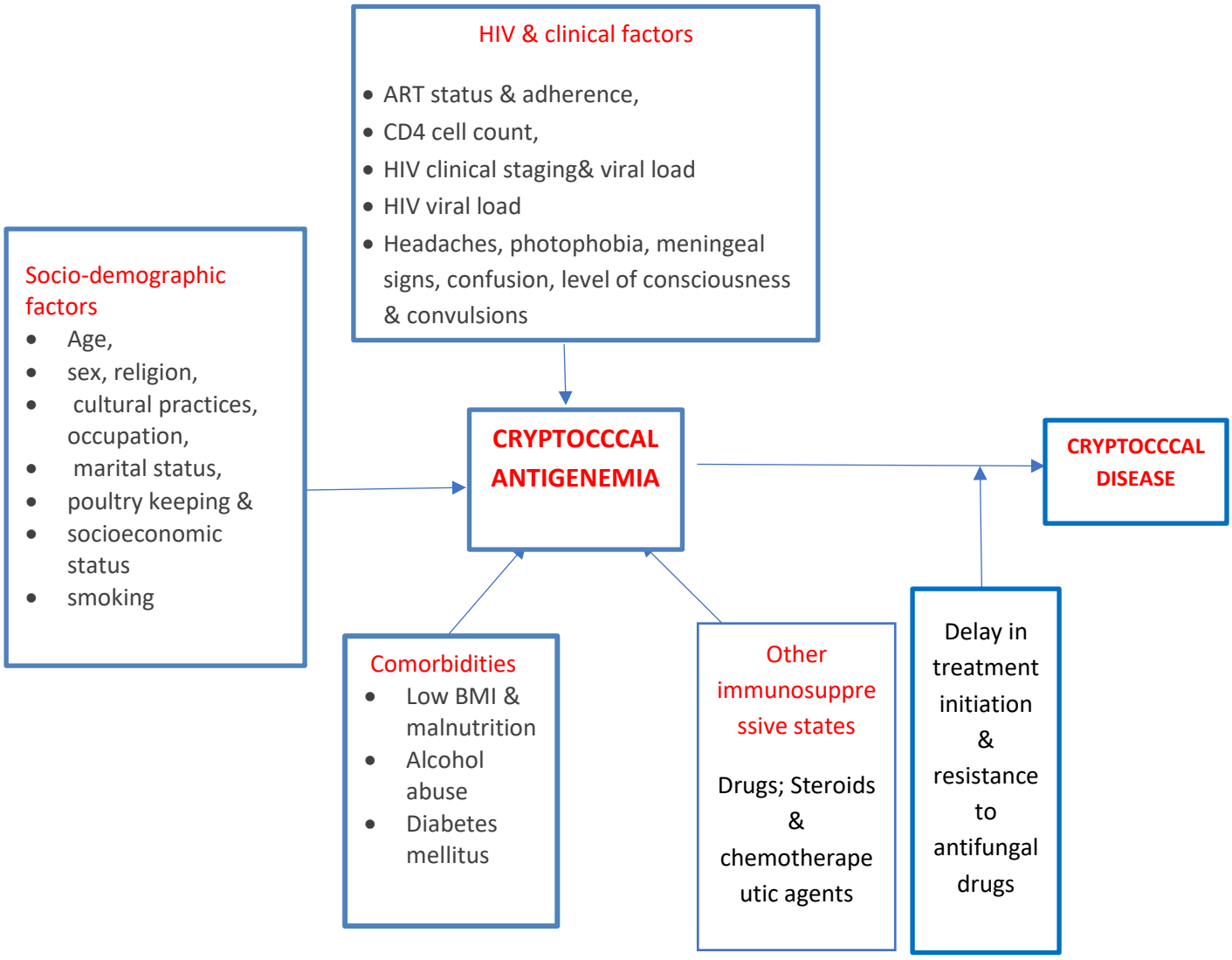
1. What is the proportion of patients with Cryptoccocal antigenemia among PLHIV with advanced HIV disease in Mbale Regional Referral Hospital?

