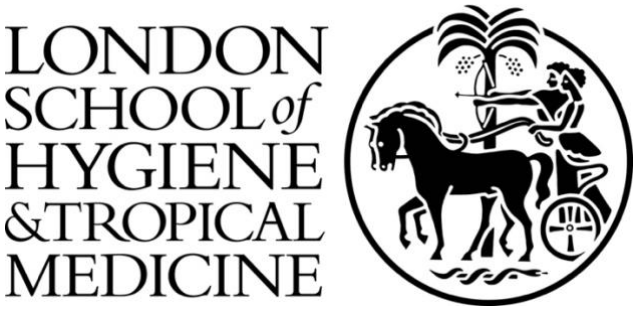

Strategies to improve diagnosis of central nervous system infections in high HIV-prevalence African settings

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Declaration of own work

I, James Edward Milburn, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

James Edward Milburn

10th November 2024

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None of this work would have been possible without substantial contributions from many colleagues and collaborators in Botswana, UK and Zimbabwe but most importantly from the patients involved in this study.

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Abstract

Central nervous system infections (CNSI) are a major cause of morbidity and mortality in low-resource, high HIV-prevalence African settings. HIV has drastically altered the epidemiology of CNSI in high HIV-prevalence African settings and cryptococcal, tuberculous and pneumococcal meningitis comprise the majority of microbiologically-confirmed diagnoses. However, in high HIV-prevalence African settings up to half of patients with a CNSI do not receive a diagnosis when conventional diagnostics are used alone. The mortality in this group is high, 40% at 10 weeks, indicating the presence of significant undiagnosed pathology that needs improved diagnostic strategies to enable to the rapid initiation of appropriate and targeted treatment to achieve better outcomes.

Enhanced diagnostics for CNSI, including monoplex PCRs for key CNS pathogens or rapid multiplex PCR platforms such as BioFIRE FilmArray-ME, are available and have transformed the investigation of CNSI in high-resource settings. However, access to these platforms is extremely limited in routine care in high HIV-prevalence Southern Africa where there is a continued reliance on conventional techniques, such as culture or microscopy with gram and Ziehl-Neelsen stains, which often lack sensitivity. Diagnostic delays also result from other components of the diagnostic pathway including delayed lumbar punctures due to a lack of consumables or concerns about potential contraindications to lumbar puncture prompting clinicians to perform often unnecessary neuroimaging. Data on the role of neuroimaging in resource-limited, high HIV-prevalence settings are extremely scarce although clinicians in the region face distinct challenges, including a broader differential diagnosis alongside severely restricted access to neuroimaging. In addition to the lack of a diagnosis meaning rapid diagnostic-driven treatment is not possible, the large burden of CNSI with no diagnosis results in a limited understanding of CNSI epidemiology in Southern Africa. This means empiric treatment choices are impossible, public health decisions regarding resource allocations are unguided, optimal drug procurement is challenging and both the impact and need for vaccination and prevention strategies

is largely unknown. Robust data from comprehensive CSF analysis could determine optimal empiric treatment strategies in patients without a microbiologically confirmed CNSI diagnosis in low-resource settings, as well as supporting resource allocation and drug procurement choices, and evaluating the success of vaccination strategies.

The overall aim of this thesis is to identify strategies to improve the diagnosis of CNSI in high HIV-prevalence settings. The specific aims of this thesis are to define the current epidemiology of CNSI in a high HIV-prevalence African setting, to determine the increase in diagnostic yield of CNSI following expansion of diagnostic capabilities, to describe the changes in epidemiology of common CNSIs in the region over time and to characterise the effect of computed tomography performed prior to lumbar puncture on patients with suspected CNSI.

The epidemiology of CNSI in Botswana and Zimbabwe and the impact of enhanced diagnostics on diagnostic yield will be described using clinical and laboratory data from two prospective meningitis surveillance networks where enhanced diagnostics including BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra were implemented into routine care alongside additional retrospective CSF analysis. Individual patient level data from other high HIV-prevalence African countries that have used BioFIRE FilmArray-ME will be combined in a systematic review and meta-analysis to further contextualise the impact of enhanced diagnostics in high HIV-prevalence African settings. These data will be complemented by national meningitis surveillance data from Botswana spanning 8 years which will be used to describe the changes in the detection of the most common CNSIs, cryptococcal and tuberculous meningitis, over time, following expansion of CNSI diagnostic capacity in routine care and increased ART coverage in Botswana. Finally, this thesis will use data from a tertiary hospital in Botswana to evaluate how computed tomography is used in routine clinical practice in high HIV-prevalence settings and characterise the effect of computed tomography performed prior to lumbar

puncture on patients with suspected CNSI on delay to diagnostic lumbar puncture and treatment initiation.

This thesis will be presented in 7 chapters including 4 published manuscripts and 2 chapters in the style of a research paper. Chapter I will serve as an overarching review of current challenges in the diagnosis of CNSI in high HIV-prevalence African settings and provides an overview of currently available diagnostic platforms for use in the investigation of suspected CNSI in the form a published narrative review. Chapter II describes the aims and objectives of this thesis and the methodology used to establish the two prospective meningitis surveillance networks in Botswana and Zimbabwe. Chapter III is a systematic review and meta-analysis of individual patient level data from all available studies from high HIV-prevalence African settings that have used the rapid multiplex PCR platform BioFIRE FilmArray-ME to evaluate the clinical utility of BioFIRE FilmArray-ME when used in settings with differing CNSI epidemiology from where BioFIRE FilmArray-ME was originally developed. Chapter IV consists of 2 manuscripts that used national meningitis surveillance data from Botswana to characterise trends in CNSI epidemiology following the rollout of improved CNSI diagnostics and scale-up of ART. Chapter V is a published manuscript evaluating the role of computed tomography in the investigation of patients with suspected CNSI in Botswana and assesses the impact of prior CT on time to diagnostic lumbar puncture and treatment initiation. Chapter VI uses prospective data captured following the introduction of enhanced diagnostic packages at two sites in Southern Africa to describe the epidemiology of CNSI in the region and evaluate the impact of these enhanced diagnostics on diagnostic yield. Chapter VII is a concluding chapter that summarises the key findings from the thesis and their implications.

Table of Contents

Acronyms/Abbreviations

AHD: Advanced HIV Disease

AMBITION: AmBisome Therapy Induction Optimization trial

ART: Antiretroviral Therapy

BNMS: Botswana National Meningitis Survey

CD4: Cluster Differentiation 4 cell

CI: Confidence interval

CMV: Cytomegalovirus

CNS: Central Nervous System

CNSI: Central Nervous System Infection

CrAg: Cryptococcal Antigen test

CSF: Cerebrospinal Fluid

DNA: Deoxyribonucleic acid

DNase: Deoxyribonuclease

EBV: Epstein-Barr Virus

HarMenAeS: Harare Meningitis Aetiology Study

HRDC: Health Research and Development Committee

HHV-6: Human Herpesvirus-6

HIV: Human Immunodeficiency Virus

HSV: Herpes Simplex virus

ICP: Intracranial Pressure

JC: John Cunningham

LP: Lumbar Puncture

MC+S: Microscopy, Culture and Sensitivity

ME: Meningitis/Encephalitis

MRCZ: Medical Research Council of Zimbabwe

OR: Odds ratio

PCR: Polymerase Chain Reaction

PMH: Princess Marina Hospital

RNA: Ribonucleic acid

RNase: Ribonucleases

RPR: Rapid plasma reagin

SOP: Standard Operating Procedure

TB: Tuberculosis

TBM: Tuberculous Meningitis

TPPA: Treponema pallidum particle agglutination

USD: United States Dollar

VZV: Varicella zoster virus

Tables and figures

List of tables and figures not included in manuscripts

Chapter I – Introduction to central nervous system infections in high HIV-prevalence African settings

1.1 Summary of chapter

This chapter serves as an overarching narrative review of central nervous system infections in high HIV-prevalence African settings and an introductory chapter to my thesis. The current impact of central nervous system infections (CNSI) in high HIV-prevalence African settings, the current practices in the diagnosis of CNSI in Southern Africa and associated challenges will be discussed. The key findings from previous larger-scale epidemiological studies employing molecular diagnostics in high HIV-prevalence settings in Africa are presented.

To describe currently available platforms that have the potential for use in high HIV-prevalence African settings, including those used in the prospective studies in Botswana and Zimbabwe, a detailed published narrative review of novel and molecular diagnostics available for use in low-resource settings is included in this chapter.

This chapter will also serve to highlight the relevance of this work and the rationale for focussing on improving CNSI diagnostics in high HIV-prevalence African settings.

1.2 Overview of central nervous system infections in high HIV-prevalence African settings

HIV has had a devastating effect on the African continent. Despite huge advances in access to ART, Africa continues to be disproportionately affected by HIV. In 2022 there were an estimated 39 million people living with HIV worldwide. Although only 15% of the world's population live within the African

continent 66% of people living with HIV are in Africa and in 2022 630,000 people died of HIV-related complications with the vast majority of these being in sub-Saharan Africa[1].

Estimates of CNSI incidence from 2019 suggest there are 1,200,000 cases of CNSI annually in sub-Saharan Africa alone; nearly 50% of the global total of 2,510,000 cases/year, and mortality from CNSI in the region is high, estimated as 14% with significant morbidity and long-term disability that is often unaccounted for[2].

HIV has markedly altered the aetiology of meningitis in high HIV-prevalence African settings with cryptococcal, tuberculous and pneumococcal meningitis being responsible for over 97% of all microbiologically confirmed diagnoses in Botswana and South Africa[3,4]. Even within the "meningitis belt" in the Sahel region where CNSI epidemiology has typically been characterised by high rates bacterial meningitis, cryptococcal meningitis has been increasing reported as a consequence of high HIV prevalence[5–8]. Infections of the central nervous system are a major cause of mortality and morbidity in high HIV-prevalence settings accounting for up to 30% of early mortality in antiretroviral therapy (ART) programmes[9,10]. Cryptococcal disease alone accounts for 15% of HIV-associated deaths worldwide, and approximately 75% of these cases occur in sub-Saharan Africa[3,4,11].

However, it is unclear whether current data accurately reflect the true aetiologies of CNSI in high HIV-prevalence African settings. There are only a small number of studies implementing molecular diagnostics in high HIV-prevalence African settings and major limitations in the availability, quality and laboratory capacity of conventional diagnostics[12–18]. Outside of clinical trials there is an almost complete absence of routine molecular diagnostics for patients with suspected CNSI across Southern Africa. This results in an inability to definitively diagnose viral meningitis and, along with limitations associated with traditional CSF culture techniques, including an overall low diagnostic yield and rapid sterilisation of CSF after antibiotics[19], large numbers of suspected CNSIs in the region do not have a

confirmed diagnosis[3,4,20]. As such, many meningitis cases are treated empirically on the basis of clinical presentation and cerebrospinal fluid (CSF) cell counts without specific microbiological diagnoses, inevitably leading to misdiagnosis, sub-optimal management, and high mortality rates[21].

1.3 Current practices in the diagnosis of CNSI in Southern Africa

Across the majority of countries in Southern Africa, standard diagnostic investigations for suspected CNSIs are usually limited to CSF microscopy, culture and sensitivity, CrAg, and Gram, Ziehl-Neelsen and India ink stains. CSF TB culture is available in some settings but, as is the case in Botswana, often solely through national referral laboratories, adding delays and complexity to testing and receiving results. Whilst all these investigations have a role in the diagnosis of meningitis, with the exception of CrAg, they are relatively insensitive and/or non-specific. The advantages and disadvantages of each available modality are described in table 1.

If implementing these routine diagnostics alone, large proportions of patients do not receive a diagnosis. Data from South Africa, the best resourced country in the region which is able to perform conventional diagnostics to a high standard, analysed 1737 clinical episodes of suspected meningitis and demonstrated 52.8% of patients with abnormal CSF had no microbiological diagnosis with routine CSF analysis alone[3]. Due to the absence of robust epidemiological data about meningitis aetiology, evidence-based decisions regarding empiric treatment are impossible and likely drive increased mortality. A national retrospective surveillance study conducted in Botswana analysing all CSF samples in Botswana between 2000-2014 demonstrated that among the 8759 patients with abnormal CSF findings, 3750 (43%) did not have any microbiological diagnosis on standard laboratory testing and 66% of these patients were HIV positive. Whilst the proportion of patients without a microbiologically-confirmed diagnosis is comparable to high-resource settings where enhanced diagnostics are used more frequently the mortality rate in patients in Botswana was far higher; over 40% at 10 weeks compared to an in-hospital mortality of 1.5% in high-resource settings[22]. These figures indicate a high

prevalence of significant underlying pathology which needs appropriate diagnosis to facilitate targeted treatment for organisms likely not being covered by current empiric treatment strategies[21]. More recent data from the DREAMM study which implemented pragmatic interventions for CNSI diagnosis in high HIV-prevalence African settings are encouraging and suggest that improvements in CNSI diagnosis are feasible and result in reduced mortality[23]. However, outcomes from CNSI in the study remained poor demonstrating the need for improved diagnostic strategies in the region.

1.4 Challenges in the diagnosis of central nervous system infections in Southern Africa

To effectively reduce the high mortality rates seen in CNSI in Southern Africa it is essential to make a rapid and accurate diagnosis to facilitate prompt, targeted treatment. Most available data on the impact of delayed diagnosis on outcomes focuses on bacterial and tuberculous meningitis, where it is well established that diagnostic delay and subsequent delays in initiation of appropriate therapy contribute to increased mortality. Similar trends are likely to be present in other types of meningitis[24–27]. Diagnosis of CNSI can be delayed in resource-limited settings due to a number of reasons including limited laboratory infrastructure to deliver a rapid diagnosis, delayed lumbar punctures and late presentation to hospital. Challenges due to laboratory infrastructure and lumbar puncture delay are discussed in the following section[28]. Although delayed presentation to hospital is a major contributor to mortality it is outside the scope of this thesis.

Limitations in laboratory infrastructure across Southern Africa restrict the ability to gain prompt, accurate diagnoses for patients with suspected CNSI particularly in settings where high HIV-prevalence broadens the differential diagnosis. Routine CSF bacterial culture methods can take up to 72 hours to finalise a result, meaning rapid administration of targeted antimicrobial therapy is not feasible in the majority of Southern African healthcare facilities. Furthermore, culture requires consumables, robust laboratory infrastructure, person-time and technical skill all of which are in short supply resulting in

limited availability and variable quality of results from traditional culture techniques. Affordable platforms that allow the prompt detection of viral and bacterial CNS pathogens are not available in Southern Africa leaving large gaps in the diagnostic capability of microbiology laboratories. Rapid multiplex polymerase chain reaction (PCR) platforms, such as the BioFIRE FilmArray-ME, able to detect a broad range of bacterial and viral pathogens, are used in Europe and USA with a turnaround time of 1-2 hours[29]. However, the cost of routine use of rapid multiplex PCR on all CSF specimens is prohibitive in resource-constrained settings and would require laboratory infrastructure upgrades, such as ensuring reliable power supply and significant investment in microbiology staff training. Whilst accessibility to rapid molecular diagnostics such as Xpert MTB/RIF Ultra is improving in Southern Africa, TB diagnostics remain limited, with Ziehl-Neelsen staining for acid fast bacilli being the most widely available test for TB meningitis[30]. When TB culture is available this takes 2-6 weeks to yield a result and therefore cannot influence urgent treatment decisions. The diagnosis of cryptococcal meningitis has been revolutionised by the widespread rollout of a cheap and easy to use rapid diagnostic test, IMMY CrAg lateral flow assay, which can detect the presence of polysaccharide on the capsule of cryptococcus in CSF within 15 minutes[31]. The success of CrAg and to a lesser extent Xpert MTB/RIF Ultra demonstrates that expanded diagnostic capacity for the investigation of suspected CNSI is potentially feasible and effective in the region.

Whilst expanding diagnostic capacity will likely improve diagnostic yield, additional factors prior to CSF analysis will also impact CNSI diagnoses and these should also be considered as part of the diagnostic pathway. The initial step required to initiate investigation for CNSI, a lumbar puncture (LP), can often be delayed due to lack of consumables or concerns about potential adverse effects or contraindications to LP which may be overstated[32,33]. Clinicians face challenges identifying those patients with raised intracranial pressure who may be at risk of complications from LP, the primary concern being cerebral herniation, and who may require neuroimaging prior to LP. Guidance on the identification of patients that require neuroimaging prior to LP exists but are designed to aid decision-

making in settings with access to rapid neuroimaging which is absent in resource-limited settings[34,35]. Clinicians working in resource-constrained settings often face additional challenges including a broader differential diagnosis alongside severely restricted access to neuroimaging. Data on the role of neuroimaging prior to lumbar puncture in patients with suspected CNSI in regions where rapid neuroimaging is not available, are needed to aid clinician decision-making in the selection of patients to receive neuroimaging prior to lumbar puncture and potentially allow development of guidelines to support clinicians working without access to rapid neuroimaging.

Test	Description	Turnaround time	Advantages	Disadvantages	Access and availability
Gram stain	Stain of bacterial cell wall to determine bacterium morphology	1 hour	Cheap Rapid Relatively simple to perform Less affected by antibiotic administration than culture	Interpretation is operator dependent	Available in most microbiology laboratories
Culture	Culture for bacterial and fungal pathogens, and TB	Variable 1-10 days for bacterial and fungal cultures 6 weeks for TB culture	Easy to perform Gives information on antimicrobial sensitivities	Prior antibiotic administration decreases culture yield Results may take several days/ weeks	Available only in larger centres
Ziehl-Neelsen staining	Staining for acid-fast bacilli	1 hour	Cheap	Low sensitivity Highly operator dependent	Available only in larger centres
Cryptococcal antigen lateral flow assay	Rapid diagnostic test that detects presence of polysaccharide on cryptococcus cell wall	15 min	Highly sensitive Cheap Easy to use even with limited laboratory infrastructure/staff training	Does not distinguish between active or previously treated infection	Widely available and can be performed at bedside
India Ink	Staining of capsule of cryptococcus neoformans	15 min	Sensitive Cheap	Does not distinguish between active or previously treated infection Operator dependent	Available in most microbiology laboratories

Table 1: Description of commonly used investigations in the diagnosis of meningoencephalitis[36,37]

1.5 The use of molecular diagnostics in CNSI in high HIV-prevalence African settings

Molecular diagnostics have been used infrequently in high HIV-prevalence African settings since the 1990's in a variety of settings. Earlier trials mainly focussed on the use of PCR for bacterial meningitis surveillance in the meningitis belt or the confirmation of the presence of suspected single pathogens such as JC virus or TB rather than describing local or regional epidemiology of CNSI[38–43]. There have been a relatively small number of studies that have implemented molecular diagnostics in high HIV-prevalence trial settings with the primary aim of identifying pathogens in patients without a confirmed diagnosis. The larger studies that have been performed were in Ethiopia, Malawi, Uganda, Zambia and Botswana[13,14,16,18,44–46]. Trials before 2015 typically used either mono- or multiplex PCRs targeting a pre-determined selection of common potential CNS pathogens whereas the majority of more recent trials have used BioFIRE FilmArray-ME, either in isolation or as part of an algorithm with CrAg testing and Xpert MTB/RIF or Xpert MTB/RIF Ultra. Whilst data from these studies have demonstrated the clinical utility of detecting organisms that would have otherwise been missed, in all studies there remains a significant number of cases without a confirmed microbiological diagnosis, leaving scope for continued testing with augmented testing capacity. The persistence of significant proportions of patients without a confirmed diagnosis may be due to the limited sensitivity of some platforms (e.g. Xpert MTB/RIF) or that the targets in FilmArray-ME panels have been determined based mostly on epidemiological data of CNSI in high-income countries and therefore may have some limitations when used in high HIV-prevalence African settings with different pathogens to Europe and North America. Trials using BioFIRE FilmArray-ME are introduced in the review article in section 1.5. Only two of the larger-scale trials have used a comprehensive panel of molecular diagnostics other than FilmArray-ME to describe the epidemiology CNSI in high HIV-prevalence settings and these are discussed in detail below. More recent studies using FilmArray-ME are summarised in the review article later in the chapter.

1.5.1 Uganda

This 2014 study of HIV-positive inpatients with suspected meningitis presenting consecutively to a tertiary referral hospital in Kampala, Uganda between 2010-2012 used a commercially available multiplex PCR panel, Ibis PLEX-ID (Abbott, USA), to analyse a total of 117 CSF samples[12]. The study included 63 samples negative for cryptococcosis and 54 with confirmed cryptococcal meningitis, to evaluate for potential co-infections. Evaluation for arboviral meningoencephalitis using PCR for alphaviruses, orthobunyaviruses and flaviviruses was performed on 111 CSF samples negative for cryptococcosis and TB meningitis. In addition, serology for West Nile, Yellow Fever, dengue, Chikungunya and Zika viruses was performed on 62 serum samples. Amongst the CSF samples without a diagnosis that underwent testing with multiplex PCR 50.7% (32/63) were reported as having no confirmed microbiological diagnosis. However, EBV was considered the sole pathogen in 18 samples in this study, despite this being an uncommon sole CNS pathogen in the acute setting[47]. If EBV was not considered as a pathogen this would mean 79.4% (50/63) of those patients that underwent analysis with molecular diagnostics do not have a clinically relevant diagnosis.

