© Copyright 2019

Mark Wilson Tenforde

The Epidemiology, Outcomes, and Prevention of HIV-associated Meningitis in Southern Africa

Mark Wilson Tenforde

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2019

Reading Committee:

Carey Farquhar, Chair

Brandon L. Guthrie

Joseph N. Jarvis

Program Authorized to Offer Degree:

Public Health-Epidemiology

University of Washington

Abstract

The epidemiology, outcomes, and prevention of HIV-associated meningitis in southern Africa

Mark Wilson Tenforde

Chair of the Supervisory Committee:

Professor Carey Farquhar MD, MPH

Departments of Epidemiology, Global Health, and Medicine

Central nervous system infection are a primary cause of HIV-associated morbidity and mortality worldwide. In this dissertation, I discuss results from research conducted in Botswana, a country in southern Africa with an HIV prevalence that is among the highest in the world, including the first comprehensive meningitis national incidence estimates in a high HIV prevalence country and temporal trends over 16 years with national scale-up of ART. Linking cerebrospinal fluid (CSF) laboratory records of patients evaluated with meningitis to HIV and vital status records from national electronic registries, I provide novel insights into HIV correlates and outcomes from suspected and microbiologically-confirmed meningitis. I compare local findings in Botswana to other settings in sub-Saharan Africa through a systematic review and meta-analysis. Finally, I discuss estimates of the impact and cost-effectiveness of preventing cryptococcal meningitis - the most common cause of meningitis we observed in Botswana - through cryptococcal antigen (CrAg) screening and targeted pre-emptive antifungal therapy for individuals with advanced HIV.

In the first two studies (Chapters 2-3), I discuss results from the Botswana National Meningitis Survey, a 16-year national cross-sectional audit of patients evaluated for meningitis by lumbar puncture with CSF analysis from 2000-2015, including complete national records for 2013 and 2014. Forty-one percent (8759/21,560) of cases of suspected meningitis in adults had abnormal cerebrospinal fluid (CSF) findings, half of these receiving a diagnosis of cryptococcal meningitis. Other causes of meningitis were rarely identified. Most evaluations for meningitis were in individuals with known HIV (73%), almost half (47%) of these with a preceding history of ART use. Using UNAIDS population data, we estimated a 2013-2014 national cryptococcal meningitis incidence of 17.8 cases / 100,000 person-years (PYO), or 96.8 cases / 100,000 PYO in HIV-positive, nearly identical to estimates from Gauteng Province, South Africa in the pre-ART era of 2002-2004. Incidence was two-fold higher in HIV-positive men than women.

In the third study (Chapter 4), I evaluated outcomes of "culture-positive" (including cryptococcal, pneumococcal, or TB) and culture-negative suspected meningitis (stratified by CSF white cell count [WCC]) in a retrospective cohort study. This was done deterministic linkage of CSF to vital status records in a national death registry with a unique national identification number ("Omang"). Estimated mortality for cryptococcal meningitis under routine care settings in Botswana was 50% at 10 weeks and 65% at one year with amphotericin B-based therapy, similar to mortality estimated in sub-Saharan Africa with less potent fluconazole therapy. Missed doses of amphotericin were common and independently predicted poor outcomes, while adherence to interventions usually associated with improved outcomes such as therapeutic lumbar punctures was poor. One-year mortality for culture-negative, pneumococcal, and TB meningitis were 49%, 49%, and 56%, respectively. For HIV-associated cases without a cause identified, CSF inflammation (CSF WCC >20 cells/μL vs. ≤20 cells/μL) was not a predictor of mortality in the setting of advanced immune-deficiency (median CD4 136 cells/μL).

In the fourth study (Chapter 5), I describe results from a systematic review and meta-analysis evaluating published mortality estimates for common HIV-associated meningitides in sub-Saharan Africa, under routine care conditions and "clinical trial" settings subject to more intensive clinical management. Pooled short-term mortality (defined as ≤2 weeks or in-hospital) for cryptococcal meningitis under routine care settings was 44% (95%CI: 39-49%, 40 studies) with no difference in mortality when stratified by treatment (amphotericin vs. fluconazole monotherapy). This contrasts with pooled mortality of 21% (95%CI: 17-26%, 17 studies) from controlled trial settings. Short-term mortality from pneumococcal and TB meningitis under routine settings were estimated at 55% (95%CI: 44-66%, 8 studies) and 48% (95%CI:35-61%, 10 studies), respectively. Few studies reported long-term outcomes.

In the fifth study (Chapter 6), I discuss results from a modeling analysis. I estimated the impact and cost-effectiveness of CrAg screening and targeted pre-emptive antifungal treatment for CrAg-positive patients for the prevention of HIV-associated cryptococcal meningitis. Using local CD4 count, CrAg prevalence, outcomes, costing, and national incidence estimates, implementation of CrAg screening in patients with a CD4 T-cell count <100 cells/μL was found to be highly cost-effective and estimated to prevent one-quarter of cryptococcal meningitis deaths. I explored treatment of ART-experienced patients, not the target population in guidelines but increasingly recognized with cryptococcal infection through laboratory-based CrAg screening. Screening and pre-emptive treatment in this population remained highly cost-effective and prevented additional deaths. CrAg screening at a higher CD4 count thresholds (100-200 cells/μL), now conditionally recommended by the World Health Organization, had little impact and was significantly less cost-effective, with an estimated cost of nearly US$15,000 per death averted in Botswana (although still cost effective per World Health Organization standards).

This research has important public health implications. First, the findings highlight that cryptococcal meningitis remains a key cause of HIV-associated mortality well into the ART era in southern Africa and now commonly occurs in patients previously established in HIV care services. Despite widespread ART availability, incidence remained high through 2013-2014 and almost half of cases occurred in patients with previous ART use. Improved strategies for care and retention in HIV-treatment services are warranted, particularly for key at-risk populations (e.g. working-age men). Secondly, these findings support nationwide scale-up of CrAg screening for individuals with advanced HIV in Botswana with CD4 count <100 cells/μL, as well as new guideline recommendations for treating the emerging population of ART-experienced patients with early cryptococcal infection increasingly recognized in mature ART programs. Given the high cost and

modest impact of CrAg screening at higher CD4 counts, policy decisions will need to weigh the benefits against other health system priorities. Thirdly, under routine care settings I showed similar high mortality with amphotericin B based treatment for cryptococcal meningitis compared to less potent antifungal treatment with fluconazole. These findings suggest that simplified cryptococcal meningitis treatment strategies, such as with less toxic, shorter-course regimens, may be necessary to reduce mortality and to complement prevention efforts through CrAg screening and other upstream measures to prevent incident disease. Finally, a majority of suspected meningitis cases have no cause identified through laboratory testing. Typical prognostic markers, e.g. CSF WCC, are poor predictors of mortality in the context of advanced HIV, and mortality is estimated around 50% at one year. These novel findings support use of enhanced meningitis diagnostic strategies, particularly routine use of available TB molecular diagnostics (e.g. Xpert Ultra), and a need to better define etiologies and treatment strategies to improve survival.

TABLE OF CONTENTS

List of Figures v

List of Tables vi

Chapter 1. Introduction 11

1.1 The burden of HIV-associated meningitis and temporal changes in the era of antiretrovirla therapy 11

1.2 Recognizing advanced HIV disease to reduce HIV-related mortality 12

1.3 Understanding outcomes from HIV-associated meningitis in order to improve care .. 14

1.4 Motivation and public health impact 15

1.5 Remaining chapter 16

1.6 References 16

Chapter 2. Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern africa: results from the botswana national meningitis survey 23

2.1 Manuscript title and title page 23

2.2 Abstract 25

2.3 Introduction 26

2.4 Methods 28

2.5 Results 32

2.6 Discussion 35

2.7 Conclusion 38

2.8 Acknowledgments 38

2.9 References 39

Chapter 3. Advanced hiv disease in botswana following successful antiretroviral therapy rollout: incidence and temporal trends in cryptococcal meningitis 51

3.1 Manuscript title and title page 51

3.2 Abstract 54

3.3 Introduction 55

3.4 Methods 56

3.5 Results 60

3.6 Discussion 62

3.7 Conclusion 65

3.8 Acknowledgments 66

3.9 References 67

Chapter 4. Mortality outcomes in culture-positive and culture-negative adult meningitis in the botswana national meningitis survey: a prevalent cohort study 81

4.1 Manuscript title and title page 81

4.2 Abstract 83

4.3 Introduction 84

4.4 Materials and methods 85

4.5 Results 89

4.6 Discussion 92

4.7 Conclusion 97

4.8 Acknowledgements 98

4.9 References 99

Chapter 5. Mortality from hiv-associated meningitis in sub-saharan africa: a systematic review and meta-analysis 110

5.1 Manuscript title and title page 110

5.2 Abstract 111

5.3 Introduction 113

5.4 Materials and methods 115

5.5 Results 119

5.6 Discussion 127

5.7 Conclusion 130

5.8 Acknowledgements 131

5.9 References 131

Chapter 6. Cost-effectiveness of reflex laboratory-based cryptococcal antigen screening for the prevention of cryptococcal meningtiis in botswana 158

6.1 Manuscript title and title page 158

6.2 Abstract 159

6.3 Introduction 161

6.4 Materials and methods 163

6.5 Results 169

6.6 Discussion 171

6.7 Conclusion 173

6.8 Acknowledgements 174

6.9 References 174

Chapter 7. Conclusions and way forward 199

7.1 Addressing gaps in the HIV care cascade 199

7.2 Implementation of differentiated care models for advanced HIV disease 200

7.3 The shifting epidemiology of central nervous system infections present new challenges and a need for innovative solutions 201

7.4 Central nervous system infections are a major cause of HIV-related mortality: better strategies are needed to combat cryptococcal disease 202

7.5 Improving meningitis diagnostics and treatment strategies 204

7.6 References 205

LIST OF FIGURES

Figure 1. Overall description of 21,560 adult cases. Of 34,505 overall lumbar puncture assessments, 7,238 samples from children, 4,014 with undocumented age, and 1,693 repeat lumbar punctures excluded. 48

Figure 2. Trends in meningitis cases at Botswana national referral hospitals, 2004-2014.49

Figure 3. (A) Culture-confirmed pneumococcal meningitis cases, (B) average rainfall (centimeters), and (C) average temperature (Celsius) by month 50

Figure 4. Age distribution of cryptococcal meningitis cases in Botswana, 2000-2014.**Error! Bookmark not defined.**

Figure 5. Incidence of cryptococcal meningitis in Botswana by age category, 2013-2014. 2A) Overall incidence and interquartile range; 2B) Incidence by gender (males solid bars and females striped bars); 2C) Incidence in HIV-infected population.**Error! Bookmark not defined.**

Figure 6. Incidence of cryptococcal meningitis in Botswana in HIV-infected population by CD4 strata, 2013-2014. 3A) Overall incidence; 3B) Incidence by gender (males black lines, females grey lines). .....**Error! Bookmark not defined.**

Figure 7. Trends in diagnosed cases of cryptococcal meningitis at the 2 national referral hospitals in Botswana, 2004-2015. 4A) Cases diagnosed at referral hospitals (thick black line) and treatment gap in adults (≥15 years) [shaded grey area]; 4B) UNAIDS estimate of median CD4 count at antiretroviral therapy initiation in Botswana; 4C) UNAID estimate of number of HIV-infected individuals not on antiretroviral therapy in Botswana.**Error! Bookmark not defined.**

Figure 8. Flowchart for inclusion of culture-negative meningitis cases. The Botswana National Meningitis Survey (BNMS) includes data on 29,704 CSF samples from 2004-2015, of which 19,409 (65·3%) are in the Integrated Patient Management System (IPMS). Cases were selected from culture-negative samples recorded in the IPMS aged 16 years or over with a valid national identification number (Omang). Random selections of 1000 samples were made from those with cerebrospinal fluid (CSF) white cell counts (WCC) of 0-2μ/L and 3-20/μL. All samples with CSF WCC >20/μL were selected. Selected samples were

deduplicated to remove multiple CSF samples taken during the same hospital admission, and any culture-negative samples with organisms seen on microscopy. 107
Figure 9. Kaplan-Meier survival curves for: Cryptococcal meningitis (2012-2014), pneumococcal meningitis, TB meningitis, and culture-negative cases (no pathogen identified). Dashed vertical lines represent two weeks, ten weeks, and one year and number at risk values presented at 60-day intervals. Cryptococcal meningitis figures are previously published 2012-2014 referral hospital outcomes for comparison.**[97]** 108
Figure 10. Kaplan-Meier survival curves for A) Culture-negative cases (no pathogen identified) with CSF WCC 0-2/μL, WCC 3-20/μL, and WCC >20/μL; B) culture-negative cases with CSF WCC >20/μL by neutrophil versus lymphocyte (≥50% lymphocytes) predominance. Dashed vertical lines represent two weeks, ten weeks, and one year and number at risk values presented at 60-day intervals. Cumulative mortality at 2 weeks, 10 weeks, and one year were: WCC 0-2/μL 29% (254/888), 41% (363/888), and 48% (428/888) respectively; WCC 3-20/μL 29% (230/803), 39% (315/803), and 49% (391/803) respectively; overall WCC >20/μL 29% (589/1209), 42% (503/1209), and 49% (589/1209) respectively; WCC >20/μL and polymorphonuclear 42% (110/265), 55% (145/265), and 61% (162/265) respectively; and WCC >20/μL and lymphocytic 24% (179/734), 36% (261/734), and 43% (314/734) respectively. Note that a proportion of patients with WCC >20 cells/μL did not have a WCC differential. 109
Figure 11. PubMed search strategy..... 152
Figure 12. PRISMA diagram 153
Figure 13. Overall pooled short-term mortality of cryptococcal meningitis in routine care settings. 154
Figure 14. Pooled short-term mortality of cryptococcal meningitis in clinical trial settings155
Figure 15. Pooled short-term mortality of TB meningitis in routine care settings 156
Figure 16. Pooled short-term mortality of pneumococcal meningitis in routine care settings 157
Figure 17. Flowcharts for decision analytic model including screening and treatment modules. 186

LIST OF TABLES

Table 1. Clinical and laboratory characteristics of patients evaluated for meningitis. 45

Table 2. Botswana national incidence of adult (≥15 years) meningitis, 2013-2014..... 47

Table 3. Description of cryptococcal meningitis cases in Botswana, 2000-2014. .... **Error! Bookmark not defined.**

Table 4. National incidence of cryptococcal meningitis in Botswana, 2013-2014. .. **Error! Bookmark not defined.**

Table 5. Baseline clinical and laboratory characteristics and mortality outcomes 104

Table 6. Predictors of mortality for known HIV-infected patients without pathogen identified. 105

Table 7. Cox proportional hazard model associations with mortality in known HIV-infected patients with CSF without pathogen identified and CSF WCC >20 cells/μL 106

Table 8. Key parameters, estimates, and sources of data for base model. 180

Table 9. Included cost estimates for CrAg screening and pre-emptive treatment and for cryptococcal meningitis treatment 181

Table 10a. Screening CD4 <100 cells/μL, ART-naïve for base (white) and sensitivity (gray) models. 182

Table 11a. Screening CD4 100-200 cells/μL, ART-naïve for base (white) and sensitivity (gray) models.. 184

ACKNOWLEDGEMENTS

I would like to offer my sincere thanks and appreciation to my dissertation committee, including Chair Carey Farquhar, MD, MPH, Members Brandon Guthrie PhD, MPH and Joseph Jarvis BSc, MSc, MBBS, MRCP, PhD, DTMH, and Graduate Student Representative James Hughes PhD, as well as mentorship for the cost modeling study from Bruce Larson PhD, Research Professor at the Boston University School of Public Health.

Most of the dissertation research studies described in here were developed under the mentorship of Joseph Jarvis, Professor at the London School of Hygiene and Tropical Medicine, while I was an Afya Bora Global Health Leadership Fellow based in Gaborone from 2014-2015 with the Botswana-UPenn Partnership. Special thanks goes to the entire Afya Bora Consortium for your support and mentorship, as well as mentors at the Botswana UPenn Partnership, and particularly Andrew Steenhoff BMMCh, DCH.

This dissertation work continued as a Fellow in Infectious Diseases in the Division of Allergy and Infectious Diseases (AID) of the University of Washington School of Medicine. I am deeply appreciative of all of the support I received within AID and for AID leadership providing me with flexibility to pursue this PhD training during infectious disease fellowship training; special thanks goes to David Fredricks MD, Wesley van Voorhis MD, PhD, Paul Pottinger MD DTMH, Anna Wald MD, MPH, Alison Wald MSc, and Mikasa Morf. Within the University of Washington School of Public Health, I am grateful to have the opportunity to learn from such inspiring faculty and the commitment within Epidemiology of teaching future leaders in public health.

I would like to recognize the large number of collaborators we had for these studies at the Botswana Ministry of Health and Wellness, Botswana National Health Laboratory, Botswana hospital and laboratory facilities we visited for data collection, the Botswana-UPenn Partnership, Botswana-Harvard AIDS Initiative, and the University of Botswana.

I would also like to acknowledge funding support from the Afya Bora Consortium through a Career Development Award which in part supported this work (U91HA06801B), the National Institutes of Health (NIH) Host Defense training grant (T32AI007044, P.I. Wesley van Voorhis MD, PhD) that provided me with support from 2016-2018, and additional training grant support from the NIH through an individual post-doctoral fellowship (F32AI140511) from 2018-present.

Finally, I would like to thank my family including my parents, brother (Adam), wife (Alida), mother-in-law (Caroline), and daughter (Coraline), for their love and support through the years.

DEDICATION

This dissertation is dedicated to the people of Botswana, a country where I have spent a majority of my time over the past 5 years. I have been humbled by the kindness and open arms I have been greeted with. The dissertation is also dedicated to my daughter, Coraline, who helps me to see the world through fresh eyes.

Chapter 1. INTRODUCTION

1.1 THE BURDEN OF HIV-ASSOCIATED MENINGITIS AND TEMPORAL CHANGES IN THE ERA OF ANTIRETROVIRAL THERAPY

Central nervous system (CNS) infections are common HIV-associated opportunistic infections in resource-limited settings and a leading cause of mortality in people living with HIV/AIDS (PLWH) [1-3]. Cryptococcal meningitis, which typically occurs in the setting of profound immune deficiency from HIV/AIDS, causes an estimated 15% of global HIV deaths [1]. The risk of TB, the most common cause of mortality in PLWH, increases with advanced HIV/AIDS and meningitis complicating up to 5% of extra-pulmonary disease [4]. The risk of other opportunistic infections, as well as traditional CNS pathogens such as *Streptococcus pneumoniae*, dramatically increases with HIV-related immune deficiency [5,6].

Understanding epidemiological shifts in CNS infections corresponding with increasing ART availability can serve as an "indicator" of a country's HIV care cascade, including successful HIV diagnosis and linkage-to-care, ART initiation, and sustained viral suppression. This is particularly true for cryptococcal meningitis, which unlike many other HIV-related infections is easily diagnosed using sensitive and widely available diagnostics [7]. In high-income countries, an estimated 5-10% of HIV-infected individuals developed cryptococcal meningitis in the pre-ART era [8]. With rollout of combined ART in the 1990s, the burden of HIV-associated cryptococcal meningitis decreased dramatically. A U.S. population-based audit using State Inpatient Databases records observed a 53.6% decline in HIV-associated cryptococcal meningitis hospitalizations between 1997 and 2009 [9], with a similar 46% decline observed in a French national surveillance study from the pre-ART era (1985-1996) to early post-ART era (1997-2001)

[10]. However, similar declines are yet to be observed in sub-Saharan Africa. Single-center studies in South Africa from and Malawi have observed a relatively stable burden of microbiologically-diagnosed cryptococcal meningitis with early rollout of ART, and sub-national data in Gauteng Province, South Africa, observed only a modest decline in incidence from 172.8 cases / 100,000 PYO to 144.7 cases / 100,000 PYO between 2009 and 2012 [11].

Understanding the epidemiology of cryptococcal meningitis in Botswana serves as an important indicator for the persistence of advanced HIV disease in sub-Saharan Africa following successful ART rollout with high coverage. Botswana established the first national ART program in Africa in 2002 and serves as a regional model. Data from the Botswana Combination Prevention Project (BCPP) estimated that over 70% of HIV-infected patients in parts of the country are now accessing HIV care and virologically suppressed [12]. However, programmatic data in Botswana and recent research from our group suggest that about one-quarter of patients may delay ART initiation until they have advanced HIV/AIDS (CD4 <200 cells/μL) [13,14], with treatment default also common [15]. Important gaps therefore remain in understanding the persistence and impact of advanced HIV disease in Botswana and regionally as the HIV epidemic matures.

1.2 Recognizing advanced HIV disease to reduce HIV-related mortality

Delays in initiation of ART with advanced HIV/AIDS is associated with high mortality during early months on ART. A systematic review of published observational study data from 1996 through 2010 estimated a pooled mortality of 17% (95%CI: 10-24%) during the first year on ART in sub-Saharan Africa [16]. Cryptococcal meningitis (3-18% of deaths) and tuberculosis (5-44% of deaths) were among the most commonly reported infectious causes of death. Published programmatic data during the early ART era in Botswana supports these findings: Of patients

starting ART between 2002 and 2010, estimated mortality was 12.8 deaths / 100 PYO during the first 3 months of ART but fell to 1.6 deaths / 100 PYO by the second year on ART [17].

With recognition of the importance of advanced HIV disease as a major driver of HIV-related deaths in sub-Saharan Africa, the World Health Organization has highlighted the importance of differentiated care models for patients with advanced HIV disease, with a focus on recognizing and treating cryptococcal infection and TB as major causes of death [18]. Asymptomatic cryptococcal infection can be recognized in the blood through a highly sensitive cryptococcal antigen (CrAg) lateral flow assay [19]. Evidence suggests that CrAg screening and targeted high-dose fluconazole therapy reduces both incident cryptococcal meningitis and all-cause mortality. The REMSTART trail in Tanzania finding that adults with advanced HIV receiving CrAg screening coupled with lay community support had a 29% reduction in one-year mortality [20]. On the basis of existing evidence, the World Health Organization recommends CrAg screening in ART-naïve adults with advanced HIV/AIDS [21,22]. On the basis of these guidelines and findings from our CrAg prevalence and screening feasibility pilot study [23], CrAg screening for ART-naïve patients with a CD4 ≤100 cells/μL was recommended in Botswana national HIV guidelines although it has yet to be widely implemented [24].

To promote screening scale-up, several studies have established cost-effectiveness of CrAg screening in other African countries [25-28]. However, data on cost-effectiveness in Botswana are lacking and important for promotion of broader scale-up. Modelling the cost-effectiveness and impact of CrAg screening in Botswana is also important in light of two recent developments, including: 1) The rollout of HIV "test-and-treat" models, with a growing proportion of PLWH starting ART without baseline CD4 testing to guide CrAg screening and treatment [18]; and 2) 2018 World Health Organization guidelines conditionally recommending screening at a higher

CD4 threshold of ≤200 cells/μL [22]. There is therefore a need to consider the cost-effectiveness and impact of CrAg screening in both ART-naïve and ART-experienced patients as well as screening at a CD4 threshold of 100-200 cells/μL to inform updated guidelines for the care of patients with advanced HIV disease.

1.3 UNDERSTANDING OUTCOMES FROM HIV-ASSOCIATED MENINGITIS IN ORDER TO IMPROVE CARE

Incident cryptoccoccal meningitis has been associated with an estimated 70% one-year case-fatality rate in sub-Saharan Africa [29,30]. This high case-fatality rate is, in part, attributed to reliance on treatment with fluconazole rather than more fungicidal amphotericin B (AmB) due to low cost and oral (rather than intravenous) administration. Botswana has been using amphotericin B for over a decade and should have relatively low mortality as observed in U.S. and other high-income country settings [31,32]. A number of factors apart from antifungal therapy used, however, are important in the management of cryptococcal meningitis and have been associated with treatment outcomes. These factors include: 1) Use of therapeutic lumbar punctures to lower elevated intracranial pressure [33]; 2) pre-treatment hydration, electrolyte supplementation, and regular laboratory monitoring to prevent and manage severe amphotericin B-related toxicities [34]; and 3) appropriate initiation of ART [35]. Long-term outcomes of cryptococcal meningitis treated with amphotericin B therapy in under-resourced, routine care conditions are not well characterized and are important to understand in deriving accurate global burden estimates as amphotericin B increasingly becomes available in resource-limited settings, as well as to inform improvements in management strategies such as incorporation of less toxic, short-course regimens [36,37].

Outcomes from other HIV-associated meningitides in sub-Saharan Africa, including TB and pneumococcal meningitis, are also poorly characterized in routine care settings. Further, almost no outcomes data has been generated for cases of meningitis without an established microbiological diagnosis. Such "culture-negative" meningitis accounts for a majority of cases in cross-sectional audits from diverse African health settings [38,39]. Reported in-hospital mortality of culture-negative lymphocyte-predominant meningitis ranged from 13-35% in two small studies of HIV-infected patients, from which limited inference can be drawn [40,41]. Furthermore, in the context of severe immune suppression with advanced HIV, traditional CSF markers of infection such as elevated CSF white cell count may be blunted [42], and poorly predict serious CNS pathology and death. Through novel linkage of patient CSF laboratory data with vital status data from a national death registry, this dissertation research establishes the first robust estimates of meningitis outcomes in sub-Saharan Africa in culture-negative meningitis, with important implication for meningitis diagnostic strategies nationally and more broadly in other high HIV prevalence resource-limited settings.

1.4 MOTIVATION AND PUBLIC HEALTH IMPACT

Central nervous system infections have a devastating impact as a leading cause of mortality in morbidity in people living with HIV worldwide [1,2]. In resource-limited countries with the major burden of HIV, no nationally-representative data exits on the epidemiology of central nervous system infections, particularly with the availability and scale-up of ART over the past decade-and-a-half. Establishing a better understanding of central nervous system infections in a mature ART program can provide valuable insights into gaps in ART coverage and retention in care for HIV programs, inform preventive efforts (e.g. through cryptococcal antigen screening and immunizations against vaccine-preventable diseases), inform diagnostic pathways for

evaluation of central nervous system infections, and guide strategies to improve the clinical management of diagnosed cases of meningitis in order to reduce central nervous system-associated mortality.

1.5 REMAINING CHAPTER

The remaining chapters of this dissertation discuss the results from the mostly completed research studies, along with a concluding chapter. The second and third chapters report results from the Botswana National Meningitis Survey on the epidemiology including 2013-2014 national incidence of meningitis in Botswana, the first under review in the *Journal of Infection* and the second published in *Clinical Infectious Diseases*. The fourth chapter describes treatment outcomes of culture-positive and culture-negative meningitis in Botswana, and has been published in *Lancet Infectious Diseases*. The fifth chapter, a systematic review of mortality from HIV-associated meningitis in sub-Saharan Africa under routine care and clinical trial conditions, is currently under review in *The Journal of the International AIDS Society*. The final chapter, evaluating the cost effectiveness and impact of cryptococcal antigen screening in Botswana, will be submitted to *PLoS One* following some refinement in several model parameters.

1.6 REFERENCES

1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017.

2. Walker AS, Prendergast AJ, Mugyenyi P, Munderi P, Hakim J, Kekitiinwa A, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and

children in Uganda and Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(12):1707-18.

3. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. Aids. 2008;22(15):1897-908.

4. Nelson CA, Zunt JR. Tuberculosis of the central nervous system in immunocompromised patients: HIV infection and solid organ transplant recipients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;53(9):915-26.

5. Harboe ZB, Larsen MV, Ladelund S, Kronborg G, Konradsen HB, Gerstoft J, et al. Incidence and risk factors for invasive pneumococcal disease in HIV-infected and non-HIV-infected individuals before and after the introduction of combination antiretroviral therapy: persistent high risk among HIV-infected injecting drug users. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(8):1168-76.

6. Jordano Q, Falcó V, Almirante B, Planes AM, del Valle O, Ribera E, et al. Invasive pneumococcal disease in patients infected with HIV: still a threat in the era of highly active antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;38(11):1623-8.

7. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerging infectious diseases. 2014;20(1):45-53.

8. Powderly WG. Cryptococcal meningitis and AIDS. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1993;17(5):837-42.

9. Pyrgos V, Seitz AE, Steiner CA, Prevots DR, Williamson PR. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PloS one. 2013;8(2):e56269.

10. Dromer F, Mathoulin-Pélissier S, Fontanet A, Ronin O, Dupont B, Lortholary O, et al. Epidemiology of HIV-associated cryptococcosis in France (1985-2001): comparison of the pre- and post-HAART eras. Aids. 2004;18(3):555-62.

11. Britz E, Perovic O, von Mollendorf C, von Gottberg A, Iyaloo S, Quan V, et al. The Epidemiology of Meningitis among Adults in a South African Province with a High HIV Prevalence, 2009-2012. PloS one. 2016;11(9):e0163036.

12. Gaolathe T, Wirth KE, Holme MP, Makhema J, Moyo S, Chakalisa U, et al. Botswana's progress toward achieving the 2020 UNAIDS 90-90-90 antiretroviral therapy and virological suppression goals: a population-based survey. Lancet HIV. 2016;3(5):e221-30.

13. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. AIDS. 2016;30(3):477-85.

14. Leeme TS MM, Lechiile K, Mosepele M, Mphoyakgosi T, Muthoga C, et al. Utility of CD4 cell count monitoring in Botswana: Analysis of routine laboratory data. CROI 2019. Seattle, WA, 2019. (oral abstract).

15. Fox MP, Rosen S. Retention of Adult Patients on Antiretroviral Therapy in Low- and Middle-Income Countries: Systematic Review and Meta-analysis 2008-2013. Journal of acquired immune deficiency syndromes. 2015;69(1):98-108.

16. Gupta A, Nadkarni G, Yang WT, Chandrasekhar A, Gupte N, Bisson GP, et al. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. PloS one. 2011;6(12):e28691.

17. Farahani M, Vable A, Lebelonyane R, Seipone K, Anderson M, Avalos A, et al. Outcomes of the Botswana national HIV/AIDS treatment programme from 2002 to 2010: a longitudinal analysis. Lancet Glob Health. 2014;2(1):e44-50.

18. World Health Organization. Guidelines for managing advanced HIV disease and early initiation of antiretroviral therapy. Available at: http://www.who.int/hiv/pub/guidelines/advanced-HIV-disease/en/. Accessed on: 5 December 2017.

19. Jarvis JN, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams GN, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;53(10):1019-23.

20. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet. 2015.

21. World Health Organization. Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: WHO; 2011.

22. World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescence and children. Geneva: WHO; 2018.

23. Lechiile K, Mitchell HK, Mulenga F, et al. Prevalence of advanced HIV disease and cryptococcal infection in Gaborone, Botswana. Presented at: CROI 2017. Seattle, WA, 2017.

24. Botswana Ministry of Health. 2016 integrated HIV clinical care guidelines. Available at: http://www.moh.gov.bw/Publications/Handbook_HIV_treatment_guidelines.pdf. Accessed 28 March 2017.

25. Meya DB, Manabe YC, Castelnuovo B, Cook BA, Elbireer AM, Kambugu A, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. Clin Infect Dis. 2010;51(4):448-55.

26. Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. PLoS One. 2013;8(7):e69288.

27. Smith RM, Nguyen TA, Ha HT, Thang PH, Thuy C, Lien TX, et al. Prevalence of cryptococcal antigenemia and cost-effectiveness of a cryptococcal antigen screening program--Vietnam. PloS one. 2013;8(4):e62213.

28. Rajasingham R, Meya DB, Greene GS, Jordan A, Nakawuka M, Chiller TM, et al. Evaluation of a national cryptococcal antigen screening program for HIV-infected patients in Uganda: A cost-effectiveness modeling analysis. PloS one. 2019;14(1):e0210105.

29. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017;17(8):873-81.

30. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. Aids. 2009;23(4):525-30.

31. Botswana Ministry of Health. Botswana national HIV/AIDS treatment guidelines: 2008 version. 2008.

32. Van Der Horst CM, Saag MS, Cloud GA, Hamill RJ, Graybill JR, Sobel JD, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. New England Journal of Medicine. 1997;337(1):15-21.

33. Rolfes MA, Hullsiek KH, Rhein J, Nabeta HW, Taseera K, Schutz C, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(11):1607-14.

34. Bahr NC, Rolfes MA, Musubire A, Nabeta H, Williams DA, Rhein J, et al. Standardized electrolyte supplementation and fluid management improves survival during amphotericin therapy for cryptococcal meningitis in resource-limited settings. Open Forum Infect Dis. 2014;1(2):ofu070.

35. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. The New England journal of medicine. 2014;370(26):2487-98.

36. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

37. Lawrence DS, Youssouf N, Molloy SF, Alanio A, Alufandika M, Boulware DR, et al. AMBIsome Therapy Induction OptimisatioN (AMBITION): High Dose AmBisome for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: Study Protocol for a Phase 3 Randomised Controlled Non-Inferiority Trial. Trials. 2018;19(1):649.

38. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC infectious diseases. 2010;10:67.

39. Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, et al. Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up and Haemophilus influenzae type b vaccination, 2000-2012. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(10):e137-45.

40. Hakim JG, Gangaidzo IT, Heyderman RS, Mielke J, Mushangi E, Taziwa A, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. Aids. 2000;14(10):1401-7.

41. Békondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D, et al. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2006;10(5):387-95.

42. Cecchini D, Ambrosioni J, Brezzo C, Corti M, Rybko A, Perez M, et al. Tuberculous meningitis in HIV-infected and non-infected patients: comparison of cerebrospinal fluid findings. Int J Tuberc Lung Dis. 2009;13(2):269-71.

Chapter 2. EPIDEMIOLOGY OF ADULT MENINGITIS DURING ANTIRETROVIRAL THERAPY SCALE-UP IN SOUTHERN AFRICA: RESULTS FROM THE BOTSWANA NATIONAL MENINGITIS SURVEY

2.1 MANUSCRIPT TITLE AND TITLE PAGE

Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana National Meningitis Survey

Mark W. Tenforde,[1,2,3] Margaret Mokomane,[4] Tshepo Leeme,[3] Nametso Tlhako,[3] Katlego Tsholo,[3] Chandapiwa Ramodimoosi,[5] Bonno Dube,[6] Kelebeletse O. Mokobela,[6] Ephraim Tawanana,[7] Tony Chebani,[8] Tlhagiso Pilatwe,[8] William J. Hurt,[3] Hannah K. Mitchell,[3] Mooketsi Molefi,[9] Paul C. Mullan,[10] Brandon L. Guthrie,[2,11] Carey Farquhar,[1,2,11] Andrew P. Steenhoff,[3,9,12] Madisa Mine,[4] Joseph N. Jarvis[3,9,13,14,15]

Affiliations

[1] Division of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle, WA USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA, USA

[3] Botswana-UPenn Partnership, Gaborone, Botswana

[4] National Health Laboratory, Gaborone, Botswana

[5] National Tuberculosis Reference Laboratory, Gaborone, Botswana

[6] Nyangabwe Referral Hospital, Francistown, Botswana

[7] Selebi Phikwe Government Hospital, Selebi Phikwe, Botswana

[8] Botswana Ministry of Health and Wellness, Gaborone, Botswana

[9] University of Botswana, Gaborone, Botswana

[10] Children's Hospital of the King's Daughters, Norfolk, VA, USA

[11] Department of Global Health, University of Washington, Seattle, WA

[12] Division of Infectious Diseases & Global Health Center, Children's Hospital of Philadelphia, Philadelphia, PA USA

[13] Division of Infectious Diseases, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA USA

[14] Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

[15] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Corresponding Author

Mark W Tenforde MD, MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2066508915

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Alternative Corresponding Author

Joseph N Jarvis MBBS, PhD

Botswana Harvard AIDS Institute Partnership

Private Bag BO 320

Gaborone, Botswana

Phone: +267 2540779

Fax: +267 3932267

E-mail: joseph.jarvis@lshtm.ac.uk

Short summary: In a 16-year Botswana national audit, nearly 90% of microbiologically-confirmed adult meningitis was due to *Cryptococcus*, but microbiological yield in suspected cases was low, highlighting diagnostic limitations in resource-poor settings; 2013-2014 national incidence of culture-confirmed pneumococcal meningitis was 1.8 cases/100,000 person-years.

Key words: Cryptococcal meningitis; pneumococcal meningitis; TB meningitis; HIV; sub-Saharan Africa

Word count: 2999/3000

2.2 ABSTRACT

Background: Nationally representative data on meningitis epidemiology in high HIV-prevalence African settings following antiretroviral therapy (ART) scale-up are lacking. We described the epidemiology of adult meningitis in Botswana over a 16 year period.

Methods: Laboratory records for adults (≥15 years) undergoing lumbar puncture (LP) from 2000-2015 were collected, capturing complete national data for 2013 and 2014. Laboratory data were linked to national HIV registry data. The proportion of culture-positive and culture-negative cases and associated HIV-related data were described. National incidence estimates were derived for cryptococcal, pneumococcal, and all meningitis in 2013-2014. Temporal trends in meningitis diagnosis at referral hospitals from 2004-2014 were evaluated using Poisson regression.

Results: We collected data from 21,560 adults undergoing LP for suspected meningitis; 41% (8759/21,560) had abnormal cerebrospinal fluid (CSF) findings (positive microbiological testing in 5004/8759 (57%) and pleocytosis alone in 3750/8759 (43%)). Of microbiologically-confirmed meningitis, 89% (4432/5004) were cryptococcal, 6% (278/5004) pneumococcal, and 1% (51/5004) TB. Of those with a unique identifier for HIV registry linkage 73% (9525/13,033) had documented HIV-infection. Overall incidence of LP for meningitis evaluation in Botswana in 2013-2014 was 142.6/100,000 person-years (95% confidence interval [CI]:138.3-147.1). Pneumococcal meningitis incidence was 1.8/100,000 (95%CI:1.4-2.4), with no evidence of a significant decrease over time at the referral hospitals (in contrast to cryptococcal meningitis for which a significant downwards trend was observed ($p<0.001$).

Conclusions: Cryptococcal meningitis was the predominant identified etiology of meningitis despite ART scale-up but a high proportion of cases had abnormal CSF findings with negative microbiological evaluation, emphasizing the need for improved meningitis diagnostics.

2.3 INTRODUCTION

Central nervous system (CNS) infections are a major cause of morbidity and mortality in people living with HIV (PLWH) [1]. Studies in high HIV prevalence regions of central, east, and southern Africa have shown cryptococcal, TB, and pneumococcal meningitis to be the most common

microbiologically-confirmed etiologies of meningitis [2-5]. However, these studies have generally been limited to single referral centers [2,3,5], or have used sub-national surveillance data [4], and data regarding temporal trends in meningitis with ART scale-up and vaccine introduction are limited. Nationally-representative longitudinal data of meningitis under routine care conditions can provide important insights into the shifting epidemiology of CNS infections in sub-Saharan Africa (SSA), highlight limitations of current diagnostic strategies, and inform research on approaches to improve management for CNS infections.

Botswana, a middle-income country in southern Africa with a 23% adult HIV prevalence in 2017 [6], established the first antiretroviral therapy (ART) program in Africa in early 2002. Expanded access to ART has led to high coverage, with more than 70% of PLWH estimated to have been diagnosed, started on ART, and virologically suppressed [7]. Despite progress, late presentation with advanced HIV/AIDS [8,9], as well as care default [10], are common. We previously showed ongoing high incidence of HIV-associated cryptococcal meningitis in Botswana despite excellent ART coverage [11]. Incidence and temporal trends for other HIV-associated meningitides have not been described, including potential changes in pneumococcal meningitis following the 2012 introduction of childhood 13-valent pneumococcal conjugate vaccine (PCV13) [12].

In a nationwide meningitis study, we collected laboratory records at all facilities that performed microbiological testing of cerebrospinal fluid (CSF). We characterized cases over a 16-year period (2000-2015) in adults (≥15 years old), determined national incidence of meningitis during 2013-2014 when full national laboratory data were available, and examined temporal trends over an 11-year period at Botswana's two referral hospitals (2004-2014).

2.4 METHODS

Study design and data collection

A cross-sectional study was performed using data from the Botswana National Meningitis Survey (BNMS), as described previously [11]. BNMS included records from laboratory facilities that perform cerebrospinal fluid (CSF) testing for meningitis evaluation. Data sources included paper-based records collected during in-person laboratory visits and records from electronic medical records (EMRs) used during the study period. Records were merged and de-duplicated.

Standard CSF evaluation remained unchanged during the study period, consisting of white cell count (WCC) and differential (lymphocytes, neutrophils) when WCC ≥10 cells/μL, protein and glucose, microscopy (gram stain, India ink), and bacterial and fungal culture [13]. CSF latex agglutination cryptococcal antigen (CrAg) testing was performed infrequently. Tuberculous meningitis evaluation by acid-fast bacillus (AFB) [Ziehl-Neelsen] smear was performed in some laboratories, on request, with TB culture available as a send-out to a national reference laboratory (National Tuberculosis Reference Laboratory). XPert MTB/RIF was introduced in 2012 but was not used on CSF samples. Other molecular diagnostic testing (e.g. herpes simplex viruses or *Toxoplasma gondii* PCR) and syphilis testing have not been introduced in public-sector laboratories.

HIV-related data (HIV status, CD4 count, ART use) were obtained from EMR records and from a national HIV registry through deterministic linkage of laboratory and HIV records using a unique 9-digit national identification number ("Omang"). As Omang was not documented in paper records, linkage was restricted to individuals undergoing evaluations at centers using the EMR,

Integrated Patient Management System (IPMS). For CD4 T-cell count, the closest value within a 6-month period of lumbar puncture (LP) was used. Prior ART use was defined as documented prescription of ART or any HIV viral load testing within 3 months of CSF evaluation (as initial viral load testing only occurs after at least 3 months on ART [14]).

The study was approved by the University of Botswana, the Botswana Ministry of Health and Wellness' Health Research Development Committee, Botswana referral and district hospitals with research ethics committees, the University of Pennsylvania, and the University of Washington.

Case definitions

A "case," or episode, was defined as receipt of a LP with CSF analysis at any lab in Botswana from 2000-2015. We restricted our analysis to adults (≥15-years) because of differences in the HIV prevalence and epidemiology of CNS infections in adults and children, and for consistency with age strata in UNAIDS denominator estimates. Cases were characterized by microbiological diagnosis: Cryptococcal meningitis was defined as positive CSF microscopy (India ink), culture, and/or CrAg. TB meningitis was defined as positive CSF culture for *Mycobacterium tuberculosis* or AFB smear. Two definitions were used for pneumococcal meningitis: A restrictive definition limited to individuals with CSF culture growth of *S. pneumoniae*, and a second including positive CSF culture or gram stain showing gram-positive cocci. This definition was used due to imperfect sensitivity of culture and added diagnostic yield of gram stain [15,16], particularly in patients receiving pre-LP antibiotics. Other meningitides were defined by positive CSF culture.

Cases without positive microbiological test results (microscopy, culture, and/or CrAg) were defined as *normal* (CSF WCC 0-5 cells/μL), with *minor abnormalities* (WCC 6-20 cells/μL), or *markedly abnormal* (WCC >20 cells/μL), the latter stratified as lymphocytic (≥50% lymphocytes) or neutrophilic (<50% lymphocytes). CSF protein and glucose analyses were not performed in a sizeable proportion of samples due to frequent reagent stock-outs, thus additional classifications of culture negative-disease were not explored. To account for repeated LPs (e.g. therapeutic LPs for cryptococcal meningitis), all CSF evaluations within a ≤14-day window from a previous LP were defined as part of the same episode, and results from the first LP used in the analysis. For TB meningitis, LP evaluations within a 6-month window of diagnosis were considered part of a single episode.

Data analysis

Findings from all adult cases 2000-2015 were described, including proportion of cases with positive microbiological findings and classification of cases without positive microbiological findings by WCC strata. Patient demographic, HIV clinical characteristics, and CSF findings were evaluated using descriptive statistics (number and percentage or median and interquartile range [IQR]).

Complete CSF records were obtained from all laboratories in Botswana 2013-2014. During this two-year period the overall and HIV-specific incidence of meningitis cases in adults (≥15 years) was determined by category (any LP, cryptococcal meningitis, pneumococcal meningitis, or markedly abnormal CSF [WCC >20 cells/μL] with or without positive bacterial gram stain or culture). TB meningitis incidence was not evaluated due to the small number of confirmed cases

resulting from limitations in TB diagnostics. UNAIDS Spectrum data were used for national population denominator estimates [11]. Incidence estimates with 95% confidence intervals (95% CIs) were calculated using a Poisson distribution.

To account for missing age in a small proportion of cases (3%), when deriving incidence estimates it was assumed that the age distribution (adult vs. child) was the same in cases without documented age as in those with age data for each type of meningitis. The total number of HIV-associated cases for each meningitis type was estimated by applying the HIV prevalence data derived from the cases registered in IPMS and linked the HIV database to the entire cohort. This provides a conservative estimate of HIV-associated cases as some PLWH may have been undiagnosed.

At referral hospitals with complete longitudinal records 2004-2014 the number of cases were plotted by year to evaluate trends. Due to the lack of defined catchment populations for the two referral hospitals, it was not possible to statistically evaluate incidence trends. Rather, given consistent clinical care provision and referral pathways over the full study duration (as evidenced by a consistent proportion of cases seen at the two referral hospitals), Poisson regression was used to assess trends in case numbers over time. Finally, seasonality of culture-confirmed pneumococcal meningitis cases was assessed by plotting number of cases by month, along with average Botswana temperature and rainfall by month 2000-2015 [17]. Both adult and pediatric cases were included for seasonality evaluation as similar findings were observed for both groups. Analyses were performed in Stata (Version 13, College Station, TX) and figures generated using Stata and the ggplot2 package in R [18]. Statistical significance was defined as a p-value <0.05.