2. What are the factors associated with Cryptoccocal antigenemia among PLHIV with advanced HIV disease in Mbale Regional Referral Hospital?
3. What are the sensitivity patterns of *Cryptococcus neoformans* in CSF isolates among the CrAg positive patients to fluconazole and amphotericin B?

1.6 Conceptual Frame work for factors associated with cryptococcal antigenemia

This study's conceptual framework shows the relationship between the independent/predictor variables with the dependent/outcome variables. In Figure 1 below, we highlight the linkages between independent variables/predictor variables*;* Social demographic factors, HIV & clinical factors, Comorbidities and other immunosuppressive states. The outcome variable which is cryptococcal antigenemia/cryptococcal disease was defined as infection of a person with *Cryptococcus* species resulting in relevant clinical symptoms and signs depending on the infected organ/tissue.

Figure 1; Conceptual Frame work for factors associated will Cryptococcal antigenemia



1.7 Chapter outline:

This dissertation is organized into distinct chapters each beginning with a short introductory synopsis.

This chapter provided an introduction and background to the study describing the prevalence and factors associated with cryptococcal antigenemia.

Chapter 2: In this chapter I have reviewed current literature on Cryptococcal disease, the risk factors and outcomes under the subtopics described in the conceptual framework.

Chapter 3: In this chapter, the study area, study design and rationale, study population and sampling procedure, variables under study, methods of data collection, data management, statistical analysis, quality control, ethical considerations, results dissemination and limitations of the study are discussed.

Chapter 4: In this chapter I have presented the results of this study using tables and graphs.

Chapter 5: In this chapter I have provided an interpretation of the study's findings and comparison to results from previous research. This chapter will also cover the strength and limitations of the study as well as implications for further interventions.

Chapter 6: In this chapter I have provided a logical conclusion of the study's findings and offered recommendations for further research.

REFERENCES

Alemu, A. S., Kempker, R. R., Tenna, A., Smitson, C., Berhe, N., Fekade, D., . . . Aseffa, A. (2013). High prevalence of cryptococcal antigenemia among HIV-infected patients receiving antiretroviral therapy in Ethiopia. *PLoS One, 8*(3), e58377.

Andama, A. O., den Boon, S., Meya, D., Cattamanchi, A., Worodria, W., Davis, J. L., . . . Huang, L. (2013). Prevalence and outcomes of cryptococcal antigenemia in HIV-seropositive patients hospitalized for suspected tuberculosis in Uganda. *J Acquir Immune Defic Syndr, 63*(2), 189-194. doi:10.1097/QAI.0b013e3182926f95

Andama, A. O., den Boon, S., Meya, D., Cattamanchi, A., Worodria, W., Davis, J. L., . . . Haller, B. J. J. o. a. i. d. s. (2013). Prevalence and outcomes of cryptococcal antigenemia in HIV-seropositive patients hospitalized for suspected tuberculosis in Uganda. *63*(2), 189.

Beardsley, J., Wolbers, M., & Day, J. N. (2016). Dexamethasone in Cryptococcal Meningitis. *N Engl J Med, 375*(2), 189-190. doi:10.1056/NEJMc1605205

Bicanic, T., Harrison, T., Niepieklo, A., Dyakopu, N., & Meintjes, G. J. C. i. d. (2006). Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. *43*(8), 1069-1070.

Bicanic, T., Meintjes, G., Wood, R., Hayes, M., Rebe, K., Bekker, L.-G., & Harrison, T. (2007). Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. *Clinical infectious diseases, 45*(1), 76-80.

Bongomin, F., Oladele, R. O., Gago, S., Moore, C. B., & Richardson, M. D. (2018). A systematic review of fluconazole resistance in clinical isolates of Cryptococcus species. *Mycoses, 61*(5), 290-297. doi:10.1111/myc.12747

Boulware, D. R., Meya, D. B., Muzoora, C., Rolfes, M. A., Huppler Hullsiek, K., Musubire, A., . . . Meintjes, G. (2014). Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med, 370*(26), 2487-2498. doi:10.1056/NEJMoa1312884

Butler, E. K., Boulware, D. R., Bohjanen, P. R., & Meya, D. B. (2012). Long term 5-year survival of persons with cryptococcal meningitis or asymptomatic subclinical antigenemia in Uganda. *PLoS One, 7*(12), e51291.