There are a number of factors that likely contribute to the low diagnostic yield in the investigation of suspected CNSI in low-resource settings. It is not clear whether the samples analysed with the multiple PCR had a high clinical suspicion for CNSI either through the presence of a pleocytosis or abnormal biochemistry. In addition the matrix that was used for extraction and subsequent downstream multiplex PCR analysis was the supernatant after CSF centrifugation for gram and India ink stains, and culture which presents two challenges. Firstly, the sample will likely be stored at room temperature until after the microbiology technician has processed the sample, which will often be at least several hours in the day and potentially longer if collected overnight. This delay may result in degradation of RNA especially in areas with high ambient temperatures likely restricting the detection of RNA viruses including the arboviral targets in this study[48]. Secondly, nucleic acid extracted from supernatant will likely have a lower yield than nucleic acid extracted from neat or uncentrifuged CSF. Furthermore, the

technology for detection of pathogens has improved since this study and we would now expect more positive results particularly for TB meningitis and *Toxoplasma gondii*. The sensitivity of Xpert MTB/RIF Ultra is superior to Xpert MTB/RIF which was used in this study for the diagnosis of TB meningitis and whilst the study does not describe which primers were used in the detection of *T.gondii* more recent targets have demonstrated improved sensitivity[30,49].

Pathogen(s)	Total (n=117)	Cryptococcal meningitis (n=54)	Tuberculous meningitis (n-4)
EBV	42	21	3
EBV + CMV	3	2	0
CMV	2	0	0
JC virus	3	2	0
Toxoplasma gondii	3	1	0
EBV + JC virus	3	2	0
EBV + VZV	1	0	0
CMV + *Toxoplasma gondii*	1	0	0
BK virus	1	0	0
Enterovirus	0	0	0
Negative	58	26	0

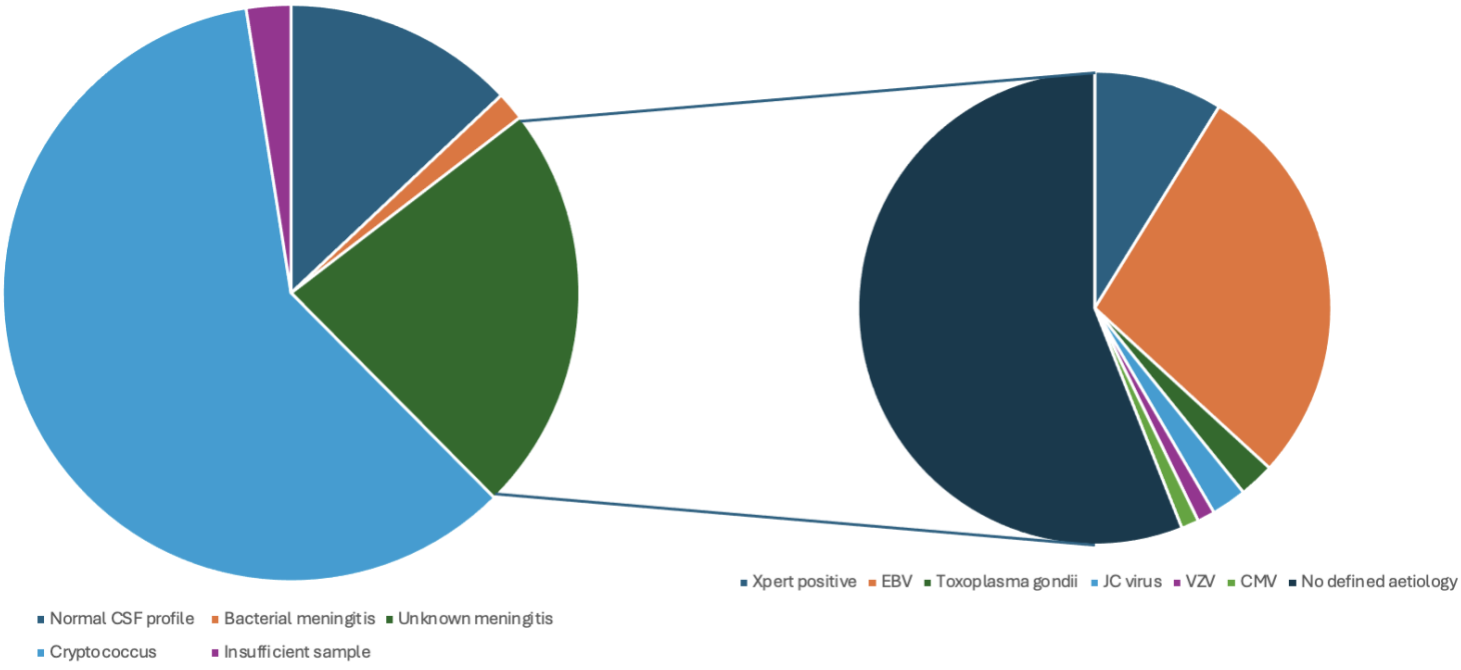

Figure 1: Results from Rajasingham et al describing proportions of patients with and without diagnoses and PCR detections from multiplex PCR analysis

1.5.2 Zambia

CSF samples from 331 HIV-positive adults with suspected CNSI were analysed at a tertiary centre in Lusaka, Zambia between 2010 and 2012[14]. Individual PCRs were performed on extracted DNA for *M.tuberculosis,* EBV, JC virus, VZV, CMV, HSV-1, HSV-2 and *T.gondii*. The study reported pathogen detection in 57.1% (189/331) samples however, similarly to the contemporaneous Uganda paper, 91 of these were EBV. Excluding EBV, 29.6% (98/331) of samples had a microbiologically confirmed diagnosis with TB (n=48), JC Virus (n=20) and CMV (n=20) being the most commonly detected pathogens.

Pathogens	Individual CSF prevalence	1 pathogen	Number of cases	2 Pathogens	Number of cases	3 Pathogens	Number of cases	4 pathogens	Number of cases
EBV	27.5% (91/331)	EBV	41	EBV/Cryptococcus	19	EBV/TB/CMV	4	EBV/Cryptococcus/ TB/VZV	1
Cryptococcus	19.3% (64/331)	Cryptococcus	35	EBV/TB	11	EBV/TB/JCV	1	Cryptococcus/TB/ VZV/CMV	1
TB	14.5% (48/331)	TB	20	EBV/VZV	3	EBV/TB/ *N. meningitidis*	1		
JC virus	6.0% (20/331)	JC virus	9	EBV/JCV	2	EBV/TB/Cryptococcus	1		
CMV	6.0% (20/331)	CMV	6	EBV/ *S.pneumoniae*	2	EBV/Cryptococcus/JCV	1		
VZV	3.9% (13/331)	VZV	4	EBV/CMV	1	EBV/JCV/CMV	1		
Streptococcus pneumoniae	2.4% (8/331)	*S.pneumoniae*	4	EBV/HSV-1	1	EBV/JCV/VZV	1		
HSV-1	1.5% (5/331)	HSV-1	2	Cryptococcus/CMV	2				
Neisseria meningitidis	0.6% (2/331)			Cryptococcus/VZV	2				
HSV-2	0			Cryptococcus/TB	1				
Toxoplasma gondii	0			Cryptococcus/HSV-1	1				
				TB/CMV	4				
				TB/JC virus	2				
				TB/VZV	1				
				JC virus/ *S.pneumoniae*	2				
				JCV/HSV-1	1				
				CMV/*N. meningitidis*	1				

Totals	271 pathogens		36/6% 121/331		16.9% (56/331)		3% (10/331)		0.6% (2/331)

Table 2: Confirmed pathogens identified from Siddiqi et al[14].

1.6 Manuscript – The diagnosis of central nervous system infections in resource-limited settings and the use of novel and molecular diagnostics platforms to improve diagnosis

This narrative review describes the currently available novel and molecular diagnostics used in the diagnosis of CNSI with an evidence base for use in resource-limited settings. It is an extremely large topic and therefore focuses on geographically relevant pathogens found in resource-limited or high HIV-prevalence settings and does not describe those commonly reported in resource-rich settings (e.g. enterovirus or herpesviruses).

The reviews highlights the importance of implementing enhanced diagnostics to improve the understanding of local and regional CNSI epidemiology that potentially could inform updates in commercial multiplex PCR panels. Furthermore, it outlines the key benefits and limitations of the most widely used molecular diagnostic platforms.

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Student ID Number	2004168	**Title**	Dr
First Name(s)	James		
Surname/Family Name	Milburn		
Thesis Title	Strategies to improve diagnosis of central nervous system infections in high HIV-prevalence African settings		
Primary Supervisor	Prof J Jarvis		

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SECTION B – Paper already published

Where was the work published?	Expert review in molecular diagnostics		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	This was an invited review. I conceptualised the structure of the work, performed the literature review and drafted and outline for the paper which was reviewed by Professor Jarvis. I wrote a first draft of the review. Dr Rachita Suresh gave input into the TBM diagnostics section using an outline which I proposed and data from studies identified from my literature review and I reviewed and edited prior to review by JJ. Dr Ronan Doyle subsequently reviewed the metagenomic section of the manuscript. JJ reviewed the entire manuscript and I addressed all comments and subsequent reviewer comments prior to submission.

SECTION E

Student Signature	
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REVIEW

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The diagnosis of central nervous system infections in resource-limited settings and the use of novel and molecular diagnostic platforms to improve diagnosis

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ABSTRACT

Introduction: Central nervous system infections (CNSI) disproportionately affect individuals in low-resource settings where diagnosis is challenging; large proportions of patients never receive a confirmed microbiological diagnosis resulting in inadequate management and high mortality. The epidemiology of CNSI varies globally and conventional diagnostics deployed in resource-limited settings have significant limitations, with an urgent need for improved diagnostic strategies.

Areas covered: This review describes molecular platforms and other novel diagnostics used in the diagnosis of CNSI that are applicable to resource-limited settings. An extensive literature search of Medline and PubMed was performed. The emphasis is on investigations targeting infections of relevance to resource-limited settings either due to variation in regional CNSI epidemiology or due to increased prevalence in patients with immunosuppression. This includes commercially available multiplex PCR platforms, mycobacterial PCR platforms, and rapid diagnostics tests. To offer a framework for the optimal implementation in clinical settings, existing evidence highlighting the advantages and limitations of available platforms is reviewed.

Expert opinion: The implementation of molecular platforms and other novel diagnostics has the potential to transform CNSI diagnosis in resource-limited settings, with several examples of successful rollout of novel diagnostics such as Xpert MTB/RIF Ultra and cryptococcal antigen testing.

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1. Introduction

Central nervous system infections (CNSI) lead to a significant burden of morbidity and mortality globally [1–4]. The burden of CNSI is highest in resource-limited settings where outcomes are often poor due restricted access to healthcare during acute infection and few resources to support survivors with long-term neurological sequelae [1,5]. Diagnosis of CNSI is challenging, and a large proportion of patients never receive a confirmed microbiological diagnosis, particularly in resource-limited settings where access to modern diagnostic platforms is limited [6,7]. The epidemiology of CNSI in resource-limited settings is therefore poorly understood making presumptive diagnosis and empirical treatment challenging. There is also significant regional variation in pathology. Japanese encephalitis, leptospirosis, scrub typhus and *Streptococcus suis* are important causes of CNSI in South-east Asia but rarely encountered in Europe or North America [8]. Meningococcal meningitis predominates in the meningitis belt of Africa extending from Senegal to Ethiopia with epidemics of meningococcal meningitis occurring in the dry season with attack rates as high as 1% of the population in major epidemics [9]. In much of Africa, HIV has drastically altered CNSI epidemiology with cryptococcal and tuberculous meningitis now being the most commonly-encountered pathogens in the region [10,11]. However, the prevalence of other central nervous system opportunistic infections associated with advanced HIV disease such as *Toxoplasma gondii* and JC virus is largely unknown outside of resource-rich settings. Patients with CNSIs who do not receive a diagnosis have an unacceptably high mortality. Data from Botswana demonstrated a 40% mortality at 10 weeks in patients with a CSF pleocytosis but no confirmed microbiological diagnosis after investigation with conventional techniques, indicating the presence of widespread pathology that needs enhanced diagnostic infrastructure in place to inform treatment decisions and improve outcomes [12].

Standard diagnostic investigations for suspected CNSIs in resource-limited settings are usually restricted to CSF microscopy, culture and sensitivity, Gram, Ziehl-Neelsen, and India ink stains, and cryptococcal antigen (CrAg) testing. However, in many locations even these routine investigations may be unavailable. CSF culture requires significant technical skill from the operator and considerable resources including dedicated laboratory space, specialized agars, centrifuges, and incubators to be performed effectively. In addition, culture techniques can take up to a week for fungal and bacterial culture and up to 6 weeks for culture of *Mycobacteria tuberculosis* and therefore have limited impact on immediate

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Article highlights

- Central nervous system infections continue to cause significant morbidity and mortality in low-resource settings with a substantial proportion of patients never receiving a confirmed diagnosis largely due to limitations in access to novel and molecular diagnostics. There is an urgent need to implement improved diagnostic platforms tailored to local epidemiology in resource-limited settings.
- Multiplex PCR platforms have the potential to improve diagnostic capability in resource-limited settings and have been successfully implemented in laboratories with little to no experience of using molecular diagnostics but financial constraints often limit their use.
- Tuberculous meningitis (TBM) is a leading cause of central nervous system infections in resource-limited settings however limitations in the diagnosis in TBM persist. Although Xpert MTB/RIF Ultra is a notable advancement in the diagnosis of TBM it remains insufficiently sensitive to exclude TBM.
- Although metagenomic sequencing costs remain high they have decreased in recent years and the COVID-19 pandemic has resulted in significantly expanded sequencing capacity. Strategic, targeted use of metagenomic sequencing on CSF samples has the potential to identify rare or novel pathogens and guide analyte selection in multiplex PCR panels deployed in resource-limited settings.

clinical decision-making. Whilst these standard investigations have a role in the diagnosis of meningitis, with the notable exception of CrAg, they all have significant limitations; there is an urgent need to improve diagnosis of CNSI in resource-limited settings.

Rapid molecular diagnostic platforms tailored to local epidemiology have the potential to significantly enhance diagnostic capabilities and are well suited for use on cerebrospinal fluid (CSF) samples. Contamination of CSF during sample collection is relatively uncommon, disease processes are usually caused by a single pathogen (other than in patients with significant immunosuppression) and common inhibitors of nucleic acid amplification are usually not present in CSF [13]. As a result their implementation in high-resource settings has redefined diagnostic strategies for CNSI [14]. However, in resource-limited settings there are significant challenges to widespread implementation of molecular diagnostics. In sub-Saharan Africa, due to high HIV-prevalence, cryptococcal and tuberculous meningitis are the most common causes of CNSI. Cryptococcal meningitis is not reliably diagnosed through current nucleic acid amplification techniques (NAAT) as most fungi have dense cell walls that are difficult to breakdown resulting in decreased yield from traditional DNA extraction techniques [15]. Due to low bacillary burdens in CSF, tuberculous meningitis has historically been challenging to diagnose using molecular platforms but some more modern platforms have improved sensitivity. Co-infections remain rare but have been increasingly reported in immunosuppressed patients, requiring a broader range of targeted investigations for suspected CNSI [11,16,17]. There are additional structural challenges such as unreliable power supplies, limited access to cold chain shipping and storage, and cost considerations. Despite these challenges, there are examples of successful implementation of novel diagnostic platforms for the investigation of suspected CNSI in resource-limited settings. Cryptococcal antigen testing is now the leading modality used to diagnose cryptococcal meningitis and Xpert MTB/RIF Ultra has emerged as an increasingly useful tool in the investigation of tuberculous meningitis (TBM), although it remains insufficiently sensitive to rule out TBM [17].

One of the primary barriers to widespread adoption of molecular diagnostics in resource-limited settings is financial. Although the cost of molecular diagnostics has decreased over the recent years, and discretionary pricing is available in resource-limited settings for some platforms such as Xpert MTB/RIF Ultra, they remain relatively expensive to implement and maintain. Understanding the advantages and limitations of these platforms, and how they can be optimally implemented as part of locally-appropriate diagnostic algorithms, is crucial to maximize their clinical- and cost-effectiveness.

To conduct this narrative review a list of low- and middle-income countries defined by the world bank was compiled, termed 'LMIC.' PubMed/Medline (1946 to 2023) were searched for: [LMIC] AND [meningitis.mp. or exp Meningitis/ or exp Encephalitis/or encephalitis.mp. or meningoencephalitis.mp. or exp Meningoencephalitis/or central nervous system infections.mp. or exp Central Nervous System Infections/] AND [exp Molecular Diagnostic Techniques/or exp Rapid Diagnostic Tests/or polymerase chain reaction.mp. or exp Polymerase Chain Reaction/or exp Metagenomics/or metagenomic sequencing.mp or sequencing.mp or diagnostics.mp or diagnosis.mp]

This review aims to describe novel and molecular platforms used in the diagnosis of CNSI that are applicable to resource-limited settings. To provide context for their optimal use in clinical settings, existing evidence highlighting the advantages and limitations of available platforms is reviewed, covering conventional CSF investigations, soluble markers of fungal and mycobacterial infection, and antigen detection techniques. The focus is on pathogen-specific investigations of particular importance in resource-limited settings either due to significant variations in CNSI epidemiology from resource-rich settings or due to increased prevalence in patients with immunosuppression. Finally, commercially available multiplex PCR platforms are discussed, focusing on their use in resource-limited settings.

2. Conventional CSF investigations

2.1. Microscopy

It provides a cell count and differential that gives initial guidance on the underlying pathogen based on the predominant white blood cell type seen in the CSF. Neutrophilic pleocytosis is typically seen more in bacterial meningitis and lymphocytic pleocytosis in viral and tuberculous meningitis [18]. The test requires laboratory infrastructure and training and must be performed quickly to prevent degeneration of cells. Interpretation is not always straightforward as there is often overlap in patterns of cell differential, for example late presentations of bacterial meningitis can be lymphocytic and early presentations of TBM neutrophilic. In addition, CSF white cell counts can vary with age, race, and immune status [19,20]. Despite its limitations cell count and differential remain crucial first-line investigations that allow other results to be contextualized.

2.2. Gram stain

It is used to classify bacteria based on morphology and cell wall properties. It is quick, relatively inexpensive, and less affected by antimicrobial administration than culture. However, the sensitivity is poor (10–90% depending on organism) and is highly operator dependent [21,22].

2.3. India ink stain

It is used to identify encapsulated yeasts, primarily *Cryptococcus neoformans*. Like gram stain it is rapid, inexpensive and highly operator dependent. The sensitivity is superior to gram stain, around 70–80% [23], but it cannot distinguish between active and past treatment as it will stay positive even after successful treatment. Therefore positive results must be interpreted in the correct clinical context.

2.4. Culture

CSF culture for bacteria can yield a result in 24–48 hours depending on the organism and pathogen burden, but results can take over a week. Time to result is usually longer in fungal cultures and most cultures are incubated for up to 10 days. Culture remains the gold standard in diagnosis of bacterial meningitis although it is only positive in 70–85% of bacterial meningitis cases [24]. It is currently the only readily available diagnostic modality that provides reliable information on antimicrobial susceptibility from CSF samples. Culture sensitivity is affected by prior antimicrobial administration and sterile CSF has been reported within the first 2 hours of administration of antibiotics in meningococcal meningitis and 4 hours in pneumococcal meningitis [25]. However, depending on the pathogen culture may still be beneficial up to 2 days after antibiotics [14].

2.5. CSF protein

Raised CSF protein is a nonspecific finding that can be suggestive of CNSI. However, There are multiple non-infectious causes of raised CSF protein such as hemorrhage, malignancy, and Guillain-Barre syndrome. Neonates and children under 1 year old also have higher CSF protein levels than adults. Access to biochemistry in resource limited settings is often limited to larger hospitals.

2.6. CSF glucose

Ideally used as a ratio with plasma glucose where a low ratio or absolute value is suggestive of active infection. Classically TBM causes extremely low glucose levels. Access to plasma glucose is often restricted in resource-limited settings but bedside tests are also available.

3. Soluble markers

3.1. Beta-D-glucan

Beta-D-glucan (BDG) is a polysaccharide component of the cell wall of multiple fungi including *Candida* species, *Histoplasma* species, *Coccidioides* species and *Aspergillus* species. It is widely used on blood in high-resource settings to diagnose or monitor treatment progress in invasive fungal infections. However, it is not specific to a particular fungal species and several factors can lead to false-positive in serum such as hemodialysis, antibiotics, and surgical gauzes although limited data exists on false-positive in CSF [26,27]. A systematic review evaluated its use on CSF and found sensitivity varied by species and organism-specific testing was still warranted in patients with a raised BDG [28]. Its main strengths are that it has a faster turnaround time than fungal cultures and has the potential to monitor treatment progress in BDG-producing fungi.

3.2. Galactomannan

Galactomannan is most commonly found in the cell wall of *Aspergillus species* and is used in a similar fashion to BDG in the diagnosis and monitoring of aspergillosis mostly in serum. False-positive from antibiotics are seen in serum and slow-growing fungal infections can lead to false-negative. Based on serum cutoff values, CSF galactomannan was shown to have a sensitivity of 100% in the diagnosis of CNS aspergillosis with a specificity of 70% [29]. It is not widely used in CNSI diagnostics in resource-limited settings.

3.3. Adenosine deaminase

Adenosine deaminase (ADA) is an enzyme involved in the metabolism of purines that plays a crucial role in the differentiation and proliferation of lymphocytes. Increased ADA activity is associated with conditions that involve a cell-mediated response including TB. ADA testing to diagnose TBM has been widely considered in low-resource settings but its utility remains unclear due to an inability to determine optimal cutoff values for use on CSF and a significant number of infectious and noninfectious conditions apart from TBM causing elevated ADA [30–32].