2.5 RESULTS

Overall description of cases

A total of 34,505 LP samples were documented from 2000-2015. After restricting to adults (≥15 years), and excluding repeat samples, 21,560 unique cases were observed (**Figure 1**). Fifty-six percent (12,143/21,560) were from referral hospitals. Median age was 36 years (IQR 30-45) and 52% (11,108/21,488) were male. Forty-one percent (8,759/21,560) had at least minor CSF WCC abnormalities and/or positive microbiological findings. A microbiological diagnosis was made in 23% (5,004/21,560) of cases. Of those with HIV-related information, 73% (9,525/13,033) were known to be HIV-infected. Median CD4 count was 91 cells/μL (IQR 37-216) with 47% (4,474/9,525) initiated on ART prior to the date of LP.

Characteristics by pathogen

Cryptococcal meningitis (CM) accounted for the majority of cases with positive microbiological findings (89%, 4,432/5,004). Although India ink and culture was performed routinely on all CSF samples, CrAg testing was performed in only 9% (1,844/21,560) of episodes. Most CM patients were males (62%, 2,720/4,421) [**Table 1**], with mild CSF inflammation (median WCC 5/μL [IQR 2-34]). Nearly all with HIV registry linkage were known HIV-infected (>99.9%, 2,772/2,775), with a median CD4 count of 42 cells/μL (IQR 19-89) and 38% (1,039/2,772) already taking ART.

TB meningitis (TBM) was diagnosed in 1% (51/5,004) of cases with positive microbiological findings; 36 by culture, 14 by AFB smear, and 1 by both. This was in the context of infrequent evaluation for TB, with smear performed in 22% (4,755/21,560) and culture in 7% (1,517/21,560) of cases. CSF was characterized by moderate lymphocytic pleocytosis but significant spread

(median WCC 48/μL [IQR 8-150]). Most patients with available HIV-related data were known HIV-infected (83%, 24/29), with a median CD4 count of 104 cells/μL (IQR 27-165).

Cases with confirmatory bacterial culture and/or gram stain accounted for 11% (552/5,004) of those with positive microbiological findings; 72% (395/552) of these were culture-positive and 28% (157/552) had a positive gram stain alone. Pneumococcal meningitis was identified in 69% (382/552), 73% (278/382) of whom were *S. pneumoniae* culture-positive. Pneumococcal meningitis cases showed highly inflammatory neutrophil-predominant pleocytosis and hypoglycorrhachia; 62% with HIV-related data (150/244) were known HIV-infected with a median CD4 count of 196 cells/μL (IQR 127-379). CSF and clinical characteristics were similar when we restricted to culture-confirmed cases alone (data not shown).

Other typical bacterial pathogens were uncommon: 11 *Haemophilus influenzae*, 6 *Group B Streptococcus*, 1 *Neisseria meningitidis*, and 1 *Listeria monocytogenes* cases. Other cultured isolates included 34 *Enterobacteriaceae* (14 *Klebsiella pneumoniae*, 13 *Escherichia coli*, 4 *Salmonella spp*, 2 Enterobacter spp, 1 *Proteus mirabilis*), 32 *Staphylococcus spp* (20 coagulase-negative *Staphylococcus*, 12 *Staphylococcus aureus*), 15 non-pneumococcal and non-group B *Streptococcus spp*, 3 *Pseudomonas aeruginosa* cases, and 12 with other pathogens. Clinical data to distinguish likely contamination versus clinical disease for atypical pathogens, e.g. coagulase-negative *Staphylococci*, were unavailable.

Cases with negative microbiological evaluation

Seventy-seven percent (16,556/21,560) of LP evaluations for meningitis had no positive microbiological findings (**Figure 1**), of which 19% (3,220/16,556) had no CSF WCC recorded. Of cases with WCC, 28% (3,755/13,336) showed elevated WCC; 10% (1,309/13,336) mildly abnormal (6-20 cells/μL) and 18% (2,446/13,336) markedly abnormal (>20 cells/μL), the majority with lymphocyte predominance (55% [1,348/2,446]) in those with a WCC differential. Demographic and HIV-related characteristics were similar between WCC categories. Of all cases with negative evaluation, 66% (6,526/9,897) with HIV registry linkage were known HIV-infected with a median CD4 count of 120 cells/mL (IQR 49-269) and 51% (3,343/6,526) were on ART before evaluation.

National incidence and temporal trends

Over the two-year period with complete national data (2013-2014), an estimated 142.6 LPs / 100,000 person-years (95%CI: 142.6-147.1) were performed in adults overall, and 405.7 LPs /100,000 person-years (95% CI…) among those known to be HIV-infected (**Table 2**). Using the combined definition for pneumococcal meningitis (positive CSF culture and/or gram stain), the estimated overall incidence of pneumococcal meningitis was 2.7/100,000 person-years (95%CI: 2.4-3.1) and 5.9 /100,000 person-years (95%CI: 4.3-7.9) in the HIV-infected population. Restricted to culture-confirmed pneumococcal meningitis, incidence was 1.8 /100,000 person-years (95%CI: 1.4-2.4) overall and 4.3/100,000 person-years (95%CI: 2.9-6.0) among those known to be HIV-infected. Incidence of cryptococcal meningitis was 25.0/100,000 person-years (95%CI: 23.2-26.9) overall and 92.9/100,000 person-years (95%CI: 86.2-99.9) in the HIV-infected.

As previously described [11], cases of cryptococcal meningitis declined from 2004-2014 at referral hospitals ($p<0.001$) [**Figure 2**]. However, similar trends were not observed for pneumococcal cases ($p=0.68$), cases with minor WCC abnormalities (6-20 cells/μL) [$p=0.94$], or cases with markedly abnormal CSF (WCC >20 cells/μL) [$p=0.16$]. No decrease in adult pneumococcal meningitis cases was observed 2013-2014 following childhood PCV13 introduction, compared to the earlier period. In seasonal analysis of culture-confirmed pneumococcal meningitis, cases peaked in the winter and early spring (**Figure 3**).

2.6 DISCUSSION

Using a nationally representative sample of over 20,000 adult episodes of suspected meningitis over 16 years, this study provides novel insights into the epidemiology of meningitis in high HIV-prevalence regions of Africa. About three-quarters of adult patients evaluated for CNS infection were known to be HIV-infected. Evaluation for CNS infections was common in the HIV-infected adults, with over 400 LPs/100,000 person-years. Most HIV-infected individuals undergoing LP were profoundly immunocompromised (median CD4 <100 cells/μL) despite almost half having a history of ART use. This mirrors findings from recent studies showing approximately half of patients with cryptococcal meningitis are now ART-experienced [19-21], reflecting a combination of late presentation to care with severe immune-suppression [9], default from care, and ART failure from resistance. Interpreted alongside recent data showing that approximately half of patients with culture-negative meningitis and two-thirds diagnosed with cryptococcal meningitis die within one year of presentation in Botswana [13,22], these findings demonstrate that CNS infections remain a principal cause of mortality in HIV-infected individuals over a decade into the ART era.

Our findings highlight severe limitations in routine strategies for the diagnosis of meningitis common throughout much of sub-Saharan Africa. Consistent with previous studies from South Africa [3,4], Zambia [23], and Uganda [24], cryptococcal meningitis was the most common etiology of meningitis accounting for about 90% of cases. This high case ascertainment reflects both the still-high burden of advanced HIV/AIDS and excellent diagnostic yield combining rapid cryptococcal testing (usually India ink) with more sensitive fungal culture in all cases.

Microbiological diagnoses other than cryptococcal meningitis were infrequent despite a significant proportion of cases with abnormal CSF WCC. TB accounted for only 1% of cases with positive microbiological findings. This contrasts with surveillance data from South Africa 2009-2012, where almost 25% of microbiologically-confirmed adult meningitis cases had TB confirmed by smear, culture and/or Xpert. Of cases in Botswana with pleocytosis and/or a positive microbiological finding, 15% (1348/8759) had a WCC >20/μL with lymphocytic predominance and negative standard work-up, much of which likely reflects undiagnosed TB meningitis. The small number of confirmed TB diagnoses in Botswana almost certainly reflects infrequent TB testing despite culture availability. Reasons for infrequent TB diagnostic testing are probably multifactorial, including lack of decentralized testing and intermittent TB laboratory stoppages; however a major factor driving clinicians' low testing rates is likely the poor sensitivity of AFB smear and delays in culture results with lack of actionable data on which to base clinical decisions [25]. Patients with TB meningitis and advanced immune suppression due to HIV also often lack significant CSF pleocytosis or other "classic" CSF findings [26], possibly reducing clinical suspicion. Rapid next-generation Xpert (Ultra) has recently shown improved yield over CSF culture in HIV-infected Ugandan patients [27], and gives actionable results within hours, providing

an attractive diagnostic option for TB meningitis which may overcome many of the existing diagnostic challenges if widely implemented.

Bacterial meningitis accounted for 11% of cases with positive microbiological findings with a predominance of *Streptococcus pneumoniae*. Even using a conservative definition restricted to culture-confirmed cases the adult national incidence of 1.8 cases / 100,000 person-years is approximately 6 times higher than the rate estimated in the United States in 2010 [28], or 9 times higher with an expanded case definition combining culture and gram stain results. HIV-specific incidence was approximately 15-20-fold higher than in the general U.S. population. Our pneumococcal meningitis incidence estimates in Botswana are certainly an underestimate of true population rates. First, we found a large number of culture-negative cases with markedly elevated CSF white cell count (>20/μL) and neutrophil predominance (632 cases versus 278 culture-confirmed pneumococcal meningitis cases), probably in-part representing rapid CSF sterilization and decreased culture sensitivity with pre-LP antibiotic administration [29]. Pneumococcal meningitis occurred year-round but peaked during winter and early spring, as previously described [30]. Cases of pneumococcal meningitis over a >10-year period at the two referral hospitals were largely unchanged over time and did not show any appreciable decline following introduction of PCV13 vaccination in 2012, although our analysis was restricted to just two full years following introduction. Pneumococcal vaccination trials in HIV-infected adults in Africa have shown mixed results [31,32], but pediatric vaccination may provide some protection through herd immunity [33], and further surveillance, preferably with serotype data, is warranted.

Our study has several limitations. We relied on routinely collected laboratory data, did not have detailed clinical information, and lacked HIV-related clinical data for some patients (those with only paper records). As most records with national identification used for HIV database linkage were from referral hospitals, HIV prevalence data may not be fully representative of the general population. HIV prevalence was likely under-ascertained in our study; although 73% of adults evaluated for meningitis were documented as HIV-infected, this is lower than observed in other high HIV prevalence African settings [3,34]. This results in conservative HIV-associated meningitis incidence estimates. Finally, other than 2013-2014, we had incomplete CSF laboratory records and were unable to evaluate national incidence trends over the full study period.

2.7 CONCLUSION

In conclusion, using a nationally representative sample of suspected meningitis cases, we provide insights into the epidemiology of meningitis in high HIV prevalence sub-Saharan African settings. Cryptococcal meningitis remains the predominant etiology identified. However, our findings highlight a large burden of culture-negative meningitis, some of which likely represents partially-treated pyogenic bacterial meningitis or TB meningitis, but may also include a large number of other potential pathogens. Our findings support the need for scale-up of molecular diagnostic platforms, particularly for TB evaluation, and to better characterize pathogens to inform treatment approaches.

2.8 ACKNOWLEDGMENTS

FUNDING AND CONFLICTS OF INTEREST

This work was supported by the National Institutes of Health (NIH) Office of AIDS Research, Health Resources and Services Administration (HRSA), and President's Emergency Plan for

AIDS Relief (PEPFAR) [grant number U91HA06801B] and NIH National Institutes of Allergy and Infectious Disease (NIAID) grant support (T32AI007044 and F32AI140511) to MWT, and the Penn Center for AIDS Research, a NIH-funded program (grant number P30 AI 045008), to JNJ. The research was commissioned in part by the National Institute for Health Research using Official Development Assistance (ODA) funding through a Global Health Professorship to JNJ (RP-2017-08-ST2-012). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. All authors declare no relevant conflicts of interest to disclose.

ACKNOWLEDGEMNTS

We thank the Afya Bora Consortium, Diakanyo Moalosi and Kabelo Mokgacha at the Botswana Ministry of Health for their assistance in obtaining electronic laboratory records, Freedom Ernest at the Botswana National Health Laboratory for arranging laboratory visits, Doreen L Banda, Elizabeth A Williams, and Raju KK Patel, for their assistance with data entry and cleaning, and Hunter Stone and personnel at Botswana laboratories for assistance with data retrieval.

2.9 REFERENCES

1. Walker AS, Prendergast AJ, Mugyenyi P, Munderi P, Hakim J, Kekitiinwa A, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(12):1707-18.

2. Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, et al. Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up

and Haemophilus influenzae type b vaccination, 2000-2012. Clin Infect Dis. 2014;58(10):e137-45.

3. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC Infect Dis. 2010;10:67.

4. Britz E, Perovic O, von Mollendorf C, von Gottberg A, Iyaloo S, Quan V, et al. The Epidemiology of Meningitis among Adults in a South African Province with a High HIV Prevalence, 2009-2012. PloS one. 2016;11(9):e0163036.

5. Marais S, Pepper DJ, Schutz C, Wilkinson RJ, Meintjes G. Presentation and outcome of tuberculous meningitis in a high HIV prevalence setting. PLoS One. 2011;6(5):e20077.

6. UNAIDS. AIDSinfo. Available at: aidsinfo.unaids.org. Accessed 23 Nov 2018.

7. Gaolathe T, Wirth KE, Holme MP, Makhema J, Moyo S, Chakalisa U, et al. Botswana's progress toward achieving the 2020 UNAIDS 90-90-90 antiretroviral therapy and virological suppression goals: a population-based survey. Lancet HIV. 2016;3(5):e221-30.

8. Lechiile K, Mitchell HK, Mulenga F, et al. Prevalence of advanced HIV disease and cryptococcal infection in Gaborone, Botswana. Presented at: CROI 2017. Seattle, WA, 2017.

9. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. AIDS. 2016;30(3):477-85.

10. Fox MP, Rosen S. Retention of Adult Patients on Antiretroviral Therapy in Low- and Middle-Income Countries: Systematic Review and Meta-analysis 2008-2013. J Acquir Immune Defic Syndr. 2015;69(1):98-108.

11. Tenforde MW, Mokomane M, Leeme T, Patel RKK, Lekwape N, Ramodimoosi C, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis. Clin Infect Dis. 2017.

12. World Health Organization. Global Health Observatory data: Pneumococcal conjugate 3rd dose (PCV3) immunization coverage. Available at: http://www.who.int/gho/immunization/pneumococcal/en/. Accessed 4 December 2018.

13. Tenforde MW, Mokomane M, Leeme TB, Tlhako N, Tsholo K, Chebani T, et al. Mortality outcomes in culture-positive and culture-negative adult meningitis in the Botswana National Meningitis Survey: A prevalent cohort study. Lancet Infect Dis. **2019**. (in press).

14. Botswana Ministry of Health. 2016 integrated HIV clinical care guidelines. Available at: http://www.moh.gov.bw/Publications/Handbook_HIV_treatment_guidelines.pdf. Accessed 28 March 2017.

15. Wu HM, Cordeiro SM, Harcourt BH, Carvalho M, Azevedo J, Oliveira TQ, et al. Accuracy of real-time PCR, Gram stain and culture for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae meningitis diagnosis. BMC infectious diseases. 2013;13:26.

16. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23(3):467-92.

17. World Bank Group. Climate Change Knowledge Portal: Average monthly temperature and rainfall for Botswana from 1901-2015. Available at: http://sdwebx.worldbank.org/climateportal/index.cfm?page=country_historical_climate&ThisCCode=BWA. Accessed 5 December 2018.

18. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

19. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

20. Jarvis JN, Leeme TB, Molefi M, Chofle AA, Bidwell G, Tsholo K, et al. Short Course High-dose Liposomal Amphotericin B for HIV-associated Cryptococcal Meningitis: A phase-II Randomized Controlled Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.

21. Beardsley J, Wolbers M, Kibengo FM, Ggayi ABM, Kamali A, Cuc NTK, et al. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. New England Journal of Medicine. 2016;374(6):543-54.

22. Patel RKK, Leeme T, Azzo C, Tlhako N, Tsholo K, Tawanana EO, et al. High Mortality in HIV-Associated Cryptococcal Meningitis Patients Treated With Amphotericin B-Based Therapy Under Routine Care Conditions in Africa. Open Forum Infect Dis. 2018;5(11):ofy267.

23. Siddiqi OK, Ghebremichael M, Dang X, Atadzhanov M, Kaonga P, Khoury MN, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(12):1771-7.

24. Rajasingham R, Rhein J, Klammer K, Musubire A, Nabeta H, Akampurira A, et al. Epidemiology of meningitis in an HIV-infected Ugandan cohort. Am J Trop Med Hyg. 2015;92(2):274-9.

25. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis. 2010;10(11):803-12.

26. Cecchini D, Ambrosioni J, Brezzo C, Corti M, Rybko A, Perez M, et al. Tuberculous meningitis in HIV-infected and non-infected patients: comparison of cerebrospinal fluid findings. Int J Tuberc Lung Dis. 2009;13(2):269-71.

27. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, et al. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis. 2018;18(1):68-75.

28. Castelblanco RL, Lee M, Hasbun R. Epidemiology of bacterial meningitis in the USA from 1997 to 2010: a population-based observational study. Lancet Infect Dis. 2014;14(9):813-9.

29. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. Pediatrics. 2001;108(5):1169-74.

30. Paireau J, Chen A, Broutin H, Grenfell B, Basta NE. Seasonal dynamics of bacterial meningitis: a time-series analysis. Lancet Glob Health. 2016;4(6):e370-7.

31. French N, Nakiyingi J, Carpenter LM, Lugada E, Watera C, Moi K, et al. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. Lancet. 2000;355(9221):2106-11.

32. French N, Gordon SB, Mwalukomo T, White SA, Mwafulirwa G, Longwe H, et al. A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. N Engl J Med. 2010;362(9):812-22.

33. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med. 2014;371(20):1889-99.

34. Scarborough M, Gordon SB, Whitty CJ, French N, Njalale Y, Chitani A, et al. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. N Engl J Med. 2007;357(24):2441-50.

Table 1. Clinical and laboratory characteristics of patients evaluated for meningitis.

Pathogen	Age (years), Median (IQR)	Sex, % (n) male	CSF WCC (/µL), median (IQR) *	Lymphocyte % (IQR)	Protein (g/dL), median (IQR)	Glucose, mmol/L, median (IQR)	HIV +ve, % (n/N) †	CD4 count (cells/µL) if HIV +ve, median (IQR)	On ART if HIV +ve, % (n)
With microbiological diagnosis									
Cryptococcus (n = 4432)	36 (31-43)	61.5% (2720/4421)	5 (2-34)	90% (60-95)	0.91 (0.49-1.75)	2.10 (1.23-2.86)	99.9% (2772/2775)	42 (19-89)	37.5% (1039/2772)
TB (n = 51)	36 (31-45)	54.9% (28/51)	48 (8-150)	90% (20-96)	2.56 (0.93-3.27)	0.83 (0.61-1.36)	82.8% (24/29)	104 (27-165)	20.8% (5/24)
Pneumococcus (n = 382)	36 (30-45)	43.2% (164/380)	250 (40-946)	10% (5-24)	3.66 (2.42-6.66)	0.07 (0.01-0.90)	61.5% (150/244)	196 (127-379)	39.3% (59/150)
Without microbiological diagnosis									
CSF WCC 0-5 (n = 9581)	36 (30-46)	47.6% (4546/9557)	----	----	0.47 (0.26-0.92)	3.20 (2.60-3.85)	66.2% (4052/6119)	113 (44-268)	54.5% (2207/4052)
CSF WCC 6-20 (n = 1309)	36 (30-45)	50.2% (654/1302)	----	----	0.84 (0.40-1.80)	2.84 (2.00-3.72)	66.8% (514/770)	129 (55-254)	52.0% (267/514)

CSF WCC >20, no differential (n = 466)	36 (30-45)	53.7% (249/464)	70 (32-230)	----	1.58 (0.80-3.18)	2.10 (1.12-3.17)	64.8% (136/210)	138 (69-317)	46.3% (63/136)
CSF WCC >20, lymphocytic (n = 1348)	36 (29-43)	52.6% (708/1345)	123 (54-312)	90% (80-96)	1.83 (1.00-3.53)	1.92 (1.10-2.89)	70.7% (662/936)	156 (77-284)	48.0% (318/662)
CSF WCC >20, pyogenic (n = 632)	36 (30-44)	57.1% (358/627)	244 (75-810)	15% (9-30)	2.37 (1.18-4.21)	1.46 (0.90-2.43)	60.1% (217/361)	116 (53-214)	43.3% (94/217)

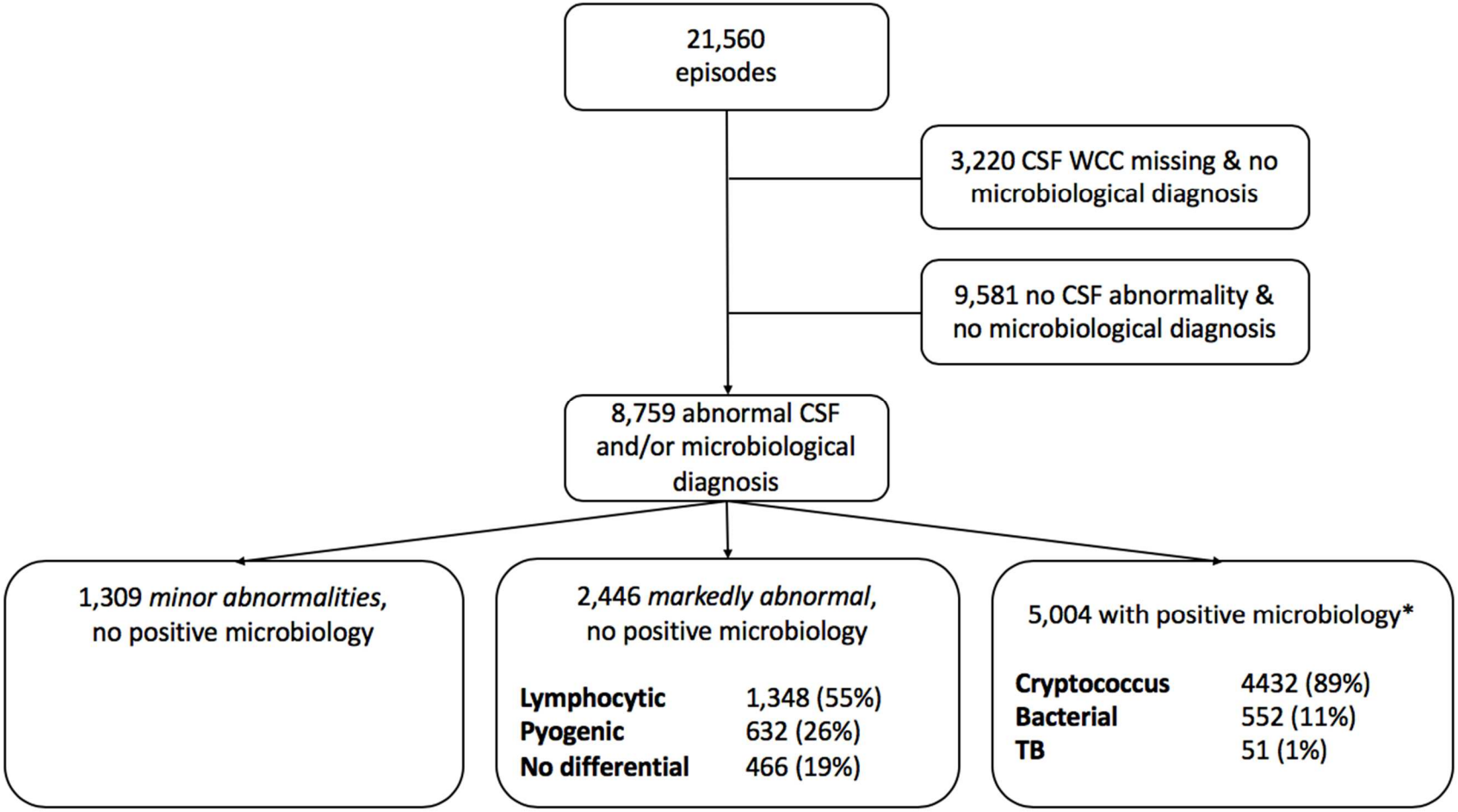
+ve - positive; ART - antiretroviral therapy; IQR - interquartile range; WCC - white cell count
* Upper limit recorded for WCC 2000/μL
† Among patients with documented HIV status

Table 2. Botswana national incidence of adult (≥15 years) meningitis, 2013-2014.

Strata	Category	Number of cases	Person-years	Incidence (per 100,000 PYO)	95% confidence interval (per 100,000 PYO)
Underwent lumbar puncture	Overall population	4114	2,884,278	142.6	138.3 - 147.1
	Known HIV-infected	3150	776,523	405.7	391.6 - 420.1
Cryptococcal meningitis	Overall population	721	2,884,278	25.0	23.2 - 26.9
	Known HIV-infected	721	776,523	92.9	86.2 - 99.9
Pneumococcal meningitis	Overall population	73	2,884,278	2.7	2.4 - 3.1
(+ve culture or GPCs)	Known HIV-infected	46	776,523	5.9	4.3 - 7.9
Pneumococcal meningitis	Overall population	53	2,884,278	1.8	1.4 - 2.4
(+ve culture)	Known HIV-infected	33	776,523	4.3	2.9 - 6.0
Any abnormal CSF (WCC >20 or	Overall population	313	2,884,278	10.9	9.7 - 12.1
non-*Cryptococcus* pathogen)	Known HIV-infected	213	776,523	27.4	23.9 - 31.4

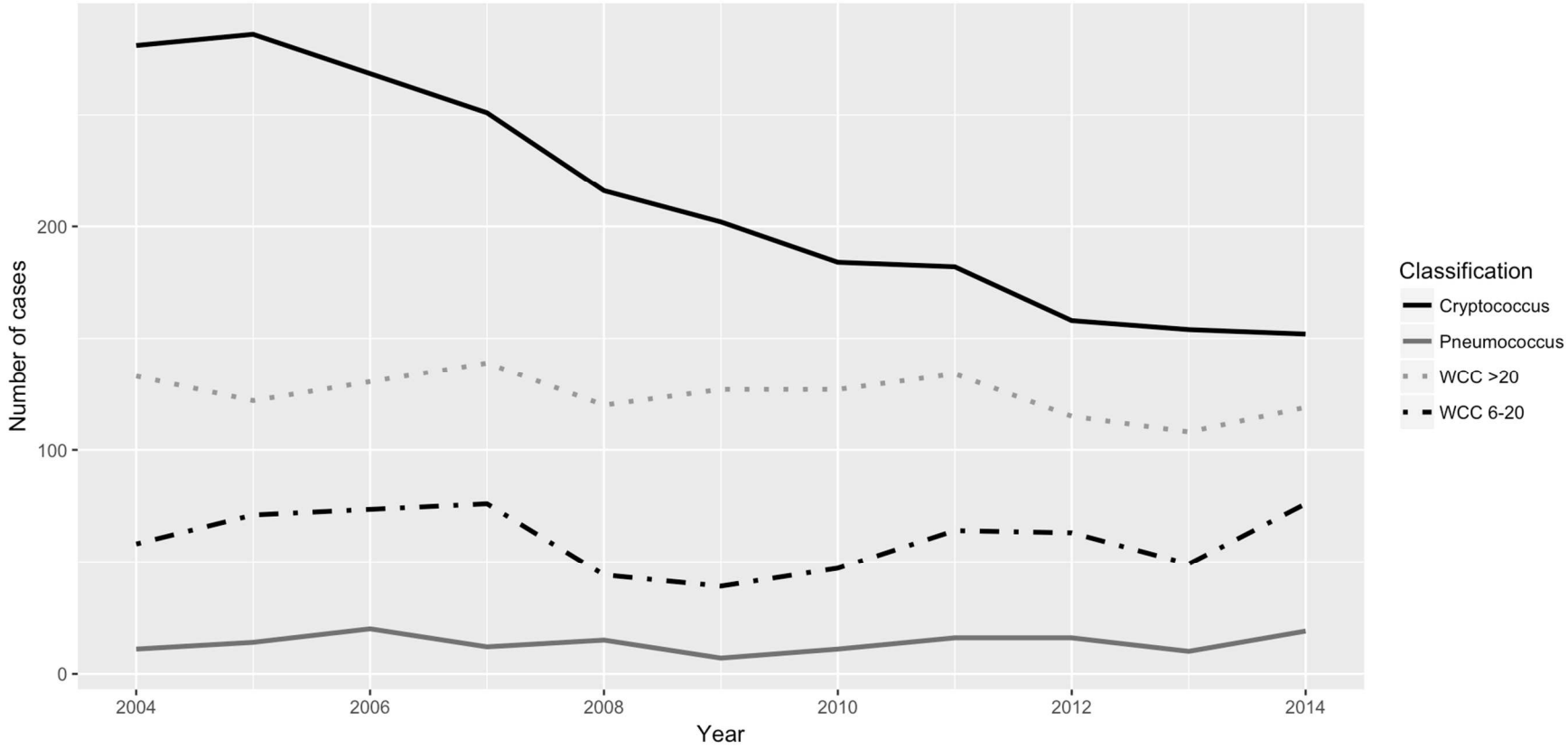
+ve - positive; CSF - cerebrospinal fluid; GPC - gram positive cocci; WCC - white cell coun

Figure 1. Overall description of 21,560 adult cases. Of 34,505 overall lumbar puncture assessments, 7,238 samples from children, 4,014 with undocumented age, and 1,693 repeat lumbar punctures excluded.



CSF = cerebrospinal fluid; TB = tuberculosis; WCC = white cell count
*>100 percent due to 31 mixed cryptococcal and bacterial meningitis cases and 2 mixed cryptococcal and TB meningitis; 1 additional case of *Candida spp* and 1 non-tuberculous Mycobacterium without further speciation also found

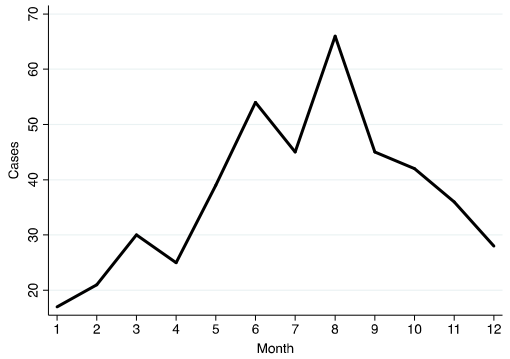
Figure 2. Trends in meningitis cases at Botswana national referral hospitals, 2004-2014.



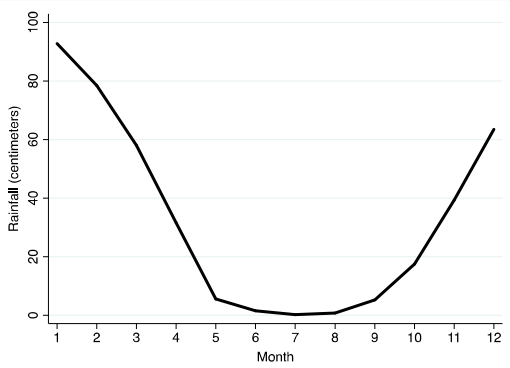
WCC - cerebrospinal fluid white cell count
Classifications for pneumococcal meningitis restricted to culture-positive cases. WCC >20 and WCC 6-20 groups excluded cases of diagnosed cryptococcal meningitis.
* Excluded 2006 data for Cryptococcus, WCC>20 and WCC 6-20; a large drop in cryptococcal meningitis cases and concurrent rise in cases with WCC 6-20 and WCC>20 likely represented missed diagnoses of cryptococcal meningitis in this year

Figure 3. (A) Culture-confirmed pneumococcal meningitis cases, (B) average rainfall (centimeters), and (C) average temperature (Celsius) by month

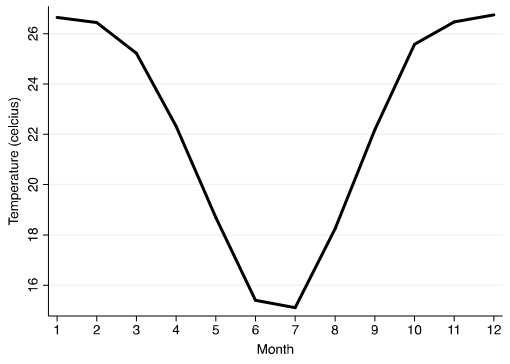
(A)



(B)



(C)



* Month: 1=January, 2=February, etc.

Chapter 3. ADVANCED HIV DISEASE IN BOTSWANA FOLLOWING SUCCESSFUL ANTIRETROVIRAL THERAPY ROLLOUT: INCIDENCE AND TEMPORAL TRENDS IN CRYPTOCOCOCAL MENINGITIS

3.1 MANUSCRIPT TITLE AND TITLE PAGE

Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis

Mark W Tenforde MD MPH[1,2], Margaret Mokomane MSc [3], Tshepo Leeme MBBS DTMH [4], Raju KK Patel MBBS DTMH [5], Nametso Lekwape [4], Chandapiwa Ramodimoosi [3], Bonno Dube [6], Elizabeth A Williams MBBS [7], Kelebeletse O Mokobela MSc [6], Ephraim Tawanana BSc [8], Tlhagiso Pilatwe [9], William J Hurt BMchB DTMH [4], Hannah Mitchell BMBS DTMH [4], Doreen L Banda [4], Hunter Stone MD [10], Mooketsi Molefi MBChB [11], Kabelo Mokgacha MD [9], Heston Phillips PhD [12], Paul C Mullan MD MPH [13], Andrew P Steenhoff MBBCh DCH [4,14], Yohana Mashalla MD PhD [11], Madisa Mine PhD [3], Joseph N Jarvis MBBS PhD [4,11,15,16]

Affiliations

[1] Division of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle, WA USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA, USA

[3] Botswana National Health Laboratory, Gaborone, Botswana

[4] Botswana-UPenn Partnership, Gaborone, Botswana

[5] Imperial College London, London, UK

[6] Nyangabwe Referral Hospital, Francistown, Botswana

[7] St. George's University, London, UK

[8] Princess Marina Hospital, Gaborone, Botswana

[9] Botswana Ministry of Health, Gaborone, Botswana

[10] University of Texas Southwestern Medical Center, Dallas, TX USA

[11] University of Botswana, Gaborone, Botswana

[12] UNAIDS, Botswana Country Office

[13] Children's National Health System, Washington, DC USA

[14] Division of Infectious Diseases, Children's Hospital of Philadelphia, Philadelphia, PA USA

[15] Division of Infectious Diseases, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA USA

[16] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Corresponding Author

Mark Tenforde MD MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2069406927

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Alternative Corresponding Author

Joseph N Jarvis MBBS PhD

244G Botswana-UPenn Partnership

University of Botswana Main Campus

PO Box AC 157 ACH

Gaborone, Botswana

Phone: +267 2540779

Fax: +267 3932267

E-mail: drjoejarvis@gmail.com

Short summary:

In a Botswana national meningitis survey, we observed a high 2013-2014 incidence of cryptococcal meningitis, particularly in men in their 4^{th}-5^{th} decade, highlighting the need to target populations at high risk for advanced HIV and adopt HIV differentiated care models.

Key words: Cryptococcal meningitis, incidence, HIV, Botswana, sub-Saharan Africa

3.2 ABSTRACT

Background: Botswana has a well-developed antiretroviral therapy (ART) program which serves as a regional model. With wide ART availability, the burden of advanced HIV and associated opportunistic infections would be expected to decline. We performed a nationwide surveillance study to determine the national incidence of cryptococcal meningitis, and describe characteristics of cases 2000-2014 and temporal trends at two national referral hospitals.

Methods: Cerebrospinal fluid data from all 37 laboratories performing meningitis diagnostics in Botswana were collected 2000-2014 to identify cases of cryptococcal meningitis. Basic demographic and laboratory data were recorded. Complete national data from 2013-2014 were used to calculate national incidence using UNAIDS population estimates. Temporal trends in cases were derived from national referral centers 2004-2014.

Results: 5296 episodes of cryptococcal meningitis were observed in 4702 individuals; 60·6% were male, and median age was 36 years. Overall 2013-2014 incidence was 17·8 cases/100,000 person-years (95%CI 16·6 – 19·2). In the HIV-infected population, incidence was 96·8 cases/100,000 person-years (95%CI 90·0 – 104·0); male predominance was seen across CD4 strata. At national referral hospitals, cases decreased 2007-2009 but stabilized 2010-2014.

Conclusions: Despite excellent ART coverage in Botswana, there is still a substantial burden of advanced HIV, with 2013-2014 incidence of cryptococcal meningitis comparable to pre-ART era rates in South Africa. Our findings suggest a key population of individuals, often men, are developing advanced disease and associated opportunistic infections due to a failure to effectively engage in care, highlighting the need for differentiated care models.

3.3 INTRODUCTION

Botswana was the first country in Africa to establish a national ART program and began providing treatment free-of-charge to its HIV-infected population in early 2002 [1]. National HIV care guidelines in 2012 recommended a CD4 T-cell threshold of <350 cells/μL for ART initiation, up from <200 cells/μL in 2008 [2,3], and recent population level data suggest that, in rural regions, the program is close to achieving "90-90-90" targets set by the Joint United Nations Programme on HIV/AIDS (UNAIDS).[4] Despite the success of the national ART program, Botswana, a country of approximately two million people, still has high incidence of new HIV infections in certain populations and an HIV prevalence among the highest in the world, with an estimated adult HIV prevalence of 25% in 2014 [5,6].

In sub-Saharan Africa as a whole, as in Botswana, although millions now access life-saving antiretroviral therapy (ART), overall HIV prevalence is still not declining and ART is initiated at low CD4 T-cell counts in a majority of HIV-infected individuals [7-9], resulting in a large population at substantial risk of opportunistic disease and with high rates of mortality [10]. Care default and treatment failure further increase the size of this high-risk population, with pooled observational cohort data from African settings suggesting only 65% of patients starting first-line ART remain in care at 36 months and virological failure and acquired ART resistance frequently observed [11-13]. Movement toward an HIV "test and treat" strategy in sub-Saharan Africa, which was adopted in Botswana in June 2016, will necessitate streamlining models of care to quickly expand treatment programs, which has the potential of leaving vulnerable patients further behind.

Cryptococcal meningitis (CM) is a severe fungal infection primarily seen in individuals with defective cell-mediated immunity [14], with the vast majority of cases occurring in the context of advanced HIV infection [15]. CM typically affects individuals with CD4 cell counts <100 cells/μL, and is now frequently described following late ART initiation and ART default, as well as in ART naïve individuals [16-18]. As countries move toward expanded HIV treatment programs, CM serves as an important indicator disease for national programs. An understanding of advanced HIV disease burden and temporal trends in sub-populations will guide program delivery and differentiated models of HIV care targeting groups most at risk for delayed HIV diagnosis, ART initiation, and care default, with the associated morbidity and mortality and high transmission risks.

We collected laboratory records from all facilities in Botswana that perform meningitis diagnostics and UNAIDS country-level HIV prevalence estimates to determine the 2013-2014 national incidence rate of cryptococcal meningitis, describe characteristics of CM cases over a 15-year period, and define temporal trends in cases diagnosed at the two national referral hospitals.

3.4 METHODS

Study design

The Botswana National Meningitis Survey was a 15-year retrospective review of routine cerebrospinal fluid (CSF) laboratory records to determine the etiologies of meningitis in Botswana and temporal trends with maturation of the local HIV epidemic. The study was conducted in collaboration with the Botswana Ministry of Health (MoH) and the National Health Laboratory. Institutional review board (IRB) approval was obtained from the University of Botswana, University of Pennsylvania, University of Washington, Health Research Development Council

(Botswana Ministry of Health), and at hospitals with independent IRB committees (Letsholathebe Memorial Hospital, Mahalapye Hospital, Nyangabwe Referral Hospital, Princess Marina Hospital, Scottish Livingstone Hospital, and Sekgoma Memorial Hospital).

Participating laboratories and CSF data collection

Thirty-seven laboratories are registered by the Botswana National Health Laboratory (NHL) to perform cerebrospinal fluid (CSF) testing for the diagnosis of cryptococcal meningitis by India ink stain (II), fungal culture, and/or cryptococcal antigen (CrAg) testing, including two national referral hospitals, seven district hospitals (six medical, one psychiatric), and 28 primary, private, mining, and military hospital-based, clinic-based, or standalone laboratories. Facilities were visited by the study team for collection of meningitis-related laboratory data. Most visits took place between February 2015 and May 2015. All available CSF records from 1 January 2000 through 1 January 2015 were located at facilities. Records were scanned on a password-protected study laptop, then numbered and entered into a REDCap database. To ensure data accuracy during transcription of paper-based records into the REDCap database, a minimum of two study team members reviewed each entry and discrepancies were adjudicated through consensus.

In addition to paper records, a majority of hospital-based laboratories maintain records on a government-financed electronic medical record (EMR), Integrated Patient Management System (IPMS). At facilities with IPMS, clinical data is entered directly into IPMS with laboratory books used to keep records during internet downtime or entered both in laboratory notebooks and IPMS. IPMS was queried centrally at the MoH to obtain all Botswana CSF-related electronic laboratory records and HIV-related data (CD4 T-cell count nearest to the date of cryptococcal meningitis

diagnosis). Paper-based records from the REDCap database were merged with IPMS records using Stata (version 12, College Station, TX), matching by date, testing location, and patient identifiers (name, age, gender), and duplicate entries removed. Three hospitals used additional EMR platforms for limited periods during the study period. Electronic records from these EMR platforms were also queried using CSF-related search terms and this data entered into the REDCap database.

Population denominator data

The UNAIDS Spectrum model version 5·41 was used to determine population numbers [19], national HIV prevalence, new HIV infections, and the distribution of CD4 count. Two module sets were activated for this projection. The demographic set included DEMPROJ – to estimate the population by age and sex, and FAMPLAN – to estimate the impact of family planning services on population size. The HIV set included AIM – to estimate the impact of HIV, GOALS – to estimate the funding needed to achieve the national HIV response targets, and RNM – to estimate the costs of implementing the national HIV program. Model inputs were derived from published country reports, strategic plans, costing studies, programmatic data, and expert opinion [20].

Patients and case definition

Records were obtained of any patient who received a lumbar puncture (LP) for CSF analysis without an age cut-off. In Botswana, routine CSF testing includes gram stain and bacterial culture, acid-fast Bacillus (AFB) smear, India ink stain and fungal culture, cell count, white blood cell (WBC) differential, and protein and glucose concentrations. AFB culture, when ordered, is performed centrally at the Botswana National Tuberculosis Reference Laboratory in Gaborone.

CSF testing for CrAg was performed by several centers and routinely at referral hospital laboratories.

A "case" (or episode) of cryptococcal meningitis was defined as CSF with positive India ink stain, Cryptococcus culture, and/or positive CSF CrAg. As CSF analysis may have been repeated on patients receiving multiple LPs for lowering of intracranial pressure or other indications during an admission, all positive CSF samples for a unique patient within a single documented hospitalization (or in the absence of admission dates, within a ≤ 14-day window of any previous CSF sample) were considered part of the same "case".

Data analysis

Data were analyzed using Stata (version 12, College Station, TX). Cases of cryptococcal meningitis in Botswana were enumerated using the case definition. The number of cases, recurrent cases, patient age and gender, and month and season of diagnosis were described using frequencies, percentage or median and interquartile range (IQR), as appropriate. It was not possible to calculate national incidence rates over the full 15-year study period, as records prior to 2013 were partially complete. Complete CSF laboratory data from all laboratories were obtained during 2013 and 2014 calendar years. For this 2013-2014 national data, UNAIDS spectrum model HIV estimates were used as a population denominator to determine cryptococcal meningitis incidence rate in person-years of observation with 95% confidence intervals (95%CI) derived using a Poisson distribution. Incidence was estimated by gender and ordinal age categories as well as by CD4 T-cell count category stratified by gender in individuals ≥15-years old.

The two national referral hospitals (Princess Marina Hospital and Nyangabwe Referral Hospital) adopted IPMS in 2004 and also maintained paper-based records, yielding a complete dataset for the 11-year period 2004 to 2014. At these two hospitals, annual cases diagnosed over this 11-year period were displayed graphically in relation to the "treatment gap" (UNAIDS-estimated HIV-infected population ≥15 years of age minus population receiving ART) and median CD4 cell count at ART initiation.

3.5 RESULTS

15-year cryptococcal meningitis data

We identified 5296 unique cases of cryptococcal meningitis in 4702 individuals from 2000-2014 (Table 3). Of these individuals, 90·3% (4248/4702) experienced a single episode, 7·7% (360/4702) two episodes, 1·0% (48/4702) three episodes, and 1·0% (46/4702) four or more episodes of disease. Median age at diagnosis was 36 years (IQR 30-43 years) (Figure 4) and more cases were diagnosed in males than females (60·6% males versus 39·4% females). There was no evidence of seasonality. Case numbers did not differ with respect to month or season at diagnosis.