Castelnuovo, B., Manabe, Y. C., Kiragga, A., Kamya, M., Easterbrook, P., & Kambugu, A. (2009). Cause-specific mortality and the contribution of immune reconstitution inflammatory syndrome in the first 3 years after antiretroviral therapy initiation in an urban African cohort. *Clin Infect Dis, 49*(6), 965-972. doi:10.1086/605500

Chang, Y. C., Khanal Lamichhane, A., & Kwon-Chung, K. J. (2018). Cryptococcus neoformans, Unlike Candida albicans, Forms Aneuploid Clones Directly from Uninucleated Cells under Fluconazole Stress. *MBio, 9*(6). doi:10.1128/mBio.01290-18

Chimowa, T., King, I., Tam, P. I., & Gonzalez-Martinez, C. (2017). Cryptococcal meningitis in a previously healthy child. *Malawi Med J, 29*(4), 330-331. doi:10.4314/mmj.v29i4.10

French, N., Gray, K., Watera, C., Nakiyingi, J., Lugada, E., Moore, M., . . . Gilks, C. F. (2002). Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *Aids, 16*(7), 1031-1038.

Hajjeh, R. A. (1999 febuary). cryptococcosis; population based multistate active surveillance and risk factors in human deficiency virus infected persons.cryptococcal active surveillance group. *the journal of infectious diseases, 179*(2), 449-454.

Hajjeh, R. A., Group, C. A. S., Conn, L. A., Group, C. A. S., Stephens, D. S., Group, C. A. S., . . . Group, C. A. S. (1999). Cryptococcosis: Population-Based Multistate Active Surveillance and Risk Factors in Human Immunodeficiency Virus—Infected Persons. *The Journal of Infectious Diseases, 179*(2), 449-454. doi:10.1086/314606 %J The Journal of Infectious Diseases

Hakim, J. G., Gangaidzo, I. T., Heyderman, R. S., Mielke, J., Mushangi, E., Taziwa, A., . . . Mason, P. R. (2000). Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *Aids, 14*(10), 1401-1407.

Hansen, J., Slechta, E. S., Gates-Hollingsworth, M. A., Neary, B., Barker, A. P., Bauman, S., . . . Hanson, K. E. (2013). Large-scale evaluation of the immuno-mycologics lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccine Immunol, 20*(1), 52-55. doi:10.1128/cvi.00536-12

Harrison T R, W. R., MM Wintrobe, JB Martin, RG Petersdorf. (2016). HARRISONS PRINCIPLES OF INTERNAL MEDICINE. *1*.

INSTITUTE, C. A. L. S. (2016). PERFOMANCE STANDARDS FOR ANTIMICROBIAL TESTING. *AMERICAN, 26*.

Jarvis, J. N., Lawn, S. D., Vogt, M., Bangani, N., Wood, R., & Harrison, T. S. (2009). Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis, 48*(7), 856-862. doi:10.1086/597262

Jarvis, J. N., Lawn, S. D., Vogt, M., Bangani, N., Wood, R., & Harrison, T. S. (2009). Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clinical infectious diseases, 48*(7), 856-862.

Jarvis, J. N., Lawn, S. D., Vogt, M., Bangani, N., Wood, R., & Harrison, T. S. J. C. I. D. (2009). Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *48*(7), 856-862.

Joshua Rheins, K. H. H., Emily E Evans, Lillian Tugume, Edwin Nuwagira, Kenneth S. (2017). Detrimental outcomes of unmasking cryptococcal meningitis with recent ART initiation.