4. Antigen testing

4.1. Cryptococcal meningitis

Cryptococcal antigen (CrAg) is a polysaccharide found on the cell wall of *Cryptococcus* species. There are several commercially available CrAg lateral flow assays (LFA) but by far the most used is the IMMY CrAg LFA; an immunochromatographic dipstick that has revolutionized the diagnosis of cryptococcal meningitis worldwide. It is cheap, does not require significant laboratory capacity and can give results in 15 minutes. In HIV-positive patients it has a sensitivity of 98.8% in CSF; superior to both India ink and culture [33,34]. Data from HIV-negative patients is limited but the incidence of cryptococcal meningitis in HIV negative patients is increasing mostly due to more widespread use of immunosuppressive therapies. A recent systematic review demonstrated a similar sensitivity in CSF in HIV-negative patients, 99% [33]. A key limitation to CSF CrAg testing is that it is unable to differentiate between active and treated infection. Newer CrAg LFAs allow semi quantification

of CrAg titer that may predict disease burden and culture positivity thereby mitigating one of the limitations of CrAg LFA. Further studies are needed to determine if semi quantification of CrAg titers could also be useful to determine disease severity and potentially inform management [35]. False-negative in both CrAg LFA and semiquantitative CrAg LFA can occur in high fungal burdens due to the prozone effect, although this can be overcome with serial dilutions.

4.2. Bacterial meningitis

Latex agglutination tests have been employed to detect common bacteria causing CNSI. They provided only a small increase in sensitivity above culture and required significant laboratory infrastructure and cold chains which restricted their use in resource-limited settings [22]. Similar lateral flow immunochromatographic technologies to CrAg testing have been developed to detect the presence of a number of bacteria. Primarily these target *Streptococcus pneumoniae* and *Neisseria meningitidis* and have been used more widely than latex agglutination tests. The Abbott BinaxNOW *Streptococcus pneumoniae* antigen card and BioSpeedia Pneumospeed both have sensitivities and specificities over 90% for the detection of *S. pneumoniae* [36]. Rapid diagnostic tests that can detect *N. meningitidis* are crucial for surveillance and early detection of meningococcal disease in the meningitis belt as despite the introduction of large-scale vaccination programmes outbreaks of meningococcal meningitis still occur [37]. The LFA BioSpeedia Meningospeed detects the five serogroups in currently licensed meningococcal vaccines, serogroups A, C, W, X and Y. It has a sensitivity of 92.7% and can partially differentiate between serotypes, which is potentially beneficial for monitoring of vaccination strategy success [37].

5. Mycobacterial testing

Tuberculous meningitis (TBM) is the most severe form of tuberculosis (TB) and is a leading cause of meningitis in resource-limited, high HIV-prevalence settings [4,17,38,39]. A modeling study estimated that 164,000 people developed tuberculous meningitis in 2019, with 70% of the global incidence occurring in South-East Asia and Africa. TBM occurs in 1.5% of tuberculosis cases in HIV-negative patients and up to 5% of cases in HIV-positive adults and carries a significant mortality with approximately 78,000 deaths globally in 2019. Early diagnosis is crucial to avoid poor outcomes but due to inadequacies in current diagnostics approximately 59% of patients with TBM died without a confirmed diagnosis and therefore patients are often either treated empirically on clinical suspicion alone with no understanding of potential antimicrobial resistance or do not receive appropriate antituberculous therapy at all [4,40].

5.1. Conventional diagnostics for tuberculous meningitis

The routinely available investigations in resource-limited settings to detect *Mycobacterium tuberculosis* in CSF are microscopy with Ziehl-Neelsen (ZN) stain and culture for *M. tuberculosis*. Microscopy with ZN staining is the cheapest and most rapid form of smear microscopy available to identify *M. tuberculosis*. It can be performed with less laboratory infrastructure than culture for *M. tuberculosis* and is widely used for pulmonary samples in both high-resource and resource-limited settings. However, the sensitivity of ZN staining on paucibacillary samples such as CSF is poor with estimates on CSF as low as 9% [41,42]. However considerably higher sensitivity of microscopy with ZN staining has been reported in centers where samples are analyzed by skilled microscopists for a minimum of 30 minutes suggesting the sensitivity is highly operator dependent [43].

TB culture using solid or liquid media remains the gold standard for TBM diagnosis despite significant limitations. The limit of detection for TB culture is approximately 10 CFU/ml and has a sensitivity of 30–60% [17,44]. To produce a positive result, it requires viable bacteria to be present in a sample and can take up to 6 weeks to produce a positive result meaning it cannot inform immediate treatment decisions [17,43].

5.2. Molecular diagnostics for mycobacterium tuberculosis

In 2010, the WHO approved Xpert MTB/RIF (Cepheid, U.S.A.) for use in pulmonary and extrapulmonary samples [38]. Xpert MTB/RIF is a cartridge-based, fully automated system that allows the rapid detection of *M. tuberculosis* and associated rifampicin resistance through nucleic acid amplification of the rpoB gene, a region that has been demonstrated to be highly predictive of rifampicin resistance [17]. The test provides a marked improvement in accessibility to TB diagnostics and turnaround time when compared to culture with results available in under 2 hours. However, the sensitivity of Xpert MTB/RIF is dependent on multiple factors including sample type, CSF volume, prior centrifugation and the patient's HIV status [44]. A large multicentre study in 2018 performed across Indonesia, South Africa and Vietnam evaluated the performance of Xpert MTB/RIF, Ziehl-Neelsen stain and CSF culture of 618 adults with suspected TB meningitis [4]. Xpert MTB/RIF was positive in 95 out of 379 patients with diagnosis of TB based on a uniform case definition [4]. The sensitivity of Xpert MTB/RIF assay was 25.1% (95% CI 21.0–29.7%) and specificity 72.3% (95% CI not reported) when compared with culture [4]. A meta-analysis from 2021 included studies performed in both resource-limited and resource-rich settings and compared the use of Xpert MTB/RIF on CSF against a composite reference standard. 14 studies were included with a total of 2203 participants and it demonstrated a pooled sensitivity 42.3% (95% CI 32.1 to 52.8) and pooled specificity of 99.8% (95% CrI 99.3 to 100.0) [45]. As such, whilst Xpert MTB/RIF provided some improvements in TBM diagnosis above conventional investigations there remained significant limitations and it was insufficiently sensitive to be implemented as a reliable standalone test for the diagnosis of TBM.

Xpert MTB/RIF Ultra is now the current initial diagnostic test for TBM recommended by WHO in 2017 [38]. Similar to Xpert MTB/RIF, Xpert MTB/RIF Ultra provides semiquantitative categories and information on rifampicin resistance. Xpert MTB/RIF

Ultra has incorporated two additional PCR targets (IS6110 and IS1081), alongside a fully nested real time PCR assay and a DNA reaction chamber twice the size of Xpert MTB/RIF. These changes combine to allow Xpert MTB/RIF Ultra to have a lower limit of detection than Xpert MTB/RIF, 16 CFU/ml vs 114 CFU/ml respectively, an almost a 7-fold improvement [17,38]. Furthermore, the melting temperature-based analysis increases the accuracy of rifampicin resistance detection using four probes that identify rifampicin resistance mutations in the rpoB gene. The entire process takes less than one and a half hours thus producing faster and more accurate results [17,38,43].

Most data surrounding the use Xpert MTB/RIF Ultra in resource-limited settings have been generated from two large prospective trials. The first study from Vietnam randomized 205 adults with suspected TBM to testing with either Xpert MTB/RIF and Xpert MTB/RIF Ultra [43]. Using a uniform case definition as a reference standard Xpert MTB/RIF Ultra was not superior to Xpert MTB/RIF, sensitivities were 39.6% (95% CI 27.6 to 53.1) and 47.2% (95% CI 34.4 to 60.3) for Xpert MTB/RIF and Xpert MTB/RIF Ultra respectively. The sensitivity of both assays was higher in HIV positive patients but there remained no improvement of Xpert MTB/RIF Ultra over Xpert MTB/RIF. Xpert MTB/RIF Ultra did perform better in samples with a lower bacillary load and in those who had started treatment [43].

The second study conducted in Uganda analyzed CSF samples from 204 patients with Xpert MTB/RIF Ultra, Xpert MTB/RIF and mycobacterial growth indicator tube culture 196 of whom were HIV positive [17]. The sensitivity was significantly higher in Xpert MTB/RIF Ultra compared to Xpert MTB/RIF and MGIT culture against 'probable' and 'definite' categories within the uniform clinical definition. The sensitivities were 55.6% (95% CI 44.0 to 70.4%) for Xpert MTB/RIF, 61.4% (95% CI 45.5 to 75.6) for MGIT culture and 76.5% (95% CI 62.5 to 96.2) for Xpert MTB/RIF Ultra [17]. Individuals with 'possible' TBM based on a uniform case definition were not included as this category is less specific in patients with HIV co-infection due to the broad differential in patients with advanced immunosuppression. Importantly 36% of positive Xpert MTB/RIF Ultra were in the lowest semiquantitative detection category which correlates to < 100 CFUs/ml. This would be below the limit of detection for Xpert MTB/RIF and potentially also TB culture highlighting a potential population in whom Xpert MTB/RIF Ultra is of particular benefit.

The difference in performance of Xpert MTB/RIF Ultra in these studies is likely due to difference in sample handling, CSF volume submitted for analysis, HIV co-prevalence and study design (specimens were divided for use across all modalities in the Uganda study and randomized to individual analysis in the Vietnam study) [46]. A meta-analysis including both these studies and two others demonstrated a pooled sensitivity of 62.7% (95% CI 45.7 to 77.0) and 42.3% (95% CI 32.1 to 52.8) against a composite reference standard for Xpert MTB/RIF Ultra and Xpert MTB/RIF respectively[34].

These studies all suggest that although Xpert MTB/RIF Ultra is a significant improvement it remains insufficiently sensitive to exclude TBM with a negative result. The Uganda study reported nine adults with a negative Xpert MTB/RIF Ultra that had clinical features and CSF results highly suggestive of TBM with one adult having macroscopic features suggestive of TBM on postmortem [17]. As such Xpert MTB/RIF Ultra results should be interpreted in the context of clinical presentation, surrogate investigations suggestive of TBM such as routine CSF analysis, imaging and local TB epidemiology.

5.3. Lateral flow lipoarabinomannan assays

Xpert MTB/RIF Ultra does have some additional limitations beyond its imperfect sensitivity. These include the need for a reliable power source, laboratory infrastructure and staff training. Lateral flow lipoarabinomannan assays (LAM) are well established for use in the urine to detect a constituent part of the cell wall of the TB bacillus in patients with TB. A recent meta-analysis demonstrated that a newly developed TB-LAM assay, SILVAMP TB-LAM, has a superior sensitivity compared to the most widely used assay Alere Determine TB LAM, when used to diagnose pulmonary and extrapulmonary TB in HIV-positive adults [47]. SILVAMP TB-LAM combines a pair of high-affinity monoclonal antibodies for *M. tuberculosis*-specific LAM epitopes and has a silver-amplification step to improve band visibility and detect LAM at concentrations 30 times lower than Alere Determine TB LAM. Previous trials using Alere Determine TB LAM on CSF to diagnose TBM have not been promising but there is encouraging data from Uganda investigating the use of SILVAMP TB-LAM [48]. In a prospective cohort study of 101 adults, including 95 HIV-positive patients, 34 participants had definite TBM and 24 had probable TBM. Amongst the 58 individuals with definite or probable TBM, 30/58 tested positive with SILVAMP TB-LAM compared to 32/58 for Xpert MTB/RIF Ultra. Against a uniform case definition, SILVAMP TB-LAM had a sensitivity comparable to Xpert MTB/RIF Ultra of 52% (95% CI 38–65%) and 55% (95% CI 42–68%) respectively [49]. However, due to manufacturing challenges the availability of this test has been severely limited. A newer version has been recently released but is awaiting validation.

5.4. Truenat MTB and MTB plus

Truenat MTB assay (Molbio Diagnostics, India) is a chip-based nucleic acid amplification platform originally developed to meet the requirements of Indian primary health centers for near point-of-care TB testing [50]. It is portable and battery operated therefore useful in remote locations, and able to operate at temperatures up to 40°C. A second-generation platform is now in circulation, Truenat MTB Plus. The test takes approximately 2 hours but is broken down in to three discrete states, DNA extraction, *M. tuberculosis* detection and rifampicin resistance testing. A study conducted in 2018 in India compared Truenat MTB Plus with Xpert MTB/RIF Ultra using CSF. Truenat MTB Plus had an overall sensitivity of 78.7%, while Xpert MTB/RIF Ultra had an overall sensitivity of 67.6% in diagnosing probable and definite TBM [50]. Although the sensitivity was comparable to Xpert MTB/RIF Ultra in the study, rifampicin resistance detection was inferior, with 10 rifampicin resistant cases missed by Truenat MTB Plus [50].

6. Targeted testing for selected single pathogens

6.1. Progressive multifocal leukoencephalopathy

John Cunningham Virus (JCV) is the cause of progressive multifocal leukoencephalopathy (PML) a demyelinating disorder that prior to the rollout of ART in North American and European communities, affected up to 5% of patients with advanced HIV. PML is usually caused by the reactivation of latent JCV in immunocompromised patients due to conditions such as HIV or the use immunomodulatory agents [51–53]. Alongside classic PML, immune reconstitution inflammatory syndromes (IRIS) associated with PML are increasingly recognized. This results from the reversal of profound immunosuppression, such as a rapid rise in CD4 following ART initiation or cessation of immunosuppressive drugs such as natalizumab in multiple sclerosis [54]. The clinical features of PML are highly heterogenous and diagnosis can only be confirmed with a positive JCV PCR from CSF or brain biopsy alongside supportive imaging and clinical features, or typical neuropathologic findings. Whilst older PCRs had a sensitivity of approximately 75% modern PCRs have a sensitivity of over 95% and have prognostic value, with higher CSF JCV DNA loads shown to be associated with poorer outcomes [53,55]. Despite improvements in PCR sensitivity, it is still possible to have a negative PCR with clinical and radiological features compatible with PML. As such PML diagnosis remains challenging and consensus guidelines have suggested repeating a lumbar puncture and JCV PCR if the first is negative. Even with adequate resources the diagnosis of PML is often delayed with the median time to diagnosis reported as 74 days in a single US center [55,56].

The epidemiology of PML in resource-limited settings is poorly understood. Prevalence estimates are more difficult to establish as molecular diagnostics are less readily available and therefore the diagnoses of PML are often based on clinical or radiological features which are less specific than JCV PCR [57,58]. This has led to significant variability in the frequency of reported PML in previous studies performed in resource-limited settings. Retrospective data from India and Brazil described PML in 2.8% and 0.3% of HIV positive patients respectively [59]. Whilst these differences might be related to genetic or viral factors it more likely reflects the limitations in availability of reliable diagnostics. When JCV PCR testing has been implemented programmatically on CSF samples it has demonstrated similar or higher PML prevalence when compared to pre-ART studies from U.S.A./Europe. Over 15% of CSF (19/121) samples in South Africa and 6% (20/331) of samples in Zambia were positive for JCV on PCR [10,60]. To allow emerging treatment options for PML to be best utilized in resource-limited settings expanded JCV testing capacity or inclusion in multiplex PCR panels will be necessary [61].

6.2. Cerebral toxoplasmosis

Toxoplasma gondii is an obligate intracellular protozoan which causes toxoplasmosis, a common self-limiting condition in immunocompetent individuals. However, in patients with immunosuppression toxoplasma can reactivate; most commonly causing cerebral toxoplasmosis in patients not receiving ART and appropriate toxoplasma prophylaxis [62,63].

Approximately 30% of the world's population is estimated to be infected with *T. gondii* and the highest burden of *T. gondii* infection in individuals with HIV is in lower income countries with 87.1% of HIV and *T. gondii* co-infections being in sSA [64]. In resource-limited settings cerebral toxoplasmosis is usually diagnosed presumptively based on compatible clinical features and imaging, and positive toxoplasma serology when available. PCR techniques exist but their use is mostly limited to resource-rich settings. The sensitivity estimates of available PCRs is broad due to the highly heterogenous PCR methods used for the detection of toxoplasma, with estimates between 50–98% [65]. These includes using different primer sets, DNA targets, and hybridization probes [66]. Furthermore, pre-analytic steps such as different extraction techniques and administration of anti-toxoplasma treatment also influence assay performance making comparisons between PCR modalities challenging. PCR targets have evolved over recent years with the most recently proposed target being the GRA7 gene. This region is expressed during all infectious stages of the parasite's lifecycle and has been shown to have superior sensitivity to previous targets [67]. A study in Zambia using an older target, B1 gene, identified no cases of Toxoplasma amongst 331 HIV positive adults [10]. Accurate, reliable and more accessible platforms are needed to diagnose toxoplasma. Loop-mediated isothermal amplification (LAMP) techniques to detect *T. gondii* have been developed with some promise but there is limited data from CSF. They present a more portable, rapid diagnostic test that requires less infrastructure and may be practical in resource-limited settings.

6.3. Japanese encephalitis

Japanese encephalitis virus (JEV) is a vector borne *Flavivirus* transmitted by *Culex spp* mosquitoes. JEV is endemic in most of Asia and some areas in the Western Pacific with more recent detection of the virus in Australia [68,69]. It is the most important cause of viral encephalitis in Asia with an estimated 68,000 cases each year with 20,000 deaths [68]. The majority of JEV infections occur in children living in rural areas when JEV-infected mosquitoes feed on humans rather than swine, their principal amplifying host.

Diagnosis is typically made through the detection of anti-JEV IgM antibodies in blood or CSF. However cross reactivity of immune-assays targeting flaviviruses is well-described [70]. This is of particular relevance for dengue virus that is co-endemic in the region and exhibits similar seasonality to JEV. In some cases, vaccination against JEV can lead to prolonged IgM detection further challenging diagnostic certainty when relying on immune-assay testing alone [71]. Direct detection of JEV RNA would provide a valuable indication of infection as it would be able to overcome some limitations of immune-assay based testing and allow improved specificity. However widespread use of JEV PCR assays has been challenging due to the low sensitivity of currently available techniques, a short period of JEV viremia and low CSF concentrations of JEV in infected humans [70,71].

6.4. Neurosyphilis

The WHO estimated the prevalence of syphilis to be 1.1% in Asia and 4–6.5% in Africa in 2016 [72]. Neurosyphilis is relatively rare but is more commonly seen in patients living with HIV with neurosyphilis described in 3% of all cases of syphilis [73,74]. The laboratory diagnosis of neurosyphilis remains imperfect and relies upon a high index of clinical suspicion alongside the presence of a CSF pleocytosis or elevated protein and a combination of non-treponemal or treponemal tests [75]. The introduction of a PCR for *T. pallidum* was hoped to improve syphilis diagnostics however sensitivity estimates so far have been relatively modest. A systematic review reported the sensitivity of PCR for definite neurosyphilis of between 40–70% compared to reference testing for neurosyphilis [76]. A commercially available immunochromatographic test, DPP Chembio syphilis assay, has been trialed with some early success demonstrating a sensitivity of 80% against a reference standard of reactive CSF-Venereal Disease Reference Laboratory that improved with treatment [77]. At present the diagnosis of neurosyphilis remains reliant on a combination of multiple testing modalities and careful clinical interpretation.

7. Multiplex PCR assays

Multiplex polymerase chain reaction (PCR) panels can reduce the time to diagnosis, test for a number of pathogens that would not be detected through routine analysis alone and increase sensitivity above conventional techniques whilst using smaller sample volumes. Until recently the only commercially available multiplex PCR platform for use on CSF was BioFIRE FilmArray-Meningitis /Encephalitis panel (bioMerieiux, France) which has been proven to be effective in the diagnosis of CNSI in a number of large-scale trials in resource-rich settings [78,79]. In 2022 QIAGEN introduced the QIAstat-Dx meningitis/encephalitis panel that has recently been shown to have a comparable sensitivity to BioFIRE FilmArray-Meningitis/Encephalitis (FilmArray-ME) for analytes included in both panels [80]. Both panels use 200 µL of neat CSF with no need for prior extraction and results are available in under 1.5 hours; analytes included in both panels are described in Table 1 [81].

Studies from resource-limited settings have solely used the FDA-approved FilmArray-ME that tests for 14 common CNS pathogens, the major studies are outlined in Table 2 [11,16,82–86]. FilmArray-ME was successfully implemented in multiple laboratories which often had little to no experience of using molecular diagnostics. In all studies FilmArray-ME increased the diagnostic yield of CSF analysis and in some cases viral pathogens were detected in the CSF for the first time in routine clinical practice. The number of bacterial meningitis diagnoses made was also increased above traditional culture techniques. FilmArray-ME has also been used to differentiate cerebral malaria by excluding other CNSI in pediatric febrile neurological presentations in malaria endemic areas [16,87]. Whilst the overall sensitivity of FilmArray-ME was high in all studies there was some reported intrapanel variation between the sensitivity of analytes with the performance of *Cryptococcus neoformans/gattii* being poorer than other analytes in FilmArray-ME, particularly in low fungal burden cryptococcal meningitis [16]. There was only one positive *Cryptococcus neoformans/gattii* tested in the comparison of QIAstat-Dx meningitis/encephalitis to FilmArray-ME, so no reliable conclusions regarding diagnostic sensitivity for this pathogen can yet be made [52]. In contrast to high-resource settings where the diagnosis of cryptococcal meningitis is often opportunistic the sensitivity of the cryptococcus analyte is of particular importance in high HIV-prevalence settings where cryptococcal meningitis is the most common cause of CNSI. Multiplex PCR testing should not be performed in isolation in patients with suspected cryptococcal meningitis due superior accuracy of CrAg testing.

The cost of multiplex PCR panels will be a barrier to more widespread implementation but data from Ethiopia suggests that multiplex PCR testing decreased antibiotic use which would mitigate some of the increased cost [83]. Furthermore, diagnostic yield varied between studies which had heterogenous inclusion criteria suggesting the use of these platforms could be targeted to higher yield groups as part of a clinically- and cost-effective diagnostic algorithm.