2013-2014 incidence of cryptococcal meningitis

National incidence of diagnosed cryptococcal meningitis 2013-2014 was 17·8 cases/100,000 person-years (95%CI 16·6 – 19·2 cases/100,000 person-years), with higher incidence observed in males than in females (22·0 cases/100,000 person-years in males [95%CI 20·1 – 24·1 cases/100,000 person-years] versus 13·7 cases/100,000 person-years [95%CI 12·1 – 15·3 cases/100,000 person-years] in females) (Table 4, Figure 5A-B). Peak incidence was observed in 40-44 year olds, with an earlier peak among females (30-34 years) and a later peak among males (40-44 years) mirroring national age trends in HIV prevalence.

In the HIV-infected population, overall incidence was 96·8 cases/100,000 person-years (95%CI 90·0 – 104·0 cases/100,000 person-years), or 135·4 cases/100,000 person-years (95%CI 123·4 – 148·3 cases/100,000 person-years) in males and 66·3 cases/100,000 person-years (95%CI 58·9 – 74·4 cases/100,000 person-years) in females. Incidence rate was relatively uniform across adult age categories, peaking in the 4th through 5th decade of life (Figure 5C). Although the absolute number of CM cases in children was relatively low, incidence was comparatively high in the two youngest age categories (0-4 years and 5-9 years), declining in the 10-14 year age group, then increasing to adult levels in the adolescent category (15-19 years). Note that the high incidence in the 0-4 year age category may be spuriously high due to an underestimation of the denominator in the UNAIDS figures and low overall numbers in both the numerator and denominator categories. Incidence increased markedly with declining CD4 T-cell count strata. In those with CD4 T-cell counts <50 cells/μL, a nearly 2% annual incidence was observed, of 1,854 cases/100,000 person-years (95%CI 1,680·5 – 2,042·0 cases/100,000 person-years), with higher incidence in males than females across CD4 count strata (Figure 6).

Referral hospital temporal trends

At the two national referral hospital laboratories, processing 60% of CSF samples, the number of cases of cryptococcal meningitis cases decreased between 2004 and 2014, with a marked decrease 2007-2009 but only a modest decline in 2010-2014, despite a shift in national ART guidelines recommending ART initiation at a higher CD4 count (<350 cells/μL in 2012 guidelines versus <200 cells/μL in 2008 guidelines).[2,3] Figure 7A shows cases of cryptococcal meningitis diagnosed at the referral hospitals, with total HIV-infected population in Botswana ≥15 years of

age and population on ART over time. Over this period, the median CD4 count at ART initiation increased from 2007-2011 but remained stable 2012-2014 (Figure 7B), with similar trends observed in the total population of HIV-infected individuals with CD4 counts <200 cells/μL (Figure 7C).

3.6 DISCUSSION

These are the first robust national cryptococcal meningitis incidence estimates from a resource-limited country. Collection of complete records from all laboratories in Botswana performing CSF testing allowed us to accurately estimate 2013-2014 national incidence rates, revealing a high incidence of cryptococcal meningitis in Botswana in the context of the highest population antiretroviral therapy coverage in Africa. The 2013-2014 Botswana national cryptococcal meningitis incidence is almost identical to the pre-ART era (2002-2004) laboratory surveillance-based incidence estimates from Gauteng Province, South Africa (17.8 cases/100,000 person-years versus 15·6 cases/100,000 person-years, respectively), a period when estimated adult (15-49 years) HIV prevalence in South Africa was 21·5% [21,22]. Limited to only HIV-infected populations, 2013-2014 Botswana incidence of 96·8 cases/100,000 person-years is again comparable to South African incidence estimates from the pre-ART era (95 cases/100,000 person-years), and greater than 100-fold higher than the 2009 estimated United States HIV-associated cryptococcal meningitis rate of 7·7 cases/1,000,000 person-years [15].

The rate of decline in cryptococcal meningitis cases diagnosed at referral center laboratories has stagnated in recent years, despite national data showing high rates of HIV testing, ART uptake, and viral suppression [4]. These findings reflect a number of factors, including high ongoing national HIV prevalence, only modest improvements in median CD4 T-cell counts at ART

initiation in recent years, from 191 cells/μL (IQR 115 – 239 cells/μL) in 2010 to 258 cells/μL (IQR 147 – 337 μL) in 2013 despite a shift in national guidelines promoting earlier therapy [1,23-25], and high annual incidence of new HIV infection estimated at 1·5-2·5% in adults [26]. Importantly, these findings suggest that, despite a decade and a half of free and widely available HIV testing and treatment services in Botswana, a population of vulnerable individuals are not being effectively reached, engaged, or retained by current testing and treatment services. This key population is now the main driver of HIV-related morbidity and mortality, and likely to be maintaining the high ongoing incidence of HIV acquisition in this setting.

With stabilizing or rising adult HIV prevalence in Botswana and the African region, eliminating the remaining treatment gap and advanced HIV-disease will require innovative solutions to effectively reach key populations who are being missed by current testing and treatment models, diagnose and treat early asymptomatic infection, and promote lifelong engagement with treatment services. Updated 2016 guidelines in Botswana, in line with 2015 World Health Organization guidelines, now recommend the "test and treat" strategy of ART at any CD4 count for people living with HIV [27,28]. This represents a critical step toward reducing advanced HIV disease and opportunistic infections like cryptococcal meningitis and tuberculosis. However, the latest Botswana AIDS Indicator Survey data estimated 30% of the population aged 10-64 years had never undergone HIV testing in 2013 and ART coverage [26], at about 250,000 HIV-infected individuals by 2015,[23] needs to expand by 100,000, which will put a strain on existing services [29]. Similar scale-up will likely be even more disruptive throughout the rest of Africa, where the number of people receiving ART needs to double to cover all HIV-infected individuals [30]. To avoid leaving vulnerable individuals behind, differentiated care models should be considered to

streamline care for populations with well-controlled disease and focus more intensive resources on those with higher need who are now driving the epidemic.

Our study provides important insights into the characteristics of individuals presenting with advanced HIV and cryptococcal meningitis in Botswana. We found peak incidence of disease in adults in the 4th and 5th decade of life, suggesting a need to better engage young, working-age adults through work- or community-based care models that minimize lost opportunity costs. Strikingly, we observed a greater than two-fold higher incidence of disease in HIV-infected males than HIV-infected females. Although this did not completely correct when the analysis was stratified by CD4 count, perhaps in part reflecting a true gender-related biological predisposition to cryptococcal disease for which the pathogenesis is incompletely understood [31,32], it highlights the high numbers of men presenting late to care or failing to engage with testing and treatment services.

This study had several important limitations. First, these estimates likely represent the lowest range of true cryptococcal meningitis incidence rates in Botswana due to the limitations of deriving incidence estimates from laboratory-based surveillance, including missing individuals who died before seeking medical care or who sought care but went undiagnosed due to misdiagnosis, stock out of lumbar puncture equipment or laboratory reagents, or lumbar puncture refusal [33]. Although we applied a rigorous approach to data collection and estimated incidence only for 2013-2014 when we believed laboratory data to be complete, missed case ascertainment may also have led to spuriously low incidence estimates. A small number of individuals may have sought care from neighboring countries, but this is not likely to have significantly impacted our findings. Over

85% of cases in the IPMS dataset had a Botswana national identification number and the vast majority of the remainder could be identified as citizens using available identifying data. Uncertainty in UNAIDS Spectrum model denominator estimates could lead to either overestimation or underestimation, although figures are based on the most robust and contemporary data available and are validated against reliable national census and HIV population prevalence survey results [26]. Secondly, as a laboratory-based surveillance study we did not have national data on ART treatment history and were unable to stratify incidence based on ART status. However, single-center data from Princess Marina Hospital indicates that 51% of cryptococcal meningitis patients are now presenting on ART [34], in keeping with other regional data [16,17]. Thirdly, we were unable to ascertain outcomes, although 2012-2014 data from Princess Marina Hospital showed a 10-week cryptococcal meningitis mortality of 50% and 1-year mortality of 65% [35]. The competing risk of high mortality could explain, in part, the relatively low observed relapse rate. Finally, we were unable to evaluate temporal trends in cryptococcal meningitis outside of the two referral hospitals due to incomplete records, which might differ from urban settings where referral centers are located. However, as almost two-thirds of cases were diagnosed at referral centers, this provides meaningful information regarding national trends.

3.7 CONCLUSION

In summary, we provide evidence for a high ongoing burden of advanced HIV disease in Botswana, a country with a mature ART program and the highest population ART coverage in Africa. Our findings highlight a need not only to adopt disease-specific measures to reduce cryptococcal meningitis, such as CrAg screening in patients with advanced disease [36,37], but the broader need to engage and link key populations to care who are being missed with current strategies. Adoption of HIV test and treat could substantially reduce advanced HIV disease but

must be coupled with paradigm shifts in testing, care linkage, and ART delivery such as differentiated care models.

3.8 ACKNOWLEDGMENTS

FUNDING

This work was supported by the Penn Center for AIDS Research, a National Institutes of Health (NIH)–funded program (grant number P30 AI 045008 to JNJ) as well as the NIH Office of AIDS Research and Centers for Disease Control and Prevention (CDC) / President's Emergency Plan for AIDS Relief (PEPFAR) grant support (grant number U91HA06801B to MWT).

ACKNOWLEDGMENTS

We thank the Afya Bora Consortium, Diakanyo Moalosi at the Botswana Ministry of Health for her assistance with querying IPMS records, Freedom Ernest at the Botswana National Health Laboratory for assistance with arranging laboratory visits, as well as multiple health facility and laboratory personnel at Athlone Hospital, Bobonong Primary Hospital, Deborah Retief Memorial Hospital, Ghanzi Primary Hospital, GoodHope Primary Hospital, Gumare Primary Hospital, Gweta Primary Hospital, Hukuntsi Primary Hospital, Kasane Primary Hospital, Lethlakane Primary Hospital, Lobatse Mental Hospital, Mahalapye Hospital, Masunga Primary Hospital, Letsholathebe Memorial Hospital, Mmadinare Primary Hospital, Nyangabwe Referral Hospital, Palapye Primary Hospital, Princess Marina Hospital, Rakops Primary Hospital, Scottish Livingstone Hospital, Sefhare Primary Hospital, Selibe-Phikwe Hospital, Sekgoma Memorial Hospital, Thamaga Primary Hospital, Tshabong Primary Hospital, Tutume Primary Hospital, Extension 2 Clinic, Charles Hill Clinic, Area W Clinic, Thebephatshwa Air Base, Bamalete Lutheran Hospital, Kanye Seventh-Day Adventist Hospital, Jwaneng Mine Hospital, Diagnofirm

Medical Center, Gaborone Private Hospital Lancet Laboratory, Tati River Clinic Laboratory, and Bokamoso Hospital Laboratory for their assistance. Individually, we would like to thank Thato Mogapi, Bothwell Muviiwa, Kago Gofamodimo, Olefile Bickie Bagwasi, Tlotlo Bogatsu, David Lubasi, Nobley Chunda, H Farrar Otieno, Felix C Banda, Joy Mangilazi, Koabetswe Ramalepa, Modisaotsile Emdee Ramaologa, Caroline Kekana, Stephen Motlhagodi, Ephraim Dambe, Keoratile Ntshambiwa, George Simwanza, Phillip Joseph, Cynthia M Kuate, Steven Tsima, Jenny Tommy, Patrick Rancholo, Finini Biyangidiki, Asini Mosango, Zein Sasikwa, Jacqueline Kasambala, Keontse Nyamadzabo, Keteng Mphoyakgosi, Olivier Betu, Frank Nyimbili, Moemedi Banda, Leabaneng Molomo, and Gabriel Malomo, among others who provided assistance to our study team during laboratory visits.

3.9 REFERENCES

1. Farahani M, Vable A, Lebelonyane R, Seipone K, Anderson M, Avalos A, et al. Outcomes of the Botswana national HIV/AIDS treatment programme from 2002 to 2010: a longitudinal analysis. Lancet Glob Health. 2014;2(1):e44-50.

2. Botswana Ministry of Health. 2012 Botswana national HIV & AIDS treatment guidelines. Available at: https://hivpolicywatch.org/duremaps/data/guidelines/BotswanaARTguidelines2012.pdf. Accessed 28 March 2017.

3. Botswana Ministry of Health. Botswana national HIV/AIDS treatment guidelines: 2008 version. Available at: http://www.moh.gov.bw/Publications/HIVAIDS%20TREATMENT%20GUIDELINES.pdf. Accessed 28 March 2017.

4. Gaolathe T, Wirth KE, Holme MP, Makhema J, Moyo S, Chakalisa U, et al. Botswana's progress toward achieving the 2020 UNAIDS 90-90-90 antiretroviral therapy and virological suppression goals: a population-based survey. Lancet HIV. 2016;3(5):e221-30.

5. Karim SA. Is the UNAIDS target sufficient for HIV control in Botswana? Lancet HIV. 2016;3(5):e195-6.

6. UNAIDS. HIV and AIDS estimates - Botswana. Available at: http://www.unaids.org/en/regionscountries/countries/botswana/. Accessed 28 March 2017.

7. Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, Gakidou E, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. Lancet HIV. 2016;3(8):e361-87.

8. Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002-2013: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(7):1120-7.

9. Avila D, Althoff KN, Mugglin C, Wools-Kaloustian K, Koller M, Dabis F, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. Journal of acquired immune deficiency syndromes. 2014;65(1):e8-16.

10. Brennan AT, Long L, Useem J, Garrison L, Fox MP. Mortality in the First 3 Months on Antiretroviral Therapy Among HIV-Positive Adults in Low- and Middle-income Countries: A Meta-analysis. Journal of acquired immune deficiency syndromes. 2016;73(1):1-10.

11. Fox MP, Rosen S. Retention of Adult Patients on Antiretroviral Therapy in Low- and Middle-Income Countries: Systematic Review and Meta-analysis 2008-2013. Journal of acquired immune deficiency syndromes. 2015;69(1):98-108.

12. Boender TS, Hoenderboom BM, Sigaloff KC, Hamers RL, Wellington M, Shamu T, et al. Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;61(11):1749-58.

13. Group TS. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. Lancet Infect Dis. 2016;16(5):565-75.

14. Jarvis JN, Harrison TS. HIV-associated cryptococcal meningitis. Aids. 2007;21(16):2119-29.

15. Pyrgos V, Seitz AE, Steiner CA, Prevots DR, Williamson PR. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PloS one. 2013;8(2):e56269.

16. Scriven JE, Lalloo DG, Meintjes G. Changing epidemiology of HIV-associated cryptococcosis in sub-Saharan Africa. Lancet Infect Dis. 2016;16(8):891-2.

17. Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, et al. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. Lancet Infect Dis. 2016;16(7):809-18.

18. Jarvis JN, Harrison TS. Forgotten but not gone: HIV-associated cryptococcal meningitis. Lancet Infect Dis. 2016;16(7):756-8.

19. Avenir Health. Spectrum. Available at: http://www.avenirhealth.org/software-spectrum.php. Accessed 28 March 2017.

20. Avalos A, Phillips H, Jefferis K. Botswana investment case: Investment towards effective HIV prevention, health system strengthening and the end of AIDS. Presented at: Botswana Ministry of Health. Gaborone, Botswana, 2016.

21. McCarthy KM, Morgan J, Wannemuehler KA, Mirza SA, Gould SM, Mhlongo N, et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. Aids. 2006;20(17):2199-206.

22. UNAIDS. 2004 report on the global AIDS epidemic. 2004.

23. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. Aids. 2016;30(3):477-85.

24. Botswana Ministry of Health. 2012 Botswana national HIV & AIDS treatment guidelines. 2012.

25. Thurey J, Molyneux E. Evidence behind the WHO guidelines: Hospital Care for Children: the usefulness of azole prophylaxis against cryptococcal meningitis in HIV-positive children. J Trop Pediatr. 2008;54(6):361-3.

26. Botswana Ministry of Health. Botswana AIDS impact survey IV 2013. Available at: http://botswana.microdatahub.com/index.php/catalog/14. Accessed 28 March 2017.

27. Monari C, Retini C, Casadevall A, Netski D, Bistoni F, Kozel TR, et al. Differences in outcome of the interaction between Cryptococcus neoformans glucuronoxylomannan and human monocytes and neutrophils. Eur J Immunol. 2003;33(4):1041-51.

28. Kawakami K. [The role of natural killer T cells in host defense to cryptococcal infection--a bridge from innate to acquired immunity]. Nihon Ishinkin Gakkai Zasshi. 2002;43(3):137-41.

29. McGovern S, Phillips H, Mosime W, et al. Test results of testing cost-yield prioritization model for test and treat in Botswana. Presented at: 21st International AIDS Conference. Durban, South Africa, 2016.

30. World Health Organization. Global health sector response to HIV, 2000-2015: Focus on innovations in Africa. 2015. http://apps.who.int.offcampus.lib.washington.edu/iris/bitstream/10665/198065/1/9789241509824_eng.pdf (accessed 15 Nov 2016). 2015.

31. McClelland EE, Hobbs LM, Rivera J, Casadevall A, Potts WK, Smith JM, et al. The role of host gender in the pathogenesis of Cryptococcus neoformans infections. PloS one. 2013;8(5):e63632.

32. Mitchell DH, Sorrell TC, Allworth AM, Heath CH, McGregor AR, Papanaoum K, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1995;20(3):611-6.

33. Thakur KT, Mateyo K, Hachaambwa L, Kayamba V, Mallewa M, Mallewa J, et al. Lumbar puncture refusal in sub-Saharan Africa: A call for further understanding and intervention. Neurology. 2015;84(19):1988-90.

34. Jarvis JN, Leeme T, Chofle, et al. High dose liposomal amphotericin for HIV-infected cryptococcal meningitis. Presented at: CROI 2017. Seattle, WA, 2017.

35. Leeme TB PR, Azzo C, Lekwape N, Tsholo K, Tawanana E, Molefi M, Mokomane M, Tenforde MW, Jarvis JN, editor Mortality due to HIV-associated cryptococcal meningitis in Botswana in the ART era. CROI (accepted abstract); 2017; Seattle, WA.

36. Meya D, Rajasingham R, Nalintya E, Tenforde M, Jarvis JN. Preventing Cryptococcosis-Shifting the Paradigm in the Era of Highly Active Antiretroviral Therapy. Curr Trop Med Rep. 2015;2(2):81-9.

37. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet. 2015.

Table 3. Description of cryptococcal meningitis cases in Botswana, 2000-2014.

Total Cryptococcal Meningitis Episodes			5296
Total Cryptococcal Meningitis Patients			4702
	Single episode	4248	90·3%
	Two episodes	360	7·7%
	Three episodes	48	1·0%
	Four or more episodes	46	1·0%

Variable	Value	Number with data	Result (N, %)*	
Age**	(Median, IQR)	*4056*	36 years	30 – 43 years
Sex**	Male	*4407*	2670	60·6%
	Female		1737	39·4%
Site†	Tertiary	*5296*	3161	59·7%
	Primary/Secondary		2135	40·3%
Month†	January	*5296*	430	8·1%
	February		442	8·4%
	March		481	9·1%
	April		398	7·5%
	May		432	8·2%
	June		418	7·9%
	July		449	8·4%

	August		490	9·3%
	September		435	8·2%
	October		423	8·0%
	November		456	8·6%
	December		442	8·4%
Season†	Summer (Nov-Jan)	*5296*	1328	25·1%
	Autumn (Feb-Apr)		1321	24·9%
	Winter (May-Jul)		1299	24·5%
	Spring (Aug-Oct)		1348	25·5%

*Unless otherwise indicated

**De-duplicated to represent individual patients rather than cryptococcal meningitis episodes.

†Data from all cryptococcal meningitis episodes including relapses.

IQR = interquartile range

Table 4. National incidence of cryptococcal meningitis in Botswana, 2013-2014.

Strata	Category	Case Number	Person-years	Incidence (per 100,000 PYO)	95% Confidence Interval (per 100,000 PYO)
Overall Incidence					
All	-	755	4,231,095	17·8	16·6 – 19·2
Sex	Male	466	2,115,031	22·0	20·1 – 24·1
	Female	289	2,116,063	13·7	12·1 – 15·3
Age					
(years)	0-4	10	475,589	2·1	1·0 – 3·9
	5-9	4	467,021	0·9	0·2 – 2·2
	10-14	5	458,710	1·1	0·4 – 2·5
	15-19	20	457,334	4·4	2·7 – 6·8
	20-24	37	450,494	8·2	5·8 – 11·3
	25-29	79	424,031	18·6	14·8 – 23·2
	30-34	156	371,214	42·0	35·7 – 49·2
	35-39	138	295,495	46·7	39·2 – 55·2
	40-44	141	212,707	66·3	55·8 – 78·2
	45-49	76	158,636	47·9	37·8 – 60·0
	50-54	40	125,781	31·8	22·7 – 43·3
	55-59	27	107,792	25·1	16·5 – 36·4
	60-64	10	83,350	12·0	5·8 – 22·1
	65-69	4	55,035	7·3	2·0 – 18·6

	70-74	3	42,068	7·1	1·5 – 20·8
	75+	3	45,839	6·5	1·4 – 19·1
HIV infected (Any CD4 Count)					
All	-	755	779,997	96.8	90·0 – 104·0
Sex	Male	466	344,120	135.4	123·4 – 148·3
	Female	289	435,877	66.3	58·9 – 74·4
CD4 cell count <200 cells/μL*					
All	-	690	241,445	285.8	264·9 – 307·9
Sex	Male	432	119,120	362.7	329·3 – 398·5
	Female	259	122,325	211.7	186·7 – 239·2
CD4 cell count <100 cells/μL*					
All	-	590	74,845	788.3	726·0 – 854·6
Sex	Male	370	39,304	941.4	847·9 – 1042·4
	Female	221	35,541	621.8	542·5 – 709·4
CD4 cell count <50 cells/μL*					
All	-	415	22,376	1854.7	1680·5 – 2042·0
Sex	Male	260	11,846	2194.8	1936·1 – 2478·5
	Female	156	10,530	1481.5	1258·1 – 1733·1

*Restricted to individuals aged 15 years old or greater.

PYO = person years of observation

Figure 4. Age distribution of cryptococcal meningitis cases in Botswana, 2000-2014.

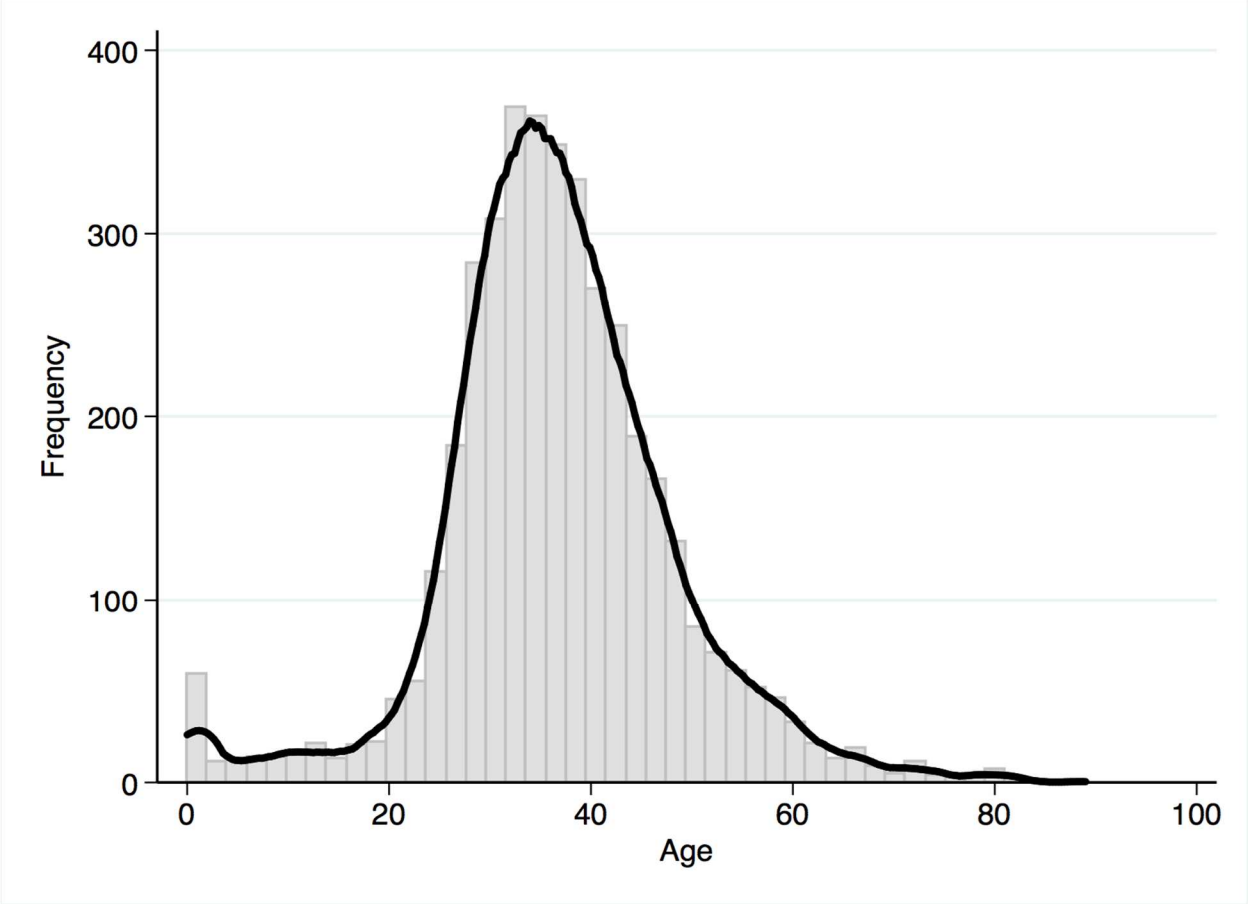


Figure 5. Incidence of cryptococcal meningitis in Botswana by age category, 2013-2014. 2A) Overall incidence and interquartile range; 2B) Incidence by gender (males solid bars and females striped bars); 2C) Incidence in HIV-infected population.

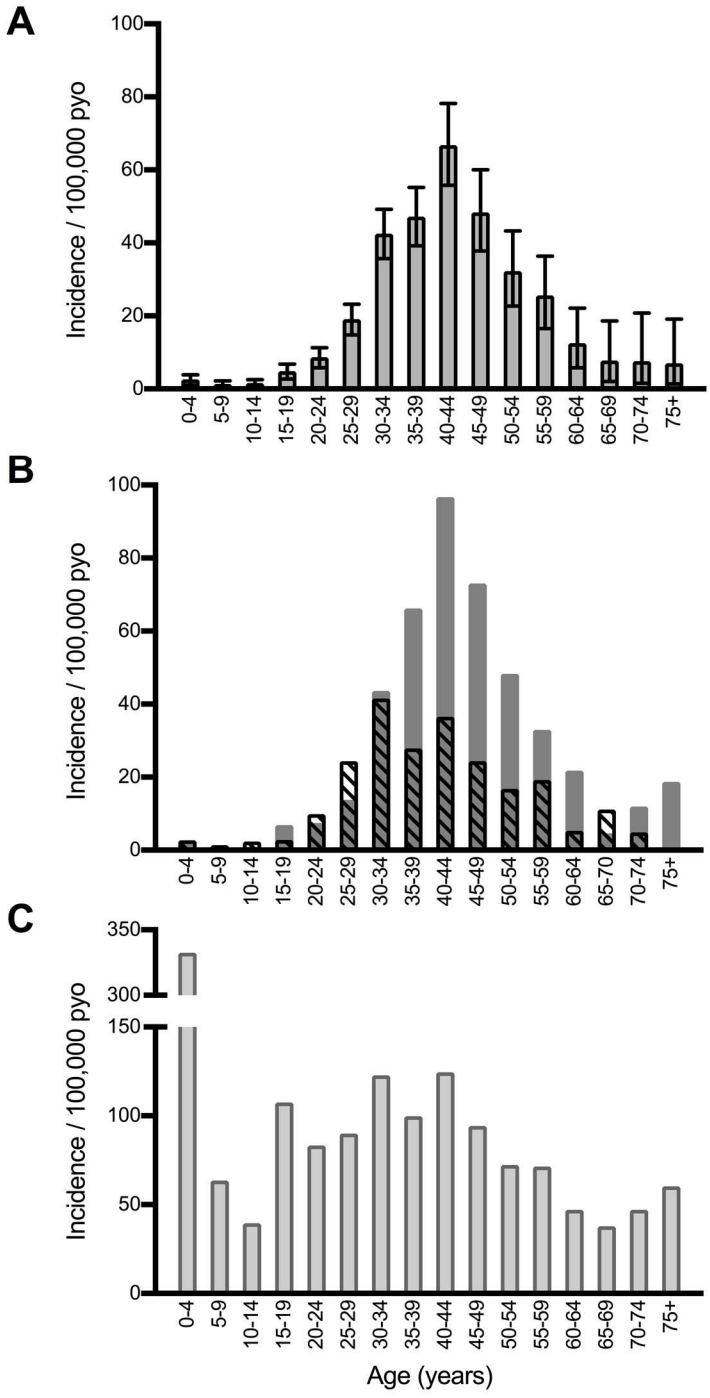


Figure 6. Incidence of cryptococcal meningitis in Botswana in HIV-infected population by CD4 strata, 2013-2014. 3A) Overall incidence; 3B) Incidence by gender (males black lines, females grey lines).

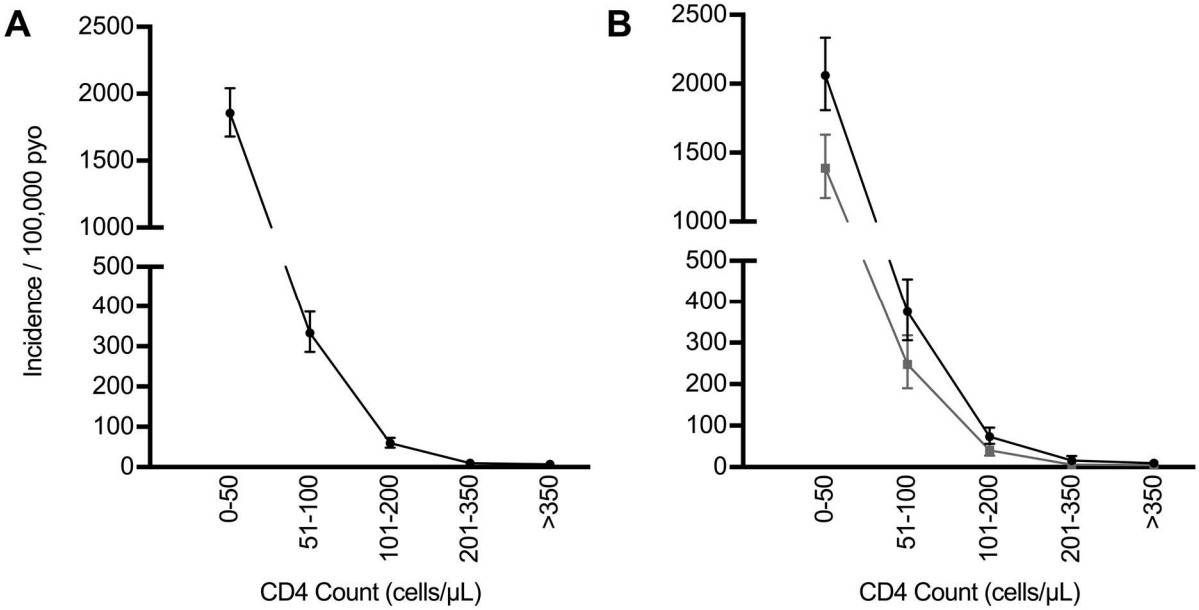
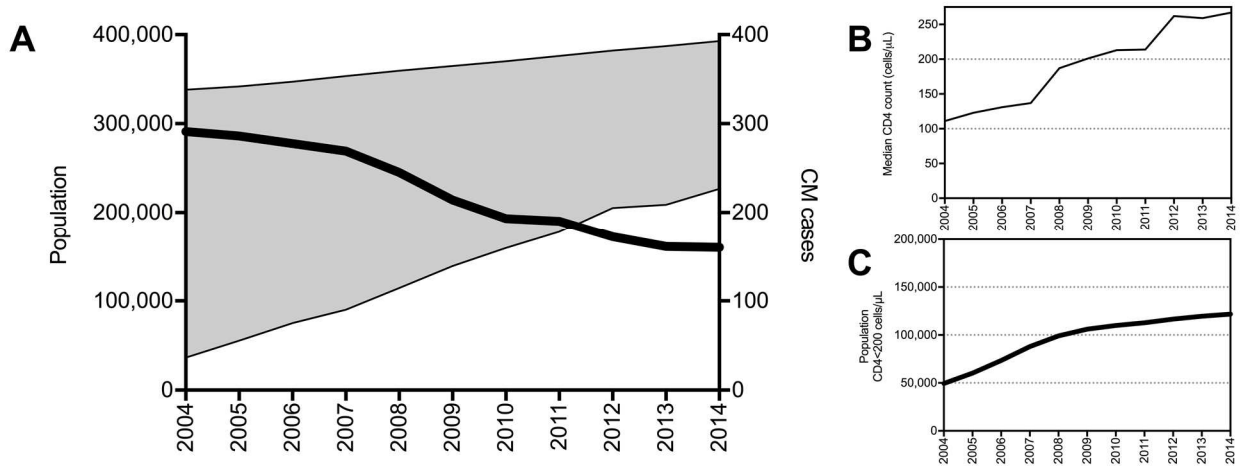


Figure 7. Trends in diagnosed cases of cryptococcal meningitis at the 2 national referral hospitals in Botswana, 2004-2015. 4A) Cases diagnosed at referral hospitals (thick black line) and treatment gap in adults (≥15 years) [shaded grey area]; 4B) UNAIDS estimate of median CD4 count at antiretroviral therapy initiation in Botswana; 4C) UNAID estimate of number of HIV-infected individuals not on antiretroviral therapy in Botswana.



Chapter 4. MORTALITY OUTCOMES IN CULTURE-POSITIVE AND CULTURE-NEGATIVE ADULT MENINGITIS IN THE BOTSWANA NATIONAL MENINGITIS SURVEY: A PREVALENT COHORT STUDY

4.1 MANUSCRIPT TITLE AND TITLE PAGE

Mortality Outcomes in Culture-positive and Culture-negative Adult Meningitis in the Botswana National Meningitis Survey: A Prevalent Cohort Study

Mark W Tenforde MD[1,2,3], Margaret Mokomane MSc[4], Tshepo B Leeme MBBS[3], Nametso Tlhako[3], Katlego Tsholo[3], Tony Chebani MSc[5], Anya Stephenson MBChB[3], Jemima Hutton MBBS[3], Hannah K Mitchell BMBS[3], Raju KK Patel MBBS[3], William J Hurt BMchB[3], Tlhagiso Pilatwe MSc[5], Tiny Masupe MD[6], Mooketsi Molefi MBChB[6], Brandon L Guthrie PhD[2,7], Carey Farquhar MD[1,2,7], Madisa Mine PhD[4], Joseph N Jarvis MD[3,8,9,10]

[1] Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington School of Medicine, Seattle, WA USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA USA

[3] Botswana-UPenn Partnership, Gaborone, Botswana

[4] Botswana National Health Laboratory, Gaborone, Botswana

[5] Botswana Ministry of Health, Gaborone, Botswana

[6] Department of Family Medicine and Public Health, University of Botswana, Gaborone, Botswana

[7] Department of Global Health, University of Washington, Seattle, WA USA

[8] Division of Infectious Diseases, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA USA

[9] Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

[10] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Corresponding author

Mark Tenforde MD, MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2066508915 (US); +267 74319965 (Botswana)

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Key Words: meningitis; sub-Saharan Africa; tuberculous meningitis; pneumococcal meningitis; lymphocytic meningitis; HIV

Word Count: 3614

4.2 ABSTRACT

Background: Central nervous system (CNS) infections are a leading cause of HIV-related deaths in sub-Saharan Africa. Aetiologies and outcomes are poorly-defined. We performed a study to determine mortality rates and predictors of mortality in adults evaluated for meningitis in Botswana.

Methods: We conducted a prevalent cohort study (2004-2015) including all culture-confirmed pneumococcal, TB, and culture-negative meningitis with cerebrospinal fluid (CSF) white cell count [WCC]>20 cells/μL, and a random selection of culture-negative cases with WCC≤20 cells/μL. Laboratory records were linked to national HIV and death registries. Predictors of mortality were evaluated using Cox proportional hazards.

Findings: We included 238 pneumococcal, 48 TB, and 2900 culture-negative cases. Median age was 37 years (interquartile range [IQR]:31-46); 50% (1605/3184) were male; 72% (2188/3023) with registry linkage had documentation of HIV-infection, median CD4 139 cells/μL (IQR 63-271). Ten-week and one-year mortality were 47% (112/238) and 49% (117/238) for pneumococcal, 46% (22/48) and 56% (27/48) for TB, and 41% (1181/2900) and 49% (1408/2900) for culture-negative cases. In known HIV-infected culture-negative cases, WCC (>20/μL vs. ≤20/μL) was not predictive of mortality (aHR 0·93, 95% confidence interval [CI]:0·80-1·09), but higher CD4 count (CD4<50 vs. >350 cells/μL aHR 2·71, 95%CI:2·06-3·56), ART use (aHR 0·74, 95%CI:0·64-0·87), and lymphocytic CSF (aHR 0·55, 95%CI:0·44-0·70) were associated with lower one-year mortality.

Interpretation: Mortality rates from pneumococcal, TB, and culture-negative meningitis were all high, with approximately half dying within one year. There is an urgent need to better define aetiologies and treatment approaches.

Funding: National Institutes of Health, President's Emergency Plan for AIDS Research.

4.3 INTRODUCTION

Central nervous system (CNS) infections are a leading cause of mortality in sub-Saharan Africa (SSA) [1,2]. The emergence of HIV markedly altered the epidemiology of meningitis in the region, with cryptococcal meningitis, tuberculous meningitis and bacterial meningitis due to *Streptococcus pneumoniae* now the most common confirmed meningitis aetiologies in adults in central, east, and southern Africa [3-5]. Outcomes from cryptococcal meningitis have been relatively well-described in clinical trials and observational studies.[6-9] However, data regarding long-term outcomes from TB or pneumococcal meningitis in routine-care settings are limited [5,10,11].

Furthermore, a majority of patients with suspected meningitis evaluated by lumbar puncture (LP) and cerebrospinal fluid (CSF) analysis in resource-limited settings have no pathogen identified through available diagnostic studies [3,4]. Even in studies that have utilised enhanced molecular diagnostics, up to half of patients still did not have a definitive microbiological diagnosis [12-14]. Aetiology of presentation therefore remains poorly understood, management recommendations for this large patient population are lacking, and no data exists on short- or long-term survival.

Botswana, a country in Southern Africa with an estimated 23% HIV prevalence among adults [15], has a national electronic death registry unique within the region, as well as an

electronic national HIV registry used for research and surveillance [16]. To determine long-term outcomes in routine care settings for culture-confirmed TB and pneumococcal meningitis and culture-negative meningitis, we linked CSF laboratory records from a national meningitis survey to patient vital registry and HIV databases using patient national identification numbers [17]. We compared outcomes for culture-confirmed TB and pneumococcal meningitis, culture-negative patients with abnormal CSF cellular findings (WCC >20 cells/μL), and culture-negative patients with a relatively normal profile (CSF WCC ≤20 cells/μL), and evaluated clinical and laboratory predictors of mortality up to one year.

4.4 MATERIALS AND METHODS

Study population and data sources.

Patients were sampled from 2004-2015 from the Botswana National Meningitis Survey (BNMS), a nationwide audit of all CSF laboratory records from patients receiving a lumbar puncture for evaluation of meningitis [17]. BNMS included both paper CSF records and electronic records from a national electronic medical record (EMR), Integrated Patient Management System (IPMS). IPMS was rolled out in Botswana's public hospital laboratory system from 2004, and by 2014 covered over 72% of patients undergoing CSF analysis (data not shown). Paper and electronic data were merged, de-duplicated and stored in a REDCap database. Standard CSF work-up included glucose, protein, and WCC with or without differential (neutrophil and lymphocyte percentages in those with elevated WCC). Microbiological testing included Gram stain, India ink stain, and standard cultures on all samples. Cryptococcal antigen (CrAg) latex agglutination testing was available in

some laboratories (performed on 4% [703/19,409] of samples registered on IPMS during the study period). TB culture was performed at the request of clinical providers at a central national TB reference laboratory (performed on 9% [1,723/19,409] of samples registered on IPMS during the study period, with AFB microscopy performed on 13% [2,524/19,409] of samples). Xpert MTB/RIF was introduced in Botswana in August 2012 but not performed on CSF samples.

Culture-negative meningitis was defined as cases with negative bacterial culture and Gram stain and negative work-up for cryptococcal meningitis (including microscopy, CrAg testing [when available], and fungal culture). For assessment of culture-negative meningitis outcomes the dataset was restricted to patients with a nine-digit national identification number ("Omang") in IPMS; a unique national identification number assigned to all Botswana citizens ≥16 years of age. Omang is recorded when a patient is registered in IPMS which occurs at the time of first hospital admission, and is verified at each subsequent admission. All patients with CSF WCCs >20 cells/μL were selected for analysis, plus a random selection of 1000 CSF samples with negative cultures and normal CSF WCCs (0-2 cells/μL), and 1000 with mildly abnormal CSF WCCs (3-20 cells/μL) [Figure 8] based on a random number sequence generated using Stata version 13·0 (College Station, TX). WCC categories were selected as mild pleocytosis, typically <20 cells/μL, is common in asymptomatic HIV-infected adults, and for consistency with previously published data [4]. We selected 1000 random samples for pragmatic reasons, based on a data agreement with the Botswana Ministry of Labour and Home Affairs which maintains the death registry. Repeat samples from the same patient were excluded, as were patients subsequently found to have positive microscopy in the absence of positive cultures.

Children usually do not have an assigned Omang; therefore, selection of culture-negative patients was restricted to adults (≥16 years). The national identification number was used for deterministic linkage of patient CSF laboratory data to HIV-related data in the national HIV registry. Antiretroviral therapy (ART) was available in Botswana starting in 2002 and HIV prevalence in adults (15-49 years) remained stable (from 25% in 2004 to 23% in 2015 [18]). Records were obtained from the HIV registry on HIV status and, for HIV-infected patients, most recent CD4 count within six months of LP and whether ART was initiated before date of LP [19]. All-cause mortality within one-year following initial lumbar puncture was determined through deterministic linkage by unique identification number to the national electronic death registry. From this registry, a prior study captured vital status up to one year for 93% of patients treated for cryptococcal meningitis at a Botswana referral hospital from 2012-2014 [6], suggesting high quality capture. In 2014, an estimated 89% of deaths were registered within one month [20].

One-year mortality was also evaluated for all cases of culture-confirmed *Streptococcus pneumoniae* meningitis in IPMS, which accounted for the majority of culture-confirmed bacterial meningitis (Figure 8), and culture-confirmed TBM cases from 2007 onward from the National Tuberculosis Reference Laboratory's EMR (Disa, which is distinct from the IPMS which is used for non-TB records). These records were linked to the death registry by Omang (when available) or by first and surname and by Omang only to the HIV registry.

Ethics Statement

The study was approved by institutional review boards at the University of Pennsylvania, University of Washington, University of Botswana, and the Ministry of Health's Health Research and Development Committee (HDRC). As the study involved only retrospective

collection of routine clinical and outcomes data, a waiver of informed patient consent was obtained.

Data analysis.

Demographic, clinical, and laboratory characteristics were described using summary statistics. The χ^2 and Wilcoxon rank sum tests were used to compare categorical and continuous characteristics between groups. Kaplan-Meier survival curves were generated for TB and pneumococcal meningitis and for culture-negative cases.

Univariable Cox proportional hazards models were constructed using ten-week and one-year mortality from first LP as the outcome to evaluate characteristics associated with short- and long-term mortality. As patients without known HIV in this dataset likely represent a mix of HIV-uninfected individuals and HIV-infected but undiagnosed individuals, an analysis restricted to patients with documented HIV was performed. Variables were dichotomised using clinically meaningful or previously established cutoffs [4,6,8]: Older age (≥50 years); elevated CSF WCC (>20 cells/μL); lymphocyte-predominant CSF (≥50%); low CSF glucose (<1 mmol/L); high CSF protein (≥1 g/dL); and facility type (referral vs. primary or district hospital). CD4 count was stratified as >350 cells/μL, 200-350 cells/μL, 100-200 cells/μL, 50-100 cells/mL, and ≤50 cells/μL. We also evaluated hazards of death by calendar year to assess temporal trends.

Multivariable Cox proportional hazards models were constructed including older age, sex, CSF WCC>20 cells/μL, CD4 strata, ART status, facility level, and calendar year as independent variables. Variables were selected *a priori* because of suspected causal association with mortality to evaluate independent predictors of death. A sub-group analysis restricted to HIV-infected patients with a CSF WCC count >20 cells/μL was

performed to evaluate additional associations between CSF profile (lymphocytes or neutrophils) and mortality. CSF chemistries (protein and glucose) and HIV viral load were excluded from multivariable models due to high degree of missingness, and in the case of viral load a high likelihood that data were not missing at random. For cases of culture-confirmed pneumococcal meningitis, only univariable regression was performed due to the limited sample size, and restricted to ten-week mortality as most deaths occurred within the first ten weeks after LP. Models estimated mortality hazard ratios per 100 cells/μL difference in WCC, 1 g/L difference in CSF protein concentration, and 1 mmol/L difference in CSF glucose concentration. P-values were obtained using conventional standard errors with a significance level set at ≤0·05. Analysis was performed in Stata Version 13.0 (College Station, TX).