Kambugu, A., Meya, D. B., Rhein, J., O'Brien, M., Janoff, E. N., Ronald, A. R., . . . Boulware, D. R. (2008). Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis, 46*(11), 1694-1701. doi:10.1086/587667

Katchanov, J., Jefferys, L., Tominski, D., Wöstmann, K., Slevogt, H., Arastéh, K., & Stocker, H. (2015). Cryptococcosis in HIV-infected hospitalized patients in Germany: Evidence for routine antigen testing. *Journal of Infection, 71*(1), 110-116.

Kizza, H. M., Oishi, K., Mitarai, S., Yamashita, H., Nalongo, K., Watanabe, K., . . . Mugerwa, R. J. C. I. D. (1998). Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. *26*(6), 1362-1366.

Letang, E., Muller, M. C., Ntamatungiro, A. J., Kimera, N., Faini, D., Furrer, H., . . . Glass, T. R. (2015). Cryptococcal Antigenemia in Immunocompromised Human Immunodeficiency Virus Patients in Rural Tanzania: A Preventable Cause of Early Mortality. *Open Forum Infect Dis, 2*(2), ofv046. doi:10.1093/ofid/ofv046

Li, Y., Fang, W., Jiang, W., Hagen, F., Liu, J., Zhang, L., . . . Pan, W. (2017). Cryptococcosis in patients with diabetes mellitus II in mainland China: 1993-2015. *Mycoses, 60*(11), 706-713. doi:10.1111/myc.12645

Liao, T. L., Chen, Y. M., & Chen, D. Y. (2016). Risk factors for cryptococcal infection among patients with rheumatoid arthritis receiving different immunosuppressive medications. *Clin Microbiol Infect, 22*(9), 815 e811-815 e813. doi:10.1016/j.cmi.2016.05.030

Lin, K. H., Chen, C. M., Chen, T. L., Kuo, S. C., Kao, C. C., Jeng, Y. C., & Ho, M. W. (2016). Diabetes mellitus is associated with acquisition and increased mortality in HIV-uninfected patients with cryptococcosis: A population-based study. *J Infect, 72*(5), 608-614. doi:10.1016/j.jinf.2016.01.016

Lortholary, O., Fontanet, A., Mémain, N., Martin, A., Sitbon, K., Dromer, F., & Aids, F. C. S. G. J. (2005). Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. *19*(10), 1043-1049.

Lortholary, O., Poizat, G., Zeller, V., Neuville, S., Boibieux, A., Alvarez, M., . . . Chêne, G. (2006). Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *Aids, 20*(17), 2183-2191.

Mathiesen, I. H., Knudsen, J. D., Gerstoft, J., Cowan, S., & Benfield, T. (2012). Outcome of HIV-1-associated cryptococcal meningitis, Denmark 1988-2008. *Scand J Infect Dis, 44*(3), 197-200. doi:10.3109/00365548.2011.611168

Mathiesen, I. H. M., Knudsen, J. D., Gerstoft, J., Cowan, S., & Benfield, T. J. S. j. o. i. d. (2012). Outcome of HIV-1-associated cryptococcal meningitis, Denmark 1988–2008. *44*(3), 197-200.

Maziarz, E. K., & Perfect, J. R. (2016). Cryptococcosis. *Infectious disease clinics of North America, 30*(1), 179-206. doi:10.1016/j.idc.2015.10.006

McKenney, J., Smith, R. M., Chiller, T. M., Detels, R., French, A., Margolick, J., & Klausner, J. D. (2014). Prevalence and correlates of cryptococcal antigen positivity among AIDS patients--United States, 1986-2012. *MMWR. Morbidity and mortality weekly report, 63*(27), 585-587.

Meya, D. B., Manabe, Y. C., Castelnuovo, B., Cook, B. A., Elbireer, A. M., Kambugu, A., . . . Boulware, D. R. J. C. I. D. (2010). Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count≤ 100 cells/μ L who start HIV therapy in resource-limited settings. *51*(4), 448-455.

Micol, R., Lortholary, O., Sar, B., Laureillard, D., Ngeth, C., Dousset, J.-P., . . . Dromer, F. (2007a). Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. *JAIDS Journal of Acquired Immune Deficiency Syndromes, 45*(5), 555-559.