8. Metagenomic sequencing

Most novel diagnostics used in CNSI are pathogen-specific and therefore rely on the current paradigm of the treating clinician instigating investigations for a specific pathogen. In contrast,

Table 1. List of pathogens detected in BioFIRE FilmArray-Meningitis/Encephalitis and QIAstat-dx meningitis/Encephalitis panel.

Potential CNS pathogen	Classification (genome type)	BioFIRE Filmrray-Meningitis /encephalitis	QIAstat-Dx Meningitis/ Encephalitis
Escherichia coli K1	Bacterium (DNA)	X	X
Haemophilus influenzae	Bacterium (DNA)	X	X
Listeria monocytogenes	Bacterium (DNA)	X	X
Mycoplasma pneumonia	Bacterium (DNA)		X
Neisseria meningitidis	Bacterium (DNA)	X	X
Streptococcus agalacticae	Bacterium (DNA)	X	X
Streptococcus pneumoniae	Bacterium (DNA)	X	X
Streptococcus pyogenes	Bacterium (DNA)		X
Cytomegalovirus	Herpesvirus (DNA)	X	
Herpes simplex virus 1	Herpesvirus (DNA)	X	X
Herpes simplex virus 2	Herpesvirus (DNA)	X	X
Human herpesvirus 6	Herpesvirus (DNA)	X	X
Enterovirus	Picornavirus (RNA)	X	X
Human parechovirus	Picornavirus (RNA)	X	X
Varicella zoster virus	Herpesvirus (DNA)	X	X
Cryptococcus neoformans/gattii	Yeast (DNA)	X	X

Table 2. Major studies implementing BioFIRE FilmArray-ME in resource-limited settings in which data on BioFIRE FilmArray-ME could be disaggregated. Diagnostic yield displayed excludes PCR detection of Cryptococcus neoformans that was concordant with conventional techniques (e.g. CSF CrAg testing) with higher sensitivity. This was to display a more representative diagnostic yield as some of the larger studies in Uganda included a high proportion of HIV-positive patients with cryptococcal meningitis.

	Criteria for CSF analysis with BioFIRE FilmArray-ME diagnostics	Number of samples analyzed	Number of patients with multiple pathogens detected in CSF	Diagnostic yield of molecular diagnostics (excluding Cryptococcus PCR detections)	Most commonly detected pathogens
Rhein et al., 2016 [82] Uganda	HIV positive individuals with suspected CNSI	69 25 negative for cryptococcosis 44 positive for cryptococcosis	2	7%	2.9% Varicella zoster virus 2.9% Cytomegalovirus 1.4% Human herpesvirus-6
Barnes et al., 2018 [83] Ethiopia	Any patient with suspected CNSI	218 216 negative for cryptococcosis 2 positive for cryptococcosis	0	9.2%	2.3% Human herpesvirus-6 1.4% Herpes simplex virus-1 0.9% Enterovirus
Tarai and Das, 2019 [85] India	Any patient with suspected CNSI	969 962 negative for cryptococcosis 7 positive for cryptococcosis*	1	9.6%	2.4% Enterovirus 1.0% Varicella zoster virus 0.9% Herpes simplex virus-1
Ellis et al., 2019 [11]. Uganda	HIV positive individuals with suspected CNSI and negative CSF CrAg and negative Xpert MTB/RIF Ultra	45 45 negative for cryptococcosis 0 positive for cryptococcosis	2	17.8%	4.4% Herpes simplex virus-1 *All other detections occurred at same frequency*
Peñata et al., 2020 [86] Colombia	Any patient with suspected CNSI	638 637 negative for cryptococcosis 11 positive for cryptococcosis	3	13.5%	2.5% *Streptococcus pneumoniae* 1.6% Herpes simplex virus-1 1.3% Varicella zoster virus
Bridge et al., 2021 [16] Uganda	HIV positive adults with suspected CNSI 106 with negative CSF CrAg and negative Xpert MTB/RIF Ultra 152 with positive CSF CrAg Any child with suspected CNSI	258 baseline/ admission samples 106 negative for cryptococcosis 152 positive for cryptococcosis	26	21.3% in HIV positive adults 7.1% in children	4.7% Human herpesvirus-6 4.3% Cytomegalovirus 4.3% *Streptococcus pneumoniae*
Debbagh et al., 2023 [84] Morocco	Any patient with suspected CNSI admitted to intensive care unit	112 111 negative for cryptococcosis 1 positive for cryptococcosis	0	17.1%	8.0% *Streptococcus pneumoniae* 3.6% Herpes simplex virus-1 2.7% *Haemophilus influenzae*

*3 negative CSF CrAg tests. Cryptococcosis diagnosed through India ink or culture.

sequencing and identification of all nucleic acid in a clinical sample, termed metagenomic sequencing for DNA sequencing and metatranscriptomic sequencing for RNA, offers an unbiased, hypothesis-free diagnostic approach for diagnosing neuroinvasive pathogens which has been employed in resource-rich settings [88,89]. Data is now emerging from resource-limited settings where it has been used to detect pathogens that would have been missed through conventional diagnostic approaches [90,91].

The majority of metagenomic sequencing has been performed on short-read sequencing platforms manufactured by Illumina, focusing on DNA fragments of less than 250 bp. However, long-read sequencing technologies are now becoming more commonplace with sequencers manufactured by Oxford Nanopore Technologies (ONT) and Pacific Biosciences sequencing long fragments of DNA (1,000 to 100,000 base pairs) [92]. ONT sequencers are of particular interest in resource-limited settings as they have potentially faster turnaround times, are easily transportable and the smaller sequencers have relatively low power consumption [93]. While short read sequencing remains the highest throughput sequencing technology with the lowest error rate, recent improvements in long-read sequencing mean that there are now comparable error rates in the data of both [94]. A previous advantage of short-read sequencing was the easy-to-use computational tools that were dedicated to the analysis of short read data only. However, pipelines such as Chan Zuckerberg ID (CZ ID) can now analyze data from ONT thereby simplifying the interpretation of the data and detection of pathogens, making this technology more accessible to countries without high-level analytical support [95].

The cost of metagenomic sequencing remains prohibitively high for routine diagnostics in resource-limited settings. However it has fallen over recent years with the expansion of DNA sequencing technologies and will continue to do so. The COVID-19 pandemic, and external funding opportunities, also greatly increased the sequencing capacity in many resource-limited countries. Therefore, despite financial challenges, targeted use of metagenomic sequencing on CSF samples to identify rare or novel pathogens could be

a crucial tool to inform routine PCR diagnostics and pathogen selection for multiplex panels.

9. Conclusion

Resource-limited settings are disproportionally affected by CNSI and prompt, accurate diagnosis is required to reduce morbidity and mortality. The diagnosis of CNSI in resource-limited settings is challenging due to an often poor understanding of regional epidemiology, high HIV-prevalence resulting in a broad differential and a reliance on traditional techniques that have poor sensitivity.

Molecular testing is widely used for CNSI diagnosis in resource-rich settings. Whilst these tests have markedly improved CNSI diagnosis certain tests have important limitations, such as the inability of currently available TB diagnostics to exclude TBM with a negative test. These need to be fully understood by clinicians to be successfully implemented.

The majority of molecular testing for CNSI in resource-limited settings is performed in trial settings and access outside of this is limited. This is mostly due to the cost of the platforms and cost-effectiveness data is lacking to support more widespread implementation. Cost-effectiveness data and involvement of policymakers is needed to identify how best these platforms can be used to improve the diagnosis of CNSI in resource-limited settings [12].

10. Expert opinion

CNSIs remain a major cause of morbidity and mortality in resource-limited settings and improved diagnostic platforms are urgently needed to provide rapid actionable results that can inform immediate treatment decisions and improve outcomes. Technologies exist that could improve diagnosis of CNSI in resource-limited settings but many are not widely used. Whilst there are multiple challenges unique to resource-limited settings that prevent widespread rollout of novel and molecular diagnostics, there are a number of success stories that demonstrate it is feasible. The primary constraint to greater adoption in resource-limited settings is financial; optimal use of these platforms potentially lies in clinically- and cost-effective algorithms tailored to local CNSI epidemiology. One example of an algorithm that would be relevant to high HIV-prevalence settings would be initial CSF testing with CrAg, if this is negative then analysis with Xpert MTB/RIF Ultra, and multiplex PCR testing performed on those samples still without a diagnosis. Whilst selective testing of patients as part of a diagnostic pathway that stops once a positive result is received is cost-effective there is a potential for missed co-infections and further data are therefore needed to identify which patients could be safely excluded. If diagnostic algorithms for investigation of CNSI were introduced, more extensive testing at sentinel sites as part of surveillance networks would still be required to identify changes in local CNSI epidemiology that may impact the clinical effectiveness of diagnostic algorithms. These networks would also be able to provide important epidemiological data that could inform future multiplex PCR panel adjustments to allow inclusion of analytes of local epidemiological importance. For example, the addition of JCV and *Toxoplasma gondii* in high HIV-prevalence settings and *M. tuberculosis* in TB endemic areas to multiple PCR panels would likely increase their clinical utility and cost-effectiveness.

Significant advancements in CNSI diagnostics have been made, but a number of pathogen-specific investigations still remain imperfect. In particular, the diagnosis is TBM remains challenging. Whilst Xpert MTB/RIF Ultra represents a notable progression it cannot be used in isolation and is unable to exclude TB. As such continued research and development in new diagnostics is crucial to reduce mortality from CNSI worldwide.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Wunrow HY, Bender RG, Vongpradith A, et al. Global, regional, and national burden of meningitis and its aetiologies, 1990–2019: a systematic analysis for the global burden of disease study 2019. Lancet Neurol [Internet]. 2023 Aug;22(8):685–711. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1474442223001953
   - **Robust estimates of global meningitis burden highlighting increased incidence and mortality of CNSI in resource-limited settings.**
2. Wang H, Zhao S, Wang S, et al. Global magnitude of encephalitis burden and its evolving pattern over the past 30 years. J Infect. 2022 Jun 1;84(6):777–787. doi: 10.1016/j.jinf.2022.04.026
3. Rajasingham R, Govender NP, Jordan A, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet Infect Dis [Internet]. 2022;22(12):1748–1755. doi: 10.1016/S1473-3099(22)00499-6
4. Dodd PJ, Osman M, Cresswell FV, et al. The global burden of tuberculous meningitis in adults: a modelling study. PLoS Glob Public Health. 2021 Dec 8;1(12):e0000069. doi: 10.1371/journal.pgph.0000069

5. Zunt JR, Kassebaum NJ, Blake N, et al. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the global burden of disease study 2016. Lancet Neurol. 2018 Dec 1;17(12):1061–1082. doi: 10.1016/S1474-4422(18)30387-9
6. Sulaiman T, Salazar L, Hasbun R. Acute versus subacute community-acquired meningitis: analysis of 611 adult patients. Open Forum Infect Dis. 2016;3(suppl_1). doi: 10.1093/ofid/ofw172.878
7. Tenforde MW, Mokomane M, Leeme T, et al. Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: results from the Botswana national meningitis survey. J Infect. 2019;79(3):212–219. doi: 10.1016/j.jinf.2019.06.013
8. Dittrich S, Rattanavong S, Lee SJ. et al. Orientia, rickettsia, and Leptospira pathogens as causes of CNS infections in Laos: a prospective study. Lancet Glob Health. 2015 Feb;3(2):e104–12
9. Lingani C, Bergeron-Caron C, Stuart JM, et al. Meningococcal meningitis surveillance in the African meningitis belt, 2004–2013. Clin Infect Dis. 2015 Nov 15;61(suppl_5):S410–5. doi: 10.1093/cid/civ597
10. Siddiqi OK, Ghebremichael M, Dang X, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. ClinI Infect Dis. 2014 Jun 15;58(12):1771–1777. doi: 10.1093/cid/ciu191
11. Ellis J, Bangdiwala AS, V CF, et al. The changing epidemiology of HIV-Associated adult meningitis, Uganda 2015–2017. Open Forum Infect Dis. 2019 Oct 1;6(10). doi: 10.1093/ofid/ofz419
12. Tenforde MW, Gertz AM, Lawrence DS, et al. Mortality from HIV-associated meningitis in sub-Saharan Africa: a systematic review and meta-analysis. J Int AIDS Soc. 2020 Jan;23(1). doi: 10.1002/jia2.25416
13. DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. Clin Microbiol Rev. 2004;17(4):903–925. doi: 10.1128/CMR.17.4.903-925.2004
14. McGill F, Heyderman RS, Michael BD, et al. The UK joint specialist societies guideline on the diagnosis and management of acute meningitis and meningococcal sepsis in immunocompetent adults. J Infect. 2016 Apr 1;72(4):405–438. doi: 10.1016/j.jinf.2016.01.007
15. Karakousis A, Tan L, Ellis D. et al. An assessment of the efficiency of fungal DNA extraction methods for maximizing the detection of medically important fungi using PCR. J Microbiol Methods. 2006 Apr;65(1):38–48
16. Bridge S, Huppler K, Nerima C, et al. Evaluation of the BioFire Ò FilmArray Ò Meningitis/Encephalitis panel in an adult and pediatric Ugandan population. J Med Mycol. 2021;31(3):101170. doi: 10.1016/j.mycmed.2021.101170
17. Cresswell FV, Tugume L, Bahr NC, et al. Xpert MTB/RIF ultra for the diagnosis of HIV-associated tuberculous meningitis: a prospective validation study. Lancet Infect Dis. 2020 Mar 1;20(3):308–317. doi: 10.1016/S1473-3099(19)30550-X
•• **Large-scale trial in high HIV-prevalence setting demonstrating clinical utility of Xpert MTB/RIF Ultra in the diagnosis of TB meningitis.**
18. Scarborough M, Thwaites GE. The diagnosis and management of acute bacterial meningitis in resource-poor settings. Lancet Neurol. 2008;7(7):637–648. doi: 10.1016/S1474-4422(08)70139-X
19. Jarvis JN, Meintjes G, Williams A, et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC Infect Dis. 2010;10(1):10. doi: 10.1186/1471-2334-10-67
20. Tenforde MW, Meintjes G, Mokomane M, et al. Low-cerebrospinal fluid white cell counts and mortality in HIV-associated pneumococcal meningitis. AIDS. 2019 Jul 15;33(9):1539–1541.
21. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. ClinI Infect Dis. 2004;1October129(9):1267–1284. doi: 10.1086/425368
22. Poplin V, Boulware DR, Bahr NC. Methods for rapid diagnosis of meningitis etiology in adults. Biomarker Med. 2020;14(6):459–479. doi: 10.2217/bmm-2019-0333
23. Jarvis JN, Bicanic T, Loyse A. et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. ClinI Infect Dis. 2014 Mar;58(5):736–745
24. Bohr V, Rasmussen N, Hansen B. et al. 875 cases of bacterial meningitis: diagnostic procedures and the impact of preadmission antibiotic therapy part III of a three-part series. J Infect. 1983 Nov;7(3):193–202
25. 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 Nov;108(5):1169–1174. doi: 10.1542/peds.108.5.1169
26. Tschopp J, Brunel AS, Spertini O, et al. High false-positive rate of (1,3)-β-D-Glucan in onco-hematological patients receiving immunoglobulins and therapeutic antibodies. ClinI Infect Dis. 2022 Aug 25;75(2):330–333. doi: 10.1093/cid/ciab1028
27. Kanamori H, Kanemitsu K, Miyasaka T, et al. Measurement of (1-3)-. BETA.-D-Glucan derived from different gauze types. Tohoku J Exp Med. 2009;217(2):117–121. doi: 10.1620/tjem.217.117
28. Davis C, Wheat LJ, Myint T, et al. Efficacy of cerebrospinal fluid beta-D-glucan diagnostic testing for fungal meningitis: a systematic review. J Clin Microbiol [Internet]. 2020;58(4). doi: 10.1128/jcm.02094-19
29. Forster J, Hoenigl M, Suerbaum S, et al. Serologic biomarkers in Candida and aspergillus infections of the central nervous system: a comparison of galactomannan, mannan and β-1,3-D-gucan testing from serum and cerebrospinal fluid. Mycoses. 2022 Jul 1;65(7):709–714.
30. Song J, Kim SH, Jung YR, et al. 10-year retrospective review of the etiologies for meningitis with elevated adenosine deaminase in cerebrospinal fluid: etiologies other than TB. Front Cell Infect Microbiol. 2022 Jul 5;12. doi: 10.3389/fcimb.2022.858724
31. Ekermans P, Dusé A, George J. The dubious value of cerebrospinal fluid adenosine deaminase measurement for the diagnosis of tuberculous meningitis. BMC Infect Dis. 2017 Dec 31;17(1):104.
32. Mishra O. Cerebrospinal fluid adenosine deaminase activity for the diagnosis of tuberculous meningitis in children. J Trop Pediatr. 1996 Jun 1;42(3):129–132.
33. Macrae C, Ellis J, Keddie SH, et al. Diagnostic performance of the IMMY cryptococcal antigen lateral flow assay on serum and cerebrospinal fluid for diagnosis of cryptococcosis in HIV-negative patients: a systematic review. BMC Infect Dis. 2023 Apr 6;23(1):209. doi: 10.1186/s12879-023-08135-w
34. Temfack E, Rim JJB, Spijker R, et al. Cryptococcal antigen in serum and cerebrospinal fluid for detecting cryptococcal meningitis in adults living with human immunodeficiency virus: systematic review and meta-analysis of diagnostic test accuracy studies. ClinI Infect Dis. 2021 Apr 8;72(7):1268–1278. doi: 10.1093/cid/ciaa1243
35. Tadeo KK, Nimwesiga A, Kwizera R, et al. Evaluation of the diagnostic performance of a semiquantitative cryptococcal antigen point-of-care assay among HIV-Infected persons with cryptococcal meningitis. J Clin Microbiol. 2021 Jul 1;59(8). doi: 10.1128/JCM.00860-21
36. Feagins AR, Ronveaux O, Taha MK. et al. Next generation rapid diagnostic tests for meningitis diagnosis. J Infect. 2020 Nov;81(5):712–718
37. Haddar C, Terrade A, Verhoeven P. et al. Validation of a new rapid detection test for detection of Neisseria meningitidis A/C/W/X/Y antigens in cerebrospinal fluid. J Clin Microbiol. 2020 Feb;58(3):e01699–19
38. WHO. Meeting report of a technical expert consultation: non-inferiority analysis of xpert MTB/RIF ultra compared to xpert MTB/RIF [Internet]. 2017. Available from: http://apps.who.int/bookorders
39. Heemskerk AD, Donovan J, Thu DDA, et al. Improving the microbiological diagnosis of tuberculous meningitis: A prospective, international, multicentre comparison of conventional and modified Ziehl–Neelsen stain, GeneXpert, and culture of cerebrospinal fluid. J Infect. 2018 Dec 1;77(6):509–515. doi: 10.1016/j.jinf.2018.09.003
40. Seid G, Alemu A, Dagne B, et al. Microbiological diagnosis and mortality of tuberculosis meningitis: systematic review and meta-analysis. PLoS One. 2023 Feb 1;18(2):e0279203.