Role of funding sources.

Funders for this study had no role in study design, data collection, analysis, or interpretation of data, writing of this report, or decision to submit this paper for publication. MWT had full access to the data and responsibility for the decision to submit this paper for publication.

4.5 RESULTS

Patient and laboratory characteristics

A description of the 2004-2015 sampled cohort is shown in Figure 8. Briefly, among all CSF records 2004-2015, 80% (21,941/27,432) had no microbiological diagnosis and 20% (5491/27,432) had a microbiological diagnosis. Cryptococcal meningitis accounted for 82% (4532/5491) of microbiological diagnoses, TB meningitis 1% (63/5491), and

pneumococcal or other bacterial meningitis 18% (977/5491), with 45 (0.8%) concurrent diagnoses. Data from 238 cases with culture-confirmed pneumococcal meningitis, 48 with culture-confirmed TB meningitis, and 2900 culture-negative meningitis cases (including 1691 with a CSF WCC ≤20/μL and 1209 with CSF WCC >20/μL) were included (Table 5). Overall, 74% (2345/3186) of cases were treated at one of the two national referral hospitals.

For culture-negative cases, 73% (2109/2900) had documented HIV infection. CD4 T-cell count testing was performed within 6 months of lumbar puncture in 75% (1578/2109) of these HIV-infected individuals, with median CD4 of 136 cells/μL (IQR 61-266 cells/μL). Compared to 1691 culture-negative cases with low CSF cell count (≤20/μL), the 1209 with elevated CSF cell count (>20/μL) were more likely to be male (55% vs. 49%, p=0·001) and less likely to be on ART before LP (43% vs. 50%, p=0·003) but with marginally higher median CD4 count (141 cells/μL vs. 133 cells/μL, p=0·010). Prior known HIV status did not differ significantly between CSF WCC strata.

Of the 83% (999/1209) of culture-negative cases with elevated CSF cell count (WCC >20/μL) and a differential cell count, 73% (734/999) had lymphocyte-predominant CSF. CSF protein concentration was higher and glucose concentration lower in patients with a CSF WCC >20/μL compared to ≤20/μL (all p<0·001).

Mortality

Kaplan-Meier survival curves are shown in Figure 9 for patients with pneumococcal meningitis, TB meningitis, and culture-negative cases, as well as previously published 2012-2014 referral hospital outcomes from cryptococcal meningitis for comparison [6]. For culture-confirmed pneumococcal meningitis, two-week mortality was 44% (105/238),

ten-week mortality 47% (112/238), and one-year mortality 49% (117/238) (Table 5). Most deaths (90% [105/117]) occurred within two weeks of CSF evaluation. For culture-confirmed TBM, two-week mortality was 38% (18/48), ten-week mortality 46% (22/48), and one-year mortality 56% (27/48). In culture-negative cases, overall two-week mortality was 29% (839/2900), ten-week mortality 41% (1181/2900), and one-year mortality 49% (1408/2900), with no significant difference between WCC strata [Figure 10].

In both the pneumococcal meningitis cohort and the cohort without a pathogen identified, mortality was higher in those without known HIV diagnosis (Table 5 footnote). However, this may represent a survival bias; a proportion of patients with early mortality were likely HIV-infected but died before HIV-testing could be performed.

Predictors of mortality

For cases of culture-confirmed pneumococcal meningitis, increased CSF WCC was associated with lower risk of mortality within ten weeks of lumbar puncture (for each 100/μL increase in WCC, HR 0·96; 95%CI: 0·92-0·99 in full model; HR 0·93, 95%CI: 0·87-0·99 restricted to those with known HIV infection). Higher CSF protein was associated with higher mortality in the full model (HR 1·04, 95%CI: 1·00-1·09 for 1 g/L increase in protein) (data not shown).

In HIV-infected patients with negative cultures, older age, male sex, low CSF glucose, lower CD4 T-cell count, and treatment at non-tertiary facilities were significantly associated with increased risk of mortality within one year in univariable analysis (Table 6). Calendar year was not associated with risk of death (data not shown). Adjusting for other covariates, older age (ten-week aHR 1·74, 95%CI: 1·41-2·14; one-year aHR 1·65, 95%CI: 1·36-2·00) and lower CD4 T-cell count were associated with higher mortality, and

baseline ART use (ten-week aHR 0·70, 95%CI: 0·59-0·83; one-year aHR 0·74, 95%CI: 0·64-0·87) with lower mortality risk.

In an adjusted analysis restricted to HIV-infected patients with clear evidence of CSF inflammation (WCC >20/μL) [Table 7], lymphocyte-predominant CSF was associated with an adjusted hazard of death of 0·56 (95%CI: 0·41-0·78) within ten weeks and 0·57 (95%CI: 0·42-0·76) within one year. Older age (ten-week aHR 1·52, 95%CI: 1·05-2·21; one-year aHR 1·52, 95%CI: 1·07-2.13) and lower CD4 T-cell count remained significantly associated with increased risk of death by ten-week and one year, and ART use by the time of lumbar puncture evaluation with lower risk (ten-week aHR 0·67, 95%CI: 0·49-0·90; one-year aHR 0·70, 95%CI: 0·53-0·91).

4.6 Discussion

In this high HIV-prevalence routine-care setting in sub-Saharan Africa, almost half of patients evaluated for CNS infection died within one year of lumbar puncture evaluation. These figures represent conservative mortality estimates as a small proportion of deaths in Botswana likely go unregistered [20]. Three-quarters of patients had known HIV infection with a median CD4 count below 150 cells/μL; however it is not possible to attribute the high mortality rates seen in this patient cohort to advanced HIV-infection alone. For comparison, in 2002-2013 the estimated one-year mortality in patients newly accessing ART in the Botswana ART programme with a median baseline CD4 count of 147 cells/μL was 4·3% [16]. Mortality was high in both culture-confirmed TB and pneumococcal meningitis, with very rapid mortality in pneumococcal meningitis patients. Importantly, our data demonstrated similarly high mortality rates in patients with culture negative

meningitis, with no pathogen identified through standard microbiological studies. Mortality did not differ in patients with clear evidence of CSF inflammation (defined as CSF WCC >20 cells/μL) and those with relatively normal CSF cell count, although neutrophil-predominant CSF predicted poor outcome in patients with pleocytosis. To our knowledge, with data from over 3000 unique patients this is the first large study to describe long-term mortality of patients evaluated for CNS infections in a high HIV prevalence setting.

Diagnosis of CNS infections is challenging in sub-Saharan Africa owing to limited laboratory and imaging infrastructure and the influence of HIV infection on CSF findings [4]. A substantial majority of individuals with meningitis in routine care settings in Botswana had a negative diagnostic evaluation, similar to previous studies from other high HIV prevalence countries in sub-Saharan Africa [3,4]. Undiagnosed cryptococcal meningitis may have accounted for some cases of culture-negative meningitis. Cryptococcal antigen (CrAg) testing was infrequently performed and the highly-sensitive IMMY lateral flow assay was not used in Botswana during the study period [21]; however, standard evaluation with combination India ink microscopy and fungal culture is likely to detect most cases. Bacterial meningitis cases may be under-diagnosed using conventional culture-based methods. Preliminary findings from an ongoing molecular diagnostics study at Botswana's largest referral hospital show that nearly 40% (6/16) of cases of *Streptococcus pneumoniae* meningitis were diagnosed by PCR alone with negative culture results. Cases may well represent undiagnosed TB, other serious opportunistic infections, or viral pathogens that require molecular diagnostics for confirmation. Our findings of

extremely high mortality within one year of evaluation strongly suggest severe underlying pathology in many patients requiring improved diagnostics to enable effective treatment.

Undiagnosed TB meningitis may have contributed to the high mortality observed in culture-negative cases, even those with relatively normal CSF cell count. Compared to HIV-uninfected hosts with TB meningitis, patients with advanced HIV are more likely to have atypical CSF findings including neutrophil predominance or relatively acellular CSF [22]. Culture-confirmed TB meningitis was uncommon in Botswana compared to previous studies in South Africa (with estimated overall TB incidence of 300 cases/100,000 person-years and 567 cases/100,000 person-years, respectively [23]) where CSF TB testing is more commonly performed.[4,5] Xpert MTB/RIF, endorsed by the World Health Organization for diagnosis of TB meningitis [24], has limited sensitivity and was not used in Botswana during the study period, and TB culture has a sub-optimal sensitivity estimated around 50-60% [25]. Recent data have demonstrated that second-generation Xpert MTB/RIF (Ultra) has improved sensitivity over culture and first-generation Xpert MTB/RIF in HIV-infected patients [26], making this an attractive diagnostic option. Our findings strongly suggest a need for an enhanced diagnostic approach including evaluation for TB meningitis for all cases of suspected meningitis without another clear underlying aetiology in high HIV prevalence, highly TB endemic settings. Evidence of TB elsewhere, coupled with CSF and imaging findings, may also suggest probable TB meningitis and guide treatment [25].

Molecular diagnostic studies in African health settings suggest that HIV-associated central nervous system infections due to treatable infections, including herpes viruses and *Toxoplasma gondii*, are common and under-diagnosed in routine care where enhanced

diagnostics are not available [12-14]. Development and scale-up of sensitive and affordable multiplex assays for meningitis may assist in guiding therapy and improving outcomes. Further detailed studies to determine underlying aetiologies are needed to inform how best to develop and deploy such diagnostics.

We found significantly higher mortality in patients evaluated for meningitis without known HIV infection, almost certainly reflecting a survival bias with patients with undiagnosed advanced-HIV dying shortly after hospital presentation before an HIV diagnosis could be established. Although almost three-quarters of patients evaluated for meningitis without a pathogen identified, and 64% of those with pneumococcal meningitis, were known to be HIV-infected, these figures likely underestimate true HIV prevalence. For example, previous studies in Malawi and South Africa have found a ≥90% HIV prevalence in patients with bacterial meningitis [4,27]. Of patients with known HIV infection in our study CD4 cell count was an important predictor of mortality, as previously described [28]. ART use before evaluation was also associated with improved survival, likely from a combination of improved outcomes for certain CNS infections (e.g. progressive multifocal leukoencephalopathy) and decreased risk death from other HIV-related co-infections or malignancies. In patients with confirmed pneumococcal meningitis, we found that lower CSF leukocyte counts were significantly associated with higher mortality, as has been described in HIV-associated cryptococcal and TB meningitis [8,29]. These findings suggest that a lack of a robust immune response may be an important predictor of mortality in HIV-related bacterial meningitis; in part, explaining why clinical trials have failed to show a mortality benefit with adjunctive glucocorticoid therapy in HIV-infected patients [27].

Lymphocyte-predominant meningitis was associated with lower mortality than neutrophil-predominant meningitis. This could be due in part to culture-negative bacterial meningitis among neutrophil-predominant cases following prior antibiotic exposure, lumbar puncture delays, and/or inadequate culture methods. However, overall mortality in patients with lymphocytic meningitis was still high at 36% (261/734) and 43% (314/734) at ten weeks and one year. This study significantly increases our understanding of outcomes of culture-negative lymphocytic meningitis. One small study in the Central African Republic found a 13% (6/46) in-hospital mortality for HIV-infected patients admitted with lymphocytic meningitis (defined as CSF white cell count [WCC] >5 cells/μL, >50% mononuclear cells, and negative bacterial and fungal microscopy and culture),[30] whereas a study from Zimbabwe found a 35% (15/43) in-hospital mortality of HIV-infected patients with lymphocytic meningitis (defined as CSF WCC >100 cells/μL with >50% mononuclear cells) [11]. Our findings highlight how lymphocytic meningitis cannot be discounted as a benign viral or other self-limited process in high HIV prevalence settings.

The study has several limitations. First, detailed information on patients' clinical presentation, evaluations including imaging studies, and clinical management including prior antimicrobial use were not available. Secondly, HIV-related data were derived from an electronic registry that is incomplete, particularly with respect to ART status and history including treatment default [16]. Thirdly, there was significantly missingness of data for some variables, including likely non-random missingness (e.g. for HIV viral load), which precluded a multiple imputation approach to missing data and inclusion of certain variables in adjusted regression models. Fourth, we restricted our analysis to a sample of adults in the EMR with available national identification numbers; potentially missing a small

proportion of non-citizens and over-representing patients receiving care at referral centres. We believe that our sampling method resulted in conservative mortality estimates compared to the full cohort of adults receiving evaluation for meningitis, as non-citizens are likely to have worse access to HIV and other health services, and patients cared for at referral centres had lower mortality than primary or district hospitals. Fifth, in any prevalent cohort study the possibility of lead-time bias exists. Most meningitis patients present to health facilities for diagnostic evaluation rapidly on becoming acutely symptomatic, thus lead-time bias is unlikely to occur. Finally, it was necessary to make certain assumptions that, although based on the best available data, may have led to misclassification for a small number of patients (e.g. in determining ART start date). We also assumed that all patients received a lumbar puncture for suspected CNS infection. This assumption is supported by data from an ongoing prospective cohort study at Botswana's main referral hospital where lumbar punctures performed between January 2017 to June 2018 were almost exclusively for evaluation of suspected CNS infection rather than cancer, autoimmune disorders, or other non-infectious work-up.

4.7 CONCLUSION

In summary, we found very high mortality for cases of pneumococcal and TB meningitis, indicating the need for earlier diagnosis and better therapies for patients treated in HIV-prevalent resource-limited settings. Patients with culture-negative meningitis and unconfirmed diagnoses, representing a large majority of cases, had similarly high mortality up to one year suggesting serious undiagnosed pathologies, with very limited ability to stratify mortality risk on cerebrospinal fluid characteristics alone. Our findings highlight

an urgent need for improved access to current diagnostics (e.g. XPert and multiplex PCR assays), for future detailed studies to better define aetiologies, and to develop novel diagnostic tools and treatment algorithms for patients evaluated for CNS infections in high HIV-prevalence settings who are at very high risk of dying.

4.8 ACKNOWLEDGEMENTS

Declarations of interests

All authors report no conflicts of interest to declare.

Author Contributions

MWT designed the study, performed data collection, performed primary data analysis, and wrote the initial draft of the manuscript; MMok and TP contributed to protocol development; TBL, NT, KT, TC, AS, JM, HKM, RKKP, WJH, TM, and MMol performed data collection; BLG and CF contributed to data analysis and interpretation and subsequent drafts of the manuscript; MMine contributed to study design and drafting the manuscript; JNJ designed the study, performed data collection, performed data analysis, and provided input on subsequent drafts of the manuscript. All authors contributed to and approved of the final version of the manuscript.

Sources of funding

This work was supported by the Penn Center for AIDS Research, a National Institutes of Health (NIH)–funded program (grant number P30 AI 045008) to JNJ, as well as the NIH Office of AIDS Research, Health Resources and Services Administration (HRSA), and

President's Emergency Plan for AIDS Relief (PEPFAR) [grant number U91HA06801B] and NIH National Institutes of Allergy and Infectious Disease (NIAID) grant support (T32AI007044 and F32AI140511) to MWT.

4.9 REFERENCES

1. Walker AS, Prendergast AJ, Mugyenyi P, Munderi P, Hakim J, Kekitiinwa A, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(12):1707-18.

2. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017.

3. Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, et al. Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up and Haemophilus influenzae type b vaccination, 2000-2012. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(10):e137-45.

4. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC infectious diseases. 2010;10:67.

5. Marais S, Pepper DJ, Schutz C, Wilkinson RJ, Meintjes G. Presentation and outcome of tuberculous meningitis in a high HIV prevalence setting. PloS one. 2011;6(5):e20077.

6. Patel RKK, Leeme T, Azzo C, Tlhako N, Tsholo K, Tawanana EO, et al. High mortality in HIV-associated cryptococcal meningitis treated with amphotericin B-based therapy under routine care conditions in Africa. Open Forum Infect Dis. 2018;5(11):ofy267, https://doi.org/10.1093/ofid/ofy267.

7. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

8. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(5):736-45.

9. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, et al. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. The New England journal of medicine. 2016;374(6):542-54.

10. Wall EC, Cartwright K, Scarborough M, Ajdukiewicz KM, Goodson P, Mwambene J, et al. High mortality amongst adolescents and adults with bacterial meningitis in sub-Saharan Africa: an analysis of 715 cases from Malawi. PloS one. 2013;8(7):e69783.

11. Hakim JG, Gangaidzo IT, Heyderman RS, Mielke J, Mushangi E, Taziwa A, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. Aids. 2000;14(10):1401-7.

12. Rajasingham R, Rhein J, Klammer K, Musubire A, Nabeta H, Akampurira A, et al. Epidemiology of meningitis in an HIV-infected Ugandan cohort. Am J Trop Med Hyg. 2015;92(2):274-9.

13. Siddiqi OK, Ghebremichael M, Dang X, Atadzhanov M, Kaonga P, Khoury MN, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(12):1771-7.

14. Rhein J, Bahr NC, Hemmert AC, Cloud JL, Bellamkonda S, Oswald C, et al. Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. Diagn Microbiol Infect Dis. 2016;84(3):268-73.

15. UNAIDS. Botswana HIV estimates. Available at: http://www.unaids.org/en/regionscountries/countries/botswana. Accessed on: 13 August 2018.

16. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. Aids. 2016;30(3):477-85.

17. Tenforde MW, Mokomane M, Leeme T, Patel RKK, Lekwape N, Ramodimoosi C, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

18. UNAIDS. HIV and AIDS estimates - Botswana. Available at: http://www.unaids.org/en/regionscountries/countries/botswana/. Accessed 28 March 2017.

19. Botswana Ministry of Health. 2012 Botswana national HIV & AIDS treatment guidelines. Available at: https://aidsfree.usaid.gov/sites/default/files/tx_botswana_2012.pdf. Accessed on: 13 August 2018.

20. Statistics Botswana. Statistics Botswana annual report 2013/2014. Available at: http://www.statsbots.org.bw/sites/default/files/documents/Statistics%20Botswana%20Annual%20Report%202013-2014.pdf. Accessed on: 13 August 2018.

21. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerging infectious diseases. 2014;20(1):45-53.

22. Cecchini D, Ambrosioni J, Brezzo C, Corti M, Rybko A, Perez M, et al. Tuberculous meningitis in HIV-infected and non-infected patients: comparison of cerebrospinal fluid findings. Int J Tuberc Lung Dis. 2009;13(2):269-71.

23. World Health Organization. Global Tuberculosis Report 2018. Available at: http://www.who.int/tb/publications/global_report/en/. Accessed 23 Nov 2018.

24. World Health Organization. WHO meeting report of a technical expert consultation: Non-inferiority analysis of XPert MTB/RIF Ultra compared to XPert MTB/RIF. Available at: http://apps.who.int/iris/bitstream/handle/10665/254792/WHO-HTM-TB-2017.04-eng.pdf?sequence=1. Accessed on: 27 April 2018.

25. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis. 2010;10(11):803-12.

26. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, et al. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis. 2018;18(1):68-75.

27. Scarborough M, Gordon SB, Whitty CJ, French N, Njalale Y, Chitani A, et al. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. The New England journal of medicine. 2007;357(24):2441-50.

28. Bowen LN, Smith B, Reich D, Quezado M, Nath A. HIV-associated opportunistic CNS infections: pathophysiology, diagnosis and treatment. Nat Rev Neurol. 2016;12(11):662-74.

29. Thwaites GE, Tran TH. Tuberculous meningitis: many questions, too few answers. Lancet Neurol. 2005;4(3):160-70.

30. Békondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D, et al. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2006;10(5):387-95.

Table 5. Baseline clinical and laboratory characteristics and mortality outcomes

Pathogen	Age (years), Median (IQR)	Sex, % (n) male	CSF WCC (/µL), median (IQR) *	Lymphocyte % (IQR) †	Protein (g/dL), median (IQR)	Glucose, mmol/L, median (IQR)	HIV +ve, % (n) §	CD4 count (cells/µL) if HIV +ve, median (IQR)	On ART if HIV +ve, % (n) ¶	2-week mortality, % (n) **	10-week mortality, % (n)	1-year mortality, % (n)
Culture-confirmed bacterial meningitis												
Pneumococcal (n=238)	32·6 (17·7-42·3)	40·8% (97)	236 (53-800)	10% (5-25)	4·14 (2·5-7·1)	0·04 (0·01-0·12)	64·2% (79)	221 (141-421)	44·3% (35)	44·1% (105)	47·1% (112)	49·2% (117)
Tuberculous (n=48)	34·1 (23·8-45·2)	52·2% (24)	65 (25-185)	93% (32-97)	3·13 (2·6-3·5)	2·73 (1·99-3·48)	76·0% (22)	105 (34-162)	unknown	37·5% (18)	45·8% (22)	56·3% (27)
Cases with no pathogen identified												
CSF WCC 0-20/µL (n=1691)	37·8 (31·2-46·8)	48·7% (823)	2 (2-5)	90% (70-98)	0·55 (0·30-1·07)	3·22 (2·57-3·83)	71.6% (1221)	133 (51-265)	50·0% (605)	28·6% (484)	40·1% (678)	48·4% (819)
CSF WCC >20/µL (n=1209)	37·0 (30·9-44·7)	54.7% (661)	115 (50-325)	88% (42-95)	1·89 (1·03-3·42)	1·85 (1·07-2·84)	74·3% (898)	141 (73-270)	43·4% (390)	29·3% (355)	41·6% (503)	48·7% (589)

ART = antiretroviral therapy; IQR = interquartile range; +ve = positive; +ve = positive; WCC = white cell count

* Upper limit recorded for WCC 2000/µL

† Cerebrospinal fluid (CSF) differential counts were rarely done for patients with total CSF white cell counts <20/µL, and were only available for 78/1691 individuals in this group.

§ Among patients with national identification number for linkage to HIV registry

¶ Initiation of ART was defined as either: ART documented in the registry prior to date of LP or, in the absence of documented ART initiation, 90 days prior to the date of first recorded HIV viral load result. This definition was chosen as ART prescription may be incompletely documented and baseline viral load testing is not performed in Botswana but recommended three months post-ART initiation.**[19]** Of individuals classified as "On ART" 64% (615/956) had a documented ART start date in IPMS, and 36% (341/956) were classified as on ART based on prior viral load testing.

** For culture-confirmed pneumococcal meningitis, of 36% (44/123) with national identification number for HIV registry linkage with unknown HIV status, 2-week mortality 77% (34/44), 10-week 82% (36/44), and 1-year 82% (36/44); Of 791 patients with unknown HIV status and no pathogen identified, 2-week mortality 43% (344/791), 10-week 55% (438/791), and 1-year 62% (487/791)

Table 6. Predictors of mortality for known HIV-infected patients without pathogen identified.

Table 6a. Univariable analyses

Variable	Category	Data	N	Mortality 10 weeks	HR	p-value	Mortality 1 year	HR	p-value
Age	<50 years	2109	1830 (86·8%)	598 (32·7%)	Ref	<0·001	752 (41·1%)	Ref	<0·001
	≥50 years	(100%)	279 (13·2%)	145 (52·0%)	1·80 (1·50-2·16)		169 (60·6%)	1·75 (1·48-2·06)	
Sex	Female	2109	1069 (50·7%)	345 (32·3%)	Ref	0·006	425 (39·8%)	Ref	0·001
	Male	(100%)	1040 (49·3%)	398 (38·3%)	1·22 (1·06-1·41)		496 (47·7%)	1·26 (1·10-1·43)	
WCC category	0-20/μL	2109	1211(57·4%)	431 (35·6%)	Ref	0·530	539 (44·5%)	Ref	0·300
	>20/μL	(100%)	898 (42·6%)	312 (34·7%)	0·95 (0·83-1·10)		382 (42·5%)	0·93 (0·82-1·06)	
CSF protein	<1 g/L	673	350 (52·0%)	102 (29·1%)	Ref	0·008	136 (38·9%)	Ref	0·026
	≥1 g/L	(31·9%)	323 (48·0%)	126 (39·0%)	1·43 (1·10-1·86)		151 (46·8%)	1·30 (1·03-1·64)	
CSF glucose	≥1 mmol/L	1031	907 (88·0%)	318 (35·1%)	Ref	0·051	394 (43·4%)	Ref	0·013
	<1 mmol/L	(48·9%)	124 (12·0%)	55 (43·4%)	1·33 (1·00-1·77)		69 (55·7%)	1·38 (1·07-1·78)	
CD4 strata	>350 cells/mL	1578	261 (16·5%)	54 (20·7%)	Ref	<0·001	73 (28·0%)	Ref	<0·001
	>200-350 cells/mL	(74·8%)	299 (179·0%)	87 (29·1%)	148 (1·05-2·07)		112 (37·5%)	1·43 (1·07-1·92)	
	>100-200 cells/mL		388 (24·6%)	131 (33·8%)	1·76 (1·28-2·41)		173 (44·6%)	1·78 (1·35-2·33)	
	>50-100 cells/mL		310 (19·7%)	144 (46·5%)	2·61 (1·91-3·57)		175 (56·5%)	2·52 (1·92-3·31)	
	≤50 cells/mL		320 (20·3%)	159 (49·7%)	2·82 (2·07-3·84)		189 (59·1%)	2·67 (2·04-3·50)	
ART status	Off ART	2109	1114 (52·8%)	409 (36·7%)	Ref	0·184	498 (44·7%)	Ref	0·315
	On ART	(100%)	995 (47·2%)	334 (33·6%)	0·91 (0·78-1·05)		423 (42·5%)	0·94 (0·82-1·07)	
Facility level	Primary/district	2109	582 (27·6%)	235 (40·4%)	Ref	0·002	287 (49·3%)	Ref	0·001
	Referral	(100%)	1527 (72·4%)	508 (33·3%)	0·78 (0·67-0·91)		634 (41·5%)	0·79 (0·68-0·90)	

Table 6b. Multivariable analyses *

Variable	Category	aHR 10 week mortality	p-value	aHR 1 year mortality	p-value
Age	<50 years	Ref	<0·001	Ref	<0·001
	≥50 years	1·74 (1·41-2·14)		1·65 (1·36-2·00)	
Sex	Female	Ref	0·733	Ref	0·675
	Male	1·02 (0·87-1·22)		1·03 (0·88-1·20)	
WCC category	0-20/μL	Ref	0·677	Ref	0·379
	>20/μL	0·96 (0·81-1·14)		0·93 (0·80-1·09)	
CD4 strata	>350 cells/mL	Ref	<0·001	Ref	<0·001
	>200-350 cells/mL	1·51 (1·08-2·13)		1·47 (1·10-1·98)	
	>100-200 cells/mL	1·80 (1·31-2·48)		1·83 (1·39-2·42)	
	>50-100 cells/mL	2·56 (1·87-3·52)		2·50 (1·90-3·30)	
	≤50 cells/mL	2·85 (2·08-3·90)		2·71 (2·06-3·56)	
ART status	Off ART	Ref	<0·001	Ref	<0·001
	On ART	0·70 (0·59-0·83)		0·74 (0·64-0·87)	
Facility level	Primary/district	Ref	0·062	Ref	0·068
	Referral	0·84 (0·70-1·01)		0·86 (0·73-1·01)	

ART = antiretroviral therapy; WCC = white cell count

* Adjusted for age, sex, WCC category, CD4 strata, ART status, year, and facility level

Table 7. Cox proportional hazard model associations with mortality in known HIV-infected patients with CSF without pathogen identified and CSF WCC >20 cells/μL

Table 7a. Univariable analyses

Variable	Category	Data	No	Mortality 10 weeks	HR	p-value	Mortality 1 year	HR	p-value
Age	<50 years	898	788 (87·8%)	260 (33·0%)	Ref	0·004	318 (40·4%)	Ref	0·001
	≥50 years	(100%)	110 (12·3%)	52 (47·3%)	1·54 (1·14-2·07)		64 (58·2%)	1·61 (1·23-2·11)	
Sex	Female	898	417 (46·4%)	131 (31·4%)	Ref	0·061	164 (39·3%)	Ref	0·069
	Male	(100%)	481 (53·6%)	181 (37·6%)	1·24 (0·99-1·55)		218 (45·3%)	1·21 (0·99-1·48)	
Lymphocyte percentage	<50%	748	178 (23·8%)	87 (48·9%)	Ref	<0·001	101 (56·7%)	Ref	<0·001
	≥50%	(83·0%)	570 (76·2%)	170 (29·8%)	0·52 (0·40-0·68)		215 (37·7%)	0·55 (0·44-0·70)	
CSF protein	<1 g/L	285	65 (22·8%)	13 (20.0%)	Ref	0·025	16 (24·6%)	Ref	0·015
	≥1 g/L	(31·7%)	220 (77·2%)	76 (34·6%)	1·96 (1·09-3·53)		91 (41·4%)	1·93 (1·14-3·29)	
CSF glucose	≥1 mmol/L	440	333 (75·7%)	118 (35·4%)	Ref	0·307	139 (41·7%)	Ref	0·085
	<1 mmol/L	(49·0%)	107 (24·3%)	44 (41·1%)	1·19 (0·85-1·69)		56 (52·3%)	1·31 (0·96-1·79)	
CD4 strata	>350 cells/mL	671	110 (16·4%)	26 (23·6%)	Ref	<0·001	32 (29·1%)	Ref	<0·001
	>200-350 cells/mL	(74·7%)	133 (19·8%)	40 (30·1%)	1·33 (0·81-2·18)		49 (36·8%)	1·34 (0·86-2·10)	
	>100-200 cells/mL		188 (28·0%)	62 (33·0%)	1·45 (0·92-2·30)		78 (41·5%)	1·52 (1.01-2·30)	
	>50-100 cells/mL		143 (21·3%)	59 (41·3%)	1·90 (1·20-3·02)		75 (52·5%)	2·08 (1·37-3·15)	
	≤50 cells/mL		97 (14·5%)	54 (55·7%)	2·80 (1·76-4.48)		64 (66·0%)	2·96 (1·94-4·54)	
ART status	Off ART	898	508 (56·6%)	186 (36·6%)	Ref	0·212	225 (44·3%)	Ref	0·229
	On ART	(100%)	390 (43·4%)	126 (32·3%)	0·87 (0·69-1·09)		157 (40·3%)	0·88 (0·72-1·08)	
Facility level	Primary/district	898	249 (27·7%)	110 (44·2%)	Ref	<0·001	124 (49·8%)	Ref	0·003
	Referral	(100%)	649 (72·3%)	202 (31·1%)	0·65 (0·52-0·82)		258 (39·8%)	0·72 (0·58-0·89)	

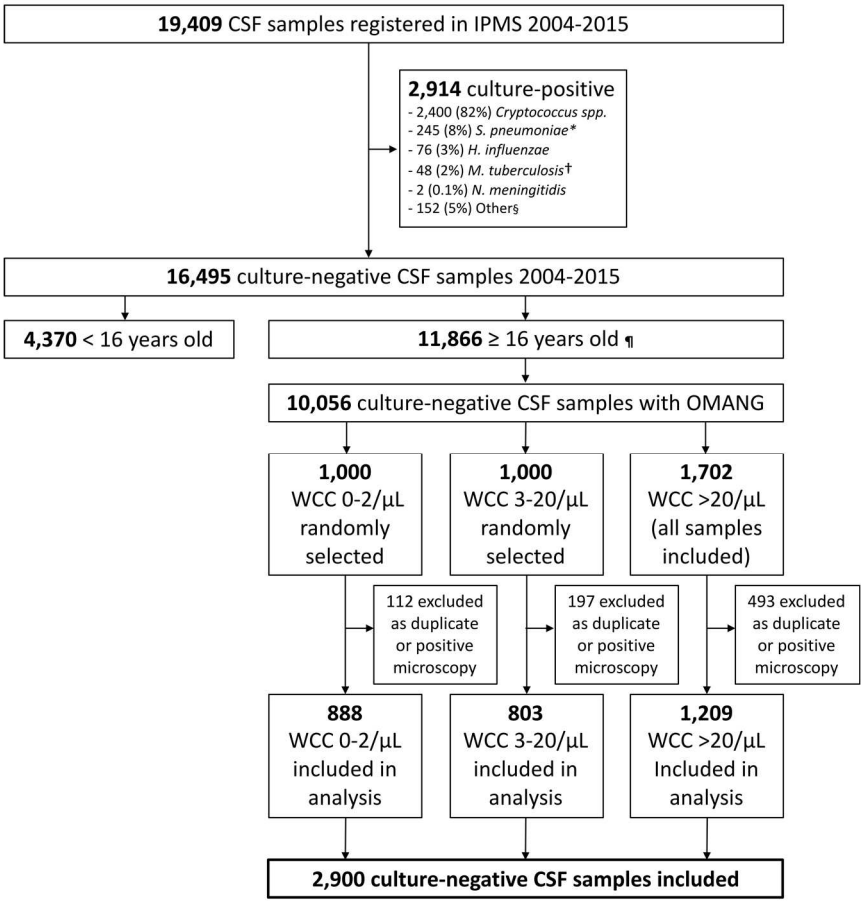
Table 7b. Multivariable analyses *

Variable	Category	aHR 10 week mortality	p-value	aHR 1 year mortality	p-value
Age	<50 years	Ref	0·027	Ref	0·017
	≥50 years	1·52 (1·05-2·21)		1·52 (1·08-2.13)	
Sex	Female	Ref	0·912	Ref	0·643
	Male	1.02 (0·76-1·36)		0·94 (0·72-1·22)	
Lymphocyte percentage	<50%	Ref	<0·001	Ref	<0·001
	≥50%	0·56 (0·41-0·78)		0·57 (0·42-0·76)	
CD4 strata	>350 cells/mL	Ref	0·054	Ref	0·002
	>200-350 cells/mL	1·10 (0·64-1·89)		1·09 (0·67-1·76)	
	>100-200 cells/mL	1·31 (0·80-2·13)		1·37 (0·88-2·11)	
	>50-100 cells/mL	1·49 (0·91-2·45)		1·63 (1·05-2·55)	
	≤50 cells/mL	1·99 (1·19-3·33)		2·26 (1·43-3·58)	
ART	Off ART	Ref	0·009	Ref	0·009
	On ART	0·67 (0·49-0·90)		0·70 (0·53-0·91)	
Facility	Primary/district	Ref	0·386	Ref	0·771
	Referral	0·86 (0·61-1·21)		1·05 (0·76-1·43)	

aHR = adjusted hazards ratio; ART = antiretroviral therapy; CSF = cerebrospinal fluid; HR = hazards ratio; WCC = white cell count

* Adjusted for age, sex, lymphocyte percentage, CD4 strata, ART status, facility level, and year

Figure 8. Flowchart for inclusion of culture-negative meningitis cases. The Botswana National Meningitis Survey (BNMS) includes data on 29,704 CSF samples from 2004-2015, of which 19,409 (65·3%) are in the Integrated Patient Management System (IPMS). Cases were selected from culture-negative samples recorded in the IPMS aged 16 years or over with a valid national identification number (Omang). Random selections of 1000 samples were made from those with cerebrospinal fluid (CSF) white cell counts (WCC) of 0-2μ/L and 3-20/μL. All samples with CSF WCC >20/μL were selected. Selected samples were deduplicated to remove multiple CSF samples taken during the same hospital admission, and any culture-negative samples with organisms seen on microscopy.



* Seven *S. pneumoniae* positive samples were repeat isolates, yielding the 238 unique patients included in subsequent analyses.

† Tuberculosis (TB) cultures were obtained from the TB specific Disa lab system. Thirty nine of the forty eight patients also had a record in the Integrated Patient Management System. These nine excess Disa patients mean the total is 2,923 rather than 2,914.

¶ Age was missing in 259 (1·6%) of cases.

Figure 9. Kaplan-Meier survival curves for: Cryptococcal meningitis (2012-2014), pneumococcal meningitis, TB meningitis, and culture-negative cases (no pathogen identified). Dashed vertical lines represent two weeks, ten weeks, and one year and number at risk values presented at 60-day intervals. Cryptococcal meningitis figures are previously published 2012-2014 referral hospital outcomes for comparison.**[6]**

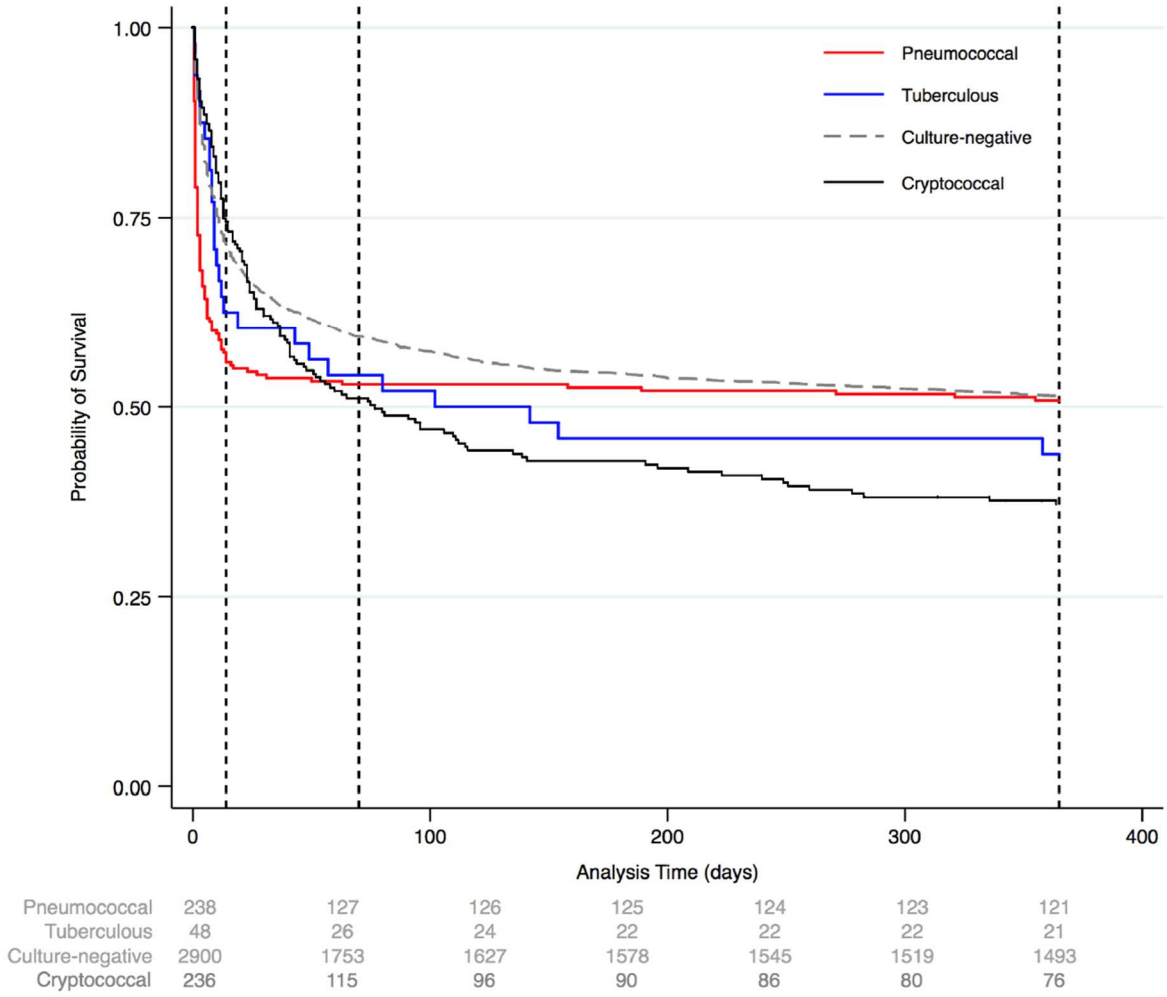
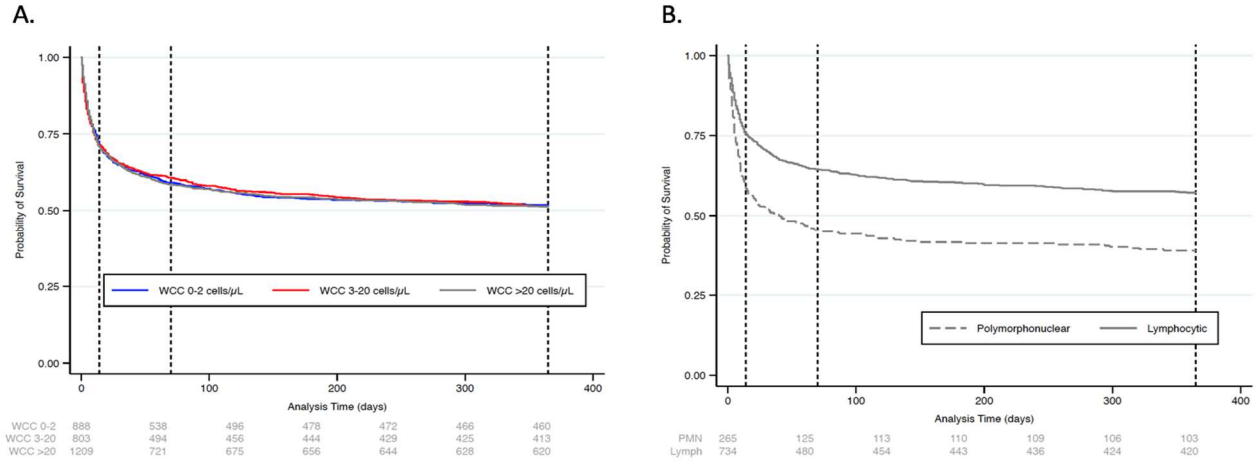


Figure 10. Kaplan-Meier survival curves for A) Culture-negative cases (no pathogen identified) with CSF WCC 0-2/µL, WCC 3-20/µL, and WCC >20/µL; B) culture-negative cases with CSF WCC >20/µL by neutrophil versus lymphocyte (≥50% lymphocytes) predominance. Dashed vertical lines represent two weeks, ten weeks, and one year and number at risk values presented at 60-day intervals. Cumulative mortality at 2 weeks, 10 weeks, and one year were: WCC 0-2/µL 29% (254/888), 41% (363/888), and 48% (428/888) respectively; WCC 3-20/µL 29% (230/803), 39% (315/803), and 49% (391/803) respectively; overall WCC >20/µL 29% (589/1209), 42% (503/1209), and 49% (589/1209) respectively; WCC >20/µL and polymorphonuclear 42% (110/265), 55% (145/265), and 61% (162/265) respectively; and WCC >20/µL and lymphocytic 24% (179/734), 36% (261/734), and 43% (314/734) respectively. Note that a proportion of patients with WCC >20 cells/µL did not have a WCC differential.



CSF = cerebrospinal fluid; WCC = white cell count

Chapter 5. MORTALITY FROM HIV-ASSOCIATED MENINGITIS IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 MANUSCRIPT TITLE AND TITLE PAGE

Mortality from HIV-associated meningitis in sub-Saharan Africa: A systematic review and meta-analysis

Mark W. Tenforde,[1,2] Alida M. Gertz,[3] David S. Lawrence,[3,4] Nicola K. Wills,[4,5] Brandon L. Guthrie,[2,6] Carey Farquhar,[1,2,6] Joseph N. Jarvis[3,4]

[1] Division of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle, WA, USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA, USA

[3] Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

[4] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

[5] Welcome Centre for Infectious Diseases Research in Africa, Infectious Disease and Molecular Medicine Unit, University of Cape Town, Cape Town, South Africa

[6] Department of Global Health, University of Washington, Seattle, WA USA

Corresponding Author

Mark W. Tenforde MD, MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2066508915

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Key words: Cryptococcal meningitis; pneumococcal meningitis; TB meningitis; sub-Saharan Africa; systematic review

Short-title: Mortality from HIV-associated meningitis in Africa

Word count: 4978

5.2 ABSTRACT

Background: HIV-associated cryptococcal, TB, and pneumococcal meningitis are the leading causes of adult meningitis in sub-Saharan Africa (SSA). We performed a systematic review and meta-analysis with a primary aim of estimating mortality from major causes of adult meningitis in routine care settings, and to contrast this with outcomes from clinical trial settings.

Methods and Findings: We searched PubMed, EMBASE, and the Cochrane Library for published clinical trials (defined as randomized-controlled trials [RCTs] or investigator-managed prospective cohorts) and observational studies that evaluated outcomes of adult meningitis in SSA from January 1, 1990 through December 31, 2018. We performed random effects modeling to estimate pooled mortality, both in clinical trial and routine care studies. Outcomes were stratified as short-term (in-hospital or two-weeks), medium-term (up to 10-weeks) and long-term (up to six-month). Seventy-seven studies met inclusion criteria. In routine care settings, pooled short-term mortality from cryptococcal meningitis was 44% (95% confidence interval [95%CI]:39-49%, 40 studies), which did not differ between amphotericin-based versus fluconazole induction therapy (41% for each), and was two-fold higher than pooled clinical trial mortality with amphotericin without flucytosine (23% [95%CI:19-28%], 12 studies). Estimated short-term mortality of TB meningitis was 48% (95%CI:35-61%, 10 routine care studies). For pneumococcal meningitis routine care studies, estimated short-term mortality was 55% (95%CI:44-66%, 8 studies), with little difference in outcomes between these studies and two published randomized-controlled trials. Few studies evaluated long-term outcomes.