Micol, R., Lortholary, O., Sar, B., Laureillard, D., Ngeth, C., Dousset, J.-P., . . . Dromer, F. J. J. J. o. A. I. D. S. (2007b). Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. *45*(5), 555-559.

Micol, R., Tajahmady, A., Lortholary, O., Balkan, S., Quillet, C., Dousset, J.-P., . . . Yazdanpanah, Y. J. P. o. (2010). Cost-effectiveness of primary prophylaxis of AIDS associated cryptococcosis in Cambodia. *5*(11), e13856.

Micol, R., Tajahmady, A., Lortholary, O., Balkan, S., Quillet, C., Dousset, J. P., . . . Yazdanpanah, Y. (2010). Cost-effectiveness of primary prophylaxis of AIDS associated cryptococcosis in Cambodia. *PLoS One, 5*(11), e13856. doi:10.1371/journal.pone.0013856

Mirza, S. A., Phelan, M., Rimland, D., Graviss, E., Hamill, R., Brandt, M. E., . . . Baughman, W. (2003). The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992–2000. *Clinical infectious diseases, 36*(6), 789-794.

MOH. (2015). UGANDA NATIONAL TUBERCULOSIS PREVALENCE SURVEY REPORT.
MoH. (2016a). uganda clinical guidelines. *4*, 256.
MoH. (2016b). Uganda clinical guidlines 2016.
MOH. (2018). UCG 2018 UPDATES.
MoH. (2020). Ugandan Consolidated HIV clinical guidelines 2020.
Mpoza, E., Rhein, J., & Abassi, M. J. M. m. c. r. (2018). Emerging fluconazole resistance: implications for the management of cryptococcal meningitis. *19*, 30-32.
Ok Atilgan, A., Ozdemir, B. H., Kirnap, M., Akdur, A., Yilmaz Akcay, E., Akar Ozkan, E., & Haberal, M. (2014). Invasive fungal infections in liver transplant recipients. *Exp Clin Transplant, 12 Suppl 1*, 110-116.
Oladele, R. O., Akanmu, A. S., Nwosu, A. O., Ogunsola, F. T., Richardson, M. D., & Denning, D. W. (2016). Cryptococcal Antigenemia in Nigerian Patients With Advanced Human Immunodeficiency Virus: Influence of Antiretroviral Therapy Adherence. *Open Forum Infect Dis, 3*(2), ofw055. doi:10.1093/ofid/ofw055
Oyella, J., Meya, D., Bajunirwe, F., & Kamya, M. R. J. J. o. t. I. A. S. (2012). Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda: a cross-sectional study. *15*(1), 15.
Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G., & Chiller, T. M. (2009a). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *Aids, 23*(4), 525-530.
Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G., & Chiller, T. M. J. A. (2009b). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *23*(4), 525-530.
Patel, S., Shin, G. Y., Wijewardana, I., Vitharana, S. R., Cormack, I., Pakianathan, M., . . . Bicanic, T. (2013). The prevalence of cryptococcal antigenemia in newly diagnosed HIV patients in a Southwest London cohort. *J Infect, 66*(1), 75-79. doi:10.1016/j.jinf.2012.09.014
Posteraro, B., Sanguinetti, M., Sanglard, D., La Sorda, M., Boccia, S., Romano, L., . . . Fadda, G. (2003). Identification and characterization of a Cryptococcus neoformans ATP binding cassette (ABC) transporter-encoding gene, CnAFR1, involved in the resistance to fluconazole. *Mol Microbiol, 47*(2), 357-371.
R.Parkes Rantanshi, B. A., R.Kwizera, A.Kambugu, D.Meya, D.W. Denning. (2015). Cryptococcal disease burden and other fungal disease in Uganda, where are the knowledge gaps and how can we fill them?
Rajasingham, R., Smith, R. M., Park, B. J., Jarvis, J. N., Govender, N. P., Chiller, T. M., . . . Boulware, D. R. (2017). Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis, 17*(8), 873-881. doi:10.1016/s1473-3099(17)30243-8
Rajasingham, R., Smith, R. M., Park, B. J., Jarvis, J. N., Govender, N. P., Chiller, T. M., . . . Boulware, D. R. (2017). Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *The Lancet infectious diseases, 17*(8), 873-881.
Ramachandran, A., Manabe, Y., Rajasingham, R., & Shah, M. (2017). Cost-effectiveness of CRAG-LFA screening for cryptococcal meningitis among people living with HIV in Uganda. *BMC Infect Dis, 17*(1), 225. doi:10.1186/s12879-017-2325-9
Rana, A. (1999). cryptococcosis; population-based multistate active surveillance and risk facors in human immune virus infected persons. *the journal of infectious diseases, 179*(2), 449-454.