41. Chen P, Shi M, Feng GD. et al. A highly efficient Ziehl-Neelsen stain: Identifying de novo intracellular Mycobacterium tuberculosis and improving detection of extracellular M. tuberculosis in cerebrospinal fluid. J Clin Microbiol. 2012 Apr;50(4):1166–1170
42. Stadelman AM, Ssebambulidde K, Buller A, et al. Cerebrospinal fluid AFB smear in adults with tuberculous meningitis: a systematic review and diagnostic test accuracy meta-analysis. SSRN Electron J. 2021. doi: 10.2139/ssrn.3770634
43. Donovan J, Thu DDA, Phu NH, et al. Xpert MTB/RIF ultra versus xpert MTB/RIF for the diagnosis of tuberculous meningitis: a prospective, randomised, diagnostic accuracy study. Lancet Infect Dis. 2020 Mar 1;20(3):299–307. doi: 10.1016/S1473-3099(19)30649-8
•• **Important study demonstrating superiority of Xpert MTB/RIF Ultra in detection of M. tuberculosis in low bacillary loads.**
44. Bahr NC, Nuwagira E, Evans EE, et al. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis. 2018 Jan 1;18(1):68–75. doi: 10.1016/S1473-3099(17)30474-7
45. Kohli M, Schiller I, Dendukuri N, et al. Xpert MTB/RIF ultra and xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2021 Jan 15;1(1):CD012768. doi: 10.1002/14651858.CD012768.pub3. PMID: 33448348; PMCID: PMC8078545.
46. Donovan J, Cresswell FV, Thuong NTT, et al. Xpert MTB/RIF ultra for the diagnosis of tuberculous meningitis: a small step forward. Clin Infec Dis. 2020 Nov 5;71(8):2002–2005. doi: 10.1093/cid/ciaa473
47. Broger T, Nicol MP, Szekely R, et al. Diagnostic accuracy of a novel tuberculosis point-of-care urine lipoarabinomannan assay for people living with HIV: a meta-analysis of individual in- a nd outpatient data. PLoS Med. 2020;17(5):1–16. doi: 10.1371/journal.pmed.1003113
48. Siddiqi OK, Birbeck GL, Ghebremichael M, et al. Prospective cohort study on performance of cerebrospinal fluid (CSF) xpert MTB/RIF, CSF lipoarabinomannan (LAM) lateral flow assay (LFA), and urine LAM LFA for diagnosis of tuberculous meningitis in Zambia. J Clin Microbiol. 2019;57(8). doi: 10.1128/JCM.00652-19
49. Quinn CM, Kagimu E, Okirworth M, et al. Fujifilm SILVAMP TB LAM assay on cerebrospinal fluid for the detection of tuberculous meningitis in adults with human immunodeficiency virus. Clinl Infect Dis. 2021;73(9):e3428–34. doi: 10.1093/cid/ciaa1910
50. Sharma K, Sharma M, Modi M, et al. Comparative analysis of TruenatE MTB plus and XpertW Ultra in diagnosing tuberculous meningitis. Int J Tuberc Lung Dis. 2021 Aug 1;25(8):626–631. doi: 10.5588/ijtld.21.0156
51. Bossolasco S, Calori G, Moretti F, et al. Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-Associated progressive multifocal leukoencephalopathy [Internet]. Available from: https://academic.oup.com/cid/article/40/5/738/365769
52. Nakamichi K, Kawamoto M, Ishii J. et al. Improving detection of JC virus by ultrafiltration of cerebrospinal fluid before polymerase chain reaction for the diagnosis of progressive multifocal leukoencephalopathy. BMC neurol. 2019 Oct 25;19(1). doi: 10.1186/s12883-019-1476-2
53. Ferretti F, Bestetti A, Yiannoutsos CT, et al. Diagnostic and prognostic value of JC virus DNA in plasma in progressive multifocal leukoencephalopathy. Clinl Infect Dis. 2018 Jun 18;67(1):65–72. doi: 10.1093/cid/ciy030
54. Tan IL, McArthur JC, Clifford DB, et al. Immune reconstitution inflammatory syndrome in natalizumab-associated PML. Neurology. 2011 Sep;77(11):1061–1067. doi: 10.1212/WNL.0b013e31822e55e7
55. Berger JR, Aksamit AJ, Clifford DB, et al. VIEWS & REVIEWS PML diagnostic criteria consensus statement from the AAN neuroinfectious disease section [Internet]. 2013. Available from: www.neurology.org
56. Miskin DP, Ngo LH, Koralnik IJ. Diagnostic delay in progressive multifocal leukoencephalopathy. Ann Clin Transl Neurol. 2016;3(5):386–391. doi: 10.1002/acn3.301
57. Sharma SK, Soneja M, Ranjan S, et al. Progressive multifocal leucoencephalopathy in HIV/AIDS: observational study from a tertiary care centre in northern India. Indian J Med Res. 2013;138(JUL 2013):72–77.
58. Arora S, Ahmad FMH, Deshwal R, et al. Study of clinical profile and outcomes in progressive multifocal leukoencephalopathy in acquired immunodeficiency syndrome patients in the highly active antiretroviral therapy era - an observational study. Indian J Sex Transm Dis AIDS. 2022;43(2):156–160. doi: 10.4103/ijstd.IJSTD_29_18
59. Vidal JE, de OA, MCDS F, et al. Aids-related progressive multifocal leukoencephalopathy: a retrospective study in a referral center in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 2008 Aug;50(4):209–212. doi: 10.1590/S0036-46652008000400004
60. Schutte CM, Ranchhod N, Kakaza M, et al. AIDS-related progressive multifocal leukoencephalopathy (PML): a retrospective study from Pretoria, South Africa. S Afr Med J. 2013;103(6):399–401. doi: 10.7196/SAMJ.6386
61. Sim BZ, Smith LK, Yarwood T, et al. Successful treatment of HIV-associated progressive multifocal leukoencephalopathy with pembrolizumab. AIDS. 2022 Mar 1;36(3):483–485.
62. Safarpour H, Cevik M, Zarean M, et al. Global status of Toxoplasma gondii infection and associated risk factors in people living with HIV. AIDS. 2020 Mar 1;34(3):469–474. doi: 10.1097/QAD.0000000000002424
63. Bokharaei-Salim F, Esteghamati A, Khanaliha K, et al. Evaluation of a PCR assay for diagnosis of toxoplasmosis in serum and peripheral blood mononuclear cell among HIV/AIDS patients. J Parasitic Dis. 2020 Mar 1;44(1):159–165. doi: 10.1007/s12639-019-01176-6
64. Wang ZD, Wang SC, Liu HH, et al. Prevalence and burden of Toxoplasma gondii infection in HIV-infected people: a systematic review and meta-analysis. Lancet HIV. 2017;4(4):e177–88. doi: 10.1016/S2352-3018(17)30005-X
65. Liu Q, Wang ZD, Huang SY, et al. Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasites Vectors. 2015 Dec 28;8(1):292. doi: 10.1186/s13071-015-0902-6
66. Roux G, Varlet-Marie E, Bastien P, et al. Evolution of toxoplasma-PCR methods and practices: a French national survey and proposal for technical guidelines. Int J Parasitol. 2018;48(9–10):701–707. doi: 10.1016/j.ijpara.2018.03.011
67. Wesołowski R, Pawłowska M, Smoguła M, et al. Advances and challenges in diagnostics of toxoplasmosis in HIV-Infected patients. Pathogens. 2023 Jan 9;12(1):110. doi: 10.3390/pathogens12010110
68. Campbell GL, Hills SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ. 2011;89(10):766–774. doi: 10.2471/BLT.10.085233
69. Mackenzie JS, Williams DT, van den Hurk AF, et al. Japanese encephalitis virus: the emergence of genotype IV in Australia and its potential endemicity. Viruses. 2022 Nov 9;14(11):2480.
70. Bharucha T, Sengvilaipaseuth O, Vongsouvath M, et al. Development of an improved RT-qPCR assay for detection of Japanese encephalitis virus (JEV) RNA including a systematic review and comprehensive comparison with published methods. PLoS One. 2018 Mar 1;13(3):e0194412. doi: 10.1371/journal.pone.0194412
71. Bharucha T, Shearer FM, Vongsouvath M, et al. A need to raise the bar — A systematic review of temporal trends in diagnostics for Japanese encephalitis virus infection, and perspectives for future research. Inter J Infect Dis. 2020 Jun 1;95:444–456.
72. Rowley J, Vander HS, Korenromp E, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ. 2019 Aug 1;97(8):548–562P. doi: 10.2471/BLT.18.228486
73. Marks M, Jarvis JN, Howlett W, et al. Neurosyphilis in Africa: a systematic review. PLoS Negl Trop Dis. 2017 Aug 1;11(8):e0005880. doi: 10.1371/journal.pntd.0005880
74. de Voux A, Kidd S, Torrone EA. Reported cases of neurosyphilis among early syphilis cases—United States, 2009 to 2015. Sex Transm Dis. 2018 Jan;45(1):39–41. doi: 10.1097/OLQ.0000000000000687

75. Ropper AH, Neurosyphilis, Longo DL editor, et al. Neurosyphilis. N Engl J Med [Internet]. 2019 Oct 3;381(14):1358–1363. doi: 10.1056/NEJMra1906228
76. Marks M, Lawrence D, Kositz C. et al. Diagnostic performance of PCR assays for the diagnosis of neurosyphilis: a systematic review. Sex Transm Infect. 2018 Dec;94(8):585–588
77. Gonzalez H, Koralnik IJ, Huhn GD, et al. A dual-platform point-of-care test for neurosyphilis diagnosis. Sex Transm Dis. 2021 May 1;48(5):353–356. doi: 10.1097/OLQ.0000000000001308
78. Leber A, Everhart K, Balada-Llasat J, et al. Multi-center clinical evaluation of a multiplex Meningitis/Encephalitis PCR panel for simultaneous detection of bacteria, yeast, and viruses in cerebrospinal fluid specimens. J Clin Microbiol. 2016;54(9):2251–2261. doi: 10.1128/JCM.00730-16
•• **Large-scale prospective study demonstrating added clinical value of rapid multiplex PCR platforms in the diagnosis of CNSI.**
79. Tansarli GS, Chapin KC. Diagnostic test accuracy of the BioFire® FilmArray® meningitis/encephalitis panel: a systematic review and meta-analysis. Clin Microbiol Infect. 2020;26(3):281–290. doi: 10.1016/j.cmi.2019.11.016
80. Humisto A, Antikainen J, Holma T, et al. Evaluation of the novel CE-IVD-Marked multiplex PCR QIAstat-dx Meningitis/Encephalitis panel. Microbiol Spectr. 2023;11(3):1–11. doi: 10.1128/spectrum.05144-22
81. QIAGEN. *Sample to insight QIAstat-dx ® Meningitis/Encephalitis (ME) panel instructions for use (handbook) 6*. 2022.
82. Rhein J, Bahr NC, Hemmert AC, et al. Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. Diagn Microbiol Infect Dis. 2016 Mar;84(3):268–273. doi: 10.1016/j.diagmicrobio.2015.11.017
• **First description of rapid multiplex PCR platform implementation in high HIV-prevalence settings.**
83. Bårnes GK, Gudina EK, Berhane M, et al. New molecular tools for meningitis diagnostics in Ethiopia - a necessary step towards improving antimicrobial prescription. BMC Infect Dis. 2018;18(1):1–14. doi: 10.1186/s12879-018-3589-4
84. Debbagh F, Harrar S, Babokh F, et al. The contribution of multiplex polymerase chain reaction in the diagnosis of central nervous system infections in intensive care units. Cureus. 2023;15(2). doi: 10.7759/cureus.35338
85. Tarai B, Das P. FilmArray® meningitis/encephalitis (ME) panel, a rapid molecular platform for diagnosis of CNS infections in a tertiary care hospital in North India: one-and-half-year review. Neurol Sci. 2019;40(1):81–88. doi: 10.1007/s10072-018-3584-y
86. Peñata A, Mesa S, Leal A, et al. Molecular diagnosis of meningitis and meningoencephalitis with an automated real-time multiplex polymerase chain reaction in a tertiary reference complex in medellín, Colombia. Rev Inst Med Trop Sao Paulo. 2020;62:e77. doi: 10.1590/s1678-9946202062077
87. Brisset J, Angendu Baki K, Watier L, et al. Non-traumatic coma in young children in Benin: are viral and bacterial infections gaining ground on cerebral malaria? Infect Dis Poverty. 2022;11(1):1–10. doi: 10.1186/s40249-022-00956-2
88. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clinl Infect Dis. 2018;66(5):778–788. doi: 10.1093/cid/cix881
89. Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med. 2019;380(24):2327–2340. doi: 10.1056/NEJMoa1803396
• **Study demonstrating the potential clinical usefulness of metagenomic sequencing.**
90. Hong NTT, Anh NT, Mai NTH, et al. Performance of metagenomic next-generation sequencing for the diagnosis of viral meningoencephalitis in a resource-limited setting. Open Forum Infect Dis. 2020;7(3):1–10. doi: 10.1093/ofid/ofaa046
91. Ramachandran PS, Ramesh A, Creswell FV, et al. Integrating central nervous system metagenomics and host response for diagnosis of tuberculosis meningitis and its mimics. Nat Commun. 2022;13(1):1–12. doi: 10.1038/s41467-022-29353-x
92. Houlihan CF, Bharucha T, Breuer J. Advances in molecular diagnostic testing for central nervous system infections. Curr Opin Infect Dis. 2019;32(3):244–250. doi: 10.1097/QCO.0000000000000548
93. Hong NTT, Anh NT, Mai NTH, et al. Performance of metagenomic next-generation sequencing for the diagnosis of viral meningoencephalitis in a resource-limited setting. Open Forum Infect Dis. 2020 Mar 1;7(3). doi: 10.1093/ofid/ofaa046
94. Wick RR, Judd LM, Holt KE, et al. Assembling the perfect bacterial genome using oxford nanopore and Illumina sequencing. PLoS Comput Biol. 2023 Mar 1;19(3):e1010905. doi: 10.1371/journal.pcbi.1010905
95. Hu T, Chitnis N, Monos D, et al. Next-generation sequencing technologies: an overview. Hum Immunol. 2021;82(11):801–811. doi: 10.1016/j.humimm.2021.02.012

1.7 References

1. UNAIDS. *UNAIDS Global AIDS Update 2022. Accessed 2/3/24*. (2022).
2. Wunrow, H. Y. *et al.* Global, regional, and national burden of meningitis and its aetiologies, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* **22**, 685–711 (2023).
3. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: Findings from 4961 suspected cases. *BMC Infect Dis* **10**, (2010).
4. Tenforde, M. W. *et al.* Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey. *Journal of Infection* **79**, 212–219 (2019).
5. Bårnes, G. K. *et al.* New molecular tools for meningitis diagnostics in Ethiopia - A necessary step towards improving antimicrobial prescription. *BMC Infect Dis* **18**, 1–14 (2018).
6. Ouedraogo, D. *et al.* Neuromeningeal Cryptococcosis and Tuberculosis Coinfection in Bamako: A One-Year Case Series. *Open Forum Infect Dis* **10**, (2023).
7. Assogba, K. *et al.* Neuromeningeal cryptococcosis in sub-Saharan Africa: Killer disease with sparse data. *J Neurosci Rural Pract* **6**, 221–224 (2015).
8. Owusu, M. *et al.* Aetiological agents of cerebrospinal meningitis: a retrospective study from a teaching hospital in Ghana. *Ann Clin Microbiol Antimicrob* **11**, (2012).
9. Walker, A. S. *et al.* Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. *Clin Infect Dis* **55**, 1707–1718 (2012).
10. Lawn, S. D., Harries, A. D., Anglaret, X., Myer, L. & Wood, R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS (London, England)* vol. 22 1897–1908 Preprint at https://doi.org/10.1097/QAD.0b013e32830007cd (2008).
11. Rajasingham, R. *et al.* Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* **17**, 873–881 (2017).

12. Rajasingham, R. *et al.* Epidemiology of meningitis in an HIV-infected Ugandan cohort. *American Journal of Tropical Medicine and Hygiene* **92**, 274–279 (2015).

13. Ellis, J. *et al.* The Changing Epidemiology of HIV-Associated Adult Meningitis, Uganda 2015-2017. *Open Forum Infect Dis* **6**, 1–8 (2019).

14. Siddiqi, O. K. *et al.* Molecular diagnosis of central nervous system opportunistic infections in HIV-infected zambian adults. *Clinical Infectious Diseases* **58**, 1771–1777 (2014).

15. Bridge, S. *et al.* Evaluation of the BioFire Ò FilmArray Ò Meningitis / Encephalitis panel in an adult and pediatric Ugandan population. *Journal of Medical Mycology* **31**, 101170 (2021).

16. Benjamin, L. A. *et al.* Detection of herpes viruses in the cerebrospinal fluid of adults with suspected viral meningitis in Malawi. *Infection* **41**, 27–31 (2013).

17. Obaro, S. *et al.* Comparison of bacterial culture with BioFire® FilmArray® multiplex PCR screening of archived cerebrospinal fluid specimens from children with suspected bacterial meningitis in Nigeria. *BMC Infect Dis* **23**, (2023).

18. Rhein, J. *et al.* Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis* **84**, 268–273 (2016).

19. Kanegaye, J. T., Soliemanzadeh, P. & Bradley, J. S. Lumbar puncture in pediatric bacterial meningitis: Defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* **108**, 1169–1174 (2001).

20. Mitchell, H. K. *et al.* Causes of Pediatric Meningitis in Botswana: Results From a 16-Year National Meningitis Audit. *Pediatr Infect Dis J* **38**, 906–911 (2019).

21. Tenforde, M. W. *et al.* Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana national meningitis survey: a prevalent cohort study. *Lancet Infect Dis* **19**, 740–749 (2019).

22. Ellis, J. *et al.* Clinical management of community-acquired meningitis in adults in the UK and Ireland in 2017: a retrospective cohort study on behalf of the National Infection Trainees Collaborative for Audit and Research (NITCAR). *BMJ Open* **12**, 1–10 (2022).

23. Mfinanga, S. *et al.* Reduction in mortality from HIV-related CNS infections in routine care in Africa (DREAMM): a before-and-after, implementation study. *Lancet HIV* **10**, e663–e673 (2023).

24. Bodilsen, J. *et al.* Early versus late diagnosis in community-acquired bacterial meningitis: a retrospective cohort study. *Clinical Microbiology and Infection* **24**, 166–170 (2018).

25. Thwaites, G. *et al.* British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *Journal of Infection* **59**, 167–187 (2009).

26. Sheu, J.-J., Yuan, R.-Y. & Yang, C.-C. Predictors for outcome and treatment delay in patients with tuberculous meningitis. *Am J Med Sci* **338**, 134–139 (2009).

27. Wall, E. C. *et al.* High Mortality amongst Adolescents and Adults with Bacterial Meningitis in Sub-Saharan Africa: An Analysis of 715 Cases from Malawi. *PLoS One* **8**, (2013).

28. Scarborough, M. & Thwaites, G. E. The diagnosis and management of acute bacterial meningitis in resource-poor settings. *Lancet Neurol* **7**, 637–648 (2008).

29. Leber, A. *et al.* Multi-Center Clinical Evaluation of a Multiplex Meningitis / Encephalitis PCR Panel for Simultaneous Detection of Bacteria, Yeast, and Viruses in Cerebrospinal Fluid Specimens. *J Clin Microbiol* **54**, 2251–2261 (2016).

30. Cresswell, F. V. *et al.* Xpert MTB/RIF Ultra for the diagnosis of HIV-associated tuberculous meningitis: a prospective validation study. *Lancet Infect Dis* **20**, 308–317 (2020).

31. Huang, H. R., Fan, L. C., Rajbanshi, B. & Xu, J. F. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: A meta-analysis and systematic review. *PLoS One* **10**, 1–10 (2015).

32. Van De Beek, D. *et al.* Community-acquired bacterial meningitis. *Nat Rev Dis Primers* **2**, 1–21 (2016).

33. Joffe, A. R. Lumbar puncture and brain herniation in acute bacterial meningitis: A review. *J Intensive Care Med* **22**, 194–207 (2007).

34. McGill, F. *et al.* The UK joint specialist societies guideline on the diagnosis and management of acute meningitis and meningococcal sepsis in immunocompetent adults. *Journal of Infection* **72**, 405–438 (2016).

35. Ettekoven, C. N. Van, Beek, D. Van De & Brouwer, M. C. Update on community-acquired bacterial meningitis : guidance and challenges. *Clinical Microbiology and Infection* **23**, 601–606 (2017).

36. Poplin, V., Boulware, D. R. & Bahr, N. C. Methods for rapid diagnosis of meningitis etiology in adults. *Biomark Med* **14**, 459–479 (2020).

37. Wu, H. M. *et al.* Accuracy of real-time PCR, Gram stain and culture for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae meningitis diagnosis. *BMC Infect Dis* **13**, (2013).

38. Chanteau, S. *et al.* Scaling up of PCR-based surveillance of bacterial meningitis in the African meningitis belt: indisputable benefits of multiplex PCR assay in Niger. *Trans R Soc Trop Med Hyg* **100**, 677–680 (2006).

39. Sidikou, F. *et al. Polymerase Chain Reaction Assay and Bacterial Meningitis Surveillance in Remote Areas, Niger*. www.cdc.gov/eid.

40. Issa, M. *et al.* PCR of cerebrospinal fluid for diagnosis of bacterial meningitis during meningococcal epidemics; an example from Sudan. *Scand J Infect Dis* **35**, 719–723 (2003).

41. Parent, I. *et al. Bacterial Meningitis in Burkina Faso: Surveillance Using Field-Based Polymerase Chain Reaction Testing Association Pour l'Aide a ` La*. (2005).

42. Bonington, A. *et al. Use of Roche AMPLICOR Mycobacterium Tuberculosis PCR in Early Diagnosis of Tuberculous Meningitis*. *JOURNAL OF CLINICAL MICROBIOLOGY* vol. 36 (1998).

43. Stoner, G. L. *et al. Detection of JC Virus in Two African Cases of Progressive Multifocal Leukoencephalopathy Including Identification of JCV Type 3 in a Gambian AIDS Patient*. *J. Med. Microbiol* vol. 47 (1998).

44. Bridge, S. *et al.* MENINGITIS / ENCEPHALITIS PANEL IN AND ADULT AND. *Journal of Medical Mycology* 101170 (2021) doi:10.1016/j.mycmed.2021.101170.

45. Bårnes, G. K. *et al.* New molecular tools for meningitis diagnostics in Ethiopia - A necessary step towards improving antimicrobial prescription. *BMC Infect Dis* **18**, 1–14 (2018).

46. Williams, C. G. *et al.* Diagnostic Yield of Adding the BioFIRE FilmArray Meningitis/Encephalitis panel to routinely available diagnostics for Central Nervous System Infections in Botswana. in *ASTMH* (2021).

47. Musukuma-Chifulo K, G. M. C. O. B. M. M. S. S. M. C. C. T. J. S. E. K. I. D. X. C. R. S. OK. Characterizing Epstein-Barr virus infection of the central nervous system in Zambian adults living with HIV. . *J Neurovirol* **29**, 706–712 (2023).

48. Heneghan, N., Fu, J., Pritchard, J., Payton, M. & Allen, R. W. The effect of environmental conditions on the rate of RNA degradation in dried blood stains. *Forensic Sci Int Genet* **51**, (2021).

49. Wesołowski, R., Pawłowska, M., Smoguła, M. & Szewczyk-Golec, K. Advances and Challenges in Diagnostics of Toxoplasmosis in HIV-Infected Patients. *Pathogens* vol. 12 Preprint at https://doi.org/10.3390/pathogens12010110 (2023).

Chapter II: Aims, Objectives and Methodology

This chapter will outline the overall aim of this research, specific hypotheses, and objectives and describe how each of these will be addressed. The overarching methods for establishing the two prospective meningitis surveillance networks, the Botswana National Meningitis Survey: protocol 3 (BNMS3) in Gaborone, Botswana and the Harare Meningitis Aetiology Survey (HarMenAeS) in Harare, Zimbabwe which generated the majority of data included in this thesis will also be described in this chapter. This will focus on the fieldwork required to set up the projects and embed them within existing healthcare systems. Specific methodologies for each individual project and relevant analyses are included in each chapter and protocols for both projects are included in the appendix.

2.1 Aim

The overall aim of this thesis is to identify strategies to improve the diagnosis of CNSI in high HIV-prevalence settings. The aim of this thesis is to describe the current epidemiology of central nervous system infections in Botswana and Zimbabwe, identify strategies to improve the diagnosis of patients with suspected central nervous system infections in low-resource, high HIV-prevalence settings and describe the impact of improved diagnostics when used in routine care.