Conclusions: Mortality from HIV-associated meningitis in SSA is in excess of 50%. Previous estimates of up to 70% one-year mortality of HIV-associated cryptococcal meningitis may be conservative, although few studies in routine care settings have reported long-term outcomes for cryptococcal meningitis. Better strategies are needed to reduce mortality from HIV-associated meningitis in the region.

5.3 INTRODUCTION

Approximately 37 million people were living with HIV worldwide in 2017, with over two-thirds in sub-Saharan Africa (SSA) [1]. Although access to combined antiretroviral therapy (ART) has improved, a large proportion of people living with HIV (PLWH) still present with advanced immune suppression and are at high risk for HIV-related infections [2,3]. Central nervous system (CNS) infections are a major cause of mortality in PLWH in SSA, with cryptococcal, TB, and pneumococcal meningitis the most common microbiologically-confirmed etiologies [4,5]. Cryptococcal meningitis alone results in approximately 15% of HIV associated deaths worldwide, with almost three-quarters of these in SSA [6].

Treatment for HIV-associated meningitis is challenging even in well-resourced settings. Cryptococcal meningitis management involves initial "induction" therapy of up to two weeks of intravenous amphotericin (ideally given with flucytosine, which remains unregistered in Africa), an effective but highly toxic drug [7], followed by consolidation and maintenance therapy with fluconazole [8]. However, less potent fluconazole monotherapy is often used in resource-constrained settings due to low cost, ease of oral administration, and better drug toxicity profile. Additionally, cryptococcal meningitis survival is associated with performance of therapeutic lumbar punctures (LPs) to reduce intracranial pressure (ICP) [9,10], intravenous fluid (IVF) hydration and electrolyte supplementation and monitoring to prevent life-threatening amphotericin-related toxicities [7,8,11], and appropriate follow-up after hospital discharge to initiate antiretroviral therapy (ART) around 4-6 weeks to reduce the risk of immune-reconstitution inflammatory syndrome [12]. Significant barriers exist to optimal management in most SSA settings, such as lack of more potent antifungal drugs, lack of manometers to measure ICP, hospital understaffing, limited laboratory services, and loss to follow-up after hospital

discharge. Similarly, effective management of TB meningitis is complicated in resource-constrained settings with challenges in management of common antituberculous drug toxicities, long duration of treatment (of a minimum of six months), poor sensitivity of diagnostic studies and delay for culture results, emergence of drug resistance, and lack of integration of TB and HIV services [13,14]. Survival from pneumococcal meningitis is negatively affected by delayed health care access and lack of timely initiation of effective antimicrobial therapy [15].

Several systematic reviews have evaluated outcomes of HIV-associated meningitides in Africa, but each has important limitations. A recent review of long-term outcomes from HIV-associated cryptococcal meningitis included only a small subset of studies with long-term outcomes data which may not be more generally representative [16]. In a previous systematic review of TB meningitis in Africa, investigators did not differentiate between short- and long-term outcomes and combined outcomes from studies with heterogeneous case definitions (e.g. microbiologically-diagnosed TB or TB diagnosed clinically using various criteria), limiting interpretation [17]. A prior well-conducted systematic review of pneumococcal meningitis in Africa focused on pediatric populations [18].

Given the large burden and mortality from HIV-associated meningitis in SSA and limitations of prior studies, we performed a systematic review and meta-analysis to evaluate short- and long-term outcomes of common HIV-associated meningitides (cryptococcal, TB, and pneumococcal). We estimated mortality in "routine care" studies (which we defined as studies in which hospital care was provided primarily by the local medical team, e.g. retrospective cohort studies), providing realistic global burden estimates important for resource allocation and prevention efforts such as cryptococcal antigen (CrAg) screening [19]. We compared outcomes from routine care and "clinical trial" studies (which we defined as randomized-controlled trials

[RCTs] or prospective cohort studies with regular management of patients by the study investigators during therapy) to characterize the outcomes gap and provide insights into potential improvement in mortality that might be realized through improved management strategies.

5.4 MATERIALS AND METHODS

Types of outcomes measures

Our overall aim was to determine the mortality associated with the most common HIV-associated meningitides in sub-Saharan Africa, captured at time points reflecting acute- and long-term outcomes. Our mortality endpoints were defined as: 1) Short-term (death ≤2 weeks after hospitalization or enrolment); 2) medium-term (death ≤10 weeks); and long-term (≤6 months). Study deviations from these pre-defined timepoints were described in the analysis.

Types of studies and participants.

We included published RCTs and observational studies (cohort studies, case-control studies, cross-sectional audits, or surveillance studies) of adults (≥18-years) treated for cryptococcal, TB, and/or pneumococcal meningitis. Inclusion criteria included: 1) Study from sub-Saharan Africa; 2) ≥15 participants treated for cryptococcal, TB, or pneumococcal meningitis; and 3) full period of observation after 1990 (to reflect emergence of HIV in SSA and modern antimicrobial therapy regimens). Exclusion criteria included: 1) Pediatric study or with a majority of patients <18-years old without disaggregated results provided in adults; 2) case report or case series; 3) antimicrobial treatment not available or treatment with therapies either not used or contraindicated (e.g. acetazolamide for cryptococcal meningitis) [20,21]; 4) non-representative

or biased sampling (e.g. significant RCT recruitment delay with immortal time bias [12,22]; 5) missing mortality data for >15% of participants; or 6) diagnostic criteria not clearly defined.

For cryptococcal meningitis, we included studies of cases confirmed by cerebrospinal fluid (CSF) testing (i.e., positive India ink, culture, or CrAg), excluding those with a significant number (>10%) of patients diagnosed through peripheral blood CrAg testing and symptoms of meningitis alone, as blood CrAg is unable to definitively confirm central nervous system involvement. For TB meningitis, we included studies of either microbiologically-confirmed cases or with diagnostic criteria that combined CSF findings and other supportive evidence, e.g. isolation of *Mycobacterium tuberculosis* at another site or suggestive imaging [23]. For pneumococcal meningitis, we included studies with mortality for microbiologically-confirmed cases or, when results were not disaggregated for confirmed *S. pneumoniae*, if a majority of cases (>50%) were confirmed. These definitions were used so that outcomes would reflect mortality from the etiologies of interest rather than other potential causes.

Search method and data collection.

We searched for studies from 1 January, 1990 through 30 April, 2018 using PubMed, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL). The search was updated through 31 December, 2018 in PubMed and EMBASE. Our search strategy combined geographic and cryptococcal, TB, and pneumococcal meningitis search terms (**Figure 11**). We excluded conference abstracts and studies that were not peer-reviewed. No restrictions were placed on age or language. The search strategy and protocol was developed by the authors before the search; the study protocol was not published.

Articles obtained in database searches were aggregated and de-duplicated in Covidence. Two reviewers (MWT and AMG) independently performed a primary title and abstract search to identify potentially eligible studies. The authors then reviewed full text articles to assess for inclusion, with data from included studies abstracted using standardized data collection forms. Secondary review was provided by DSL and NW. Discrepancies were decided through consensus or adjudication from JNJ if needed. We contacted authors as needed for clarification. Reference lists of included articles as well as relevant reviews were searched to identify other potential studies. We generated a flow diagram, following PRISMA recommendations, and summary tables of excluded full-text articles with rationale (data not known), and reporting was conducted per PRISMA recommendations [24].

Data synthesis and analysis

Data were pooled using standard random effects meta-analyses for proportions, with variance of proportions stabilized using Freeman-Tukey double arcsine transformation before pooling [25]. We estimated the pooled proportion of deaths and 95% confidence interval for each pathogen (*Cryptococcus*, TB, and pneumococcus) by study type (clinical trial vs. routine care study) and by timepoint (short-term, medium-term, and long-term mortality). Patient outcomes data were combined for multiple routine care studies from the same facility if the studies included patients from non-overlapping periods. Heterogeneity was assessed with I^2 values, representing the percentage of total variability due to between-study heterogeneity. Results were presented using forest plots, along with summary tables of study and patient characteristics (data not known). Analyses were conducted in R Studio using *metaprop* in the *meta* package.

Sub-group analyses.

We performed additional analyses to stratify estimates by clinically important confounders. For cryptococcal meningitis, we stratified outcomes by induction therapy; amphotericin B with and without flucytosine (for <2 or ≥2 weeks), fluconazole with flucytosine, fluconazole, or treatment not specified. Outcomes from induction regimens with amphotericin B alone or amphotericin B with fluconazole were combined, as no mortality difference has been documented between groups [26,27]. For TB meningitis, we stratified analyses by studies with microbiological-confirmed cases and with a majority of cases defined using combined microbiological and clinical criteria without *Mycobacterium tuberculosis* confirmed from CSF culture, smear, or PCR. For pneumococcal meningitis, we stratified analyses by treatment regimen. We further stratified outcomes by region (Southern, Eastern, Middle [Central], and Western Africa) as defined by the United Nations geoscheme [28], and studies conducted after 2000 or before 2000. We initially planned additional stratified analyses by HIV prevalence (≥50% or <50%) within a population, but most studies included a majority or only HIV-positive patients.

Dealing with missing data and assessing risk of bias.

We described missingness of data, including clinical characteristics (e.g. HIV status) and mortality. We performed intention-to-treat analysis when multiple groups were compared. Outcomes were reported for patients with known outcomes at each mortality timepoint. This was done so as not to systematically bias estimates toward higher survival as we would have done if those lost to follow-up were all assumed to have survived. If loss to follow-up exceeds 15%, we excluded the study from the analysis at that timepoint due to the high likelihood that outcomes were not missing at random (i.e. those who died were more likely to be lost to follow-up). Given

the heterogeneity of study types, we undertook a subjective assessment of study quality based on previous guidance for systematic reviews [29]. This incorporated quality of reporting of important covariates, e.g. timing of ART in ART-naïve patients treated for cryptococcal meningitis.

5.5 RESULTS

Overall search findings.

The initial database search yielded 3569 titles, with four additional articles found that met inclusion criteria. After removing duplicates, 2507 titles and abstracts were reviewed, with 265 selected for full text review. Of these, 188 were excluded (**Figure 12**). Seventy-seven studies were included from the primary database search and additional references.

Cryptococcal meningitis outcomes from treatment provided under "routine care" settings.

Forty-one observational studies with 10,139 cases of cryptococcal meningitis were included, most of HIV-positive patients treated at district or referral hospitals. The majority of studies were judged of low quality, with limited description of clinical management (e.g. performance of therapeutic lumbar punctures, antifungal therapy dose/duration, timing of ART following initiation of antifungal therapy in ART-naïve patients). Seventeen were prospective cohorts and one was a mixed prospective and retrospective cohort [30], which we classified as routine care studies. Three of these prospective cohorts described more active involvement from a study team [31-33], but at irregular intervals with routine day-to-day management under local conditions. In 10% (4/41) of included studies, the period of observation began prior to 2000. Seventeen countries were represented, with greatest representation from South Africa (14 studies) [30,33-

45], Ethiopia (three studies) [46-48], and Uganda (three studies) [49-51]; other countries had two or fewer studies [9,31,32,52-70]. Amphotericin B-based induction therapy (with or without fluconazole) was the predominant induction regimen in 16 studies, fluconazole in 13 studies, a mix of treatments (45% fluconazole and 43% amphotericin B) in one study [40], and details of antifungal induction regimens were not specified in 11 studies. In 28 studies with details on baseline ART status, 26% (1792/6777) of patients had a history of current or previous ART use. Reasons for exclusion included non-representative sampling [22,71-73], high loss to follow-up or duration of follow-up not clear [58,74], antifungal therapy not available [20,75,76], and use of treatment regimens not used or contraindicated in management [77-79].

Short-term mortality was reported in 40/41 included studies (35 in-hospital, with mean/median hospitalization approximately two weeks for most, and five studies with two-week mortality used). The overall pooled short-term mortality was 44% (95%CI: 39-49%, 40 studies), with a high between-study heterogeneity (I^2 = 89%) [**Figure 13**]. Mortality was lowest in Southern Africa (37% [95%CI: 31-43%], I^2 = 86%, 16 studies) and highest in Central Africa (55% [95%CI: 41-69%], I^2 = 75%, 3 studies) and West Africa (54% [95%CI: 40-67%], I^2 = 83%, 8 studies). Restricting to studies with a full observation after 2000, pooled mortality was 44% (95%CI: 39-49%, I^2 = 90%, 36 studies). Within these more recent studies, excluding one study in which the proportion of patients receiving amphotericin B-based and fluconazole induction therapy was roughly equivalent [40], short-term mortality was 41% (95%CI: 32-51%, I^2 = 91%, 15 studies) in studies with exclusive or predominant amphotericin B-based induction therapy, 41% (95%CI: 31-50%, I^2 = 89%, 11 studies) with exclusive or predominant fluconazole, and 50% (95%CI: 39-61%, I^2 = 78%, 9 studies) when treatment was not specified.

Few studies reported medium- or long-term mortality. Medium-term mortality (defined as death ≤10-weeks, except specified as ≤3-months in one study) was described in five studies with 422 total patients in Southern Africa (two studies) or East Africa (three studies), with pooled mortality of 51% (95%CI: 43-60%, I^2 = 60%). Long-term mortality was described in three studies (at nine months in one study and 12 months in two [31,38,52]) with 294 patients in Southern Africa (two studies) and East Africa (one study), with pooled mortality of 63% (95%CI: 46-78%, I^2 = 79%). Studies from Botswana [52], with standard amphotericin B and fluconazole induction therapy, and Malawi [31], with high-dose fluconazole (800 mg/day) induction therapy, reported one-year mortality of 65% (142/219) and 77% (43/56), respectively, in patients with known vital status. The third study, from South Africa, reported nine-month mortality of 37% (7/19) [38], comparable to acute mortality in most other studies from South Africa. Few participants in this study had baseline altered mental status (11% [2/19] noted to have confusion), an established predictor of mortality [80], and investigators may have recruited less seriously ill patients in this study. Regions in SSA with the highest acute mortality (Central and Western Africa) did not have long-term mortality data.

Cryptococcal meningitis outcomes for treatment provided under clinical trial settings.

We included 18 studies from clinical trials (randomized trials or investigator-managed cohorts that compared ≥1 treatment regimen for cryptococcal meningitis) with a total of 2048 cases, almost all HIV-positive. Studies included 10 RCTs and eight prospective cohorts with management from the study team investigators, all after 2000 and most at referral hospitals. The quality was judged to be good for a majority of studies, with clear documentation of clinical management compared to routine care studies (therapeutic lumbar puncture details in 12/18

studies, details on timing of ART initiation for ART-naïve patients in 17/18, and details of antifungal treatment in all studies). Nine countries were represented (including several multi-country RCTs); the greatest representation was from South Africa (six studies) [81-86], Uganda (six studies) [21,87-91], and Malawi (four studies) [21,92-94], with other countries represented in two or fewer studies [95-97]. Ten of 18 studies excluded patients on ART at the time of enrollment. Two studies described fluconazole monotherapy as the only regimen and a third RCT evaluated fluconazole as one treatment arm [89,93,97], two RCTs evaluated combined flucytosine and fluconazole therapy as treatment arms [93,94], and the rest described use of amphotericin B with or without flucytosine or fluconazole. Reasons for study exclusion included use of abnormal treatment regimens [98-100] and studies with non-representative sampling, e.g. delayed enrollment for one week after initiation of antifungal therapy [12]. For RCTs that compared standard regimens to experimental regimens not used in clinical practice, e.g. adjunctive interferon-gamma [83], or therapies demonstrated to be harmful, e.g. adjunctive dexamethasone [21], we restricted our analysis to patients who received standard antifungal regimens. For one RCT that recruited patients both in African and Asian health centers, we restricted our analysis to patients recruited from Africa [21].

Short-term mortality was reported in 17 studies (all reporting two-week outcome) with 1989 cases. Pooled two-week mortality was 21% (95%CI: 17-26%, I^2 = 75%, 17 studies) [**Figure 14**]. Stratified by treatment regimen, mortality was 12% (95%CI: 7-18%, I^2 = 40%, 5 studies) for amphotericin and flucytosine induction therapy, 17% (95%CI: 12-22%, I^2 = 0%, 2 studies) for flucytosine with fluconazole, 23% (95%CI: 19-28%, I^2 = 72%, 12 studies) for amphotericin with or without fluconazole, and 30% (95%CI: 21-40%, I^2 = 0%, 3 studies) for

fluconazole alone. Little mortality difference was observed for shorter (<2 weeks) and longer (2-week) amphotericin-based induction regimens.

Medium-term mortality (10-week, except four-months in one study [88]) was reported in 13 studies with 1485 cases, with pooled mortality of 37% (95%CI: 33-41%, I^2 = 43%). Stratified by induction regimen, mortality was 30% (95%CI: 25-34%, I^2 = 0%, 5 studies) for amphotericin with flucytosine, 35% (95%CI: 29-42%, I^2 = 0%, 2 studies) for flucytosine with fluconazole, 39% (95%CI: 35-44%, I^2 = 14%, 8 studies) for amphotericin with or without fluconazole, and 49% (95%CI: 39-60%, I^2 = 29%, 3 studies) for fluconazole. Long-term mortality (within six-month except 18-weeks in one) was described in six studies with 612 cases, with a pooled mortality of 44% (95%CI: 36-52%, I^2 = 71%). Stratified by regimen, mortality was 33% (95%CI: 21-45%, 1 study) for amphotericin with flucytosine, 46% (95%CI: 36-56%, I^2 = 71%, 4 studies) for amphotericin with or without fluconazole, and 50% (95%CI: 30-70%, 1 study) in a small study from Zimbabwe of fluconazole monotherapy.

TB meningitis outcomes.

We included 11 studies of TB meningitis with a total of 508 cases, all considered routine care studies and all but one study having with a majority or all HIV-positive patients (median 81%) [101]. Study quality was relatively poor, with most studies not including details of ART or antituberculous treatment or average duration of hospitalization. Five studies included cases before 2000 [30,102-105], whereas six had a full period of observation after 2000 [49,56,63,101,106,107]. Eight countries were represented, with greatest representation from South Africa (three studies) and Uganda (two studies). Six studies included exclusively definite cases (with positive CSF AFB smear, culture, or PCR) and five included predominately

probable/possible cases (0-21% of cases microbiologically-confirmed). One study specified use of adjunctive dexamethasone [104]. Several potential studies were excluded on full-text review: Reasons included TB case series, e.g. exclusively multi-drug resistant cases [108-110], high risk of selection bias [38,111-113], significant missingness of outcomes [114-116], and poor specification of diagnostic criteria [46,70,117-119].

Short-term mortality for 468 cases was reported in 10 studies (two from a single hospital with combined patient outcomes [49,106]; two-week mortality specified in one study [107], in-hospital mortality in nine studies with length of hospitalization not specified). Pooled mortality was 48% (95%CI: 35-61%, I^2 = 87%, 10 studies) [**Figure 15**], and differed marginally between studies with only definite cases (51% [95%CI: 37-65%], I^2 = 78%, 6 studies) or with predominately possible/probable cases (45% [95%CI: 22-68%], 4 studies). Restricting to studies after 2000, pooled short-term mortality was similar at 46% (95%CI: 28-64%, I^2 = 93%, 6 studies). Ten-week mortality from a national meningitis audit in Botswana of microbiologically-confirmed cases was 46% (95%CI: 31-61%) [107]. Long-term mortality was reported in two studies, within one year in the Botswana study and after completion of six months of antituberculous therapy in Nigerian study [104], both studies with exclusively definite cases. Reported mortality for these studies was 56% (27/48) in Botswana and 78% (31/40) in Nigeria. Of note, the Botswana study relied on national electronic death registry data, which has been previously shown to accurately capture deaths but might slightly under-estimate mortality [52].

Pneumococcal meningitis outcomes.

We included 11 studies for pneumococcal meningitis with 2891 cases, a majority HIV-positive in 8 studies, <50% HIV-positive in one, and with HIV prevalence not specified in two studies. Eight studies were classified as routine care studies [107,120-126] and two were RCTs

[127,128]. One study consisted of two sequential cohorts, a lead-in observational routine care cohort followed by a prospective cohort that received a bundled Goal Directed Therapy (GDT) intervention including parenteral ceftriaxone therapy within one hours of registration, airway support, and fluid resuscitation according to sepsis guidelines, among other supportive measures [129]. The clinical trial studies were judged to be of high quality, but routine care studies of relatively poor quality (including lack of description of patient ART status, details on dose and duration of antibiotic therapy). Eight countries were represented, with four studies (two RCTs, one observational cohort, and the combined sequential cohort study) from a single referral hospital in Malawi. Eight of 11 studies included a full period of observation after 2000. Nine of 11 studies provided outcomes for microbiologically-confirmed cases (CSF culture, PCR, and/or antigen testing) and two studies had *Streptococcus pneumoniae* microbiologically-confirmed for a majority of cases (64-69%). Treatment specified varied significantly between studies; four specified ceftriaxone as standard antibiotic therapy (one RCT with adjunctive dexamethasone used in a single treatment arm) [107,127-129], two included chloramphenicol-based treatment as the predominant treatment (one combined with intravenous penicillin G) [120,122], two included aminopenicillins (amoxicillin or ampicillin) with or without gentamycin as the predominant therapy given [123,125], two studies did not specify therapy [121,124], and one study included a similar mix of treatment with ceftriaxone and chloramphenicol [126]. Vancomycin was not provided with ceftriaxone in any study. Several potential studies were excluded on full text review: Reasons included a minority of cases with *Streptococcus pneumoniae* isolation [30,130-132] and high loss to follow-up / missing outcomes data [133-137].

Short-term mortality was reported in all eight routine care studies with 2436 cases (including 14-day [107], 30-day [121], in-hospital [122-125], and with length of follow-up not

specified in two surveillance studies but assumed to be short-term [120,126]). Pooled mortality was 55% (95%CI: 44-66%, I^2 = 95%, 8 studies) [**Figure 16**]. Medium- and long-term mortality was described in two routine care studies, one from Botswana and the other from Malawi [107,122]. Both found generally good long-term survival in those who survived the early period shortly after diagnosis. The study from Malawi showed a short-term (in-hospital up to nine days), medium-term (10-week), and long-term (six-month) mortality of 65% (42/65), 69% (45/65), and 69% (45/65), respectively, with one-year mortality of 75% (49/65). The study from Botswana found short-term (2-week), medium-term (10-weeks), long-term (6-month), and one-year mortality of 44% (105/238), 47% (112/238), 47% (113/238), and 49% (117/238), respectively.

The two RCTs were both conducted at the same referral hospital in Malawi, provided 40-day treatment outcomes, and provided treatment with ceftriaxone (with or without dexamethasone) [127,128]. We combined data for patients treated with or without adjunctive dexamethasone in one RCT as the trial found no difference in mortality between arms [128], and patients receiving adjunctive glycerol in one RCT were excluded from the analysis as glycerol did not improve outcomes and may have been associated with severe adverse events [127]. Reported short-term (here defined as 40-day) mortality ranged from 39% (20/51) to 51% (140/272); however, mortality from pneumococcal meningitis was certainly under-estimated in the smaller trial as 17% (63/380) of patients with suspected acute bacterial meningitis (pneumococcal or other) died before they could be recruited. In the RCT with a 51% (140/272) 40-day mortality, long-term mortality was provided at 61% (150/245) in patients with known outcomes at six-months.

The study with the combined sequential observational routine care cohort and interventional Goal Directed Therapy cohort was conducted at the same center as the two RCTs, with ceftriaxone treatment provided as standard treatment [129]. At the primary endpoint of 40 days, among patients with known outcomes 49% (28/57) had died in the lead-in observational cohort and 63% (38/60) in the subsequent clinical trial cohort.

5.6 DISCUSSION

This systematic review and meta-analysis provides comprehensive estimates of outcomes from common HIV-associated meningitides in sub-Saharan Africa and addresses important limitations of previous systematic reviews, including disaggregation of mortality from controlled-trial (prone to selection bias and non-representative management) and routine care settings, and also evaluating outcomes from different timepoints. The results highlight the poor current understanding of long-term outcomes of cryptococcal and TB meningitis under routine care conditions in sub-Saharan Africa. Furthermore, most studies included in the review reported outcomes for patients treated at referral hospitals, where staffing, pharmaceutical, and laboratory resources are likely to be better than in lower-level health facilities. Our findings also suggest significant heterogeneity between regions. Pooled acute mortality from routine care studies of HIV-associated cryptococcal meningitis in Central and West Africa was in excess of 50%, compared to 37% in Southern Africa.

Comparing mortality outcomes of patients treated under routine care conditions to clinical trial settings, there were significant differences for cryptococcal meningitis but similar mortality for pneumococcal meningitis. Of studies with observation periods after 2000, short-term mortality for cryptococcal meningitis managed under routine care conditions was two-fold

higher compared to mortality under clinical trial conditions (44% vs. 21%); a finding consistent when stratified by antifungal regimen (e.g. 41% vs. 23% with amphotericin B with/without fluconazole). This difference, at least in part, reflects some degree of selection bias, (e.g. exclusion of more severely ill patients in randomized-controlled trials). However, this also likely reflects benefits from therapeutic lumbar punctures, standardized intravenous fluid and electrolyte supplementation, and better management of intravenous catheters to prevent thrombophlebitis and catheter-associated bacteremia, among other factors, in clinical trial settings [138]. In contrast, short-term mortality from pneumococcal meningitis differed little between RCTs and routine care settings (both around 50%), perhaps reflecting the less intensive management currently recommended for pneumococcal disease, combined with late presentation to care and diagnosis, result in poor outcomes regardless of initial management. In routine care settings, more than two-thirds of patients in two studies died during the course of treatment for TB meningitis. Data was not available to compare these findings with clinical trials. One prospective cohort from South Africa with study investigator management reported a 12% (3/34) nine-month mortality in patients treated for HIV-associated TB meningitis [38]. However, this study had non-representative sampling, e.g. excluding patients with severe TB and those who did not initiate ART or were judged to have poor medication adherence.

Our findings suggest that the 70% one-year mortality used for global burden estimates of HIV-associated cryptococcal meningitis in sub-Saharan Africa may be conservative in some regions [6], particularly in Central and West Africa where acute (in-hospital) mortality exceeded 50% and long-term data was not available. Further, with a general paucity of data from primary hospitals that are relatively under-resourced compared to referral centers, survival may have been systematically over-estimated. Our findings also suggest that greater availability of more

fungicidal - but more toxic - amphotericin B therapy alone, to be used in place of fluconazole monotherapy, may not have a sizeable impact. Under routine care settings, although not compared using pairwise comparisons, reported short-term mortality for amphotericin B and fluconazole-based regimens were similar at around 40%, and long-term mortality reported at 65% even with available amphotericin B with high-dose fluconazole [52]. Recent RCT evidence suggests that when combined with flucytosine (5FC) a shortened one-week course of amphotericin B may be the most effective antifungal regimen for reducing mortality from HIV-associated cryptococcal meningitis [94], with similar rates of early fungal clearance compared to longer treatment durations and less drug toxicity [26]. Thus, alongside expanding access to amphotericin B formulations, there is an urgent need for flucytosine access in sub-Saharan Africa where it remains unregistered, and further research is needed to evaluate amphotericin B with flucytosine-based regimens in SSA under routine care settings.

Our study has a number of important limitations. First, we applied strict diagnostic criteria for included meningitis etiologies. This resulted in the exclusion of a number of studies in setting where microbiological tests (e.g. culture, or PCR) were not available, or in which a majority of cases were diagnosed based on clinical suspicion without clearly enumerated criteria. This may have over-sampled cases from clinical settings with better diagnostic capability (e.g. TB PCR) or cases of TB meningitis with a higher CSF bacillary burden. However, we found little variation in outcomes between studies with only microbiologically-confirmed cases and in which a majority of cases were diagnosed using combined laboratory, clinical, and/or imaging characteristics. Secondly, in comparing outcomes for cryptococcal meningitis between routine care and clinical trial settings, we were unable to determine the proportion of excess mortality in routine care settings attributable to various factors (e.g. lack of therapeutic lumbar punctures vs.

poor laboratory monitoring vs. poor completion of therapy). In reality, this high mortality in routine care settings is very likely multi-factorial and our findings highlight the need for better management strategies such as protocol-driven care, simplified short-course therapy with less toxicity, and better preventive strategies such as through CrAg screening for early infection in patients with advanced HIV disease [19]. Thirdly, the classification of some prospective cohort studies as clinical trials versus routine care studies required some judgment from the author team. Study enrolment in "routine care" prospective cohorts may have also led to better management of participants per local or international guidelines and some risk of selection bias. Fourthly, most studies had a majority or all HIV-positive patients, precluding our ability to compare outcomes in sub-Saharan Africa between HIV-positive versus HIV-uninfected patients. Our primary interest, however, was in describing meningitis outcomes in high HIV prevalence settings. Finally, duration of follow-up varied between studies evaluating short-term mortality, and observational studies of cryptococcal meningitis typically reporting in-hospital rather than two-week mortality. This may have biased result slightly toward a higher acute mortality in observational settings if length of stay was significantly longer on average than two weeks.

5.7 CONCLUSION

In conclusion, we found very high mortality from common HIV-associated meningitides in sub-Saharan Africa, with pooled short-term mortality for pneumococcal meningitis around 50% and long-term mortality for TB and cryptococcal meningitis well in excess of 50% but with an overall lack of robust long-term outcomes data. Importantly, we found a large degree of heterogeneity in outcomes between sub-regions, likely reflecting both relative resource-availability but also suggesting a potential benefit of enhanced training and management

strategies in regions with higher mortality. Improved management strategies are needed and prevention efforts should be prioritized, including CrAg screening for prevention of cryptococcal meningitis and childhood pneumococcal vaccination to provides herd immunity in adults [139].

5.8 ACKNOWLEDGEMENTS

The authors have no acknowledgements.

5.9 REFERENCES

1. UNAIDS. Fact sheet - latest statistics on the status of the AIDS epidemic. 2018. Accessed at: http://www.unaids.org/en/resources/fact-sheet. Accessed on: 23 Apr 2018.

2. Avila D, Althoff KN, Mugglin C, Wools-Kaloustian K, Koller M, Dabis F, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. Journal of acquired immune deficiency syndromes. 2014;65(1):e8-16.

3. Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002-2013: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(7):1120-7.

4. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC infectious diseases. 2010;10:67.

5. Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, et al. Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up

and Haemophilus influenzae type b vaccination, 2000-2012. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(10):e137-45.

6. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017.

7. Bicanic T, Bottomley C, Loyse A, Brouwer AE, Muzoora C, Taseera K, et al. Toxicity of Amphotericin B deoxycholate-based induction therapy in patients with HIV-associated cryptococcal meningitis. Antimicrob Agents Chemother. 2015.

8. World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescence and children. Geneva: WHO; 2018.

9. Meda J, Kalluvya S, Downs JA, Chofle AA, Seni J, Kidenya B, et al. Cryptococcal meningitis management in Tanzania with strict schedule of serial lumber punctures using intravenous tubing sets: an operational research study. Journal of acquired immune deficiency syndromes. 2014;66(2):e31-6.

10. Rolfes MA, Hullsiek KH, Rhein J, Nabeta HW, Taseera K, Schutz C, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(11):1607-14.

11. Bahr NC, Rolfes MA, Musubire A, Nabeta H, Williams DA, Rhein J, et al. Standardized electrolyte supplementation and fluid management improves survival during amphotericin therapy for cryptococcal meningitis in resource-limited settings. Open Forum Infect Dis. 2014;1(2):ofu070.

12. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. The New England journal of medicine. 2014;370(26):2487-98.

13. Marra F, Marra CA, Bruchet N, Richardson K, Moadebi S, Elwood RK, et al. Adverse drug reactions associated with first-line anti-tuberculosis drug regimens. Int J Tuberc Lung Dis. 2007;11(8):868-75.

14. Naidoo P, Theron G, Rangaka MX, Chihota VN, Vaughan L, Brey ZO, et al. The South African Tuberculosis Care Cascade: Estimated Losses and Methodological Challenges. J Infect Dis. 2017;216(suppl_7):S702-S13.

15. Køster-Rasmussen R, Korshin A, Meyer CN. Antibiotic treatment delay and outcome in acute bacterial meningitis. The Journal of infection. 2008;57(6):449-54.

16. Pasquier E, Kunda J, De Beaudrap P, Loyse A, Temfack E, Molloy SF, et al. Long-term Mortality and Disability in Cryptococcal Meningitis: A Systematic Literature Review. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(7):1122-32.

17. Woldeamanuel YW, Girma B. A 43-year systematic review and meta-analysis: case-fatality and risk of death among adults with tuberculous meningitis in Africa. J Neurol. 2014;261(5):851-65.

18. Ramakrishnan M, Ulland AJ, Steinhardt LC, Moïsi JC, Were F, Levine OS. Sequelae due to bacterial meningitis among African children: a systematic literature review. BMC Med. 2009;7:47.

19. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. Cryptococcal meningitis screening and community-based early adherence support in people with

advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet. 2015.

20. Heyderman RS, Gangaidzo IT, Hakim JG, Mielke J, Taziwa A, Musvaire P, et al. Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1998;26(2):284-9.

21. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, et al. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. The New England journal of medicine. 2016;374(6):542-54.

22. Sunpath H, Edwin C, Chelin N, Nadesan S, Maharaj R, Moosa Y, et al. Operationalizing early antiretroviral therapy in HIV-infected in-patients with opportunistic infections including tuberculosis. Int J Tuberc Lung Dis. 2012;16(7):917-23.

23. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis. 2010;10(11):803-12.

24. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.

25. Miller J. The inverse of the Freeman-Tukey double arcsine transformation . Am Stat. 1978; 32 :138.

26. Tenforde MW, Shapiro AE, Rouse B, Jarvis JN, Li T, Eshun-Wilson I, et al. Treatment for HIV-associated cryptococcal meningitis. Cochrane Database Syst Rev. 2018;7:CD005647.

27. Day JN, Chau TT, Lalloo DG. Combination antifungal therapy for cryptococcal meningitis. The New England journal of medicine. 2013;368(26):2522-3.

28. United Nations Statistics Division: Africa geoscheme. Accessed at: http://millenniumindicators.un.org/unsd/methods/m49/Un. Accessed on: 21 Feb 2019.

29. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.

30. Bergemann A, Karstaedt AS. The spectrum of meningitis in a population with high prevalence of HIV disease. QJM : monthly journal of the Association of Physicians. 1996;89(7):499-504.

31. Rothe C, Sloan DJ, Goodson P, Chikafa J, Mukaka M, Denis B, et al. A prospective longitudinal study of the clinical outcomes from cryptococcal meningitis following treatment induction with 800 mg oral fluconazole in Blantyre, Malawi. PloS one. 2013;8(6):e67311.

32. Gaskell KM, Rothe C, Gnanadurai R, Goodson P, Jassi C, Heyderman RS, et al. A prospective study of mortality from cryptococcal meningitis following treatment induction with 1200 mg oral fluconazole in Blantyre, Malawi. PloS one. 2014;9(11):e110285.

33. Scriven JE, Graham LM, Schutz C, Scriba TJ, Wilkinson KA, Wilkinson RJ, et al. A Glucuronoxylomannan-Associated Immune Signature, Characterized by Monocyte Deactivation and an Increased Interleukin 10 Level, Is a Predictor of Death in Cryptococcal Meningitis. J Infect Dis. 2016;213(11):1725-34.

34. Adeyemi B, Ross A. Profile and mortality outcome of patients admitted with cryptococcal meningitis to an urban district hospital in KwaZulu-Natal, South Africa. Journal of the International AIDS Society. 2014;17(4 Suppl 3):19623.

35. Hiesgen J, Schutte C, Olorunju S, Retief J. Cryptococcal meningitis in a tertiary hospital in Pretoria, mortality and risk factors - A retrospective cohort study. Int J STD AIDS. 2017;28(5):480-5.

36. Jarvis JN, Meintjes G, Harrison TS. Outcomes of cryptococcal meningitis in antiretroviral naïve and experienced patients in South Africa. The Journal of infection. 2010;60(6):496-8.

37. Lessells RJ, Mutevedzi PC, Heller T, Newell ML. Poor long-term outcomes for cryptococcal meningitis in rural South Africa. S Afr Med J. 2011;101(4):251-2.

38. Marais S, Meintjes G, Lesosky M, Wilkinson KA, Wilkinson RJ. Interleukin-17 mediated differences in the pathogenesis of HIV-1-associated tuberculous and cryptococcal meningitis. Aids. 2016;30(3):395-404.

39. McCarthy KM, Morgan J, Wannemuehler KA, Mirza SA, Gould SM, Mhlongo N, et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. Aids. 2006;20(17):2199-206.

40. Meiring ST, Quan VC, Cohen C, Dawood H, Karstaedt AS, McCarthy KM, et al. A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. Aids. 2012;26(18):2307-14.

41. Meiring S, Fortuin-de Smidt M, Kularatne R, Dawood H, Govender NP, GERMS-SA. Prevalence and Hospital Management of Amphotericin B Deoxycholate-Related Toxicities during Treatment of HIV-Associated Cryptococcal Meningitis in South Africa. PLoS Negl Trop Dis. 2016;10(7):e0004865.

42. Moosa MY, Coovadia YM. Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for human immunodeficiency

virus (HIV)-positive and HIV-negative patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1997;24(2):131-4.

43. Schutte CM, Van der Meyden CH, Magazi DS. The impact of HIV on meningitis as seen at a South African Academic Hospital (1994 to 1998). Infection. 2000;28(1):3-7.

44. Sogbanmu OO, John MA, Lalloo U. Management of cryptococcal meningitis in adults at Mthatha Hospital Complex, Eastern Cape, South Africa. Southern African Journal of HIV Medicine 2014; 15(3):104-107.

45. Von Pressentin K, Conradie HH, Mash R. A medical audit of the management of cryptococcal meningitis in HIV-positive patients in the Cape Winelands (East) district, Western Cape. South African Family Practice 2012; 54(4):339-346.

46. Berhe T, Melkamu Y, Amare A. The pattern and predictors of mortality of HIV/AIDS patients with neurologic manifestation in Ethiopia: a retrospective study. AIDS Res Ther. 2012;9:11.

47. Beyene T, Zewde AG, Balcha A, Hirpo B, Yitbarik T, Gebissa T, et al. High Dose Fluconazole Monotherapy is Inadequate for CSF Cryptococcal Antigen Positive HIV-infected Persons in an Ethiopian CrAg Screening Program. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

48. Mamuye AT, Bornstein E, Temesgen O, Blumberg HM, Kempker RR. Point-of-Care Testing for Cryptococcal Disease Among Hospitalized Human Immunodeficiency Virus-Infected Adults in Ethiopia. The American journal of tropical medicine and hygiene 2016; 95(4):786-792.

49. Majwala A, Burke R, Patterson W, Pinkerton R, Muzoora C, Wilson LA, et al. Handheld point-of-care cerebrospinal fluid lactate testing predicts bacterial meningitis in Uganda. Am J Trop Med Hyg. 2013;88(1):127-31.

50. Namutebi AM, Kamya MR, Byakika-Kibwika P. Causes and outcome of hospitalization among HIV-infected adults receiving antiretroviral therapy in Mulago hospital, Uganda. Afr Health Sci. 2013;13(4):977-85.

51. Trachtenberg JD, Kambugu AD, McKellar M, Semitala F, Mayanja-Kizza H, Samore MH, et al. The medical management of central nervous system infections in Uganda and the potential impact of an algorithm-based approach to improve outcomes. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2007;11(6):524-30.

52. Patel RKK, Leeme T, Azzo C, Tlhako N, Tsholo K, Tawanana EO, et al. High mortality in HIV-associated cryptococcal meningitis treated with amphotericin B-based therapy under routine care conditions in Africa. Open Forum Infect Dis. 2018;5(11):ofy267, https://doi.org/10.1093/ofid/ofy267.

53. Steele KT, Thakur R, Nthobatsang R, Steenhoff AP, Bisson GP. In-hospital mortality of HIV-infected cryptococcal meningitis patients with C. gattii and C. neoformans infection in Gaborone, Botswana. Medical mycology. 2010;48(8):1112-5.

54. Bamba S, Barro-Traoré F, Sawadogo E, Millogo A, Guiguemdé RT. [Retrospective study of cases of neuromeningeal cryptococcosis at the University Hospital of Bobo Dioulasso since accessibility to antiretroviral in Burkina Faso]. J Mycol Med. 2012;22(1):30-4.

55. Millogo A, Ki-Zerbo GA, Andonaba JB, Lankoandé D, Sawadogo A, Yaméogo I, et al. [Cryptococcal meningitis in HIV-infected patients at Bobo-Dioulasso hospital (Burkina Faso)]. Bull Soc Pathol Exot. 2004;97(2):119-21.

56. Luma HN, Tchaleu BC, Temfack E, Doualla MS, Ndenga DP, Mapoure YN, et al. HIV-Associated Central Nervous System Disease in Patients Admitted at the Douala General Hospital between 2004 and 2009: A Retrospective Study. AIDS research and treatment. 2013;2013:709810.

57. Mbuagbaw JN, Biholong, Njamnshi AK. La cryptococcoses neuro-meningee et l'infection au VIH dans le service de medicine du Centre Hospitalier et Universitaire de Yaounde, Cameroon. African Journal of Neurological Sciences 2006;25(2):13-20.

58. Yassibanda S, Kamalo CG, Mbolidi CD, Koffi B, Camengo SM, Akelelo N, et al. Les infections neuromeningees de l'adulte en milieu hospitalier a Bangui: Aspects étiologiques cliniques et évolutifs. Médicine de Afrique Noire 2002; 49(6):299-303.

59. Traore FA, Cissoko Y, Tounkara TM, Sako FB, Mouelle AD, Kpami DO, et al. Étiologies des méningites lymphocytaires chez les personnes vivant avec le VIH suivies dans le service des maladies infectieuses de Conakry. Médecine et Santé Tropicales 2015; 25:52-5.

60. Oumar AA, Dao S, Ba M, Poudiougou B, Diallo A. [Epidemiological, clinical and prognostic aspects of cryptococcal meningitis in hospital area of Bamako, Mali]. Rev Med Brux. 2008;29(3):149-52.

61. Agaba PA, Digin E, Makai R, Apena L, Agbaji OO, Idoko JA, et al. Clinical characteristics and predictors of mortality in hospitalized HIV-infected Nigerians. Journal of Infection in Developing Countries 2011; 5(5):377-382.

62. Kisenge PR, Hawkins AT, Maro VP, McHele JP, Swai NS, Mueller A, et al. Low CD4 count plus coma predicts cryptococcal meningitis in Tanzania. BMC infectious diseases. 2007;7:39.

63. Siddiqi OK, Ghebremichael M, Dang X, Atadzhanov M, Kaonga P, Khoury MN, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(12):1771-7.

64. Nyazika TK, Hagen F, Machiridza T, Kutepa M, Masanganise F, Hendrickx M, et al. Cryptococcus neoformans population diversity and clinical outcomes of HIV-associated cryptococcal meningitis patients in Zimbabwe. Journal of Medical Microbiology.65(11):1281-8.

65. Gbangba-Ngai E, Fikouma V, Mossoro-Kpinde CD, Tekpa G, Ouavene JO, Yangba Mongba DS, et al. [Cryptococcal neuromeningitidis in HIV-infected patients in Bangui, in the era of antiretroviral treatment]. Bull Soc Pathol Exot. 2014;107(2):106-9.

66. Kouakou GA, Ello NF, Kassi NA, Keita M, Doumbia A, Mossou C, et al. [Fluconazole 1200mg or 800mg for cryptococcal meningitis treatment in Ivory Coast]. J Mycol Med. 2017;27(1):72-8.

67. Aoussi EF, Ehui E, Dembélé JP, Kolia-Diafouka P, Elloh NF, Ouattara SI, et al. Cryptoccocal meningitis and HIV in the era of HAART in Côte d'Ivoire. Med Mal Infect. 2012;42(8):349-54.

68. Soumaré M, Seydi M, Ndour CT, Fall N, Dieng Y, Sow AI, et al. [Epidemiological, clinical, etiological features of neuromeningeal diseases at the Fann Hospital Infectious Diseases Clinic, Dakar (Senegal)]. Med Mal Infect. 2005;35(7-8):383-9.

69. Baldassarre R, Mdodo R, Omonge E, Jaoko W, Baddley J, Pappas P, et al. MORTALITY AFTER CLINICAL MANAGEMENT OF AIDS-ASSOCIATED CRYPTOCOCCAL MENINGITIS IN KENYA. East Afr Med J. 2014;91(5):145-51.

70. Siika AM, Ayuo PO, Sidle MJ, Wools-Kaloustian K, Kimaiyo SN, Tierney WM. Admission characteristics, diagnoses and outcomes of HIV-infected patients registered in an ambulatory HIV-care programme in western Kenya. East Afr Med J. 2008;85(11):523-8.

71. Wake RM, Britz E, Sriruttan C, Rukasha I, Omar T, Spencer DC, et al. High Cryptococcal Antigen Titers in Blood are Predictive of Subclinical Cryptococcal Meningitis Among HIV-Infected Patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

72. Jarvis JN, Meintjes G, Williams Z, Rebe K, Harrison TS. Symptomatic relapse of HIV-associated cryptococcal meningitis in South Africa: the role of inadequate secondary prophylaxis. S Afr Med J. 2010;100(6):378-82.

73. Ousley J, Niyibizi AA, Wanjala S, Vandenbulcke A, Kirubi B, Omwoyo W, et al. High Proportions of Patients With Advanced HIV Are Antiretroviral Therapy Experienced: Hospitalization Outcomes From 2 Sub-Saharan African Sites. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(suppl_2):S126-S31.