Reardon, C. C., Kim, S. J., Wagner, R. P., Koziel, H., & Kornfeld, H. (1996). Phagocytosis and growth inhibition of Cryptococcus neoformans by human alveolar macrophages: effects of HIV-1 infection. *Aids, 10*(6), 613-618.

Reepalu, A., Balcha, T. T., Yitbarek, T., Jarso, G., Sturegard, E., & Bjorkman, P. (2015). Screening for cryptococcal antigenemia using the lateral flow assay in antiretroviral therapy-naive HIV-positive adults at an Ethiopian hospital clinic. *BMC Res Notes, 8*, 702. doi:10.1186/s13104-015-1707-6

Rugemalila, J., Maro, V. P., Kapanda, G., Ndaro, A. J., & Jarvis, J. N. (2013). Cryptococcal antigen prevalence in HIV-infected Tanzanians: a cross-sectional study and evaluation of a point-of-care lateral flow assay. *Tropical medicine & international health, 18*(9), 1075-1079.

Sar, B., Monchy, D., Vann, M., Keo, C., Sarthou, J. L., & Buisson, Y. J. J. o. A. C. (2004). Increasing in vitro resistance to fluconazole in Cryptococcus neoformans Cambodian isolates: April 2000 to March 2002. *54*(2), 563-565.

Smith, K. D., Achan, B., Hullsiek, K. H., McDonald, T. R., Okagaki, L. H., Alhadab, A. A., . . . Nielsen, K. (2015). Increased Antifungal Drug Resistance in Clinical Isolates of Cryptococcus neoformans in Uganda. *Antimicrob Agents Chemother, 59*(12), 7197-7204. doi:10.1128/aac.01299-15

Sungkanuparph, S., Filler, S. G., Chetchotisakd, P., Pappas, P. G., Nolen, T. L., Manosuthi, W., . . . Kopetskie, H. J. C. i. d. (2009). Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococcal meningitis: a prospective multicenter study. *49*(6), 931-934.

Takahara, D. T., Lazera Mdos, S., Wanke, B., Trilles, L., Dutra, V., Paula, D. A., . . . Hahn, R. C. (2013). First report on Cryptococcus neoformans in pigeon excreta from public and residential locations in the metropolitan area of Cuiaba, State of Mato Grosso, Brazil. *Rev Inst Med Trop Sao Paulo, 55*(6), 371-376. doi:10.1590/s0036-46652013000600001

Takahara, D. T., Lazéra, M. d. S., Wanke, B., Trilles, L., Dutra, V., Paula, D. A. J. d., . . . Pereira, D. J. R. d. I. d. M. T. d. S. P. (2013). First report on Cryptococcus neoformans in pigeon excreta from public and residential locations in the metropolitan area of Cuiabá, State of Mato Grosso, Brazil. *55*(6), 371-376.

Vena, A., Munoz, P., Guinea, J., Escribano, P., Pelaez, T., Valerio, M., . . . Bouza, E. (2018). Fluconazole resistance is not a predictor of poor outcome in patients with cryptococcosis. *Mycoses*. doi:10.1111/myc.12847

WHO. (March 2018). Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV infectected adults, adoloscents and children.