2.2 Problem statement

CNSI are common in high HIV-prevalence African settings, but their epidemiology is poorly understood. HIV has altered the epidemiology of meningitis in the region and widened the differential diagnosis. Large numbers of patients with CNSI in high HIV-prevalence African settings do not receive a diagnosis, the majority of whom are people living with HIV, and this is associated with high mortality. Diagnostic capacity is limited and often only consists of basic investigations with limited sensitivity and specificity. The majority of molecular diagnostics including BioFIRE FilmArray-ME are developed in the Global North using data on regional CNSI epidemiology and it is uncertain how well these platforms translate for use in high HIV-prevalence settings where the CNSI epidemiology is different.

The reason why large numbers of patients do not receive a diagnosis is likely multifactorial and however it is unclear why this carries such a high mortality. The failure to make a diagnosis is impacted by multiple challenges within the diagnostic pathway: (1) Lack of robust transport infrastructure and financial constraints limiting ability for prompt transfer to hospital, (2) Complex household/societal networks requiring senior members to decide when to seek medical advice, (3) Under recognition of CNSI in the community, (4) Delays in lumbar punctures occurring due to several reasons including lack of resources and restricted access to neuroimaging, (5) A lack of diagnostics that can reliably diagnose

common CNSI in high HIV-prevalence African settings meaning appropriate treatment is often not administered and (6) Under-utilisation of currently available diagnostics.

2.3 Hypotheses

Hypothesis 1: The addition of enhanced diagnostics will reveal large numbers of previously undiagnosed TB meningitis and viral CNSI. Molecular platforms will also increase the number of bacterial CNSI due to an ability to overcome challenges of rapid sterilisation of CSF following antibiotic administration and subsequent low culture yield. The introduction of rapid molecular diagnostics, including those used as part of an enhanced diagnostic package, for the investigation of patients with suspected CNSI in low-resource, high HIV-prevalence settings will significantly increase the number of microbiologically confirmed diagnoses and decrease the number of patients without a confirmed microbiological diagnosis.

Hypothesis 2: BioFIRE FilmArray-ME will increase the total number of diagnoses made above routine diagnostics alone in patients with suspected CNSI in high HIV-prevalence African settings. However, if used in isolation there will be a significant proportion of patients with inflammatory CSF suggestive of CNSI and no confirmed diagnosis. Diagnostic yield from BioFIRE FilmArray-ME will vary between population groups and there may be the potential to target these to individuals where diagnostic yield will be greatest.

Hypothesis 3: Cryptococcal meningitis will remain the most common cause of CNSI in Botswana and Zimbabwe due to high HIV prevalence but incidence will decrease with increased ART coverage. Improved access to CNSI diagnostics, such as Xpert MTB/RIF, used as part of routine care will increase the number of tests performed and the number of confirmed diagnoses made.

Hypothesis 4: Computed tomography in resource-limited settings will delay diagnosis and targeted treatment for CNSI including for those CNSI which are more common in the region such as cryptococcal and tuberculous meningitis.

2.4 Objectives

Objective 1: Define the current epidemiology of central nervous system infections in a high HIV-prevalence African setting including the impact of an enhanced diagnostic package on diagnostic yield and the proportion of patients with inflammatory CSF suggestive of CNSI but no diagnosis.

Objective 2: Determine the increase in diagnostic yield of CNSI due to the addition of BioFIRE FilmArray-ME to routine diagnostics in the diagnosis of CNSI in high HIV-prevalence African settings

Objective 3: Describe changes in the detection of the most common CNSIs in Southern Africa, cryptococcal and tuberculous meningitis, over time following expansion of CNSI diagnostic capacity in routine care (CrAg and Xpert MTB/RIF Ultra) and increased ART coverage in Botswana.

Objective 4: Determine how computed tomography is used in routine clinical practice in high HIV-prevalence settings and characterise the effect of computed tomography performed prior to lumbar puncture on patients with suspected CNSI on delay to diagnostic lumbar puncture and treatment initiation.

Addressing individual objectives

Objective 1: Describing current CNSI epidemiology in Botswana and Zimbabwe

This was achieved by establishing two prospective meningitis surveillance networks where enhanced diagnostics including BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra were implemented into routine care and additional retrospective analyses of routinely collected national meningitis surveillance data were performed as part of the Botswana National Meningitis Survey: Protocol 3 (BNMS 3) and Harare Meningitis Aetiology Survey (HarMenAeS) – Chapter IV and Chapter VI

Objective 2: Clinical utility of BioFIRE FilmArray-ME in high HIV-prevalence settings

To answer this all available individual patient level data from four African countries were combined to perform a systematic review and meta-analysis evaluating CNSI diagnoses made through the use of BioFIRE FilmArray-ME stratified by clinically relevant population groups – Chapter III

Objective 3: Impact of improved CNSI diagnostics in routine care

This was addressed by using routinely collected national data captured from the electronic records of all CSF samples analysed in government sector healthcare facilities in Botswana between 2015 and 2022. The work will be presented in two manuscripts:

1) Description of trends in cryptococcal meningitis incidence in Botswana following widespread ART rollout using 8 years of retrospective national data from Botswana and the use of cryptococcal meningitis as an indicator of HIV programmatic success – Chapter IV
2) The impact of increased availability of near-person Xpert MTB/RIF testing on the proportion of patients investigated for TB meningitis and the number of confirmed cases of TBM – Chapter IV

Objective 4: Computed tomography in routine care

To determine this objective detailed clinical and management data from 711 adults recruited to the Botswana National Meningitis Survey: Protocol 2 were analysed with the key exposure of interest being computed tomography prior LP and outcome variables, time from admission to LP and antimicrobial initiation, and inpatient mortality – Chapter V

2.5 Study settings

2.5.1 Botswana

2.5.1.1 Country information

Botswana is a parliamentary republic in Southern Africa with a population of 2.3 million in 2022[1]. At independence in 1966 Botswana was one of the poorest countries in the world but due to a combination of significant mineral deposits, a small population and judicious economic management it is now an upper-middle income country. Despite its economic success there remains significant inequality in wealth with the 9th highest degree of disparity of incomes in the world and as a result Botswana has many of the same developmental challenges faced by less wealthy nations.

2.5.1.2 Healthcare infrastructure in Botswana

In comparison to other Southern African countries, Botswana has a reasonably effective public health system. Universal healthcare is offered to all citizens including referrals to private healthcare or abroad if required treatments are not available within government sector hospitals. Patients aged between 5-65 years pay 5 Botswana Pula (0.37 USD) for an initial consultation and all subsequent care is covered under the same fee. This figure has remained largely unchanged despite inflation[2]. There are 26 government-run referral, district or primary hospitals in addition to an extensive network of clinics and health posts. As a result, 85% of the population live within 5km of a government-run healthcare facility. However, although health care worker density in the population is relatively high for the region, at 4 doctors per 10,000 population, it is heavily concentrated in urban areas[3].

2.5.1.3 HIV in Botswana

The HIV epidemic had a devastating impact in Botswana where adult HIV prevalence peaked at 37% in 2003[4]. As a result, life expectancy fell from a little over 60 years in the late 1990s to 39 in 2003[4]. Botswana initiated a national programme for the treatment of HIV/AIDS and has since become a world-leader in HIV programming. It has introduced a series of innovative HIV care models, including becoming the first African country to offer free ART to its citizens in 2002, introducing universal treatment in 2016 and being an early adopter of dolutegravir as first-line ART. Subsequently, it became one of the first countries to reach the UNAIDS 95-95-95 targets[5]. Despite these advances HIV prevalence in adults aged 15-49 remains high at 18.9% in 2021 with significant numbers of patients presenting with advanced HIV disease due to late diagnosis or disengagement with care[6].

2.5.1.4 Lessons learnt from previous CNSI research in Botswana

Princess Marina Hospital in Gaborone has been well sensitised to meningitis research. It was a study site in the AMBITION trial and the sentinel site in the Botswana National Meningitis Survey protocol 1 (BNMS1) and protocol 2 (BNMS2), which have been described in the introduction[7,8]. During BNMS2 BioFIRE FilmArray-Meningitis/Encephalitis was piloted at Princess Marina Hospital. Learning from the challenges faced during this work was a crucial factor in successfully establishing BNMS 3 and HarMenAeS. I will highlight some of the lessons learnt from this work:

CSF volumes – BNMS2 was solely reliant on the CSF collected by clinicians at Princess Marina Hospital. In general CSF sample volumes were very small with usually between 1-2mls submitted for analysis in the microbiology department. In addition, volumes were not accurately ascertained or documented by the microbiology team making sample division and data capture challenging. Whilst this volume was sufficient for analysis with BioFIRE, which requires only 200microL, it was prohibitively small to

perform any additional analyses. To overcome this, I ensured appropriate regulatory approvals were in place for BNMS3 and HarMenAeS to allow the study team to consent participants for additional LPs if the volume of CSF collected by the clinical teams was insufficient to perform all analyses. I developed measuring charts and sample division flow sheets for the microbiology team that allowed technicians to quickly and accurately determine the CSF volume and divide the sample between relevant analyses. Sample volumes and how the sample was divided was recorded on laboratory worksheets using a template stamped on to worksheet using a rubber ink stamp. Alongside this I also presented to the clinical teams at Princess Marina Hospital and Parirenyatwa Hospital highlighting that the collection of larger CSF volumes (10-15mls) is safe, has therapeutic benefit in cryptococcal meningitis and is likely to increase diagnostic yield, particularly in TBM. After an initial introductory phase in BNMS3 sample volumes collected by clinical teams increased to the extent that additional LPs performed by the study team were rarely needed. In HarMenAeS with support from co-investigators working within Parirenyatwa the clinical teams started collecting sufficient CSF volumes from the outset meaning additional LPs have not been needed.

Nucleic acid extraction – as part of BNMS2 small volumes of CSF were stored for samples that had sufficient CSF to preserve an aliquot. Nucleic acid was extracted from these samples to perform metagenomic analysis in samples without a diagnosis to refine the assay planned to be used on BNMS3. Total nucleic acid was extracted using an automated platform, NucliSENS EasyMag, bioMerieux, France and sent to LSHTM to perform subsequent analyses. Two issues emerged from this extraction technique. Firstly, the extraction of total nucleic acid meant that the amount of available DNA and RNA was half of the total amount eluted from the sample. DNA and RNA were sequenced separately and therefore the sample had to be divided in 2 to perform both analyses with RNase added to one to generate purified DNA and DNase added to the other for purified RNA. This decreased diagnostic yield and therefore we planned to extract DNA and RNA separately in future work. The other issue identified during BNMS2 was that through using an automated platform a large

amount of contamination was introduced as downstream metagenomic sequencing detected a number of non-virulent waterborne organisms masking other potentially pathogenic organisms in the CSF. Whilst Botswana has comparatively robust research laboratory infrastructure in some areas equipment is not regularly used, serviced or maintained and we suspect this contamination originated from residual buffer in the instrument that was used in the extraction. As such we opted to avoid automated extraction platforms and used a manual extraction platform that can elute DNA and RNA separately with minimal risk of contamination.

Missing clinical information – In BNMS2 accompanying clinical information was often lacking from patients whose CSF underwent analysis with BioFIRE FilmArray-ME as no patient contact was permitted under BNMS2 ethics. This meant contextualising abnormal CSF results was challenging. Therefore, regulatory approvals for BNMS3 and HarMenAeS were designed to allow additional patient consultation in the case of key missing clinical data.

2.5.2 Zimbabwe

2.5.2.1 County information

Zimbabwe is a country in Southern Africa that borders Botswana, Mozambique, South Africa and Zambia with a population of 15.1 million in 2022[9]. Despite significant human capital and abundant natural resources hyperinflation has hampered economic development with an estimated 39.8% of the population living in extreme poverty in 2021[10]. Alongside economic instability the current government has faced criticism for alleged authoritarianism and corruption.

2.5.2.2 Healthcare infrastructure in Zimbabwe

National economic challenges have resulted in healthcare being funded through variety of sources with government funding comprising less than half of total health expenditure. Most of these governmental funds are directed towards salaries and other employee expenses often resulting in shortfalls in consumables[11]. As such, the healthcare system remains heavily reliant on donations and a strictly-enforced user fee policy for services to account for the deficit[11,12]. As a result, patients are often unable to receive appropriate investigations or medication unless they can afford to pay for their own treatment.

2.5.2.3 HIV in Zimbabwe

Adult HIV prevalence in Zimbabwe in 2022 was 11.0% with approximately 85% of patients on ART, which is provided free of charge through government healthcare facilities[13]. Viral load testing has been decentralised and second and third-line ART are available at national ART treatment centres[14,15]. Progress is being made towards the UNAIDS 95-95-95 targets with 95% of people living with HIV knowing their status, 94% on ART and 89% of patients on ART having a suppressed viral load[16].

2.6 Study Design – Botswana National Meningitis Survey: protocol 3 (BNMS3) and Harare Meningitis Aetiology Survey (HarMenAeS)

Prospective meningitis surveillance networks were established at two sites, Princess Marina Hospital, Gaborone, Botswana and Parirenyatwa Hospital, Harare, Zimbabwe. BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra were integrated into the routine analysis of CSF samples at both sites with additional CSF stored for retrospective analysis with additional monoplex PCRs and syphilis testing. Accompanying detailed clinical and management data were captured from patients who had CSF analysed.

	Botswana National Meningitis Survey	***Harare Meningitis Aetiology Study***
Inclusion Criteria	Patients of any age with CSF collected for investigation of suspected CNSI at Princess Marina Hospital, Gaborone, Botswana	Patients aged 18 or over with CSF collected for investigation of suspected CNSI at Parirenyatwa Hospital, Harare, Zimbabwe
Exclusion criteria	There are no specific exclusion criteria	Patients aged 18 or under will not be recruited*.
Sample size	No pre-defined sample size but rather intend to include all patients meeting the above criteria.	
Clinical and laboratory data	Routinely collected clinical and laboratory data will be retrieved from clinical and electronic patient records. If insufficient or	

	missing data the patient will be consented for an additional consultation.	
CSF Analysis: Routine diagnostics performed by local microbiology laboratory	*Microscopy, culture and sensitivity* *Cryptococcal antigen testing (using IMMY CrAg lateral flow assay)* *Gram stain* *India Ink* *CSF Protein and Glucose*	
CSF Analysis: Enhanced diagnostics performed by research team	Xpert® MTB/Rif Ultra BioFIRE FilmArray-ME Metagenomic analysis Rapid plasma reagin and Treponema pallidum particle agglutination Targeted PCRs for Toxoplasma gondii	Xpert® MTB/Rif Ultra BioFIRE FilmArray-ME Metagenomic analysis Rapid plasma reagin and Treponema pallidum particle agglutination Targeted PCRs for Toxoplasma gondii

Table 3 Overview of methodology for Botswana National Meningitis Survey in Gaborone, Botswana and Harare Meningitis Aetiology Survey in Harare, Zimbabwe

2.6.1 Study set-up

2.6.1.1 Regulatory approval

Ethical approval for this project was granted by LSHTM (17322). Approvals for Botswana remain in place from the Health Research and Development Committee of Botswana (HRDC reference number 13/18/1), the University of Botswana Institutional Review Board Committee (UBR/RES/IRB/1631) and Princess Marina Hospital (PMH 2/2A(7)/134). Approvals for Zimbabwe are in place from the Medical Research Council of Zimbabwe (MRCZ/A/2896), Research Council of Zimbabwe (MRCZ/A/2896) and local approval from the Joint Research and Ethics Committee (220/2022).

Enhanced diagnostics (BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra) were performed at the time of sample collection on all CSF samples with sufficient volume submitted to the microbiology laboratory at Princess Marina Hospital, Gaborone and all CSF samples with sufficient volume from adults ≥18 years at Parirenyatwa Hospital. This was through a waiver of consent issued by Human Research and Development Committee (Botswana) and Medical Research Council of Zimbabwe (Zimbabwe).

If additional CSF was required, then consent could be sought from the patient to perform an additional LP however this was rarely used. Consent forms were reviewed by regulatory bodies and translated to Setswana for use in Botswana and Shona for use in Zimbabwe.

The majority of clinical data was collected from the patient's hospital or electronic health records. In cases where insufficient information is available from the patient notes, consent was sought for an additional consultation with the patient.

2.6.1.3 Data collection

Patients were identified from the laboratory records in the microbiology departments at Princess Marina Hospital, Gaborone and Parirenyatwa Hospital, Harare. Any patient at Princess Marina Hospital or any adult ≥18 years old at Parirenyatwa Hospital with CSF submitted to the microbiology laboratory by the treating physicians was included. Patient demographic details were captured from laboratory records, entered on to a screening log stored on a secure database. Using the screening log the patient was assigned a unique study identification number. The patient and their medical records were then identified on medical wards. Clinical and laboratory data were then captured from the patients' medical records and entered on to a separate secure database linked through the unique study identification numbers. The majority of data was collected from patient's medical records. If insufficient clinical details were recorded in the patient notes, then consent could be sought for a consultation by the study team.

Clinical data focussed on clinical presentation including the presence or absence of features suggestive of CNS infection, HIV status including level of immunosuppression and current treatment and other co-morbidities. In addition, data on treatment including antimicrobials and adjunctive therapies such as steroids and therapeutic lumbar punctures alongside outcome data were collected.

2.6.1.4 Dissemination of results from enhanced diagnostics

Results from BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra were disseminated to clinicians using existing result reporting systems at both sites. In Botswana a standardised report and comment to aid clinical interpretation were developed and included in the CSF MC+S result (figure 2). This was available for clinicians on the electronic health records with an electronic time stamp at the time of

result release. If the electronic health record was not functioning these same standardised reports were included on stickers that were placed on paper records that were retrieved by clinicians. In Zimbabwe there are no reliable electronic health records, and all results were documented in a reporting book in the microbiology laboratory. Stickers with the result report, comment on clinical interpretation and time of result release were developed by myself and used by laboratory staff.

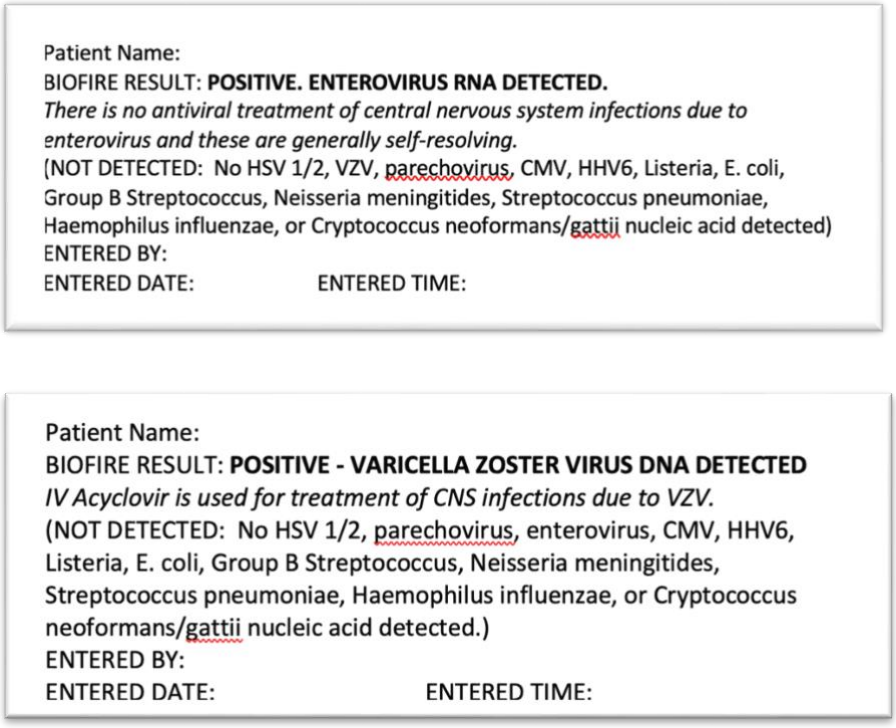

Figure 2: Examples of standardised reports for enterovirus and VZV detection on BioFIRE FilmArray-ME

2.7 Laboratory work

2.7.1 Staff training

Training sessions on the use of FilmArray-ME were performed by the manufacturer bioMerieux for microbiology staff at both sites with regular refreshers available from senior laboratory scientists. A standard operating procedure for the use of FilmArray-ME was developed using manufacturer guidelines and all staff were trained on this. Key staff personnel were identified at both sites to assist

with the implementation of FilmArray-ME and ongoing training and development of other staff. Staff at both sites were already trained to use GeneXpert platforms. Manufacturer guidelines for the handling of extrapulmonary samples were implemented, including an additional centrifugation step when processing larger volumes of CSF.

2.7.2 Sample handling

The full panel of tests was dependent on receipt of a sufficient volume of CSF, thus all tests were not performed on all patients. In such cases, the routine tests requested by the treating physician and/or mandated by the public sector laboratory were prioritized and the sample was divided using an algorithm which was agreed between the study team and microbiology departments at Princess Marina Hospital and Parirenyatwa Hospital. An SOP was developed for the division of CSF samples summarised in figure 3.