74. Park BJ, Shetty S, Ahlquist A, Greenbaum A, Miller JL, Motsi A, et al. Long-term follow-up and survival of antiretroviral-naive patients with cryptococcal meningitis in the pre-antiretroviral therapy era, Gauteng Province, South Africa. Int J STD AIDS. 2011;22(4):199-203.

75. Mwaba P, Mwansa J, Chintu C, Pobee J, Scarborough M, Portsmouth S, et al. Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal

meningitis in Zambian AIDS patients treated under local conditions. Postgrad Med J. 2001;77(914):769-73.

76. Maher D, Mwandumba H. Cryptococcal meningitis in Lilongwe and Blantyre, Malawi. The Journal of infection. 1994;28(1):59-64.

77. Wang W, Carm AR. A clinical manifestation of AIDS with cryptococcal meningitis in Equatorial Guinea. Trop Doct. 2001;31(4):221-2.

78. Seboxa T, Alemu S, Assefa A, Asefa A, Diro E. Cryptococcal meningitis in patients with acquired immunudeficiency syndrome in prehaart era at Gondar College of Medical Sciences Hospital north-west Ethiopia. Ethiop Med J. 2010;48(3):237-41.

79. Eholie SP, N'gbocho L, Bissagnene E, Coulibaly M, Ehui E, Kra O, et al. Mycoses profondes au cours du sida à Abidjan (Côte d'Ivoire). Bull Soc Path Ex 1997;90(5):307-11.

80. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(5):736-45.

81. Bicanic T, Meintjes G, Wood R, Hayes M, Rebe K, Bekker LG, et al. 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 : an official publication of the Infectious Diseases Society of America. 2007;45(1):76-80.

82. Bicanic T, Wood R, Meintjes G, Rebe K, Brouwer A, Loyse A, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected

patients: a randomized trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(1):123-30.

83. Jarvis JN, Meintjes G, Rebe K, Williams GN, Bicanic T, Williams A, et al. Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. Aids. 2012;26(9):1105-13.

84. Lightowler JV, Cooke GS, Mutevedzi P, Lessells RJ, Newell ML, Dedicoat M. Treatment of cryptococcal meningitis in KwaZulu-Natal, South Africa. PloS one. 2010;5(1):e8630.

85. Loyse A, Wilson D, Meintjes G, Jarvis JN, Bicanic T, Bishop L, et al. Comparison of the early fungicidal activity of high-dose fluconazole, voriconazole, and flucytosine as second-line drugs given in combination with amphotericin B for the treatment of HIV-associated cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(1):121-8.

86. Chang CC, Dorasamy AA, Gosnell BI, Elliott JH, Spelman T, Omarjee S, et al. Clinical and mycological predictors of cryptococcosis-associated immune reconstitution inflammatory syndrome. Aids. 2013;27(13):2089-99.

87. Kambugu A, Meya DB, Rhein J, O'Brien M, Janoff EN, Ronald AR, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(11):1694-701.

88. Katwere M, Kambugu A, Piloya T, Wong M, Hendel-Paterson B, Sande MA, et al. Clinical presentation and aetiologies of acute or complicated headache among HIV-seropositive patients in a Ugandan clinic. Journal of the International AIDS Society. 2009;12:21.

89. Longley N, Muzoora C, Taseera K, Mwesigye J, Rwebembera J, Chakera A, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(12):1556-61.

90. Muzoora CK, Kabanda T, Ortu G, Ssentamu J, Hearn P, Mwesigye J, et al. Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. The Journal of infection. 2012;64(1):76-81.

91. Tugume L, Rhein J, Hullsiek KH, Mpoza E, Kiggundu R, Ssebambulidde K, et al. HIV-associated Cryptococcal Meningitis Occurring at Relatively Higher CD4 counts. J Infect Dis. 2018.

92. Jackson AT, Nussbaum JC, Phulusa J, Namarika D, Chikasema M, Kanyemba C, et al. A phase II randomized controlled trial adding oral flucytosine to high-dose fluconazole, with short-course amphotericin B, for cryptococcal meningitis. Aids. 2012;26(11):1363-70.

93. Nussbaum JC, Jackson A, Namarika D, Phulusa J, Kenala J, Kanyemba C, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;50(3):338-44.

94. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

95. Jarvis JN, Leeme TB, Molefi M, Chofle AA, Bidwell G, Tsholo K, et al. Short Course High-dose Liposomal Amphotericin B for HIV-associated Cryptococcal Meningitis: A phase-II

Randomized Controlled Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.

96. Ochieng PO, McLigeyo SO, Amayo EO, Kayima JK, Omonge EO. Nephrotoxicity of amphotericin B in the treatment of cryptococcal meningitis in acquired immunodeficiency syndrome patients. East Afr Med J. 2009;86(9):435-41.

97. Makadzange AT, Ndhlovu CE, Takarinda K, Reid M, Kurangwa M, Gona P, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;50(11):1532-8.

98. Joly V, Aubry P, Ndayiragide A, Carrière I, Kawa E, Mlika-Cabanne N, et al. Randomized comparison of amphotericin B deoxycholate dissolved in dextrose or Intralipid for the treatment of AIDS-associated cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1996;23(3):556-62.

99. Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, et al. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. Lancet Infect Dis. 2016;16(7):809-18.

100. Mayanja-Kizza H, Oishi K, Mitarai S, Yamashita H, Nalongo K, Watanabe K, et al. Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1998;26(6):1362-6.

101. Raberahona M, Rakotoarivelo RA, Razafinambinintsoa T, Andrianasolo RL, Randria MJ. Clinical Features and Outcome in Adult Cases of Tuberculous Meningitis in Tertiary Care Hospital in Antananarivo, Madagascar. Biomed Res Int. 2017;2017:9316589.

102. Hakim JG, Gangaidzo IT, Heyderman RS, Mielke J, Mushangi E, Taziwa A, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. Aids. 2000;14(10):1401-7.

103. Karstaedt AS, Valtchanova S, Barriere R, Crewe-Brown HH. Tuberculous meningitis in South African urban adults. QJM : monthly journal of the Association of Physicians. 1998;91(11):743-7.

104. Ogun SA, Ojini F, Okubadejo N, Danesi M, Kolapo K, Osalusi B. Pattern and outcome of neurological manifestations of HIV/AIDS - A review of 154 cases in a Nigerian University Teaching Hospital - A preliminary report. African Journal of Neurological Sciences 2005; 24(1):29-36.

105. Schutte CM. Clinical, cerebrospinal fluid and pathological findings and outcomes in HIV-positive and HIV-negative patients with tuberculous meningitis. Infection. 2001;29(4):213-7.

106. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, et al. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis. 2018;18(1):68-75.

107. Tenforde MW, Mokomane M, Leeme TB, Tlhako N, Tsholo K, Chebani T, et al. Mortality outcomes in culture-positive and culture-negative adult meningitis in the Botswana National Meningitis Survey: A prevalent cohort study. Lancet Infect Dis. 2019.

108. Pepper DJ, Marais S, Maartens G, Rebe K, Morroni C, Rangaka MX, et al. Neurologic manifestations of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome: a case series. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(11):e96-107.

109. Patel VB, Padayatchi N, Bhigjee AI, Allen J, Bhagwan B, Moodley AA, et al. Multidrug-resistant tuberculous meningitis in KwaZulu-Natal, South Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;38(6):851-6.

110. Marais S, Roos I, Mitha A, Mabusha SJ, Patel V, Bhigjee AI. Spinal Tuberculosis: Clinicoradiological Findings in 274 Patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;67(1):89-98.

111. Burton NT, Forson A, Lurie MN, Kudzawu S, Kwarteng E, Kwara A. Factors associated with mortality and default among patients with tuberculosis attending a teaching hospital clinic in Accra, Ghana. Trans R Soc Trop Med Hyg. 2011;105(12):675-82.

112. Jowi JO, Mativo PM, Musoke SS. Clinical and laboratory characteristics of hospitalised patients with neurological manifestations of HIV/AIDS at the Nairobi hospital. East Afr Med J. 2007;84(2):67-76.

113. Patel VB, Burger I, Connolly C. Temporal evolution of cerebrospinal fluid following initiation of treatment for tuberculous meningitis. S Afr Med J. 2008;98(8):610-3.

114. Cresswell FV, Bangdiwala AS, Bahr NC, Trautner E, Nuwagira E, Ellis J, et al. Can improved diagnostics reduce mortality from Tuberculous meningitis? Findings from a 6.5-year cohort in Uganda. Wellcome Open Res. 2018;3:64.

115. Patel VB, Singh R, Connolly C, Coovadia Y, Peer AK, Parag P, et al. Cerebrospinal T-cell responses aid in the diagnosis of tuberculous meningitis in a human immunodeficiency virus- and tuberculosis-endemic population. Am J Respir Crit Care Med. 2010;182(4):569-77.

116. Bahr NC, Tugume L, Rajasingham R, Kiggundu R, Williams DA, Morawski B, et al. Improved diagnostic sensitivity for tuberculous meningitis with Xpert(®) MTB/RIF of centrifuged CSF. Int J Tuberc Lung Dis. 2015;19(10):1209-15.

117. Chapp-Jumbo EN. Neurologic infections in a Nigerian university teaching hospital. Afr Health Sci. 2006;6(1):55-8.

118. Gordon SB, Walsh AL, Chaponda M, Gordon MA, Soko D, Mbwvinji M, et al. Bacterial meningitis in Malawian adults: pneumococcal disease is common, severe, and seasonal. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2000;31(1):53-7.

119. Thinyane KH, Motsemme KM, Cooper VJ. Clinical Presentation, Aetiology, and Outcomes of Meningitis in a Setting of High HIV and TB Prevalence. J Trop Med. 2015;2015:423161.

120. Boisier P, Maïnassara HB, Sidikou F, Djibo S, Kairo KK, Chanteau S. Case-fatality ratio of bacterial meningitis in the African meningitis belt: we can do better. Vaccine. 2007;25 Suppl 1:A24-9.

121. Cohen C, Naidoo N, Meiring S, de Gouveia L, von Mollendorf C, Walaza S, et al. Streptococcus pneumoniae Serotypes and Mortality in Adults and Adolescents in South Africa: Analysis of National Surveillance Data, 2003 - 2008. PloS one. 2015;10(10):e0140185.

122. Gordon SB, Chaponda M, Walsh AL, Whitty CJ, Gordon MA, Machili CE, et al. Pneumococcal disease in HIV-infected Malawian adults: acute mortality and long-term survival. Aids. 2002;16(10):1409-17.

123. Manga NM, Ndour CT, Diop SA, Ka-Sall R, Dia NM, Seydi M, et al. [Adult purulent meningitis caused by Streptococcus pneumoniae in Dakar, Senegal]. Med Trop (Mars). 2008;68(6):625-8.

124. Mbelesso P, Tatangba-Bakozo A, Fikouma V. Les méningites bactériennes de l'adulte en milieu hospitalier centrafricain. Bull Soc Pathol Exot 2006; 99(4):261-3.

125. Ouattara B ES, Adoubryn KD, Kra O, Tia H, Kouadio-Yapo CG. Étude retrospective des méningites bactériennes et à cryptocoques chez des sujets adultes infectés par le VIH à Abidjan (Coˆte d'Ivoire). Journal de Mycologie Médicale. 2007, 17:82-6.

126. Yaro S, Lourd M, Traoré Y, Njanpop-Lafourcade BM, Sawadogo A, Sangare L, et al. Epidemiological and molecular characteristics of a highly lethal pneumococcal meningitis epidemic in Burkina Faso. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2006;43(6):693-700.

127. Ajdukiewicz KM, Cartwright KE, Scarborough M, Mwambene JB, Goodson P, Molyneux ME, et al. Glycerol adjuvant therapy in adults with bacterial meningitis in a high HIV seroprevalence setting in Malawi: a double-blind, randomised controlled trial. Lancet Infect Dis. 2011;11(4):293-300.

128. Scarborough M, Gordon SB, Whitty CJ, French N, Njalale Y, Chitani A, et al. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. The New England journal of medicine. 2007;357(24):2441-50.

129. Wall EC, Mukaka M, Denis B, Mlozowa VS, Msukwa M, Kasambala K, et al. Goal directed therapy for suspected acute bacterial meningitis in adults and adolescents in sub-Saharan Africa. PloS one. 2017;12(10):e0186687.

130. Békondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D, et al. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2006;10(5):387-95.

131. Boumandouki P, Oyongo A, Yala F. [Clinical, epidemiological and therapeutic aspects of purulent meningitis in the adult. Apropos of 74 cases treated at CHU of Brazzaville (Congo)]. Bull Soc Pathol Exot. 1993;86(2):141-3.

132. Letsa T, Noora CL, Kuma GK, Asiedu E, Kye-Duodu G, Afari E, et al. Pneumococcal meningitis outbreak and associated factors in six districts of Brong Ahafo region, Ghana, 2016. BMC Public Health. 2018;18(1):781.

133. Aku FY, Lessa FC, Asiedu-Bekoe F, Balagumyetime P, Ofosu W, Farrar J, et al. Meningitis Outbreak Caused by Vaccine-Preventable Bacterial Pathogens - Northern Ghana, 2016. MMWR Morbidity and mortality weekly report. 2017;66(30):806-10.

134. Bozio CH, Abdul-Karim A, Abenyeri J, Abubakari B, Ofosu W, Zoya J, et al. Continued occurrence of serotype 1 pneumococcal meningitis in two regions located in the meningitis belt in Ghana five years after introduction of 13-valent pneumococcal conjugate vaccine. PloS one. 2018;13(9):e0203205.

135. Kambiré D, Soeters HM, Ouédraogo-Traoré R, Medah I, Sangare L, Yaméogo I, et al. Nationwide Trends in Bacterial Meningitis before the Introduction of 13-Valent Pneumococcal Conjugate Vaccine-Burkina Faso, 2011-2013. PloS one. 2016;11(11):e0166384.

136. Okome-Nkoumou M, Loembe PM. [Bacterial meningitis in the adult. Study of 85 cases observed in the infectious disease unit of the Fondation Jeanne Ebori (F.J.E.), Libreville, Gabon]. Bull Soc Pathol Exot. 1999;92(5):288-91.

137. Traore Y, Tameklo TA, Njanpop-Lafourcade BM, Lourd M, Yaro S, Niamba D, et al. Incidence, seasonality, age distribution, and mortality of pneumococcal meningitis in Burkina Faso and Togo. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48 Suppl 2:S181-9.

138. Rajasingham R, Williams D, Meya DB, Meintjes G, Boulware DR, Scriven J. Nosocomial drug-resistant bacteremia in 2 cohorts with cryptococcal meningitis, Africa. Emerging infectious diseases. 2014;20(4):722-4.

139. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. The New England journal of medicine. 2014;371(20):1889-99.

Figure 11. PubMed search strategy

#1

Search (Africa[MeSH] OR Africa*[tiab] OR sub-Saharan[tiab] OR Angola[tiab] OR Benin[tiab] OR Botswana[tiab] OR Burkina[tiab] OR Burundi[tiab] OR Cameroon[tiab] OR Cape Verde[tiab] OR Cabo Verde[tiab] OR Chad[tiab] OR Comoros[tiab] OR Congo[tiab] OR Cote d'Ivoire[tiab] OR Ivory Coast[tiab] OR Equatorial Guinea[tiab] OR Eritrea[tiab] OR Ethiopia[tiab] OR Gabon[tiab] OR Gambia[tiab] OR Ghana[tiab] OR Guinea[tiab] OR Bissau[tiab] OR Guinea-Bissau[tiab] OR Kenya[tiab] OR Lesotho[tiab] OR Madagascar[tiab] OR Malawi[tiab] OR Mali[tiab] OR Mauritania[tiab] OR Mauritius[tiab] OR Mozambique[tiab] OR Namibia[tiab] OR Niger[tiab] OR Rwanda[tiab] OR Sao Tome and Principe[tiab] OR Senegal[tiab] OR Seychelles[tiab] OR Sierra Leone[tiab] OR Somalia[tiab] OR Sudan[tiab] OR Swaziland[tiab] OR Tanzania[tiab] OR Togo[tiab] OR Tunisia[tiab] OR Uganda[tiab] OR Western Sahara[tiab] OR Zambia[tiab] OR Zimbabwe[tiab])

#2

Search (Meningitis, Cryptococcal[Mesh] OR cryptococcus neoformans[mh] OR cryptococcus gattii[mh] OR cryptococcal meningitis[tiab] OR cryptococcal meningitides[tiab] OR cerebral cryptococcosis[tiab] OR cerebral cryptococcoses[tiab] OR toruloma[tiab] OR cryptococcus neoformans[tiab] OR (cryptococcal[tiab] OR cyptococcosis[tiab] OR cryptococcoses[tiab] OR Cryptococcus[tiab]) AND (meningitis[tiab])))

#3

Search (Tuberculosis, Meningeal[MeSH] OR TBM[tiab] OR tubercul* meningitis[tiab] OR TB meningitis[tiab] OR tubercul* cerebrospinal fluid[tiab] OR tubercul* CSF[tiab] OR (TB[tiab] OR tubercul*[tiab] AND (meningitis[tiab])))

#4

Search (Meningitis, Pneumococcal[MeSH] OR pneumococcal meningitis[tiab] OR streptococcus pneumon* meningitis[tiab] OR streptococcus meningitis[tiab] OR bacterial meningitis[tiab] OR (pneumococc*[tiab] OR streptococc*[tiab] AND (meningitis[tiab])))

(Searching #1 and #2, #1 and #3, #1 and #4 limit search 1 Jan 1990 through 31 Dec 2018)

Figure 12. PRISMA diagram

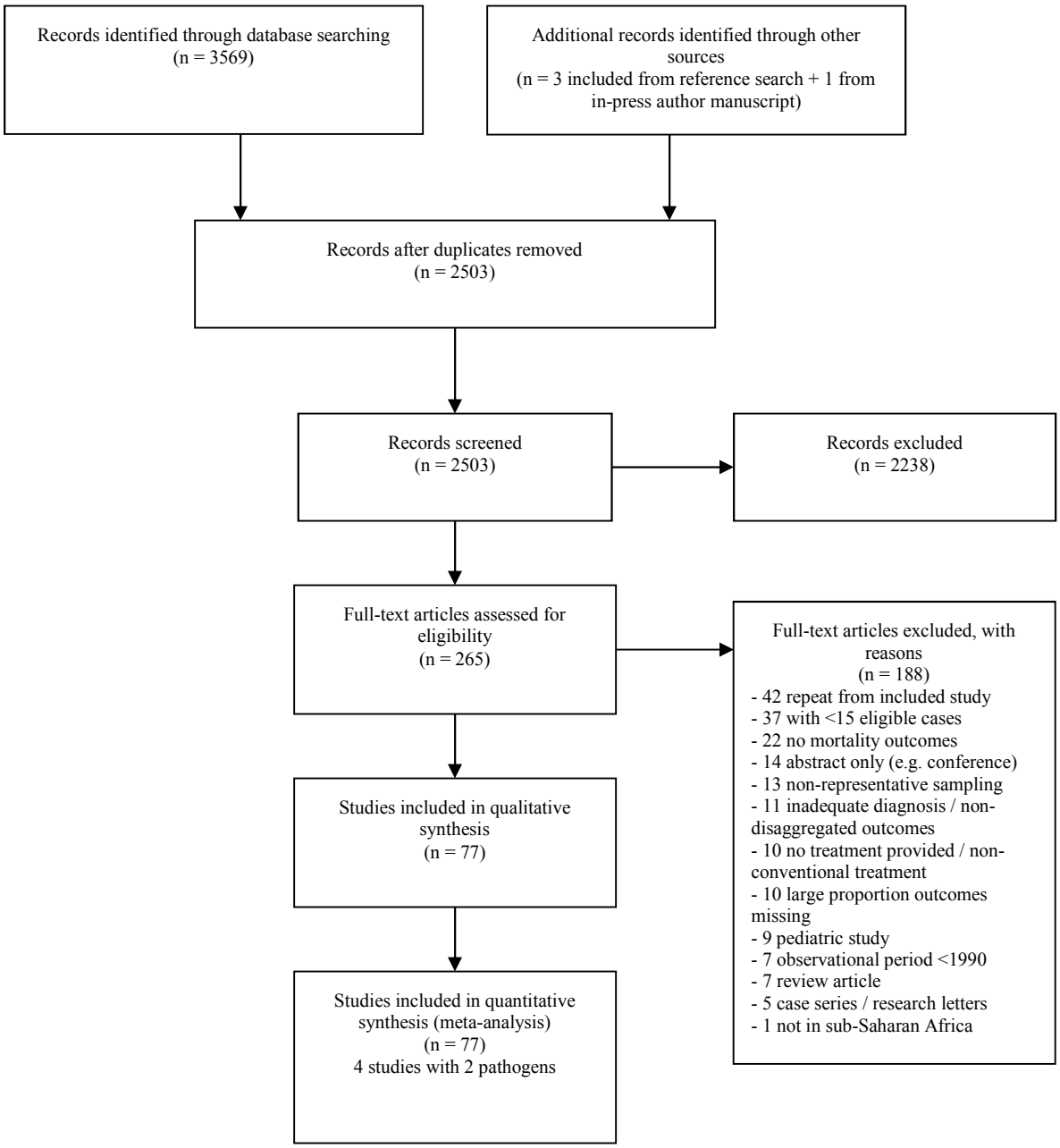


Figure 13. Overall pooled short-term mortality of cryptococcal meningitis in routine care settings.

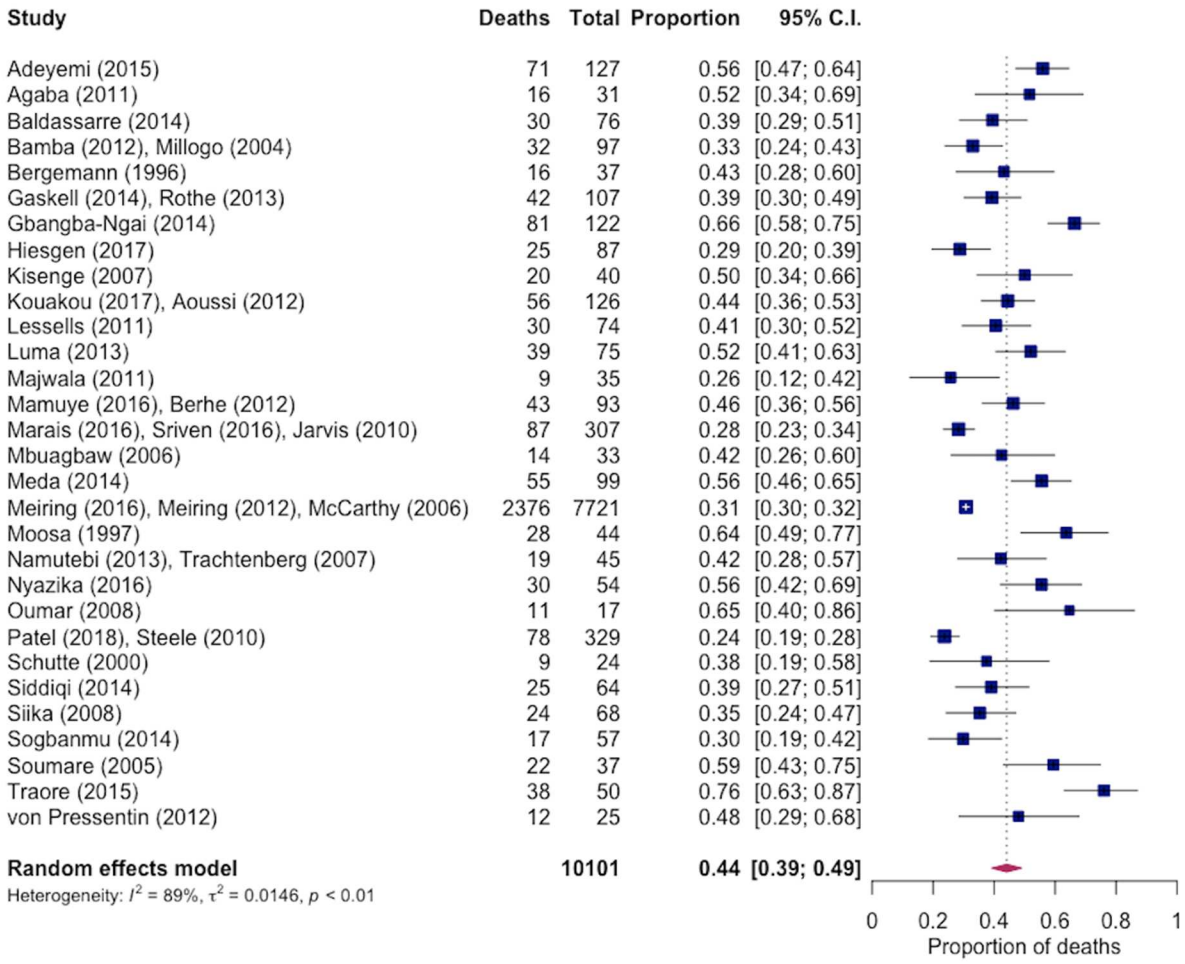


Figure 14. Pooled short-term mortality of cryptococcal meningitis in clinical trial settings

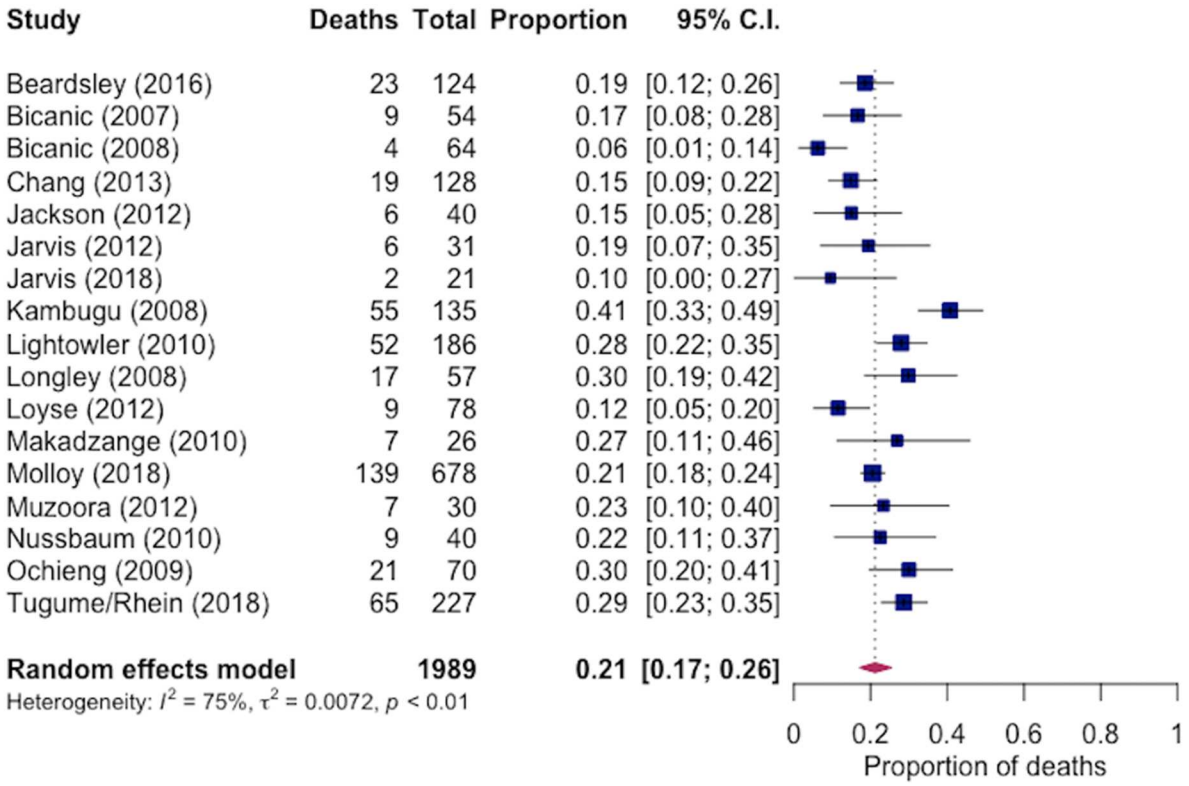


Figure 15. Pooled short-term mortality of TB meningitis in routine care settings

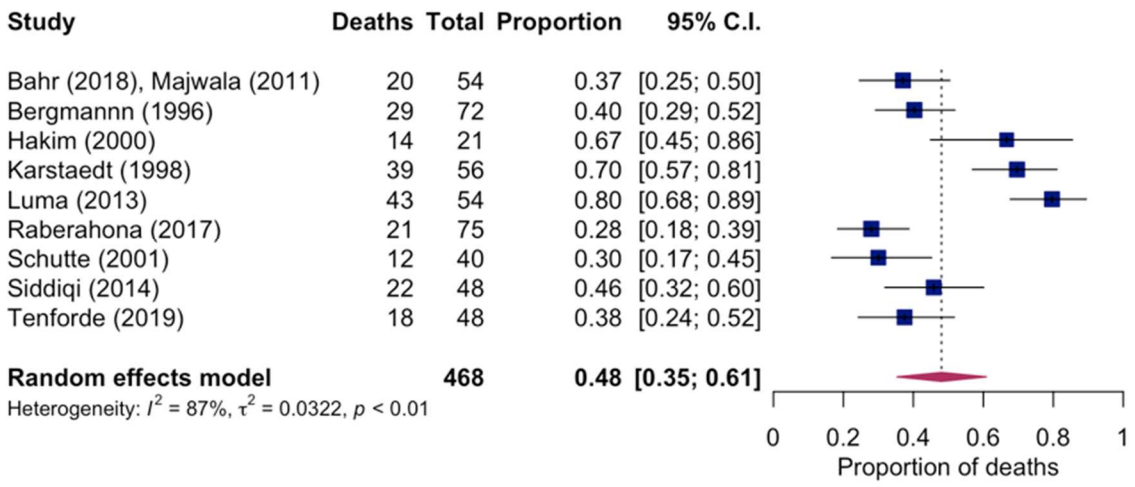
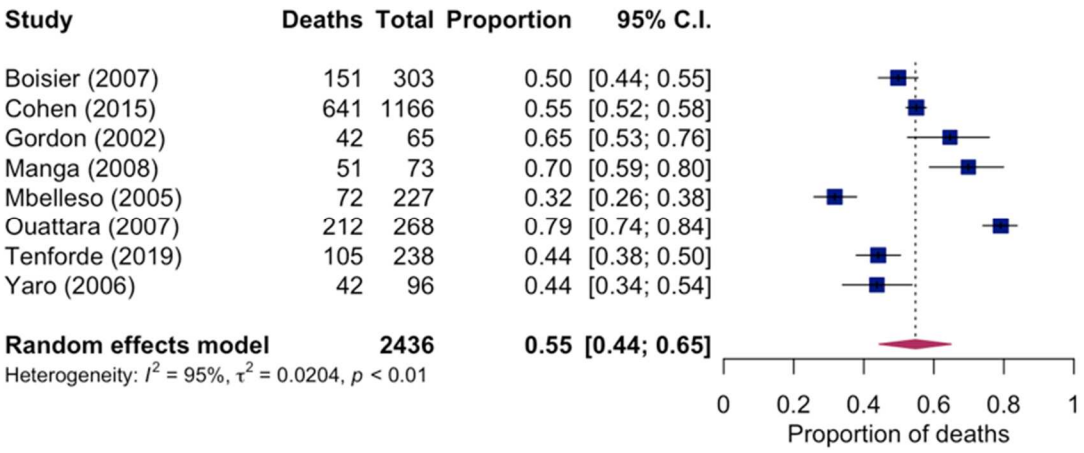


Figure 16. Pooled short-term mortality of pneumococcal meningitis in routine care settings



Chapter 6. COST-EFFECTIVENESS OF REFLEX LABORATORY-BASED CRYPTOCOCCAL ANTIGEN SCREENING FOR THE PREVENTION OF CRYPTOCOCCAL MENINGTIIS IN BOTSWANA

6.1 MANUSCRIPT TITLE AND TITLE PAGE

Cost-effectiveness of reflex laboratory-based cryptococcal antigen screening for the prevention of cryptococcal meningitis in Botswana

Mark W. Tenforde,[1,2] Charles Muthogo,[3] Andrew Callaghan,[3] Ponego Ponetshego,[4], Bruce Larson,[5*] Joseph N. Jarvis[4,6*]

* Authors contributed equally

Affiliations

[1] Division of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle, WA USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA, USA

[3] Botswana-UPenn Partnership, Gaborone, Botswana

[4] Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

[5] Boston University School of Public Health, Boston, MA, USA

[6] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Corresponding Author

Mark W Tenforde MD, MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2066508915

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Key Words

Cryptococcal antigen, CrAg, HIV, cost-effectiveness, modelling

6.2 ABSTRACT

ABSTRACT

Background and Aims: Cryptococcal antigen (CrAg) screening of antiretroviral therapy (ART)-naïve adults with advanced HIV/AIDS (CD4 <100 cells/μL) reduces the incidence of cryptococcal meningitis (CM) and all-cause mortality, but implementation of CrAg screening has not been evaluated in Botswana. We modeled the cost-effectiveness and impact of laboratory-based CrAg screening, including screening at different CD4 count thresholds and treatment of both ART-naïve and ART-experienced CrAg-positive patients.

Methods: We used a decision analytic model was used to evaluate CrAg screening. Based on local CD4 count and CrAg prevalence data, we modeled the number of CrAg tests performed, the proportion of patients started on pre-emptive antifungal treatment, and the number of incident CM cases and CM-related deaths with CrAg screening compared versus no screening. We evaluated screening and treatment costs and estimated the cost per death or disability-adjusted life year (DALY) averted across various assumptions. Separate analyses were performed at CrAg screening thresholds of <100 cells/μL and 100-200 cells/μL.

Results: At 650,000 CD4 tests performed annually, we estimate 16,364 CrAg tests at a CD4 <100 cells/μL and 33,036 at a CD4 100-200 cells/μL, with half of patients ART-experienced. Under base model assumptions, CrAg screening in ART-naïve patients with a CD4 <100 cells/μL prevented 20% (47/240) of CM-related deaths and was highly cost-effective (cost per DALY averted US$1). Treatment of ART-experienced patients with a CD4 <100 cells/μL resulted in further deaths averted (52/240) and was cost-saving. Screening at a CD4 <100 cells/μL remained highly cost-effective across different model assumptions. Screening in a population with a CD4 100-200 cells/μL with treatment of CrAg-positive ART-naive saved an estimated 13 lives at a cost of US$14,547 per death averted, with higher costs and few lives saved in sensitivity models.

Conclusions: Our findings support CrAg screening in Botswana at a CD4 threshold of <100 cells/μL and benefit of pre-emptive treatment for both CrAg-positive ART-naïve and ART-experienced patients. Screening at a higher CD4 count threshold of 100-200 cells/μL has a small public health impact and policies around screening in this population should be weighed against other health systems priorities.

6.3 INTRODUCTION

Cryptococcal meningitis (CM) is a leading cause of mortality in people living with HIV/AIDS (PLWH) worldwide, resulting in an estimated 15% of HIV deaths [1]. HIV-associated CM predominantly occurs in the setting of advanced HIV disease (usually at a CD4 T-cell count <100 cells/μL [2], although also seen at higher CD4 counts in a minority of cases [3]). In patients with advanced HIV initiating antiretroviral therapy (ART), detection of cryptococcal antigen (CrAg) in the blood is highly predictive of incident CM [4], with clinical symptoms usually developing after in weeks [4,5]. CrAg screening with pre-emptive fluconazole therapy in CrAg-positive ART-naïve adults has been shown to reduce all-cause mortality and is recommended by the World Health Organization (WHO) in adults starting ART with a CD4 <100 cells/μL [6,7]. Recently, the WHO conditionally recommended screening ART-naïve PLWH with a CD4 count of 100-200 cells/μL [7].

Botswana, a country of approximately 2.3 million with a 2017 adult HIV prevalence of 23% [8], has a mature HIV program with free ART provision to citizens that started in 2002. Despite ART scale-up, advanced HIV/AIDS and CM remain common with almost 400 confirmed cases diagnosed per year in 2013 and 2014 [2,9,10]. In 2016, national HIV guidelines recommended CrAg screening in ART-naïve CrAg-positive patients with a CD4 <100 cells/μL [11]. For CrAg-positive ART-naïve patients, fluconazole 1200 mg/day for 2 weeks, then 800 mg/day for 8 weeks with ART initiation, then 200 mg/day until CD4 recovers to >200 cells/μL for at least 6 months is recommended. Currently, "reflex" screening (screening of any sample sent for CD4 testing below the CD4 count threshold) is conducted at the Botswana-Harvard HIV Reference Laboratory (BHHRL), which performs most CD4 testing in the urban Gaborone region.

The cost-effectiveness and impact of CrAg screening has not been evaluated in Botswana. There is a need to both evaluate screening for ART-naïve patients with a CD4 count <100 cells/μL, as recommended in current national guidelines, and to evaluate screening in those with a CD4 count of 100-200 cells/μL to inform future guidelines. Previous models have assumed that all or most CrAg-screened patients are ART-naïve [12-14]. However, about half of cases of CM now occur in ART-experienced patients [15-17], and - in the context of a mature ART program in Botswana - reflex laboratory-based CrAg screening is now identifying a large number of ART-experienced CrAg-positive patients. This population represents a mix of individuals: 1) Those recently started on ART without baseline CD4 and CrAg screening results following adoption of the HIV "test-and-treat" strategy [18]; 2) those on ART but with treatment failure; and 3) those started ART with subsequent ART default. Current guidelines don't address management of this growing population of patients unintentionally screened through reflex laboratory-based screening strategies, although there is a plausible benefit of treating CrAg-positive patients.

The primary aim of our study was to model the impact, cost, and cost-effectiveness of CrAg screening and targeted pre-emptive fluconazole treatment in Botswana using different screening strategies. First, we modeled screening and pre-emptive treatment of ART-naïve patients with a CD4 <100 cells/μL, according to current guidelines [11]. Secondly, we evaluated screening of ART-naïve patients with a CD4 count of 100-200 cells/μL to inform future guidelines. Finally, for the CD4 <100 cells/μL and 100-200 cells/μL model, we generated models in which ART-experienced CrAg-positive patients are also given pre-emptive fluconazole. Given uncertainty of risk of CM progression in this population and the benefit of pre-emptive treatment, we evaluated these models under a range of assumptions. A decision analytic model was employed, as described before [14,19], with local CD4 distribution [9], CrAg prevalence [20], treatment outcomes [15],

and costing data used to estimate national costs for screening, cases and deaths averted through screening, and incremental cost effectiveness ratio (ICER) as determined by cost per death averted and cost per disability-adjusted life year (DALY) averted comparing screening with no screening.

6.4 MATERIALS AND METHODS

Overview.

Our models use CD4 count distribution and CrAg prevalence data from the Botswana-Harvard HIV Reference Laboratory (BHHRL) in Gaborone, including data from a completed CrAg screening prevalence study conducted 2015-2016 and a second cohort that started in 2018 [20]. CrAg testing is performed using the highly-accurate CrAg lateral flow assay (LFA) [IMMY, Norman, OK] [21], assumed here to be 100% sensitive / specific. Approximately 65,000 CD4 tests are performed annually at the BHHRL in 35,000 unique patients. With an estimated population of 370,000 adults living with HIV and high level of engagement [22,23], this covers nearly 10% of the adult HIV-positive population in Botswana. We therefore chose to start with a model of 650,000 CD4 tests performed annually on an population of 350,000 adults to provide an estimate of the annual cost and impact of CrAg screening implementation at a national level (not accounting for sub-national variability). Our models include two components: 1) A reflex CrAg *screening module*; and 2) a *treatment module*, both described below.

Screening module.

The screening model estimates the proportion of patients who receive CD4 testing who are CrAg-positive and pre-emptive treatment-eligible and the proportion of these patients targeted for pre-emptive therapy or diagnosed with CM on urgent clinic follow-up after a positive CrAg test,

factoring a proportion of eligible patients who may not receive CrAg screening or who receive screening but do not receive timely evaluation. Flowcharts for the screening module are shown in **Figure 17**, and sources for estimates in **Table 8**.

From 2015-2017, 2.65% of CD4 samples from clinics had a CD4 count <100 cells/μL, with a CrAg prevalence cells/μL of 4.6% at CD4 <100 cells/μL in non-hospitalized patients [20]; 5.37% of samples had a CD4 100-200 cells/μL, with an estimated CrAg prevalence of 2% from a published systematic review [24]. Of CrAg-positive samples, approximately 25% are from patients with prior treated cryptococcal meningitis or positive CrAg result in the blood, which result in a persistently positive test result. These patients are not targeted for pre-emptive therapy. The remaining 75% of CrAg-positive patients are eligible for pre-emptive fluconazole.

We used local CrAg titer data to estimate the risk of ART-naïve CrAg-positive patients progressing to CM [20]. A high CrAg titer (>1:160) is associated with a high risk of CM without pre-emptive treatment in ART-naïve patients [25,26]. From local data, 59% of CrAg-positive patients with a CD4 <100 cells/μL have a high CrAg titer. At a CD4 of 100-200 cells/μL, we estimate that a lower proportion (10%) of CrAg-positive patients have high titers due to comparatively less immunodeficiency in this population. A lumbar puncture is offered to all CrAg-positive patients evaluated for pre-emptive therapy to rule out *prevalent* CM, as up to one-third of even relatively asymptomatic CrAg-positive patients have prevalent meningitis diagnosed when evaluated by lumbar puncture (LP) and cerebrospinal fluid (CSF) testing [25]. As LP refusal is common [27], we estimate that only 25% of patients consent to an LP. Eighty-five percent of high-titer and 15% of low-titer patients who undergo LP are diagnosed with prevalent CM and hospitalized [25,28]. The remaining CrAg-positive patients (i.e. those who did not undergo LP or had negative CSF testing) are targeted for pre-emptive fluconazole.

A proportion of CrAg-positive patients who should receive CrAg screening are not tested (5%), e.g. due to laboratory error or CrAg assay stockout. Without CrAg testing and pre-emptive fluconazole, these patients are at an high risk of incident CM, with risk influenced by a patient's CrAg titer and how quickly ART is initiated [4,25,29]. Of screened CrAg-positive patients, an estimated 25% with CD4 <100 cells/μL (10% at CD4 100-200 cells/μL) do not return to clinic quickly for evaluation and initiation of pre-emptive fluconazole and/or LP evaluation; these patients are also at a high risk of progression to CM.

We estimate that ART-experienced patients constitute approximately 50% of patients receiving CrAg screening (laboratory-based "reflex" CrAg screening is performed based on CD4 count threshold regardless of a patient's ART status which is typically unknown by the laboratory) [20]. For ART-experienced CrAg positive patients, given high uncertainty of the risk of progression to CM without pre-emptive fluconazole we performed several analyses with a range of assumptions. In our base model, we estimate a risk of progressing to CM in CrAg-positive patients with a CD4 <100 cell/μL of 59% without pre-emptive fluconazole (assuming 25% of patients are ART defaulters with a high CM risk [90%] and - among the other 75% of patients currently on ART - 40% have treatment failure with a high risk [90%] and 60% recently started on ART with a lower risk [21%] of incident CM [29]). In the ART-experienced population with a CD4 100-200 cells/μL, the risk of progression to CM is assumed to be lower (21%) than the CD4 <100 cells/μL group (25% of patients are ART defaulters with a CM risk of 30% and - among the 75% of patients on ART - 40% with treatment failure with a high risk 30% and 60% recently started on ART with a lower risk 10% of incident CM).

Treatment module.

The treatment module estimates outcomes for patients who should receive CrAg screening but do not and progress to CM, those who receive screening but progress to CM without urgent follow-up and pre-emptive therapy, and those who start but "fail" pre-emptive by developing CM. Flowcharts for the treatment module are shown in **Figure 17**, and sources for estimates detailed in **Table 8**.

For ART-naïve CrAg-positive patients, success of pre-emptive treatment is derived from local estimates and a published systematic review [20,29]. For ART-naïve patients, we estimate that 20% of high-titer patients and 5% of low titer patients started on pre-emptive fluconazole will fail pre-emptive fluconazole. For ART-experienced patients, given lack of published outcomes data in this population we assume that there is a 75% reduction in the risk of progression to CM with pre-emptive therapy (i.e. from 59% to 14.8% in the base model for CD4 <100 cells/μL).

For patients who progress to CM, case-fatality rates are derived from local data [15]. Ten-week mortality for patients with a CD4 <100 cells/μL hospitalized for cryptococcal meningitis and treated with amphotericin B-based induction therapy is estimated to be 50%, with 17% of survivors experiencing relapse. We assumed lower mortality (40%) for patients with a CD4 of 100-200 cells/μL, as low CD4 count is a predictor of higher mortality [30]. CrAg-positive patients who are diagnosed with early CM on urgent follow-up or hospitalized after failing pre-emptive fluconazole are assumed to have a lower 10-week mortality (25% instead of 50% for those missed and not started on pre-emptive treatment) based on regional data [25], with early recognition of infection and timely initiation of antifungal treatment associated with better survival. In our models, we assume that a minority of patients (20%) with incident CM die at home without being diagnosed, with a lower proportion who start on pre-emptive fluconazole (10%) or are diagnosed with CM at their initial follow-up (0%).

CrAg screening and treatment costs.