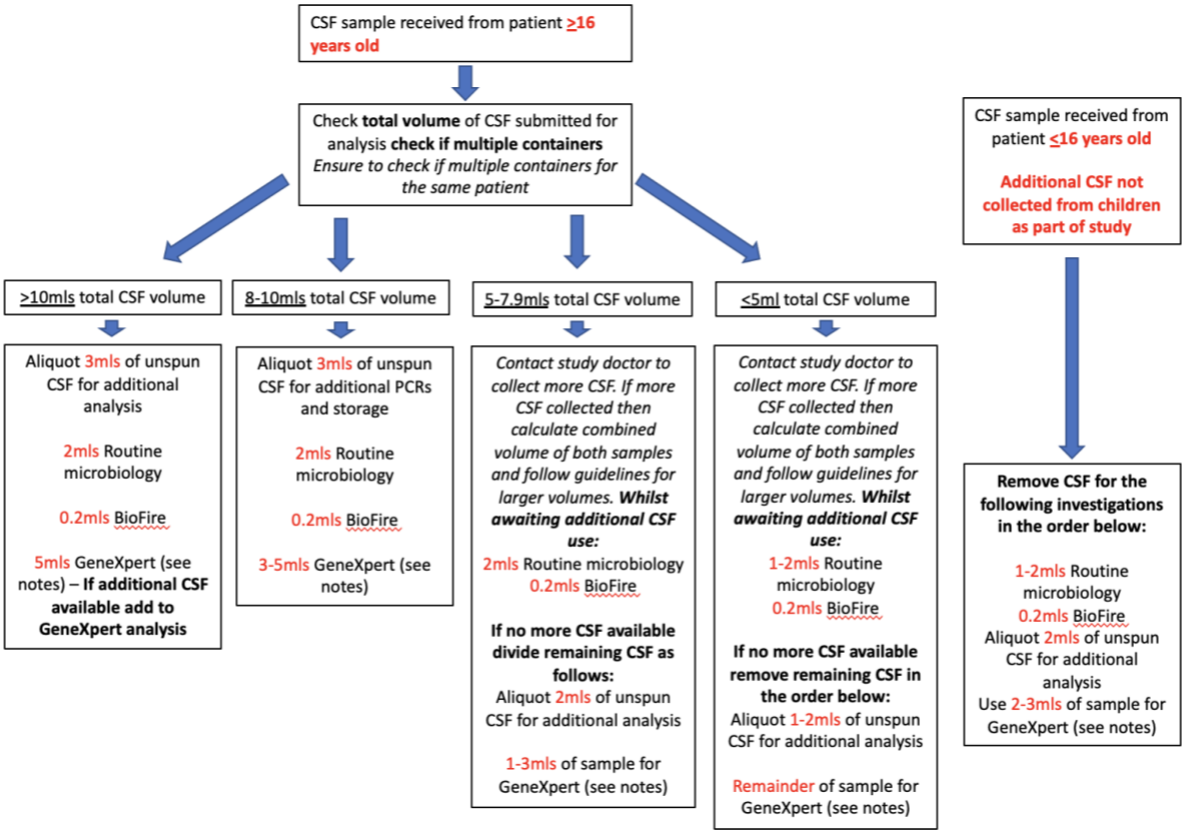

Figure 3: CSF sample division algorithm for use in Princess Marina Hospital, Botswana

*Repeat GeneXpert if not performed on CSF in last 2 weeks **and** no positive GeneXpert on **CSF** in last 2 months (unless volume submitted for analysis with is greater than the previous episode).*

2.7.3 Quality control

Verification was performed for each FilmArray instrument with Zeptometrix NATrol™ verification panel and quality control was performed with INTROL® ME Control Panel M262 for each new lot of FilmArray-ME pouches. Verification and quality control of GeneXpert platforms was performed by the microbiology teams at both sites.

2.7.4 Nucleic acid extraction from stored samples

Nucleic acid extraction was performed at Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana using QIAGEN AllPrep Mini kits, a commercially available manual extraction kit that allowed separate extraction of RNA and DNA. A protocol for the extraction of total RNA and genomic DNA was established based on the manufacturer's guidelines and previous experience from the study team. This included the addition of a bead-beating step to optimise the additional sample analysis. This is of particular importance for fungal pathogens that have a more robust cell wall. Extracted nucleic acid was stored at -80^{o}C.

2.7.5 Additional analysis

A commercially available monoplex PCR for *Toxoplasma gondii* was performed on all stored CSF samples from HIV positive patients. Rapid plasma reagin and Treponema pallidum particle agglutination was performed on all stored CSF samples. However due to challenges obtaining consumables and some samples with limited CSF volumes not all analyses have been performed on all samples. Additional analyses are planned including an additional multiplex PCR platform including analytes for *Rickettsia* species, *Leptospira* spp., Arboviruses and *Salmonella* spp.

2.8 References

1. Statistics Botswana. 2022 Population and Housing Census. *https://www.statsbots.org.bw/census-2022 (accessed 6/11/23)* (2022).
2. Pagiwa, V., Shiell, A., Barraclough, S. & Seitio-Kgokgwe, O. A Review of the User Fees Policy for Primary Healthcare Consultations in Botswana: Problems With Effective Planning, Implementation and Evaluation. *Int J Health Policy Manag* 11, 2228–2235 (2022).
3. Nkomazana, O., Peersman, W., Willcox, M., Mash, R. & Phaladze, N. Human resources for health in Botswana: The results of in-country database and reports analysis. *Afr J Prim Health Care Fam Med* 6, (2014).
4. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. *Botswana: Epidemiological Fact Sheet on HIV/AIDS and Sexually Transmitted Infections*. http://www.who.int/hiv (2004).
5. Vinikoor, M. J. & Hachaambwa, L. Advanced HIV disease during the 'Treat All' era in Botswana. *AIDS* 34, 2321–2323 (2020).
6. Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Botswana. Available at: https://www.unaids.org/en/regionscountries/countries/botswana.
7. Jarvis, J. N. *et al.* Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. *New England Journal of Medicine* 386, 1109–1120 (2022).
8. Tenforde, M. W. *et al.* Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey. *Journal of Infection* 79, 212–219 (2019).
9. Zimbabwe National Statistics Agency. 2022 Population and Housing Census. (2022).
10. World Bank. Zimbabwe 'poverty headcount ratio'. https://data.worldbank.org/country/zimbabwe.
11. Mhazo, A. T. & Maponga, C. C. The political economy of health financing reforms in Zimbabwe: a scoping review. *International Journal for Equity in Health* vol. 21 Preprint at https://doi.org/10.1186/s12939-022-01646-z (2022).

12. Mhazo, A. T., Maponga, C. C. & Mossialos, E. Inequality and private health insurance in Zimbabwe: history, politics and performance. *Int J Equity Health* 22, (2023).

13. Benade, M. *et al.* Economic evaluation of a cluster randomized, non-inferiority trial of differentiated service delivery models of HIV treatment in Zimbabwe. *PLOS Global Public Health* 3, e0000493 (2023).

14. Apollo, T., Takarinda, K. C., Phillips, A., Ndhlovu, C. & Cowan, F. M. Provision of HIV viral load testing services in Zimbabwe: Secondary data analyses using data from health facilities using the electronic Patient Monitoring System. *PLoS One* 16, (2021).

15. Chimbetete, C., Shamu, T. & Keiser, O. Zimbabwe's national third-line antiretroviral therapy program: Cohort description and treatment outcomes. *PLoS One* 15, (2020).

16. Joint United Nations Programme on HIV/AIDS (UNAIDS). Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Zimbabwe. Accessed 6/11/23. (2022).

Chapter III - Determining the clinical utility of BioFIRE FilmArray-Meningitis/Encephalitis in the diagnosis of central nervous system infections in high HIV-prevalence settings: systematic review and individual patient data meta-analysis

3.1 Introduction

Central nervous system infections (CNSI) caused by bacteria, fungi and protozoa have a high morbidity and mortality. For viral CNSI, outcomes depend on the causative organisms, disease caused by non-polio enteroviruses being largely self-limiting whilst in contrast neonatal herpes simplex virus CNSI have significant mortality and high rates of long-term neurologic sequelae[1,2]. Large proportions of patients presenting with CNSI never receive a microbiologically confirmed diagnosis and this is associated with significant mortality[3–7]. Failure to obtain a diagnosis can be due to several or often a combination of reasons. Diagnostic infrastructure, particularly in low- and middle-income settings, is often absent or limited to only basic microbiological investigations. Commonly used diagnostics lack the sensitivity to achieve a diagnosis either due to innate limitations of the platform, limited presence of pathogen in CSF, such as in tuberculous meningitis, or due to pre-analytical factors such as reduction in bacterial culture positivity after administration of antibiotics[8,9]. Failure to detect a pathogen can also result from CNSI caused by rare or novel pathogens that were not investigated for or non-infectious causes such as inflammatory conditions and malignancy. Empirical antibiotic treatment usually with third-generation cephalosporins is widely used in patients with CNSI without a confirmed diagnosis. Whilst this may appear a valid treatment strategy, particularly in some high-resource settings where tuberculous and cryptococcal meningitis are uncommon, it will inevitably lead to increased unnecessary antimicrobial use and length of stay in cases of self-limited viral meningitis[4], increased mortality in cases of viral, cryptococcal or tuberculous CNSI where alternative treatment is warranted and in a significant proportion of bacterial meningitis cases will not be adequate given the growing rates of antimicrobial resistance[10–12].

Platforms designed to improve the diagnosis of infectious diseases are invariably developed in the Global North targeting the disease spectrum in these low HIV-prevalence, high-income countries. The epidemiology of infectious diseases including CNSI is very different in low-income countries but the impact is often more marked and the need for improved diagnostics greater. As such it is important to evaluate how tests developed in the Global North perform in low- and middle-income settings.

The BioFIRE FilmArray-Meningitis/Encephalitis (FilmArray-ME) is a multiplex PCR platform routinely used in the diagnosis of patients with suspected CNSI in high-resource settings[13–16]. Analytes included in the panel were primarily informed by epidemiological data on CNSI aetiology from high-resource settings including the USA and Europe (figure 4). However, in large parts of Africa the epidemiology of CSNI is vastly different to these regions. HIV has markedly altered the aetiology of CNSI and cryptococcal, tuberculous and pneumococcal meningitis predominate[5,6,17]. Viral CNSI are rarely diagnosed, mostly due to a lack of reliable diagnostics, contrasting with high-income settings where viral CNSI, usually caused by non-polio enteroviruses are the most common cause of CNSI[4].

FilmArray-ME has been used in research settings in 6 countries in the WHO African Region [18–24] (Benin, Botswana, Ethiopia, Nigeria, Uganda and Zimbabwe [unpublished]). Whilst FilmArray-ME has increased the total number of diagnoses made in all studies, most of the published studies recruited relatively small numbers for FilmArray-ME analysis (40-300 patients) limiting subgroup analyses disaggregated by HIV status, routine CSF analysis results and individual pathogens. Although there are potentially important differences between the research contexts and study populations included, these studies are performed in countries that have important similarities. Income level, HIV prevalence and disease burden in the included countries are far closer aligned across these variables with each other than with the developed countries where most new diagnostics are developed.

FilmArray-ME has demonstrated the potential to improve the diagnosis of CNSI in resource-rich settings. Assessing the value of FilmArray-ME above conventional diagnostics in low-resource, high HIV-prevalence settings with differing CNSI epidemiology from where FilmArray-ME was originally developed is crucial before widespread implementation can be considered.

Viruses	**Bacteria**	**Yeast**
Cytomegalovirus	*Escherichia coli K1*	*Cryptococcus neoformans/Cryptococcus gattii*
Enterovirus	*Haemophilus influenzae*	
Herpes simplex virus 1	*Listeria monocytogenes*	
Herpes simplex virus 2	*Neisseria meningitidis*	
Human Herpes Virus 6	*Streptococcus agalactiae*	
Human parechovirus	*Streptococcus pneumoniae*	
Varicella Zoster Virus		

Figure 4 Potential CNS pathogens detected by FilmArray-ME

3.2 Methods

We performed a systematic review and meta-analysis of individual patient data from studies that have used FilmArray-ME for the analysis of CSF in patients of all ages with suspected CNSI in the WHO African region. The search was conducted in MEDLINE using the search criteria detailed in figure 5. We also obtained information from the test manufacturer about what countries had ordered FilmArray-ME kits alongside a list of publications from Africa that used FilmArray-ME generated by the manufacturer. Forward and reverse citation chaining was performed on included studies to identify additional studies. The lead investigators for relevant studies were approached to share anonymised raw data[18–20,24].

Studies were included if FilmArray-ME was used on CSF to investigate patients of any age with suspected CNSI in the WHO African Region. Exclusion criteria included: (1) CNSI diagnoses assigned using molecular diagnostics on samples other than CSF, (2) case series or report with <20 participants, (3) results solely describing a single pathogen or (4) study reporting solely repeat data from another

included study. Titles were screened to reject papers clearly out of scope, remaining abstracts were then reviewed and full texts accessed if it met inclusion/exclusion criteria.

The primary outcome measure was the proportion of suspected CNSI with and without confirmed diagnoses resulting from CSF analysis using both conventional techniques and FilmArray-ME. Secondary outcomes were the spectrum of diagnoses resulting from CSF analysis, the relative increase in diagnostic yield above routine diagnostics alone stratified by age group, HIV status, CSF pleocytosis and CSF cryptococcal antigen positivity, the frequency of co-detections, and in-hospital mortality stratified by diagnosis made through CSF analysis.

Search strategy MEDLINE (2015-2023)
Central Nervous System Infections meningitis.mp. OR exp Meningitis OR exp Encephalitis OR encephalitis.mp. OR meningoencephalitis.mp. OR exp Meningoencephalitis OR central nervous system infection.mp. OR exp Central Nervous System Infections
BioFIRE FilmArray-Meningitis/Encephalitis exp Molecular Diagnostic Techniques/ OR polymerase chain reaction.mp. OR molecular diagnostics.mp. or Pathology, Molecular OR polymerase chain reaction.mp. or Polymerase Chain Reaction
Region Central Africa.ti,ab. OR Eastern Africa.ti,ab. OR Southern Africa.ti,ab. OR Western Africa.ti,ab. OR Algeria/ OR Algeria.ti,ab OR Angola/ OR Angola.ti,ab. OR Cameroon/ OR (Cameroon or Kamerun or Cameroun).ti,ab. OR Cape Verde/ OR (Cape Verde or Cabo Verde).ti,ab. OR Comoros/ OR (Comoros or Glorioso Islands or Mayotte).ti,ab. OR Congo/ OR (Congo not ((Democratic Republic adj3 Congo) or congo red or crimean-congo)).ti,ab. OR Cote d'Ivoire/ OR (Cote d'Ivoire or Cote

dIvoire or Ivory Coast).ti,ab. OR Eswatini/ OR (eSwatini or Swaziland).ti,ab. OR Ghana/ OR (Ghana or Gold Coast).ti,ab. OR Kenya/ OR (Kenya or East Africa Protectorate).ti,ab. OR Lesotho/ OR (Lesotho or Basutoland).ti,ab. OR Mauritania/ OR Mauritania.ti,ab. OR Nigeria/ OR Nigeria.ti,ab. OR (Sao Tome adj2 Principe).ti,ab. OR Senegal/ OR Senegal.ti,ab. OR Zambia/ OR (Zambia or Northern Rhodesia).ti,ab. OR Zimbabwe/ OR (Zimbabwe or Southern Rhodesia).ti,ab. OR Botswana/ OR (Botswana or Bechuanaland or Kalahari).ti,ab. OR Equatorial Guinea/ OR (Equatorial Guinea or Spanish Guinea).ti,ab. OR Gabon/ OR (Gabon or Gabonese Republic).ti,ab. OR Mauritius/ OR (Mauritius or Agalega Islands).ti,ab. OR Namibia/ OR (Namibia or German South West Africa).ti,ab. OR South Africa/ OR (South Africa or Cape Colony or British Bechuanaland or Boer Republics or Zululand or Transvaal or Natalia Republic or Orange Free State).ti,ab. OR Benin/ OR (Benin or Dahomey).ti,ab. OR Burkina Faso/ OR (Burkina Faso or Burkina Fasso or Upper Volta).ti,ab. OR Burundi/ OR (Burundi or Ruanda-Urundi).ti,ab. OR Central African Republic/ OR (Central African Republic or Ubangi-Shari).ti,ab. OR Chad/ OR Chad.ti,ab. OR "Democratic Republic of the Congo"/ OR (((Democratic Republic or DR) adj2 Congo) or Congo-Kinshasa or Belgian Congo or Zaire or Congo Free State).ti,ab. OR Eritrea/ OR Eritrea.ti,ab. OR Ethiopia/ OR (Ethiopia or Abyssinia).ti,ab. OR Gambia/ OR Gambia.ti,ab. OR Guinea/ OR (Guinea not (New Guinea or Guinea Pig* or Guinea Fowl or Guinea-Bissau or Portuguese Guinea or Equatorial Guinea)).ti,ab. OR Guinea-Bissau/ OR (Guinea-Bissau or Portuguese Guinea).ti,ab. OR Liberia/ OR Liberia.ti,ab. OR Madagascar/ OR (Madagascar or Malagasy Republic).ti,ab. OR Malawi/ OR (Malawi or Nyasaland).ti,ab. OR Mali/ OR Mali.ti,ab. OR Mozambique/ OR (Mozambique or Mocambique or Portuguese East Africa).ti,ab. OR Niger/ OR (Niger not (Aspergillus or Peptococcus or Schizothorax or Cruciferae or Gobius or Lasius or Agelastes or Melanosuchus or radish or Parastromateus or Orius or Apergillus or Parastromateus or Stomoxys)).ti,ab. OR Rwanda/ OR (Rwanda or Ruanda).ti,ab. OR Sierra Leone/ OR (Sierra Leone or Salone).ti,ab. OR South Sudan/ OR South Sudan.ti,ab. OR Tanzania/ OR (Tanzania or Tanganyika or Zanzibar).ti,ab. OR Togo/ OR (Togo or

Togolese Republic or Togoland).ti,ab. OR Uganda/ OR Uganda.ti,ab. OR Seychelles/ OR Seychelles.ti,ab

Figure 5: Search strategy for MEDLINE restricted to 2015-2023

Datasets were cleaned and variables recoded prior to being merged for analysis. If a patient had more than one sample analysed either from multiple clinical episodes or multiple lumbar punctures during the same episode only the first sample from the first episode was included.

Minimum variables required for inclusion were:

- Age
- Sex
- HIV status
- Routine CSF analysis – microscopy for white cell count and gram and India stains, CSF cryptococcal antigen testing and fungal and bacterial culture
- Result of analysis with FilmArray-ME
- In-hospital mortality

Patients were stratified by the presence of inflammatory CSF suggestive of CNSI. Inflammatory CSF was defined as either an age-adjusted pleocytosis or both a CSF protein >1mg/mL and a CSF glucose <2.2mmol/mL.

Data were analysed using STATA version 18.0. Patient demographics, CSF analysis results including from FilmArray-ME, HIV-related data and in-hospital mortality were described using frequencies, percentages, or median and interquartile range (IQR) as appropriate. Multivariate logistic regression analysis was performed to assess for associations between inpatient mortality and detection of pathogens in CSF.

Missing data was seen in the context of this observational research from the WHO African Region and this occurs for a number of reasons. Routine testing is often not performed due to reagent stockouts, technician error or lack of consumables and data on patient age or gender was also occasionally missing given the observational nature of some of these studies. We describe the missingness of this data in our analyses. Our aim was to capture the diagnostic yield of routine CSF testing algorithms in real-world settings and therefore if a diagnostic test such as CrAg or culture was not performed this was considered a negative test result for the purpose of determining diagnostic yield. For other variables we performed complete case analysis and only included cases with a result in the analysis. Importantly there was no missing FilmArray-ME data. We performed an assessment of study quality to assess for risk of bias using established guidance for systematic reviews[25].

3.3 Results

Included studies

98 titles were screened by JM, of these 24 abstracts were reviewed and 8 studies met inclusion criteria for full text review. After full text review 2 studies were excluded due to insufficient participants or repeat data from other studies being used leaving 6 studies for inclusion. The authors of the included studies were contacted to request sharing of data. There were 2 studies not included as data has not yet been shared, leaving 4 studies for inclusion. Unpublished data generated by 3 studies involving JM, KK and JJ were also included giving a total of 7 included studies.