The costs of CrAg screening implementation and CM treatment are estimated from a payer-perspective using local supply costs from Botswana Central Medical Stores (CMS) for most parameter estimates (**Table 9**). For CrAg screening, the cost per CrAg test is estimated at 50 Pula (US$4.71); to the wholesale price (~US$2), we factor in additional mark-ups from local distributors, shipment, and laboratory personnel costs. Fluconazole is relatively expensive in Botswana (at ~US$0.50 / 200 mg tablet through CMS) compared to other countries in the region and we assumed procurement through CMS rather than a pharmaceutical company drug donation program. Average length of maintenance therapy is estimated at 6 months, assuming some incomplete adherence.

We do not have reliable local hospital "hotel" costs in Botswana for the treatment of CM so used inflation-adjusted WHO-CHOICE estimates for cost per day of hospital admission [31]. Length of hospital stay was estimated at 17 days for survivors for local data (75% of this time in those who die within 10 weeks) [15]. We assume a standard 14-day course of amphotericin B with high-dose fluconazole, intravenous fluid and electrolyte supplementation, and routine laboratory monitoring. Patients who survive hospitalization receive 8 weeks of fluconazole consolidation and 6 months of maintenance fluconazole on average. Patients who die within 10 weeks of treatment have lower utilization of treatment, laboratory, and post-discharge care and fluconazole.

Outcomes.

We estimate annual costs of CrAg screening implementation nationally in Botswana and the costs of treatment (both for pre-emptive fluconazole and cryptococcal meningitis) for ART-naïve

patients at CD4 <100 cells/μL and 100-200 cells/μL. Comparing these to a counterfactual scenario with no screening, we estimate the number of cryptococcal meningitis cases averted from screening, number of deaths prevented, and the cost per death averted as the incremental cost effectiveness ratio (ICER). We also evaluated cost per disability-adjusted life year (DALY) avoided, with average age of death from CM from local data [15], life expectancy determined from World Factbook estimates [32], and with future years discounted at a 3% rate to give a DALY estimate of 21.4 years. We then re-evaluated models with both ART-naïve (the target population for CrAg screening) *and* ART-experienced CrAg-positive patients being offered pre-emptive treatment. We do not factor in additional costs or deaths from CM relapse as these are small and unlikely to have a significant public health or health system cost impact.

Sensitivity analyses.

We performed a series of sub-analyses (SA) to account for areas of model uncertainty. In the ART-naïve models we performed the following analyses: 1) 50% vs. 75% in the base model return quickly for follow-up to start treatment (CD4 <100 cells/μL group) and 85% vs. 90% in the CD4 100-200 cells/μL group; 2) A higher proportion (75% vs. 50% in the base model) of patients are ART-experienced; and 3) 40% vs. 59% in the base model with positive CrAg have a high titer (CD4 <100 cells/μL group) and 5% vs. 10% in the CD4 100-200 cells/μL group.

For ART-experienced CrAg-positive patients, we performed additional sub-analyses assuming lower risk of progression to CM without fluconazole as well as less benefit of pre-emptive fluconazole: 1) 30% vs. 59% in the base model risk of CM progression without pre-emptive fluconazole in the CD4 <100 cells/μL group and 10% vs. 20% in the CD4 100-200 cells/μL group, assuming again a 75% reduction in risk of CM progression with pre-emptive

therapy and no patients diagnosed with CM at urgent follow-up; and 2) 20% vs. 59% in the base model risk of CM progression without pre-emptive fluconazole in the CD4 <100 cells/μL group and 10% vs. 20% in the CD4 100-200 cells/μL group, now assuming a 50% reduction in risk of CM progression with pre-emptive therapy and with no patients diagnosed with CM at urgent follow-up.

6.5 RESULTS

Cryptococcal meningitis cases and costs without screening.

Without CrAg screening, our base model estimates 52,002 CD4 counts ≤200 cells/μL (17,225 with CD4 <100 cells/μL and 34,773 with CD4 100-200 cells/μL). In the base model without CrAg screening, 401 patients with CD4 <100 cells/μL and 121 patients with a CD4 100-200 cells/μL develop CM without screening, 44% (228/522) of cases in ART-naïve. Assuming about 20-25% of patients die without being diagnosed, this is consistent with national incidence estimates [2], as well as local estimates of 45% of cases occurring in ART-experienced individuals [15]. The proportion of screened CD4 100-200 cells/μL patients who develop CM (23%) is higher than the ~15% diagnosed with cryptococcal meningitis in Botswana with a CD4 >100 cells/μL [2], although some proportion of these are likely to experience a drop in CD4 count before diagnosis of CM. The overall cost for treatment is estimated at $985,129, including costs of hospital and post-discharge care.

CrAg screening at CD4 <100 cells/μL, ART-naïve and ART-experienced.

In our CD4 <100 cells/μL base model, 16,364 CrAg tests are performed at a cost of US$77,073 (**Table 10a**). Without screening, we estimate 240 CM-related deaths annually in this population.

For ART-naïve only, with CrAg screening and treatment, we estimate 47 lives saved, at a cost savings of US$26 per death averted or US$1 per DALY averted (**Table 10a**). In sensitivity analyses, including evaluation of a lower proportion of CrAg-positive patients who return quickly for evaluation for pre-emptive treatment, a lower proportion of CrAg-positive ART-naïve patients, and a lower proportion of patients with high CrAg titers, screening saved 31-47 lives and remained highly cost effective at a cost per DALY averted <US$125 under all assumptions.

We next considered treating *both* ART-naïve as well as ART-experienced patients recognized as CrAg-positive through reflex screening. No additional costs are accrued for screening ART-experienced patients. Under base model assumptions, the number of lives saved was 98 with a cost-savings of US$904 per death averted or US$42 per DALY averted (**Table 10b**). In sensitivity analyses, treatment for both ART-naïve and ART-experienced saved lives and remained highly cost-effective with an estimated cost per DALY averted <US$125 under all assumptions (**Table 10c**).

CrAg screening at CD4 100-200 cells/μL, ART-naïve and ART-experienced.

In our CD4 100-200 cells/μL model population, 33,036 CrAg tests are performed at a cost of US$155,601 (**Figure 11a**). Without screening, we estimate 63 CM-related deaths in this population. For ART-naïve, with CrAg screening and treatment, we estimate 13 lives save, at a cost of US$14,547 per death averted or US$680 per DALY averted. In sensitivity analyses, screening saved an estimated 6-8 lives saved at a cost ranging from US$26,063 to US$29,213 per death averted.

No additional screening cost is included for screening ART-experienced patients with a CD4 100-200 cells/μL. Under base model assumptions, extending treatment to ART-experienced

as well as ART-naïve CrAg-positive patients resulted in an estimated 20 lives saved at a cost of US$11,572 per death averted, or US$541 per DALY averted (**Table 11b**). In sensitivity analyses comparing various assumptions, the impact of CrAg screening and treatment diminished with a range of 1-18 lives saved and a cost per life saved ranging from US$12,734 to US$265,506 (**Table 11c**).

6.6 DISCUSSION

Using local costing and clinical estimates, we provide the first estimates of the cost-effectiveness and national impact of CrAg screening implementation in Botswana. This analysis was intended to be pragmatic, reflecting current CD4 testing practice is real world settings in Botswana, a country where national guidelines currently recommend CD4 testing at baseline, after 3 months on ART, at 12 months and yearly thereafter in stable clients [11]. This strategy, coupled with adoption of HIV "test-and-treat" [33], contributes to a growing proportion of ART-experienced CrAg-positive patients recognized through laboratory-based reflex screening. Existing screening guidelines and modeling studies have not generally considered the impact of this emerging population or benefits of pre-emptive treatment. As in other studies from sub-Saharan Africa [12,14,19], we found CrAg screening in ART-naïve patients with a CD4 <100 cells/μL to be highly cost-effective or cost-saving. Targeting only CrAg-positive ART-naïve patients for pre-emptive therapy had a modest public health impact, preventing approximately 20% of CM-related deaths under base model assumptions. Also treating CrAg-positive ART-experienced patients resulted in additional deaths averted (41% reduction in CM-related deaths overall), and was highly cost-effective or cost-saving across assumptions.

In contrast to CrAg screening of patients with a CD4 <100 cells/μL, we found high costs and a marginal public health impact of CrAg screening in patients with a CD4 count of 100-200

cells/μL. Under base model assumptions, pre-emptive treatment for CrAg-positive ART-naïve patients was estimated to prevent 13 deaths at a cost per death averted of nearly US$15,000. Considering treatment of both ART-naïve and ART-experienced CrAg-positive patients, an estimated 20 deaths were averted at a cost in excess of US$11,000 per death averted. In sensitivity analyses assuming lower risk of CM progression without treatment, worse follow-up, and less benefit from pre-emptive therapy, cost-effectiveness was further diminished with few lives saved. Future policy decisions will need to consider the cost and impact of screening against competing health system priorities for the population with CD4 count of 100-200 cells/μL. The high cost per death averted was attributable to a number of factors. First, at an estimated cost of US$4.71 per CrAg test and a large population with a CD4 count 100-200 cells/μL based on local CD4 data, screening costs was high. However, the cost used in our model is comparable to US$4.28 reported at a high volume central laboratory in South Africa [34]. Our finding of high ICER was also impacted by high costs for fluconazole in Botswana, or US$0.51 per 200 mg tablet versus US$0.06 in South Africa [14]. However, even with lower costs of CrAg screening and pre-emptive fluconazole therapy, the estimated impact of implementing screening for patients with a CD4 of 100-200 cells/μL is small (<10% of CM-related deaths averted).

Our study has a number of limitations. First, we assume most patients access HIV care before being diagnosed of CM. This results in an estimated total number of CM cases in our base model that is congruent with published incidence data [2]. However, it's likely that a proportion of patients are diagnosed with CM without previously accessing services and receiving CrAg screening. However, we found that CrAg screening in patients with a CD4 <100 cells/μL remained highly cost effective across a variety of assumptions, even if the public health impact is diminished with fewer patients accessing care and receiving CrAg screening. Secondly, as a pragmatic study

using real world data, we did not evaluate other screening strategies such as point-of-care CD4 and/or CrAg testing, and we focused on CrAg screening in clinic settings without considering hospitalized patients [35,36]. Thirdly, we had a high level of uncertainty for certain parameters, particularly outcomes of CrAg-positive ART-experienced patients and those with a CD4 of 100-200 cells/μL. We addressed this uncertainty by testing benefits of screening across a range of assumptions. We also had highly robust local data for many cost and clinical parameters, particularly CD4 distribution, CrAg prevalence, and CM treatment outcomes data [15,20]. Fourthly, our analysis may fail to account for future changes in CD4 testing practices, including a possible shift away from CD4 testing with greater emphasis on HIV viral load testing [18]. Finally, pre-emptive antifungal therapy for CrAg-positive ART-experienced clients may benefit certain sub-populations more than others (e.g. those with ART treatment failure who may have prolonged immune suppression while awaiting a switch to second-line or salvage therapy). Given uncertainty, we used an averaged risk of progression to CM without pre-emptive therapy and benefit of pre-emptive therapy in CrAg-positive ART-experienced patients for simplicity.

6.7 CONCLUSION

In summary, using robust local estimates and accounting for uncertainty through sensitivity analyses, we found strong support for CrAg screening in Botswana for patients with advanced HIV at a threshold of <100 cells/μL, with high costs and small impact of screening at a higher CD4 count strata of 100-200 cells/μL. Our findings provide support for pre-emptive treatment of CrAg-positive ART-experienced patients at a CD4 <100 cells/μL recognized in laboratory-based reflex CrAg screening programs. Future outcomes data is needed for this growing population and guidelines should consider the evidence for pre-emptive treatment in this group.

6.8 ACKNOWLEDGEMENTS

We have no acknowledgements.

6.9 REFERENCES

1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017.

2. Tenforde MW, Mokomane M, Leeme T, Patel RKK, Lekwape N, Ramodimoosi C, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

3. Tugume L, Rhein J, Hullsiek KH, Mpoza E, Kiggundu R, Ssebambulidde K, et al. HIV-associated Cryptococcal Meningitis Occurring at Relatively Higher CD4 counts. J Infect Dis. 2018.

4. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(7):856-62.

5. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. Aids. 2002;16(7):1031-8.

6. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. Cryptococcal meningitis screening and community-based early adherence support in people with

advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet. 2015.

7. World Health Organization. Guidelines for the diagnosis, management and prevention of cryptococcal disease. *WHO press*. 2018. Available at: http://www.who.int/hiv/pub/guidelines/cryptococcal-disease/en/. Accessed on: 31 Mar 2019.

8. UNAIDS. Botswana HIV estimates. Available at: http://www.unaids.org/en/regionscountries/countries/botswana. Accessed on: 13 August 2018.

9. Leeme TS MM, Lechiile K, Mosepele M, Mphoyakgosi T, Muthoga C, et al. Utility of CD4 cell count monitoring in Botswana: Analysis of routine laboratory data. CROI 2019. Seattle, WA, 2019. (oral abstract).

10. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. Aids. 2016;30(3):477-85.

11. Botswana Ministry of Health. 2016 integrated HIV clinical care guidelines. Available at: http://www.moh.gov.bw/Publications/Handbook_HIV_treatment_guidelines.pdf. Accessed 28 March 2017.

12. Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. PloS one. 2013;8(7):e69288.

13. Meya DB, Manabe YC, Castelnuovo B, Cook BA, Elbireer AM, Kambugu A, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-

limited settings. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;51(4):448-55.

14. Larson BA, Rockers PC, Bonawitz R, Sriruttan C, Glencross DK, Cassim N, et al. Screening HIV-Infected Patients with Low CD4 Counts for Cryptococcal Antigenemia prior to Initiation of Antiretroviral Therapy: Cost Effectiveness of Alternative Screening Strategies in South Africa. PloS one. 2016;11(7):e0158986.

15. Patel RKK, Leeme T, Azzo C, Tlhako N, Tsholo K, Tawanana EO, et al. High mortality in HIV-associated cryptococcal meningitis treated with amphotericin B-based therapy under routine care conditions in Africa. Open Forum Infect Dis. 2018;5(11):ofy267, https://doi.org/10.1093/ofid/ofy267.

16. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

17. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, et al. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. The New England journal of medicine. 2016;374(6):542-54.

18. Tenforde MW, Walker AS, Gibb DM, Manabe YC. Rapid antiretroviral therapy initiation in low- and middle-income countries: A resource-based approach. PLoS medicine. 2019;16(1):e1002723.

19. Rajasingham R, Meya DB, Greene GS, Jordan A, Nakawuka M, Chiller TM, et al. Evaluation of a national cryptococcal antigen screening program for HIV-infected patients in Uganda: A cost-effectiveness modeling analysis. PloS one. 2019;14(1):e0210105.

20. Lechiile K, Mitchell HK, Mulenga F, et al. Prevalence of advanced HIV disease and cryptococcal infection in Gaborone, Botswana. Presented at: CROI 2017. Seattle, WA, 2017.

21. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerging infectious diseases. 2014;20(1):45-53.

22. UNAIDS. HIV and AIDS estimates - Botswana. Available at: http://www.unaids.org/en/regionscountries/countries/botswana/. Accessed 28 March 2017.

23. Gaolathe T, Wirth KE, Holme MP, Makhema J, Moyo S, Chakalisa U, et al. Botswana's progress toward achieving the 2020 UNAIDS 90-90-90 antiretroviral therapy and virological suppression goals: a population-based survey. Lancet HIV. 2016;3(5):e221-30.

24. Ford N, Shubber Z, Jarvis JN, Chiller T, Greene G, Migone C, et al. CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(suppl_2):S152-S9.

25. Wake RM, Britz E, Sriruttan C, Rukasha I, Omar T, Spencer DC, et al. High Cryptococcal Antigen Titers in Blood are Predictive of Subclinical Cryptococcal Meningitis Among HIV-Infected Patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

26. Beyene T, Zewde AG, Balcha A, Hirpo B, Yitbarik T, Gebissa T, et al. Inadequacy of High-Dose Fluconazole Monotherapy Among Cerebrospinal Fluid Cryptococcal Antigen (CrAg)-Positive Human Immunodeficiency Virus-Infected Persons in an Ethiopian CrAg Screening Program. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;65(12):2126-9.

27. Thakur KT, Mateyo K, Hachaambwa L, Kayamba V, Mallewa M, Mallewa J, et al. Lumbar puncture refusal in sub-Saharan Africa: A call for further understanding and intervention. Neurology. 2015;84(19):1988-90.

28. Beyene T, Zewde AG, Balcha A, Hirpo B, Yitbarik T, Gebissa T, et al. High Dose Fluconazole Monotherapy is Inadequate for CSF Cryptococcal Antigen Positive HIV-infected Persons in an Ethiopian CrAg Screening Program. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017.

29. Temfack E, Bigna JJ, Luma HN, Spijker R, Meintjes G, Jarvis JN, et al. Impact of routine cryptococcal antigen screening and targeted pre-emptive fluconazole therapy in antiretroviral naive HIV-infected adults with less than 100 CD4 cells/μL: a systematic review and meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.

30. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(5):736-45.

31. World Health Organization. CHOosing Interventions that are Cost Effective (WHO-CHOICE). Available at: https://www.who.int/choice/country/country_specific/en/. Accessed on: 5 Mar 2019.

32. Central Intelligence Agency. The World Factbook. Available at: https://www.cia.gov/library/publications/the-world-factbook/fields/2054.html. Accessed on: 5 December 2017.

33. World Health Organization. Guidelines for managing advanced HIV disease and early initiation of antiretroviral therapy. Available at: http://www.who.int/hiv/pub/guidelines/advanced-HIV-disease/en/. Accessed on: 10 May 2019.

34. Cassim N, Schnippel K, Coetzee LM, Glencross DK. Establishing a cost-per-result of laboratory-based, reflex Cryptococcal antigenaemia screening (CrAg) in HIV+ patients with CD4 counts less than 100 cells/μl using a Lateral Flow Assay (LFA) at a typical busy CD4 laboratory in South Africa. PloS one. 2017;12(2):e0171675.

35. Manabe YC, Nonyane BA, Nakiyingi L, Mbabazi O, Lubega G, Shah M, et al. Point-of-care lateral flow assays for tuberculosis and cryptococcal antigenuria predict death in HIV infected adults in Uganda. PloS one. 2014;9(7):e101459.

36. Mamuye AT, Bornstein E, Temesgen O, Blumberg HM, Kempker RR. Point-of-Care Testing for Cryptococcal Disease Among Hospitalized Human Immunodeficiency Virus-Infected Adults in Ethiopia. The American journal of tropical medicine and hygiene 2016; 95(4):786-792.

Table 8. Key parameters, estimates, and sources of data for base model.

Screening Module			
Parameter	**CD4 <100 cells/µl**	**CD4 100-200 cells/µL**	**Source(s)**
% within CD4 strata	2.65%	5.37%	BHHRL
CrAg prevalence within CD4 strata (outpatient), %	4.6%	2.0%	[20,24]
Prior CM or Crag+ result among screened CrAg+, %	25%	25%	[20]
High CrAg titer (≥1:180), %	59%	10%	[20]; assumption for CD4 100-200
Pre-ART of CrAg+, %	50%	50%	Local cohort [9]
Return quickly enough of CrAg+, %	75%	90%	Assumption
Treatment Module			
Parameter	**CD4 <100 cells/µl**	**CD4 100-200 cells/µL**	**Source(s)**
Hospitalized if missed CrAg+ and develops CM, %	80%	80%	Assumption
10-week CM mortality	50%	40%	[15]; higher mortality with lower CD4 [30]
CM relapse	17%	17%	[15]
High CrAg titer and fail pre-emptive therapy (if receive fluconazole)	20%	20%	[25,28]
Low CrAg titer and fail pre-emptive therapy (if receive fluconazole)	5%	5%	[25,28]
Hospitalized if fail pre-emptive therapy and develop CM	90%	90%	Assumption
10-week mortality	25%	25%	[25]
CM relapse	17%	17%	[15]
Hospitalized if diagnosed with CM at urgent follow-up visit	100%	100%	Assumption
10-week mortality	25%	25%	[25]
CM relapse	17%	17%	[15]

BHHRL = Botswana-Harvard HIV Reference Laboratory; CM = cryptococcal meningitis

Table 9. Included cost estimates for CrAg screening and pre-emptive treatment and for cryptococcal meningitis treatment

CrAg screening and pre-emptive therapy *		
Parameter	**Estimate (USD)**	**Source(s)**
CrAg LFA	$4.71	IMMY wholesale plus additional costs
Pre-emptive fluconazole 1200 mg/day x2 weeks 800 mg/day x8 weeks 200 mg/day x26 weeks	$0.51 / 200 mg tablet x 490 tablets = $247.54	CMS; proportion with treatment failure or partial adherence
Urgent return evaluation	$18.85	Assumption
Treatment Module *		
Parameter	**Estimate (%)**	**Source(s)**
Hotel costs 17-day hospital stay	$126.77 / hospital day	[15,31]
Hospital drug costs 14 days AmBd and FLU, 2 lumbar punctures	$201.87 (survives), $141.31 (dies)	CMS; [15]
Post-admission costs FLU consolidation/maintenance Extra clinic visit	$226.37	CMS
Laboratory costs 2 FBC, 4 U/E, 1 ALT	$71.00	BHHRL; WHO guidelines [7]

* See Supplementary Excel File for detailed costing estimates
ALT = alanine aminotransferase; AmBd = amphotericin B deoxycholate; BHHRL = Botswana Harvard HIV Reference Laboratory; CM = cryptococcal meningitis; CMS = Central Medical Stores; FBC = full blood count; FLU = fluconazole; KCl = potassium choloride; Mg = magnesium supplementation; NS = normal saline; U/E = urea and electrolyte testing; WHO = World Health Organization

Table 10a. Screening CD4 <100 cells/μL, ART-naïve for base (white) and sensitivity (gray) models.

Summary Results (screening < 100) ART-naive	No screening; base model	Screening < 100; base model	No screening; 50% return	Screening < 100; 50% return	No screening; 25% ART-naive	Screening < 100; 25% ART-naive	No screening; 40% high CrAg titer	Screening; 40% high CrAg titer
Number Screened	0	16,364	0	16,364	0	16,364	0	16,364
Cost of screening	0	77,073	0	77,073	0	77,073	0	77,073
Cost of treatment	750,271	671,504	750,271	732,971	703,087	663,704	688,171	641,407
Total Cost	750,271	748,577	750,271	810,044	703,087	740,777	688,171	718,480
CM deaths	240	193	240	209	225	193	226	179
ICER (US$ per death avoided)		**-26**		**1,928**		**1,178**		**645**
ICER (US$ per DAILY avoided)		**-1**		**90**		**55**		**30**
CM relapse	27	24	27	25	26	25	25	23

Table 10b. Combined ART-naïve and ART-experienced base models for CD4 <100 cells/μL.

Summary Results (screening < 100) -- base model assumptions	No screening	Screening < 100; preempt txt naïve only	Screening < 100; preempt txt for ART-naïve and ART-experienced
Number Screened	0	16,364	16,364
Cost of screening	0	77,073	77,073
Cost of treatment	750,271	671,504	584,602
Total Cost	750,271	748,577	661,675
CM deaths	240	193	142
ICER (cost per death averted)		-26	-904
ICER (cost per DALY averted)		-1	-42
CM relapse	27	24	20

Table 10c. Combined ART-naïve and ART-experienced base models for CD4 <100 cells/μL under different combinations of assumptions. Estimated deaths averted, cost per death averted, and cost per disability-adjusted life year averted presented.

		ART-experienced assumptions		
		Base model assumptions	**30% risk w/o preempt txt**	**20% risk w/o preempt tx, 50% risk reduction w/txt**
ART-naïve assumptions	**Base model assumptions**	**Deaths averted: 98** **US$ / death averted: -904** **US$ / DALY averted: -42**	**Deaths averted: 73** **US$ / death averted: -1,170** **US$ / DALY averted: -55**	**Deaths averted: 60** **US$ / death averted: -583** **US$ / DALY averted: -27**
	50% CrAg+ return quickly	**Deaths averted: 54** **US$ / death averted: 34** **US$ / DALY averted: 2**	**Deaths averted: 48** **ICER death averted: 83** **ICER DALY averted: 4**	**Deaths averted: 29** **US$ / death averted: 1,297** **US$ / DALY averted: 61**
	25% ART-naive	**Deaths averted: 82** **US$ / death averted: -1,131** **US$ / DALY averted: -53**	**Deaths averted: 40** **ICER death averted: -679** **ICER DALY averted: -32**	**Deaths averted: 21** **US$ / death averted: 2,311** **US$ / DALY averted: 108**
	40% CrAg+ high titer	**Deaths averted: 89** **US$ / death averted: -568** **US$ / DALY averted: -27**	**Deaths averted: 63** **ICER death averted: -572** **ICER DALY averted: -27**	**Deaths averted: 50** **US$ / death averted: 297** **US$ / DALY averted: 14**

Table 11a. Screening CD4 100-200 cells/µL, ART-naïve for base (white) and sensitivity (gray) models..

Summary Results (screening 100-200) ART-naive	No screening; base model	Screening 100-200; base model	No screening; 85% return	Screening 100-200; 85% return	No screening; 25% ART-naive	Screening 100-200; 25% ART-naive	No screening; 5% high CrAg titer	Screening 100-200; 5% high CrAg titer
Number Screened	0	33,036	0	33,036	0	33,036	0	33,036
Cost of screening	0	155,601	0	155,601	0	155,601	0	155,601
Cost of treatment	234,859	268,374	234,859	283,749	220,244	237,001	214,196	267,102
Total Cost	234,859	423,975	234,859	439,350	220,244	392,602	214,196	422,703
CM deaths	63	50	63	56	59	53	58	50
ICER (US$ per death avoided)		**14,547**		**29,213**		**28,726**		**26,063**
ICER (US$ per DAILY avoided)		**680**		**1,365**		**1,342**		**1,218**
CM relapse	10	10	10	10	9	9	9	10

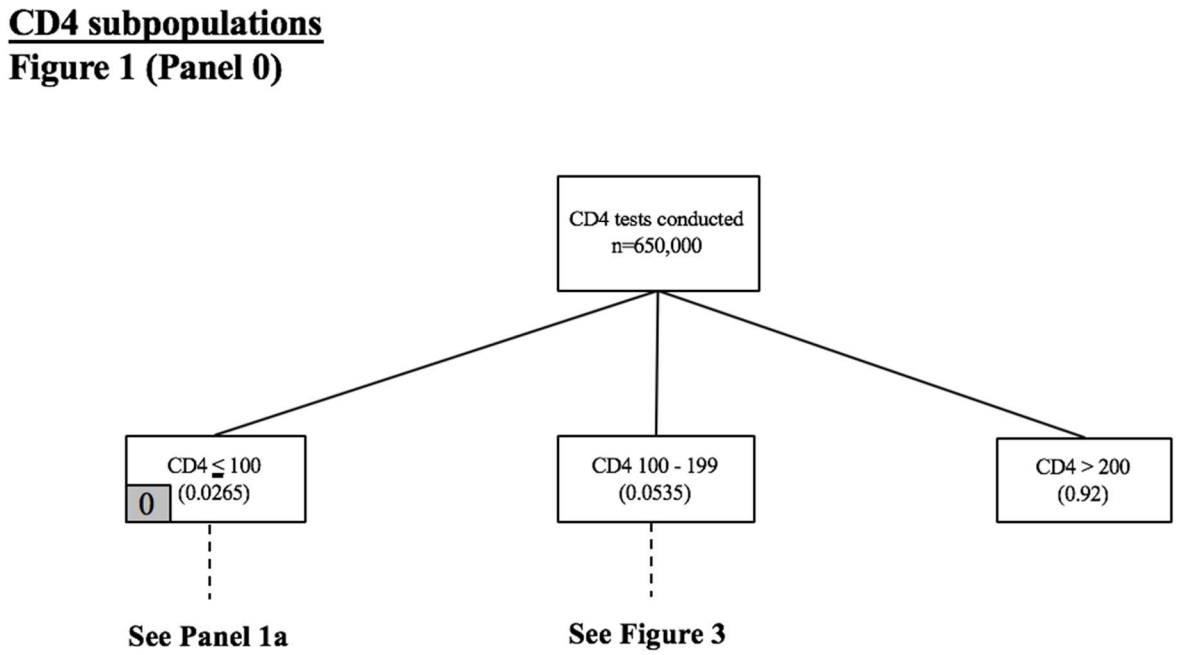
Table 11b. Combined ART-naïve and ART-experienced base models for CD4 100-200 cells/µL.

Summary Results (screening 100-200) -- base model assumptions	No screening	Screening 100-200; preempt txt naïve only	Screening 100-200; preempt txt all
Number Screened	0	33,036	33,036
Cost of screening	0	155,601	155,601
Cost of treatment	234,859	268,374	345,473
Total Cost	234,859	423,975	466,303
CM deaths	63	50	43
ICER (cost per death averted)		14,547	11,572
ICER (cost per DALY averted)		680	541
CM relapse	10	10	8

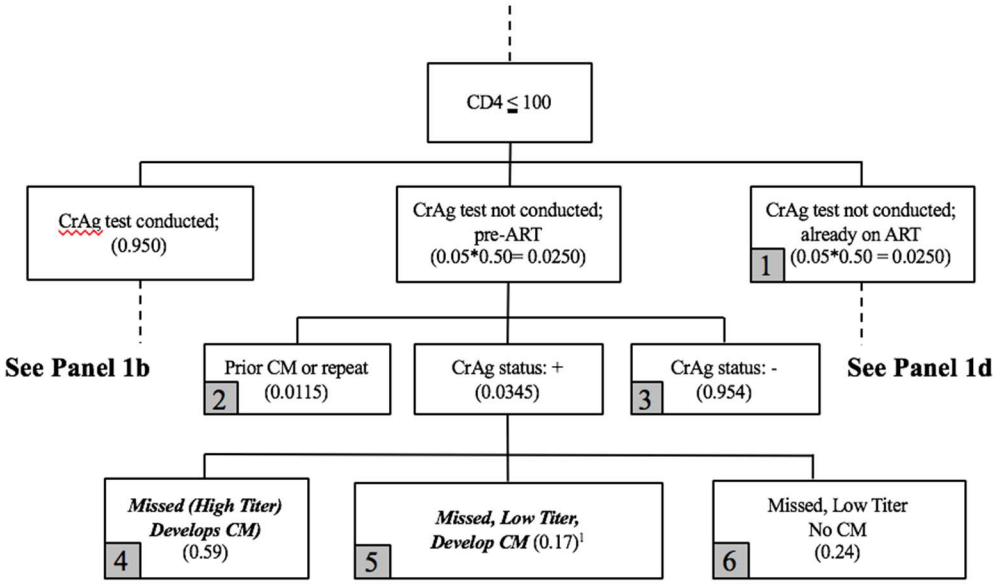
Table 11c. Combined ART-naïve and ART-experienced base models for CD4 100-200 cells/μL under different combinations of assumptions. Estimated deaths averted, cost per death averted, and cost per disability-adjusted life year averted presented.

		ART-experienced assumptions		
		Base model assumptions	**10% risk w/o preempt txt**	**10% risk w/o preempt tx, 50% risk reduction w/txt**
ART-naïve assumptions	**Base model assumptions**	**Deaths averted: 20** **US$ / death averted: 11,572** **US$ / DALY averted: 541**	**Deaths averted: 14** **US$ / death averted: 15,926** **US$ / DALY averted: 744**	**Deaths averted: 12** **US$ / death averted: 20,038** **US$ / DALY averted: 936**
	80% CrAg+ return quickly	**Deaths averted: 9** **US$ / death averted: 32,043** **US$ / DALY averted: 1,497**	**Deaths averted: 3** **US$ / death averted: 83,322** **US$ / DALY averted: 3,894**	**Deaths averted: 1** **US$ / death averted: 265,506** **US$ / DALY averted: 12,407**
	25% ART-naive	**Deaths averted: 17** **US$ / death averted: 12,734** **US$ / DALY averted: 595**	**Deaths averted: 5** **US$ / death averted: 48,547** **US$ / DALY averted: 2,269**	**Deaths averted: 2** **US$ / death averted: 130,600** **US$ / DALY averted: 6,103**
	5% CrAg+ high titer	**Deaths averted: 18** **US$ / death averted: 13,391** **US$ / DALY averted: 626**	**Deaths averted: 11** **US$ / death averted: 21,600** **US$ / DALY averted: 1,009**	**Deaths averted: 9** **US$ / death averted: 28,353** **US$ / DALY averted: 1,325**

Figure 17. Flowcharts for decision analytic model including screening and treatment modules.

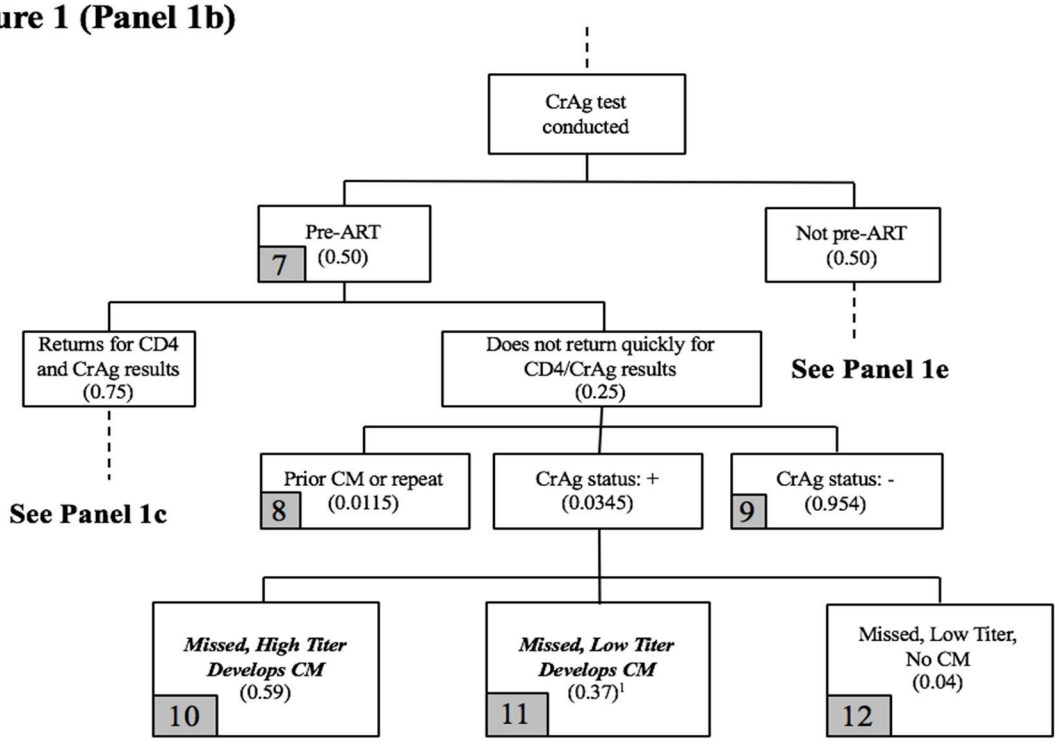


Screening module
(<100)
Figure 1 (Panel 1a)



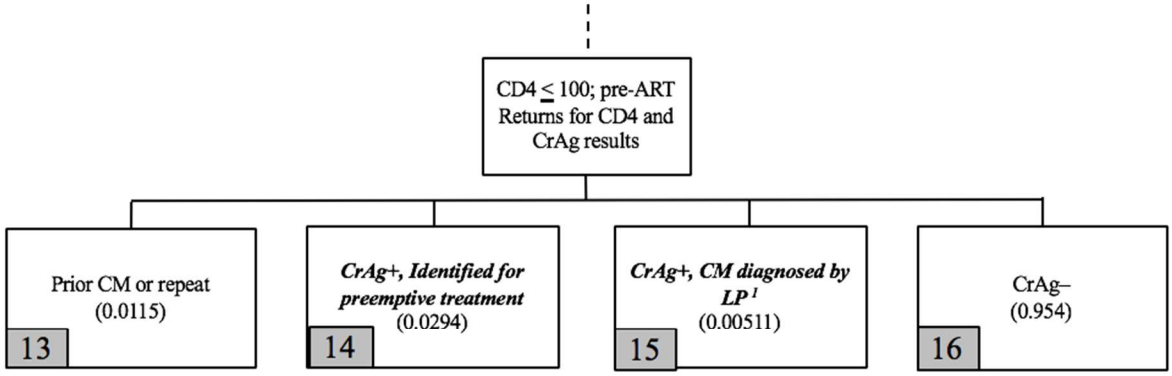
(41% low titer * 75% start ART quickly * 25% develop CM) + (41% low titer * 25% don't start quickly * 90% develop CM) = 17%
All patients with higher titer develop CM

Figure 1 (Panel 1b)



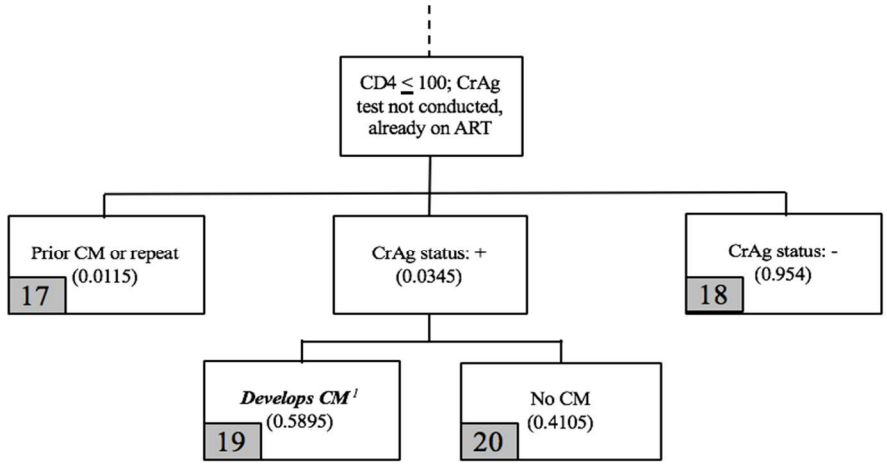
[1] 41% low titer * 90% develop CM = 37%
All patients with high titer develop CM

Figure 1 (Panel 1c)



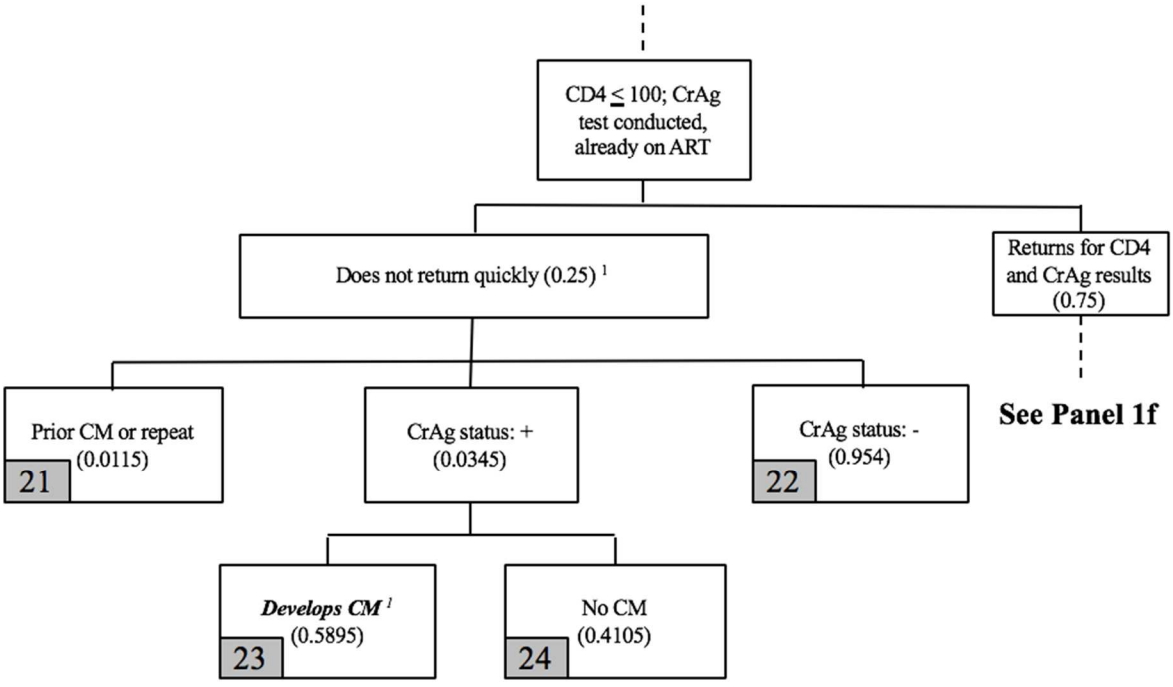
[1] 59% high-titer * 25% consent to LP * 90% with CM +
41% low-titer * 25% consent to LP * 15% with CM = **14.8% (0.00511/0.0345)** diagnosed with CM on return clinic visit
in ART-naïve, assume same for ART-experienced base model

Figure 1 (Panel 1d)



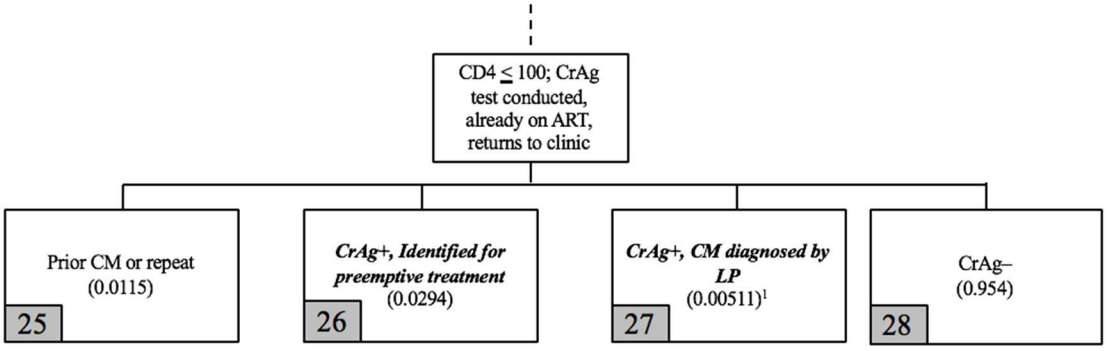
[1] 0.25 defaulted on ART * 0.90 develop CM + 0.75 on ART * 0.40 with treatment failure * 0.90 develop CM + 0.75 on ART * 0.60 recently started * 0.21 develop unmasking CM = 0.5985

Figure 1 (Panel 1e)



[1] 21-24 same as screening modules 17-20

Figure 1 (Panel 1f)



[1] Same as screening module 14 and 15; assume similar % with high- and low-titer as ART-naïve in base model

Treatment module (<100)

Figure 2 (Panel 2a)

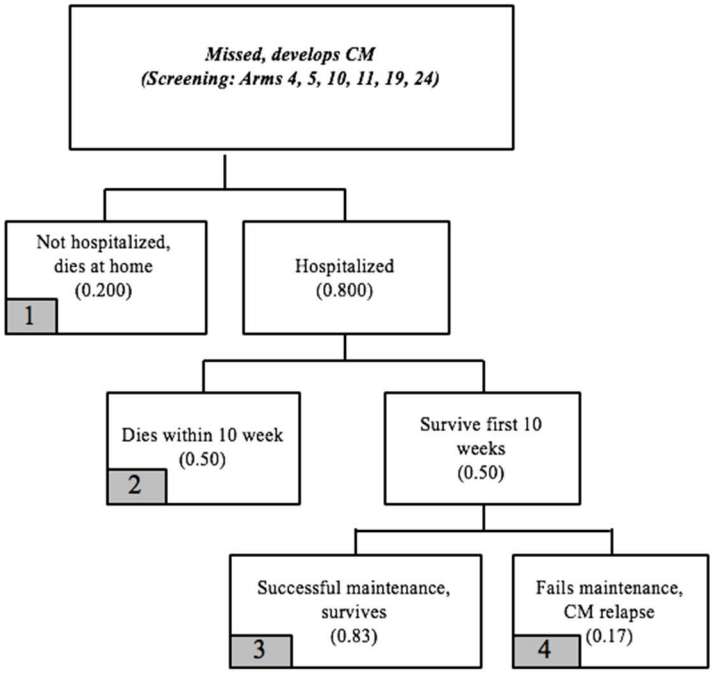


Figure 2 (Panel 2b)

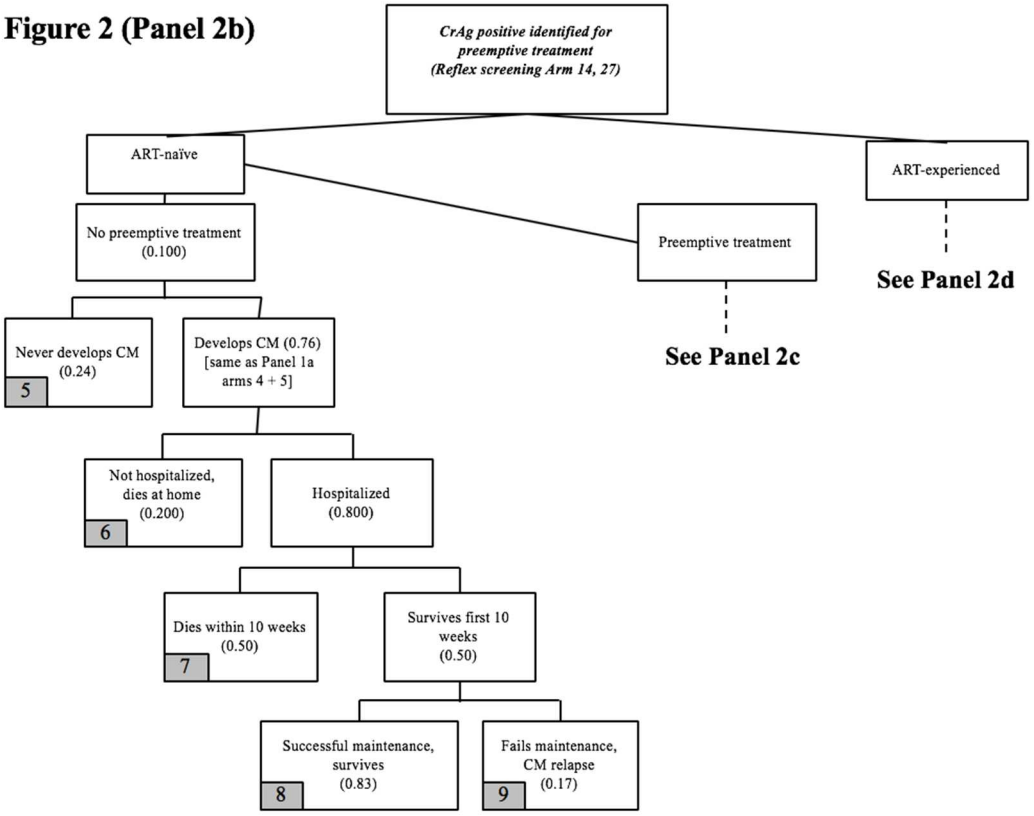


Figure 2 (Panel 2c)

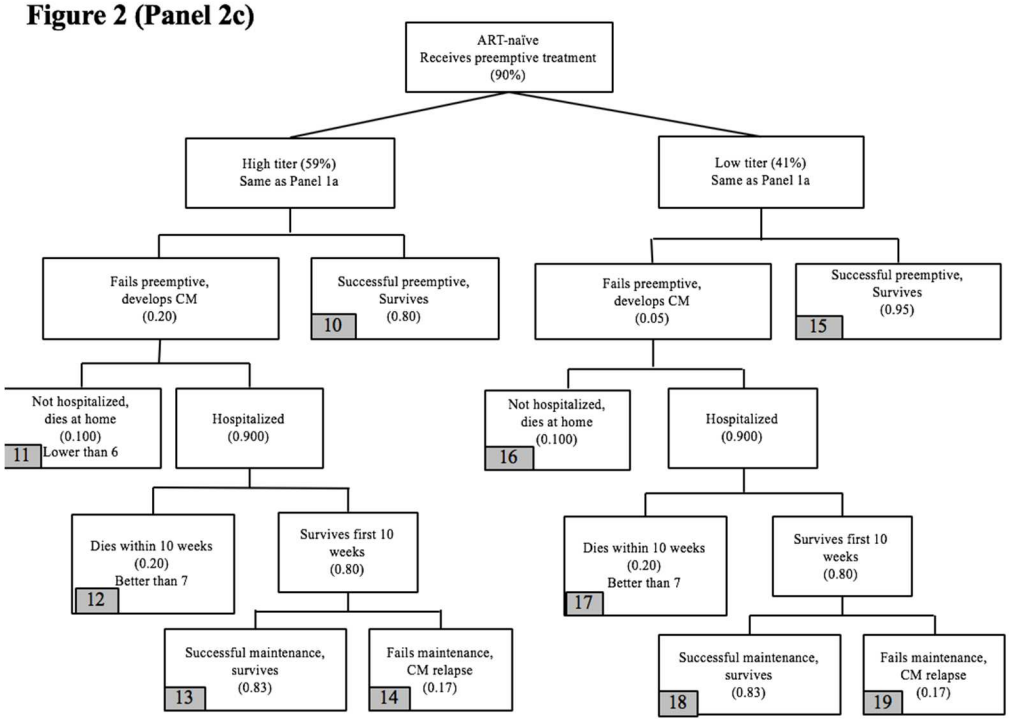


Figure 2 (Panel 2d)

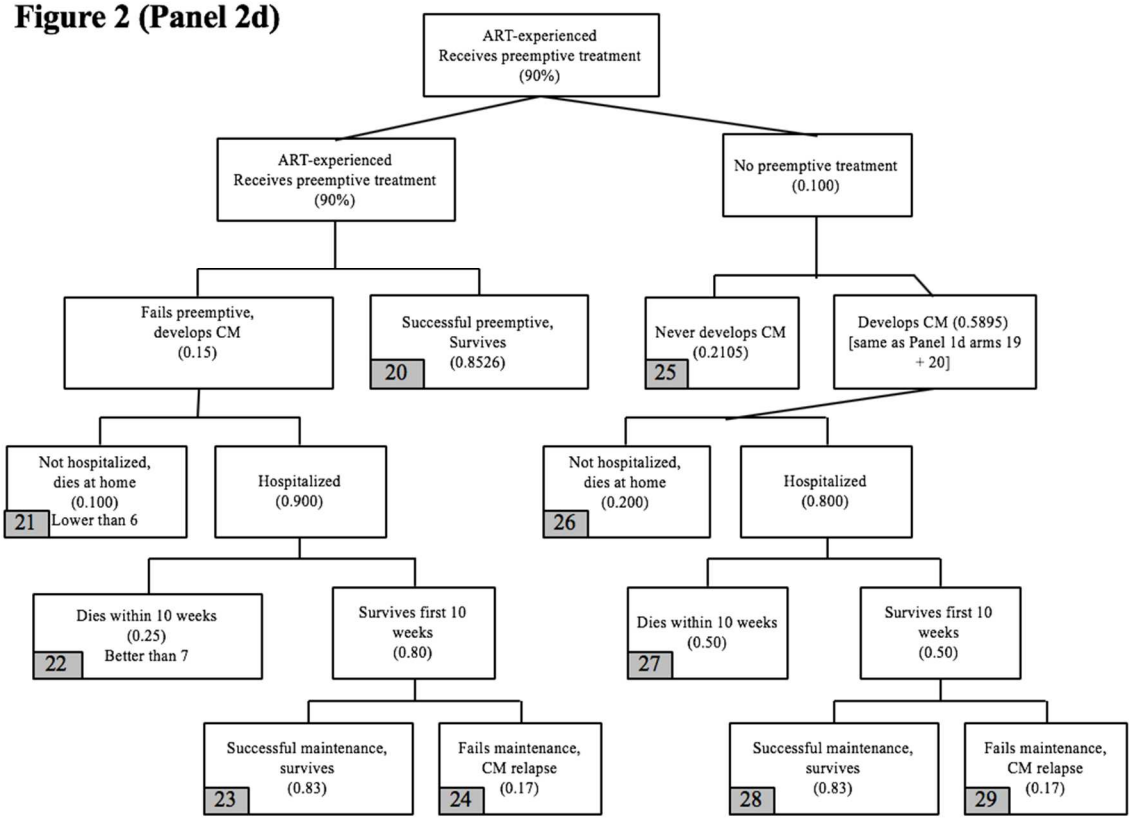
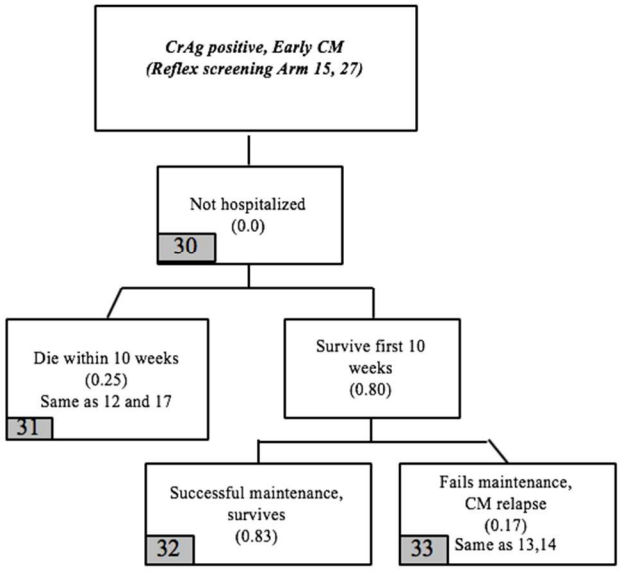
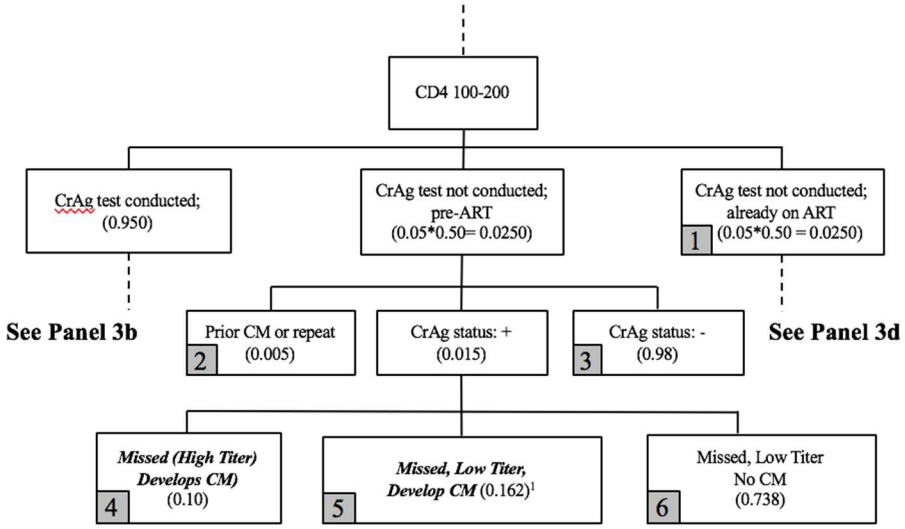


Figure 2 (Panel 2e)



Screening module (100-200)

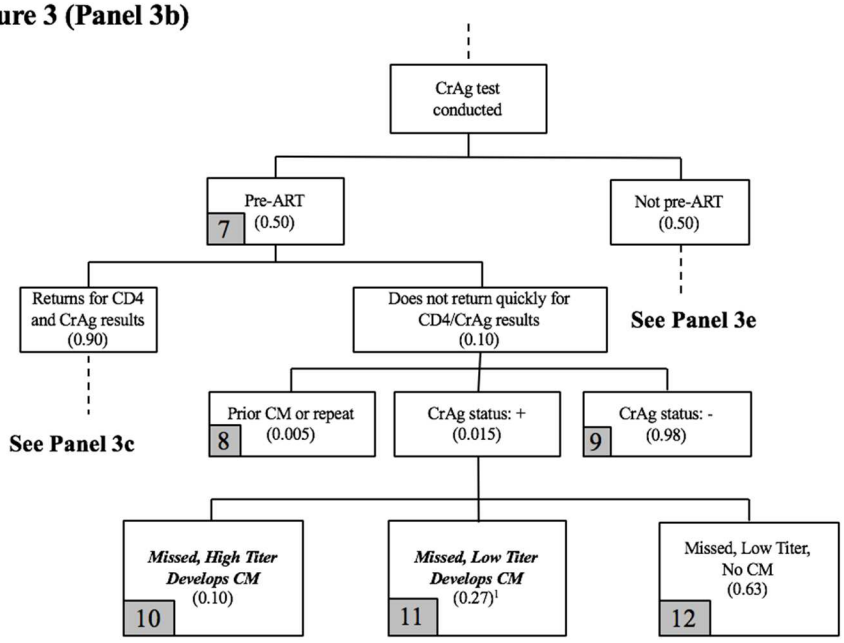
Figure 3 (Panel 3a)



(90% low titer * 90% start ART quickly enough * 10% develop CM) + (90% low titer * 10% don't start quickly * 90% develop CM) = 16.2%

All patients with higher titer develop CM

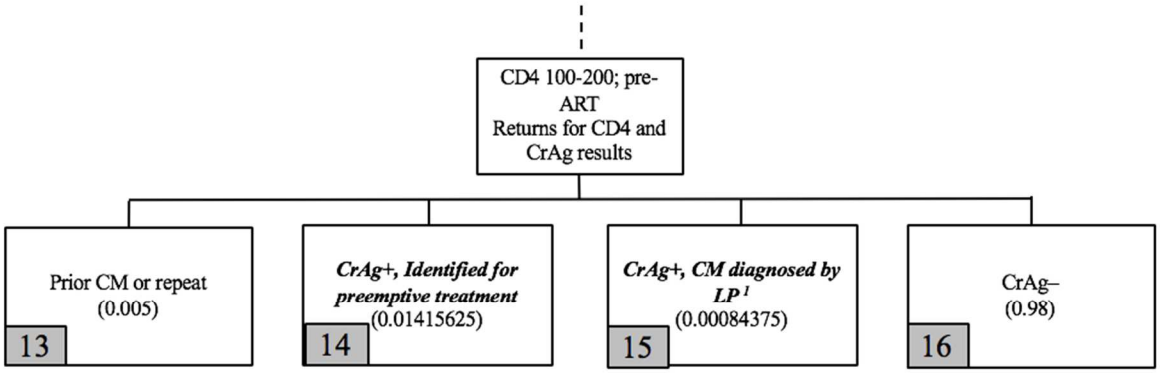
Figure 3 (Panel 3b)



[1] 90% low titer * 0.9% develop CM = 81%

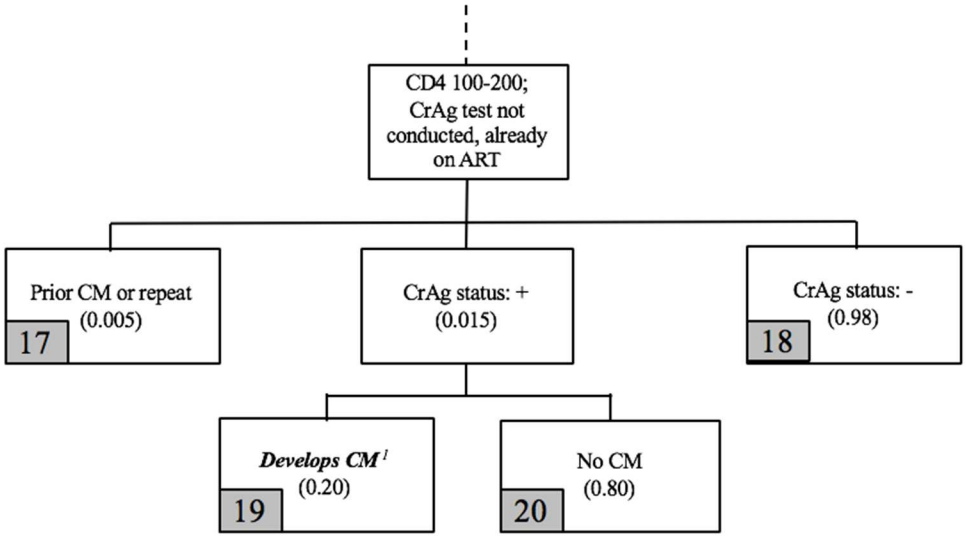
All patients with high titer develop CM

Figure 3 (Panel 3c)



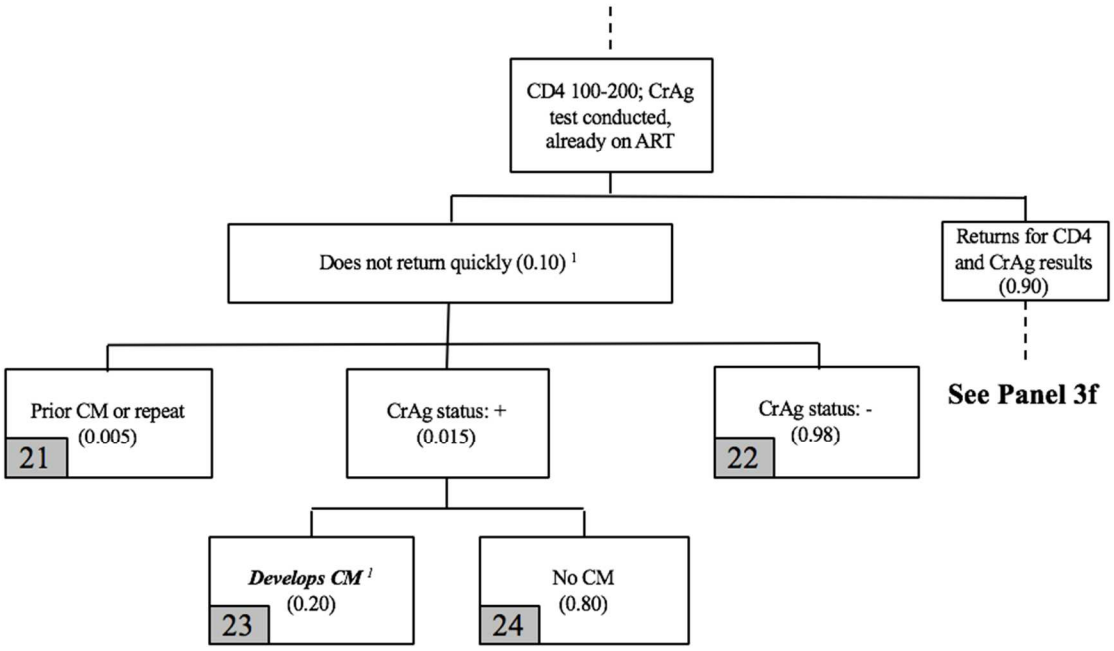
[1] (10% high-titer * 25% consent to LP * 90% with CM +
90% low-titer * 25% consent to LP * 15% with CM) = 5.6% diagnosed with CM on return clinic visit

Figure 3 (Panel 3d)



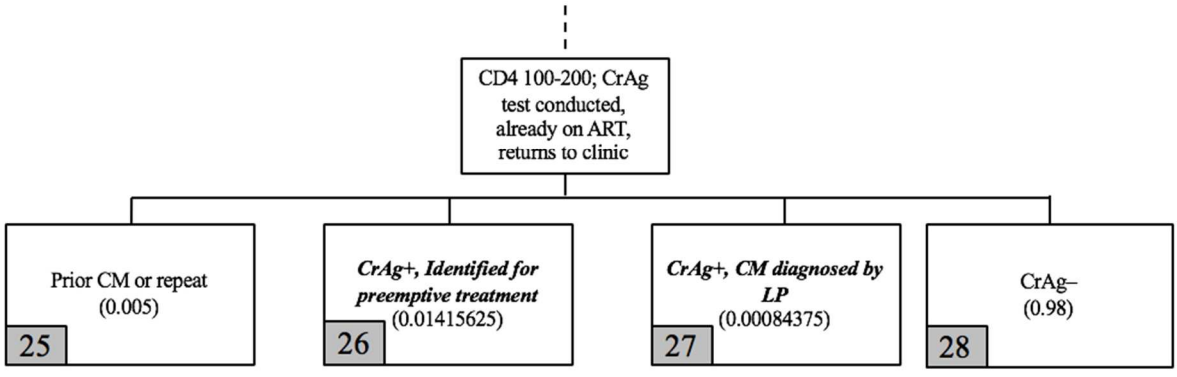
[1] 0.25 defaulted on ART * 0.3 develop CM + 0.75 on ART * 0.40 with treatment failure * 0.3 develop CM + 0.75 on ART * 0.60 recently started * 0.10 develop unmasking CM = ~0.20

Figure 3 (Panel 3e)



[1] 21-24 same as screening modules 17-20

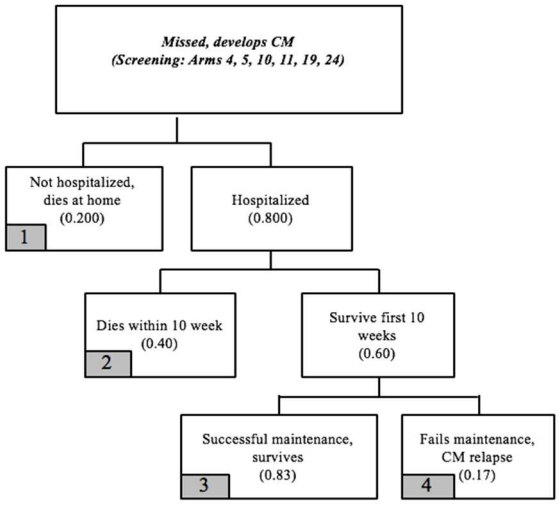
Figure 3 (Panel 3f)



[1] Same as screening module 14 and 15

Treatment module (100-200)

Figure 4 (Panel 4a)



[1] low CD4 count is a predictor of poor survival - assume better 10-week survival in CD4 100-200 cells/µL group than CD4 <100 cells/µL group (60% and 50%, respectively)

Figure 4 (Panel 4b)

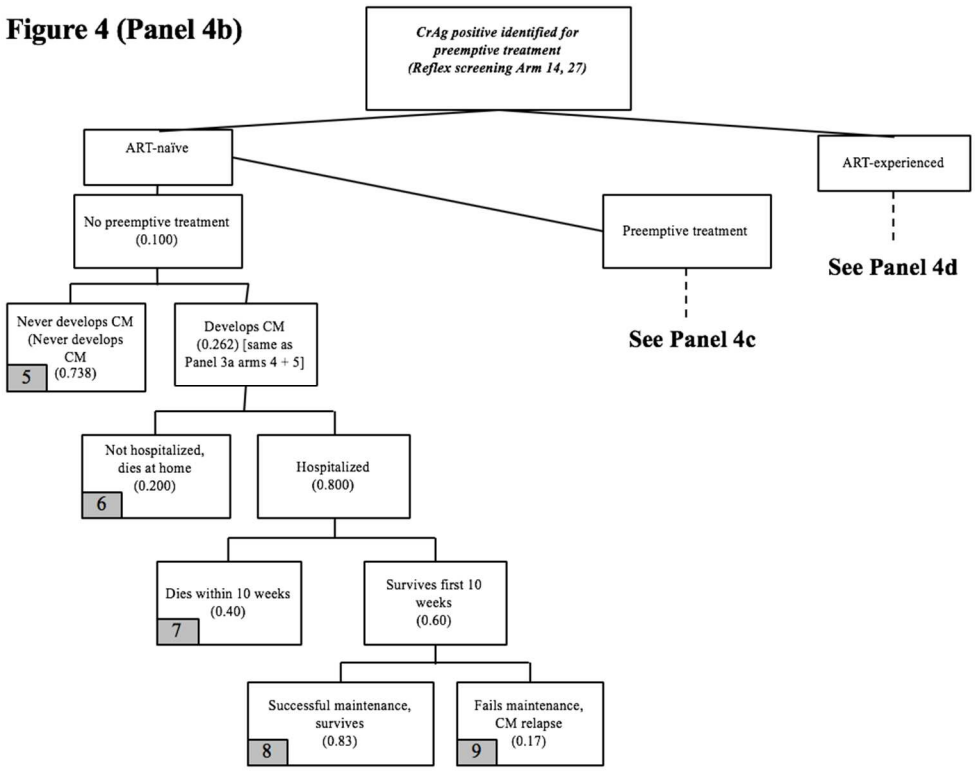


Figure 4 (Panel 4c)

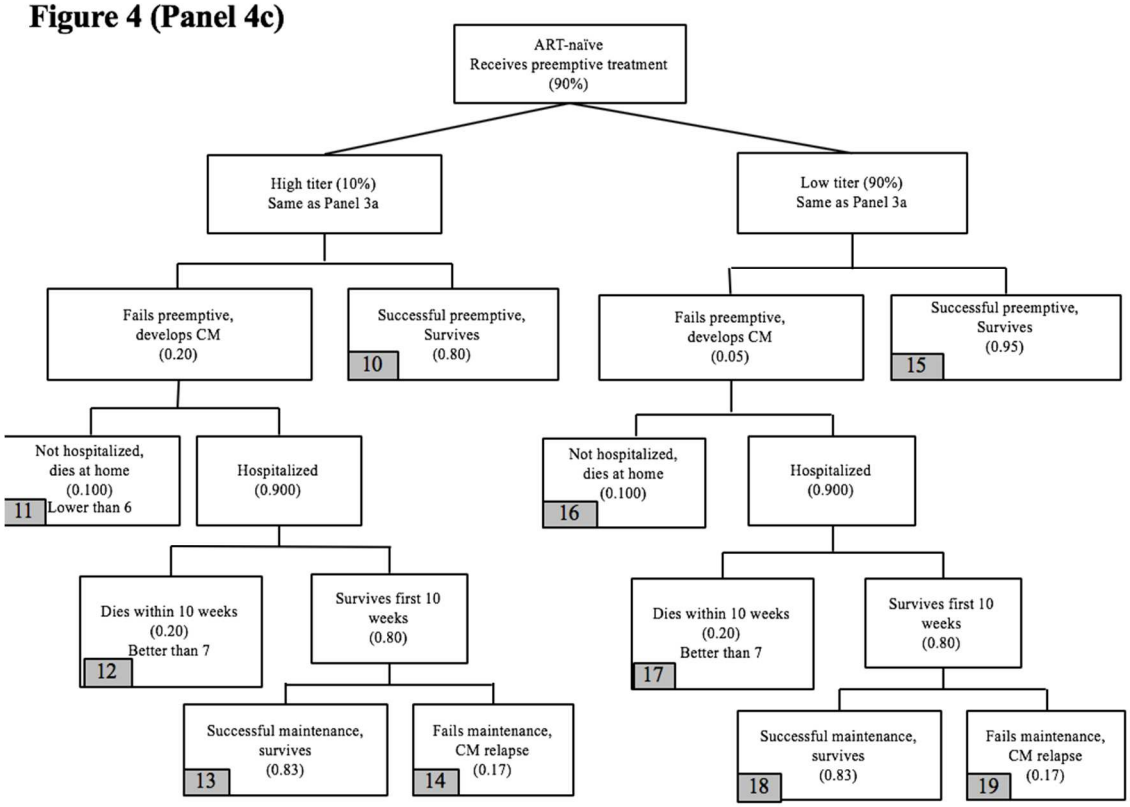


Figure 4 (Panel 4d)

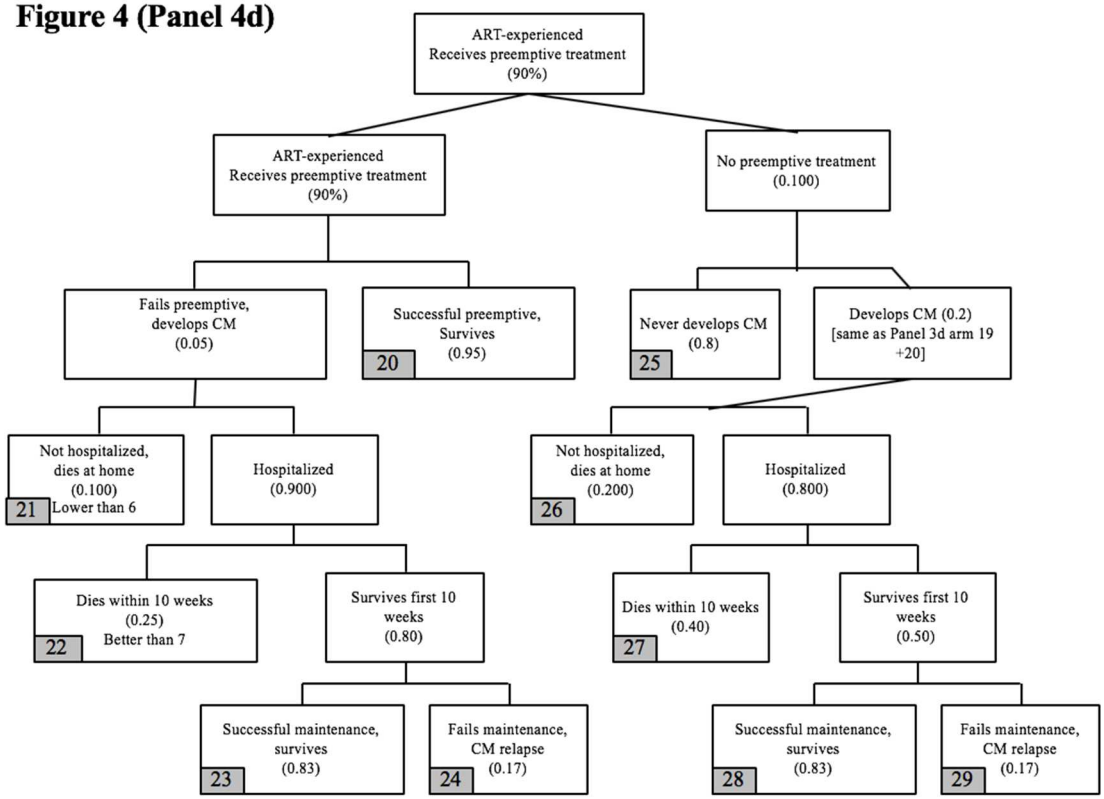
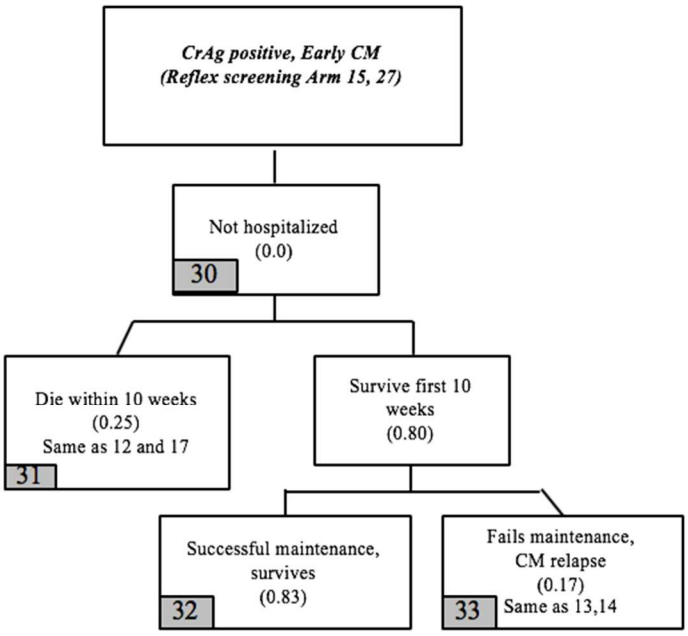


Figure 4 (Panel 4e)



Chapter 7. CONCLUSIONS AND WAY FORWARD

The studies in this dissertation describe the first nationally representative estimates of the burden and outcomes of HIV-associated central nervous system infections in a high HIV prevalence country, with national incidence estimates over a decade after initial ART rollout in Botswana. An in-depth examination of central nervous system infections in southern Africa has important implications with respect to understanding the HIV care cascade and informing HIV differentiated care models as well as future interventions to improve prevention, diagnostics, and treatment strategies to reduce deaths from central nervous system infections in populations living with HIV.

7.1 ADDRESSING GAPS IN THE HIV CARE CASCADE

Using cryptococcal meningitis as an "indicator disease," I highlight gaps in the HIV care cascade and key at-risk populations that need to be effectively targeted to reduce deaths from advanced HIV and to reduce ongoing HIV transmission. In Chapters 2 and 3, I show an initial decline in cases of cryptococcal meningitis cases but with the number of cases at referral centers flatlining in more recent years of observation. This reflects both the early gains achieved with greater availability of ART and subsequent challenges in reaching key high-risk populations through traditional care models which have not changed substantially over the past two decades. The meningitis incidence estimates were derived during a period shortly before the HIV "Treat All" strategy in Botswana (ART recommended for all persons living with HIV, regardless of CD4 count or WHO clinical stage) [1]. However, Botswana was the first country in Africa to offer ART starting in 2002. Further, pre-Treat-All guidelines until 2016 recommending treatment in HIV-positive individuals with a CD4 count <350 cells/μL [2] and over 70% of persons living

with HIV were estimated to have been diagnosed, on therapy, and with sustained viral suppression in parts of the country [3]. By itself, adoption of a HIV Treat All strategy is unlikely to eliminate advanced HIV disease, and this is further corroborated by our ongoing prospective cohort and clinical trials work we are conducting in Botswana. Young, working-age men are a key at-risk population, with HIV-infected men having a 2-fold higher incidence of cryptococcal meningitis then women peaking in the 4th and 5th decades of life. Innovative strategies tailored to the population, such as targeted education and engagement (e.g. through adherence clubs or short message services), decentralized care and simplified care in stable patients (differentiated care), as well as preventive efforts including voluntary male medical circumcision (VMMC) and pre-exposure prophylaxis (PrEP) and improved community-based diagnostics, could be utilized in order to make further gains needed toward reducing HIV mortality [4,5].

7.2 IMPLEMENTATION OF DIFFERENTIATED CARE MODELS FOR ADVANCED HIV DISEASE

Despite high ART availability and overall coverage, these findings highlight the ongoing need for differentiated care models targeting individuals with advanced HIV disease in an effort to reduce HIV-related mortality. These models should include screening for early cryptococcal infection for the prevention of cryptococcal meningitis, which I estimate to be highly cost-effective in Botswana across a wide range of assumptions for those with advanced HIV/AIDS (CD4 <100 cells/μL). These efforts should also target prevention and early diagnosis of other major infectious causes of mortality, such as TB and *Pneumocystis* disease, and intensified case management for individuals with advanced HIV to provide needed support and education for the promotion of life-long engagement in HIV care. Informed by his dissertation research, I am the Clinical Lead for an ongoing CrAg screening cohort study in Botswana and, with development

partners, have been designing the content for a package for the management of patients with advanced HIV that will be used for training health care workers at a national level.

7.3 THE SHIFTING EPIDEMIOLOGY OF CENTRAL NERVOUS SYSTEM INFECTIONS PRESENT NEW CHALLENGES AND A NEED FOR INNOVATIVE SOLUTIONS

This work highlights a shifting epidemiology of HIV-associated meningitis in the ART era. For example, almost half of cases of HIV-associated cryptococcal meningitis now occur in ART-experienced individuals. These findings challenge current guidelines for cryptococcal meningitis prevention, with WHO and national guidelines recommending CrAg screening for ART-naïve individuals with advanced HIV disease but providing no guidance on the management of the emerging population of ART-experienced patients recognized with cryptococcal infection by laboratory-based reflex CrAg screening [6,7]. The costing models (Chapter 5) suggest that CrAg screening and pre-emptive antifungal therapy for ART-naïve individuals with a CD4 <100 cells/μL is cost-effective but has a modest impact alone in reducing cryptococcal meningitis-related deaths (~20% reduction in deaths in our base models). Also treating ART-experienced patients recognized with cryptococcal infection (i.e. CrAg-positive) was estimated to save additional lives and was highly cost-effective or cost-saving across a range of assumptions. The impact of cryptococcal antigen screening programs is likely to be further challenged with shifts in CD4 testing practices with rollout of HIV test-and-treat strategies [1], often started on ART immediately without laboratory testing, as well as a shift in resource allocation from CD4 testing to HIV viral load testing in HIV-positive patients already established in care, and suggests the continued importance of CD4 testing services [8]. Innovative implementation strategies, such as point-of-care CD4 and CrAg screening strategies [8] at the time of HIV diagnosis or in patients with treatment default or failure, may be needed to maximize the impact of life-saving

preventive interventions, and future guideline will need to reflect epidemiolocal changes observed in mature ART programs like Botswana.

7.4 CENTRAL NERVOUS SYSTEM INFECTIONS ARE A MAJOR CAUSE OF HIV-RELATED MORTALITY: BETTER STRATEGIES ARE NEEDED TO COMBAT CRYPTOCOCCAL DISEASE

My work supports previous estimates and show that central nervous system infections remain a major cause of HIV-related mortality in southern Africa [9]. Approximately 4,500 HIV-related deaths occur in Botswana annually. Of approximately 2,000 annual lumbar punctures evaluations for meningitis (most in persons living with HIV), one-year mortality exceeded 50%. Although we lacked detailed clinical information of patients evaluated for meningitis including presenting clinical history and cause of death, our findings strongly suggest that central nervous system infections are a major cause of deaths in persons living with HIV in Botswana.

Cryptococcal meningitis was implicated in almost 90% of microbiologically-confirmed cases of meningitis and diagnosed in ~20% of all patients evaluated for meningitis by lumbar puncture. Despite use of more potent amphotericin B and high-dose fluconazole induction therapy - rather than fluconazole monotherapy used in much of sub-Saharan Africa - one-year mortality was high with about two-thirds of patients dying within one year. Further, the systematic review (Chapter 5) found similar pooled short-term (primarily in-hospital) mortality with amphotericin B-based induction therapy and fluconazole induction therapy (both 41%), as well as similar long-term outcomes in a limited number of studies. These findings indicate that, in real world settings, improving access to amphotericin B therapy alone is unlikely to lead to significant progress in reducing cryptococcal meningitis-related deaths in high HIV burden settings in Africa.

Interventions are urgently needed to improve outcomes from HIV-associated cryptococcal meningitis. *First*, effective implementation of cryptococcal antigen screening for individuals with advanced HIV disease has the potential to save lives by reducing the number of incident cases. *Secondly*, improved local training, as well as access to lumbar puncture kits with manometers, laboratory testing and other services, could reduce mortality. In a retrospective cohort, we recently found that performance of therapeutic lumbar punctures to reduce intracranial pressure and laboratory monitoring of common amphotericin B-related drug toxicities were uncommonly performed, and over half of patients treated for HIV-associated cryptococcal meningitis missed one or more doses of intravenous amphotericin B therapy at a Botswana referral hospital despite resource availability[10]. Although prone to temporal confounding, an implementation study found that adoption of a protocol-driven therapeutic lumbar puncture schedule significantly reduced the case-fatality rate from HIV-associated cryptococcal meningitis at a center in Tanzania when compared to a historic cohort [11]. *Thirdly*, access to short-course and less toxic therapies for incident cryptococcal meningitis may help to improve survival. The landmark ACTA trial recently showed that 1-week amphotericin induction therapy combined with flucytosine was associated with lower mortality at 10 weeks than 2-week amphotericin B regimens, with less severe toxicity and similar rate of fungal clearance [12,13]. However, flucytosine remains unregistered and unavailable throughout sub-Saharan Africa, with ongoing efforts to register the drug and for affordable pricing. Our group recently showed in a phase 2 RCT that a single high dose of liposomal amphotericin combined with fluconazole resulted in the same rate of early fungal clearance as two weeks of amphotericin B with fluconazole and was safe, with an ongoing phase 3 clinical trial evaluating single-dose liposomal amphotericin [14,15].

7.5 IMPROVING MENINGITIS DIAGNOSTICS AND TREATMENT STRATEGIES

Similar to other studies in southern and eastern Africa [16,17], I showed that a majority of cases of suspected meningitis did not have a confirmed microbiological diagnosis to guide therapeutic management using routinely available diagnostic tests. This included a sizeable minority of cases with evidence of inflammation by CSF testing suggesting true pathology. In the context of common advanced HIV disease, CSF white cell count surprisingly did not predict short-term or one-year mortality with approximately one-half of HIV-positive patients evaluated for meningitis dying with one year regardless of CSF white cell strata. The study has important limitations, including lack of clinical data on clinical presentation. For example, although most adults were likely evaluated for suspected meningitis, a small proportion may have been evaluated with altered mental status from severe sepsis of unknown etiology with a poor short-term prognosis. Nevertheless, these findings suggest a need to better characterize CSF pathology and tailor diagnostic and therapeutic management strategies.

Our research group is currently completing a prospective cohort evaluating use of enhanced diagnostics for meningitis evaluation at the major referral hospital in Botswana, evaluating central nervous system pathogens using a 14-pathogen multiplex polymerase chain reaction assay to better characterize infectious pathogens and evaluating changes in management following molecular diagnostic testing. Future studies using next-generation metagenomic sequencing and other methods could better characterize pathogens to guide therapy for suspected meningitis [18]. Further, the dissertation research suggests a need for better implementation of currently available meningitis diagnostics. In particular, I showed few cases of culture-confirmed TB meningitis compared to other high HIV prevalence, high TB endemicity settings [19]. A recent validation study suggested improved sensitivity of next-generation Xpert MTB/RIF (Xpert Ultra) over

traditional culture in the diagnosis of HIV-associated TB meningitis, with results available within a couple of hours. With Xpert testing already available in Botswana, future research is planned to characterize the prevalence of TB meningitis and modeling studies will be needed to evaluate the cost-effectiveness and impact of universal Xpert Ultra testing with current routine studies in the evaluation of meningitis in high HIV prevalence settings.

7.6 REFERENCES

1. World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Available at: http://apps.who.int/iris/bitstream/10665/186275/1/9789241509565_eng.pdf?ua=1. Accessed 28 March 2017.

2. Botswana Ministry of Health. 2012 Botswana national HIV & AIDS treatment guidelines. Available at: https://hivpolicywatch.org/duremaps/data/guidelines/BotswanaARTguidelines2012.pdf. Accessed 28 March 2017.

3. Gaolathe T, Wirth KE, Holme MP, Makhema J, Moyo S, Chakalisa U, et al. Botswana's progress toward achieving the 2020 UNAIDS 90-90-90 antiretroviral therapy and virological suppression goals: a population-based survey. Lancet HIV. 2016;3(5):e221-30.

4. Haberer JE, Sabin L, Amico KR, Orrell C, Galárraga O, Tsai AC, et al. Improving antiretroviral therapy adherence in resource-limited settings at scale: a discussion of interventions and recommendations. Journal of the International AIDS Society. 2017;20(1):21371.

5. Mulubwa C, Hensen B, Phiri MM, Shanaube K, Schaap AJ, Floyd S, et al. Community based distribution of oral HIV self-testing kits in Zambia: a cluster-randomised trial nested in four HPTN 071 (PopART) intervention communities. Lancet HIV. 2019;6(2):e81-e92.

6. World Health Organization. Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: WHO; 2011.

7. World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescence and children. Geneva: WHO; 2018.

8. Tenforde MW, Walker AS, Gibb DM, Manabe YC. Rapid antiretroviral therapy initiation in low- and middle-income countries: A resource-based approach. PLoS medicine. 2019;16(1):e1002723.

9. Walker AS, Prendergast AJ, Mugyenyi P, Munderi P, Hakim J, Kekitiinwa A, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(12):1707-18.

10. Patel RKK, Leeme T, Azzo C, Tlhako N, Tsholo K, Tawanana EO, et al. High mortality in HIV-associated cryptococcal meningitis treated with amphotericin B-based therapy under routine care conditions in Africa. Open Forum Infect Dis. 2018;5(11):ofy267, https://doi.org/10.1093/ofid/ofy267.

11. Meda J, Kalluvya S, Downs JA, Chofle AA, Seni J, Kidenya B, et al. Cryptococcal meningitis management in Tanzania with strict schedule of serial lumber punctures using intravenous tubing sets: an operational research study. Journal of acquired immune deficiency syndromes. 2014;66(2):e31-6.

12. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. The New England journal of medicine. 2018;378(11):1004-17.

13. Tenforde MW, Shapiro AE, Rouse B, Jarvis JN, Li T, Eshun-Wilson I, et al. Treatment for HIV-associated cryptococcal meningitis. Cochrane Database Syst Rev. 2018;7:CD005647.

14. Jarvis JN, Leeme TB, Molefi M, Chofle AA, Bidwell G, Tsholo K, et al. Short Course High-dose Liposomal Amphotericin B for HIV-associated Cryptococcal Meningitis: A phase-II Randomized Controlled Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.

15. Lawrence DS, Youssouf N, Molloy SF, Alanio A, Alufandika M, Boulware DR, et al. AMBIsome Therapy Induction OptimisatioN (AMBITION): High Dose AmBisome for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: Study Protocol for a Phase 3 Randomised Controlled Non-Inferiority Trial. Trials. 2018;19(1):649.

16. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC infectious diseases. 2010;10:67.

17. Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, et al. Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up and Haemophilus influenzae type b vaccination, 2000-2012. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(10):e137-45.

18. Wilson MR, O'Donovan BD, Gelfand JM, Sample HA, Chow FC, Betjemann JP, et al. Chronic Meningitis Investigated via Metagenomic Next-Generation Sequencing. JAMA Neurol. 2018;75(8):947-55.

19. Britz E, Perovic O, von Mollendorf C, von Gottberg A, Iyaloo S, Quan V, et al. The Epidemiology of Meningitis among Adults in a South African Province with a High HIV Prevalence, 2009-2012. PloS one. 2016;11(9):e0163036.

VITA

Dr. Mark Tenforde earned his Medical Degree (MD) with Special Qualifications in Biomedical Research at the Cleveland Clinic Lerner College of Medicine, also earning a Masters in Public Health (MPH) through Case Western Reserve University. He was the recipient of several fellowships in medical school, including the CDC-Hubert Global Health Fellowship and Duke Sanford School of Public Policy's Global Health Fellowship, and became committed to a career in global public health with a focus on improving health in developing countries. He completed internal medicine training at the Johns Hopkins Hospital. Following residency, he spent a year in Botswana as an Afya Bora Fellow leading several studies on the epidemiology, prevention, and treatment of HIV-associated cryptococcal meningitis. As a Fellow in Infectious Diseases and Epidemiology student at the University of Washington, he has continued working in Botswana with support from an Afya Bora Career Development Award and National Institutes of Health (NIH) T32 and F32 individual post-doctoral fellowship grants. He led a Cochrane systematic review to inform World Health Organization guidelines on the treatment of cryptococcal meningitis and his work in cryptococcal infection and advanced HIV disease has been published in journals including *Lancet Infectious Diseases*, *Clinical Infectious Diseases*, *PLoS Medicine*, and the *Cochrane Database of Systematic Reviews*. He will join the Epidemic Intelligence Service of the U.S. Centers for Disease Control and Prevention (CDC) in July 2019 to continue his career in public health research and applied epidemiology.