Study and author	**Study period**	**Study setting** *Country* *Hospital name* *Hospital category*	**Adult HIV prevalence at time of study (aged 15-49)**	**CSF analysis performed***	**Criteria for CSF analysis with FilmArray-ME**	**Number of baseline/admission samples analysed with FilmArray-ME**
Botswana National Meningitis Survey: Protocol 2.	May 2017-Aug 2018	Botswana Princess Marina Hospital Tertiary hospital	22.8% in 2017	CSF cell count and differential Protein Glucose CrAg LFA Gram and India ink stains Bacterial and fungal cultures	Any inpatient without age restriction presenting with signs and symptoms of suspected CNSI with a CSF sample taken as part of routine care	628
Botswana National Meningitis Survey: Protocol 3.	March 2022-ongoing	Botswana Princess Marina Hospital Tertiary hospital	16.4% in 2022	CSF cell count and differential Protein Glucose CrAg LFA Gram and India ink stains Bacterial and fungal cultures	Any inpatient without age restriction presenting with signs and symptoms of suspected CNSI with a CSF sample taken as part of routine care	661

Barnes et al, 2018 New molecular tools for meningitis diagnostics in Ethiopia – a necessary step towards improving antimicrobial prescription	March 2017-June 2017	Ethiopia Jimma University Specialised Hospital Tertiary hospital	1% in 2017	CSF cell count and differential Protein Glucose Gram stain Bacterial and fungal cultures **On clinician request:** CrAg LFA India ink stain	Any inpatient without age restriction presenting with signs and symptoms of suspected CNSI with a CSF sample taken as part of routine care	218
Rhein et al, 2016 Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population	January 2014-May 2014	Uganda Mulago National Referral Hospital Tertiary hospital	6.1% in 2014	**All samples:** CrAg LFA **On CrAg negative samples only:** CSF cell count and differential Protein Glucose Gram stain Bacterial and fungal cultures	HIV positive adults ≥18 years old with suspected CNSI as part of ASTRO-cm pilot trial	69
Ellis et al, 2019. The Changing epidemiology of HIV-	March 2015-	Uganda Mulago National	5.9% in 2016	**All samples:** CrAg LFA	HIV positive adults ≥18 years old with suspected CNSI as part of ASTRO-cm trial	45

associated adult meningitis, Uganda 2015-2017	September 2017	Referral Hospital **and** Mbarara Regional Referral Hospital Tertiary hospitals		**On CrAg negative samples only:** CSF cell count and differential Protein Gram stain Bacterial and fungal cultures	Stepwise diagnostic algorithm implemented. FilmArray-ME performed on patients with negative CSF CrAg and Xpert MTB/RIF Ultra	*842 HIV positive adults screened. 733 excluded.* *45/109 patients suitable for analysis with FilmArray-ME received testing due to limitations in test availability.*
Bridge et al, 2021 Evaluation of the BioFIRE FilmArray-ME Meningitis/Encephalitis panel in an adult and pediatric Uganda population	September 2016-April 2019	Uganda Mulago National Referral Hospital **and** Mbarara Regional Referral Hospital Tertiary hospitals	5.7% in 2018	CSF cell count and differential Protein Glucose CrAg LFA Gram stain Bacterial and fungal cultures	HIV positive adults ≥18 years with suspected CNSI as part of ASTRO-cm trial Children age 0-17 years with suspected CNSI	300
Harare Meningitis Aetiology Survey. **Unpublished**	September 2022 ongoing	Zimbabwe Parirenyatwa Hospital Tertiary hospital	11.0% in 2022	CSF cell count Protein Glucose CrAg LFA	Any adult inpatient ≥18 presenting with signs and symptoms of suspected CNSI with a CSF sample taken as part of routine care	398

				Gram and India ink stains Bacterial and fungal cultures		

Table 4: Summary of included studies describing HIV prevalence at time of study, additional CSF analyses performed, inclusion criteria and number of samples analysed with FilmArray-ME

*TB testing was not performed universally and often inconsistently or on clinician request. In Rhein et al, Xpert MTB/RIF or TB culture was performed on clinician request. In Ellis et al and Bridge et al Xpert MTB/RIF Ultra was performed on CrAg LFA negative CSF samples and if it was positive FilmArray-ME was not routinely performed in conjunction to FilmArray-ME, unless on clinician request. In Botswana National Meningitis Survey: Protocol 3 and Harare Meningitis Aetiology Xpert MTB/RIF Ultra is performed on all patients recruited with sufficient CSF. In Botswana National Meningitis Survey: Protocol 2 TB testing was performed either on clinician request or retrospectively on a subset of 170 patients and in Barnes et al it was performed solely on clinician request. Due to overall low numbers of testing performed in the included patients TB testing was not included in the analysis.

Study	Study type	Assessment	Risk of bias
Botswana National Meningitis Survey: Protocol 2 and Botswana National Meningitis Survey: Protocol 3 (aggregated IPD included in analysis)	Prospective cohort study	Consecutive enrolment of participants with clear inclusion criteria as part of routine practice	Low
Barnes et al, 2019 Ethiopia	Prospective cohort study	Consecutive enrolment of participants with clear inclusion criteria as part of routine practice HIV testing performed infrequently, and CrAg testing performed only on clinician request	Low
Rhein et al, 2015 Uganda	Prospective cohort study with retrospective testing of stored samples	Risk of selection bias - Excluded HIV negative patients and patients who had clinician directed positive TB test due to biosafety concerns. FilmArray-ME testing performed retrospectively therefore may not influence treatment decision and therefore impact outcome	Medium
Ellis et al, 2019 Uganda	Prospective cohort study	Potential selection bias. Excluded HIV negative patients or HIV positive patients with positive CrAg or CSF Xpert Ultra	Medium
Bridge et al, 2019 Uganda	Prospective cohort study	Variable inclusion criteria. Potential selection bias. Excluded subset of HIV negative patients or HIV positive patients with positive CrAg or CSF Xpert Ultra and analysed proportion of patients with positive CSF CrAg	Medium
Harare Meningitis Aetiology Survey	Prospective cohort study	Consecutive enrolment of adults. Risk of selection bias Children <18 years old excluded	Low

Table 5 Risk of bias assessment for included studies. Risk of bias for unpublished studies determined from study protocols.

A total 2242 CSF samples were analysed using FilmArray-ME from 4 sites in Africa; 57.5% (1289/2242) were from Botswana, 17.8% (398/2242) from Zimbabwe, 15.0% (337/2242) from Uganda and 9.7% (218/2242) from Ethiopia. Adults made up 68.0% (1525/2242) of the study population with neonates, infants and children comprising 12.0% (270/2242), 11.7% (262/2242) and 5.8% (131/2242) respectively with 2.4% (54/2242) age unknown. No neonates were included in Uganda and no children of any age were included in Zimbabwe (table 6).

HIV status was known in 91.4% (1394/1525) of adults and 73.6% (1123/1525) of all included adults were HIV positive. The proportion of patients with HIV varied between sites with 96.0% of adults in Uganda being HIV positive compared to 30.0% of those in Ethiopia. The proportion of HIV positive adults with available CD4 counts was 62.2% (340/547), 25% (3/12), 64.6% (184/285), 45.9% (128/279) across Botswana, Ethiopia, Uganda and Zimbabwe respectively. The level of immunosuppression was different across sites with median CD4 counts of 212 (IQR 59-434), 6 (IQR 6-14), 28 (IQR 9-71) and 86 (IQR 42-220) cells/mm^3 in Botswana, Ethiopia, Uganda and Zimbabwe respectively. ART status was well captured across all studies except for Ethiopia where this was not recorded, 46.8-54.5% of people were recorded as receiving ART at time of CNSI.

Using age-adjusted parameters for CSF pleocytosis 13.8% (n=309) of all patients had a CSF pleocytosis. Among those with recorded white cell counts, incorporating CSF biochemistry with cell counts, 19.2% had inflammatory CSF, table 6.

A diagnosis was made in 18.5% of patients (n=414) using routine diagnostics alone, in 22.7% (n=508) with FilmArray-ME alone and in 26.4% (n=592) of patients with the combination of routine diagnostics and FilmArray-ME. *Cryptococcus neoformans/gattii* was the most commonly detected pathogen found in 61.2% (n=358) of patients with a confirmed microbiological diagnosis, FilmArray-ME was positive for *Cryptococcus neoformans/gattii* in 84.1% (301/358) of these cases. 10 samples without a

positive CSF CrAg, India ink or culture for *Cryptococcus* spp had a positive FilmArray-ME for *Cryptococcus neoformans/gattii*. *Streptococcus pneumoniae* was the second most common in 11.1% (n=65) of all patients with a confirmed microbiological diagnosis. The diagnosis of *S. pneumoniae* was made through culture in 16.9% of cases (n=11) all of which were positive on FilmArray-ME, the remainder were made through FilmArray-ME alone. Cytomegalovirus (CMV) was the third, 9.4% (n=55) of patients with a confirmed microbiological diagnosis; 34.5% (n=19) of these detections were as a co-detection with another organism.

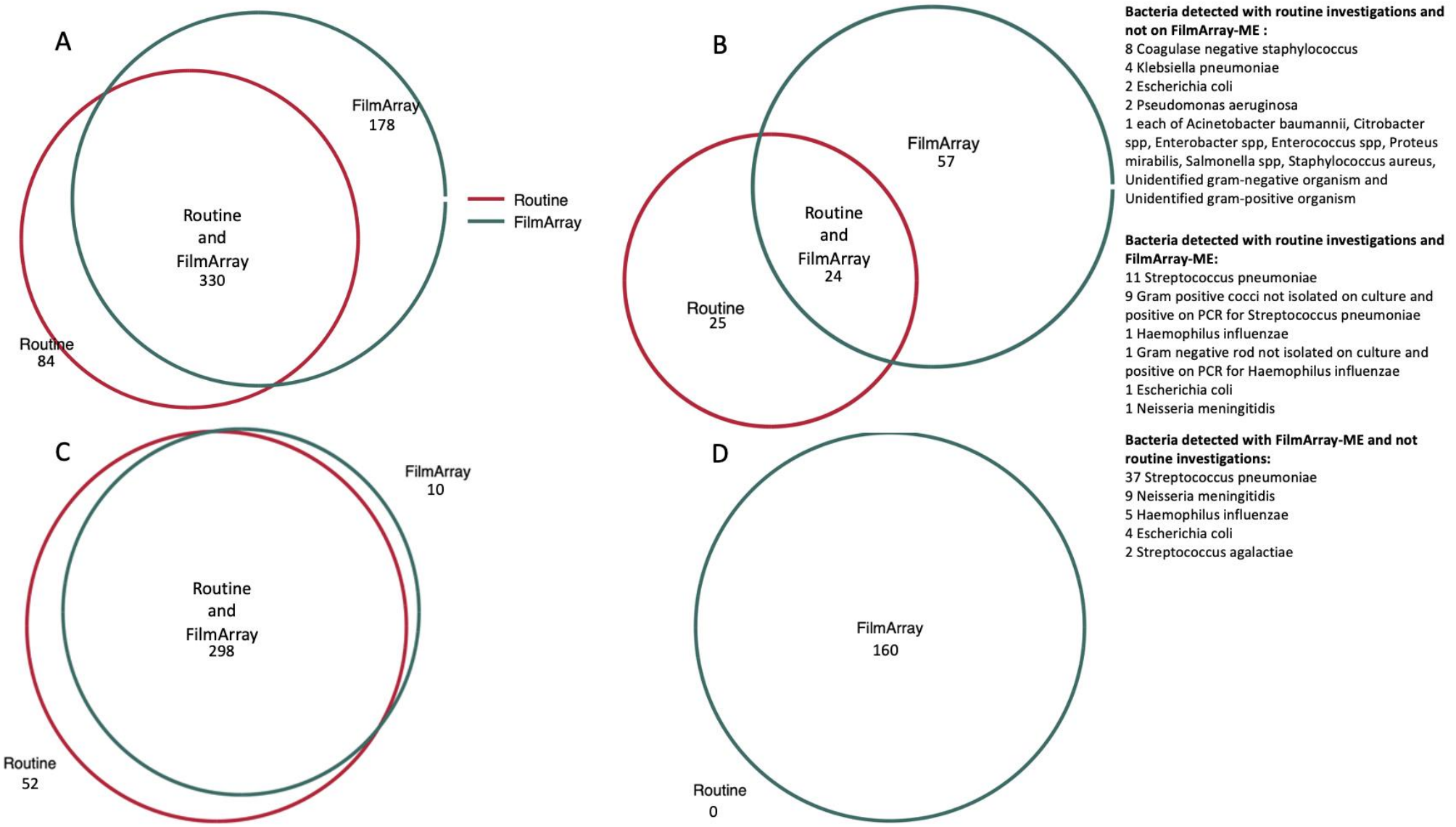

Figure 6a-6d: Proportions of diagnoses made with routine diagnostics and FilmArray-ME. Clockwise from top left (A) Overall (B) Bacteria (C) Fungi (D) Viruses.

Diagnostic yield, the proportion of patients with a microbiologically confirmed diagnosis, with routine diagnostics and FilmArray-ME was stratified by age, sex, HIV status and prior CSF analysis. Diagnostic yield after analysis with both routine and FilmArray-ME varied between groups, the highest diagnostic yield was in HIV positive adults, 41.7% (n=468) and the lowest in HIV negative adults with no CSF pleocytosis, 3.3% (7/214). The relative increase in diagnostic yield through the addition of FilmArray-ME to routine diagnostics was highest in HIV positive adults with a negative CrAg and uninflammatory CSF where the yield increased from 3.3% with routine diagnostics alone to 14.4% with the addition of FilmArray-ME, a relative increase of 432.1%. The lowest relative increase in diagnostic yield was 27.8% in HIV negative adults with uninflammatory CSF.

In-hospital mortality was 16.4% (n=368/2242) overall. Uganda had the highest mortality, 30.3% (n=102) and Ethiopia the lowest 10.1% (n=22). Mortality rates also varied by pathogen detection 46.2% of patients (6/13) with HSV-2 detected in CSF died in hospital compared to 10% of patients (1/10) with *Escherichia coli* or *Neisseria meningitidis*.

54 patients (2.5%) had more than one organism detected, 53 patients had 2 organisms detected and 1 patient had 3 organisms detected, a full description of co-detections is in Table 10. HIV prevalence in this group was 87.0% (47/54) with 7.4% (4/54) patients having an unknown HIV status with only 5.6% (3/54) being confirmed HIV negative. Median CD4 was 30 cells/mm^3 (IQR 12-63). Odds ratio for adjusted mortality adjusted for age, sex, HIV status and CD4 count for patients with one pathogen was 1.68 (95% 1.09-2.61, p=0.02) and 6.05 (95% CI 2.59-14.13, p<0.01) for two or more organisms detected compared to patients with no pathogens detected.

	Total (n=2242)	Botswana (n=1289)	Ethiopia (n=218)	Uganda (n=337)	Zimbabwe (n=398)
Demographics					
Age category, % (n)					
Neonate *0-28 days*	12.0% (270)	12.0% (155)	52.8% (115)	-	-
Infant *1-23 months*	11.7% (262)	15.9% (205)	19.3% (42)	4.5% (15)	-
Child *2-14 years*	5.8% (131)	6.8% (88)	8.3% (18)	7.4% (25)	-
Adult *>14 years*	68.0% (1525)	61.3% (790)	18.4% (40)	88.1% (297)	100 (398)
Unknown	2.4% (54)	3.9% (51)	1.4% (3)	-	-
Sex, % (n)					
Female	44.9% (1007)	45.9% (592)	42.2% (92)	41.3% (139)	46.2% (184)
Male	54.4% (1219)	52.9% (682)	57.8% (126)	58.7% (198)	53.5% (213)
Unknown	0.7% (16)	1.2% (15)	-	-	0.25% (1)
Adult HIV details					
Adult HIV status, % (n)					
Positive	73.6% (1123)	69.2% (547)	30.0% (12)	96.0% (285)	70.1% (279)
Negative	17.8% (271)	24.8% (196)	37.5% (15)	2.7% (8)	13.1% (52)
Unknown	8.6% (131)	6.0% (47)	32.5% (13)	1.4% (4)	16.8% (67)
Median CD4 If HIV positive, cells/mm^3 (IQR)	93 (28-318)	212 (60-435)	6 (6-14)	28 (9-71)	86 (42-220)
Proportion of adults living with HIV with CD4 <200 cells/mm^3	37.9% (425)	29.8% (163)	25% (3)	59.9% (168)	32.6% (91)
Unknown CD4 if HIV positive, % n	41.7% (468)	37.7% (207)	75% (9)	35.4% (101)	54.1% (151)

Currently on ART	50.0% (519)	46.8% (256)	-	53.7% (153)	54.5% (152)
ART status unknown	5.0% (56)	5.7% (31)	100% (12)	0.4% (1)	4.3% (12)
CSF Analysis					
Age-adjusted CSF WCC pleocytosis	13.8% (309)	13.0% (168)	7.3% (16)	26.7% (90)	8.8% (35)
CSF protein (mg/mL) (IQR)*	0.45 (0.25-0.94)	0.45 (0.25-0.88)	-	0.38 (0.21-0.84)	0.54 (0.36-1.2)
CSF glucose (mmol/mL) (IQR)	3.27 (2.49-4.0)	3.28 (2.60-3.98)	2.84 (2.62-3.31)	2.72 (1.72-4.70)	3.4 (2.5-4.2)
Inflammatory CSF*, % (n)**	19.2% (386)	18.1% (220)	9.7% (16)	31.5% (91)	17.3% (59)
Diagnoses with routine analysis alone, % (n)	18.5% (414)	13.0% (168)	2.8% (6)	49.6% (167)	18.3% (73)
Diagnoses with FilmArray-ME alone, % (n)	22.7% (508)	15.6% (201)	9.2% (20)	52.2% (176)	27.9% (111)
Diagnoses with routine and FilmArray-ME, % (n)	26.4% (592)	18.8% (242)	9.6% (21)	60.2% (203)	31.7% (126)
Diagnoses with routine analysis alone excluding cryptococcal meningitis, % (n)	2.9% (66)	4.1% (53)	1.8% (4)	0.3% (1)	2.0% (8)
Diagnoses with routine and FilmArray-ME excluding cryptococcal meningitis, % (n)	10.3% (231)	9.5% (123)	8.7% (19)	9.5% (32)	14.3% (57)
Inflammatory CSF* with no diagnosis (routine only), % (n/N)**	60.6% (234/386)	66.4% (146/220)	81.3% (13/16)	37.4% (34/91)	69.5% (41/59)
Inflammatory CSF* with no diagnosis (routine and FilmArray-ME), % (n)**	45.1% (174)	55.5% (122)	31.3% (5)	25.3% (23)	40.7% (24)

Outcome					
Discharged alive	68.3% (1532)	71.4% (920)	60.6% (132)	67.1% (226)	62.8 (254)
Died in hospital	16.4% (368)	10.2% (131)	10.1% (22)	30.3% (102)	28.4% (113)
Unknown	15.3% (342)	18.5% (238)	29.4% (64)	2.7% (9)	7.8% (31)

Table 6: Summary of patient demographics, HIV status, CSF analysis and outcome by site

*CSF protein was unknown in 41.6% (933) of participants overall. This varied by region with 34.4% (443), 100% (218), 23.4% (78) and 48.7% (194) of participants having an unknown CSF protein in Botswana, Ethiopia, Uganda and Zimbabwe respectively.

**CSF glucose was unknown in 33.7% (756) of participants overall. This varied by region with 20.2% (269), 80.7% (176), 48.7% (164) and 32.9% (131) of participants having an unknown CSF protein in Botswana, Ethiopia, Uganda and Zimbabwe respectively.

***Inflammatory CSF was defined as either the presence of an age-adjusted CSF white cell pleocytosis **or** CSF protein >1mg/mL **and** CSF glucose <2.2mmol/mL

	CMV	Enterovirus	HSV-1	HSV-2	Human Herpesvirus-6	VZV	Escherichia coli	Haemophilus influenzae	Neisseria meningitidis	Streptococcus agalactiae	Streptococcus pneumoniae	Cryptococcus neoformans/ gatti	Other microbiological diagnosis	No diagnosis	Co-detections
Number of detections	55	14	13	13	38	29	10	9	10	2	65	358	22	1657	54
Age distribution															
Neonates (0-28 days), % (n)	3.6% (2/55)	14.3% (2/14)	7.7% (1/13)	-	7.9% (3/38)	-	10.0% (1/10)	-	-	-	-	-	13.6% (3/22)	15.6% (259/1657)	-
Infant (1-23 months), % (n)	9.1% (5/55)	35.7% (5/14)	7.7% (1/13)	-	31.6% (12/38)	-	40.0% (4/10)	11.1% (1/9)	-	50% (1/2)	7.7% (5/65)	-	18.2% (4/22)	13.6% (226/1657)	1.9% (1/54)
Paediatrics (2-14 years), % (n)	3.6% (2/55)	21.4% (3/14)	-	-	5.3% (2/38)	-	20.0% (2/10)	11.1% (1/9)	11.1% (1/9)	-	6.2% (4/65)	0.3% (1/358)	13.6% (3/22)	6.8% (112/1657)	3.7% (2/54)
Adults (15+ years), % (n)	83.5% (46/55)	28.6% (4/14)	84.6% (11/13)	100% 13/13	55.3% (21/38)	100% (29/29)	30.0% (3/10)	77.8% (7/9)	88.9% (8/9)	50% (1/2)	86.2% (56/65)	99.7% (357/358)	54.5% (12/22)	60.7% (1006/1657)	94.4% (51/54)
Age unknown, % (n)	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3% (54/1657)	-
Sex															
Female, % (n)	43.6% (24/55)	42.8% (6/14)	38.5% (5/13)	38.5% (5/13)	39.5% (15/38)	37.9% (11/29)	30.0% (3/10)	33.3% (3/9)	40% (4/10)	-	41.5% (27/65)	35.5% (127/358)	31.8% (7/22)	46.8% (881/1657)	42.6% (23/54)
Male, % (n)	56.4% (31/55)	57.1% (8/14)	61.5% (8/13)	61.5% (8/13)	39.5% (15/38)	62.1% (18/29)	70.0% (7/9)	66.7% (6/9)	60% (6/10)	100% (2/2)	58.5% (38/65)	64.4% (231/358)	68.2% (15/22)	52.4% (988/1657)	57.4% (31/54)

Unknown, % (n)	-	-	-	-	21.1% (8/38)	-	-	-	-	-	-	-	-	0.9% (15/1657)	
HIV characteristics of adult population															
HIV positive, % (n)	95.7% (44/46)	75% (3/4)	81.8% (9/11)	100% (13/13)	85.7% (18/21)	89.7% (26/29)	100% (3/3)	85.7% (6/7)	33.3% (3/9)	100% (1/1)	75.0% (42/56)	96.6% (345/357)	33.3% (4/12)	64.9% (650/1001)	92.2% (47/51)
HIV negative, % (n)	2.2% (1/46)	-	18.2% (2/11)	-	4.8% (1/21)	3.5% (1/29)	-	14.3% (1/7)	55.6% (5/9)	-	16.1% (9/56)	1.1% (4/357)	58.3% (7/12)	23.9% (239/1001)	2.0% (1/51)
HIV status unknown, % (n)	2.2% (1/46)	25.0% (1/4)	-	-	9.5% (2/21)	6.9% (2/29)	-	-	11.1% (1/9)	-	8.9% (5/56)	2.2% (8/357)	8.3% (1/12)	11.2% (112/1001)	5.9% (3/51)
Not on ART (either ARV naïve or defaulted)	43.2% (19/44)	66.7% (2/3)	33.3% (3/9)	53.8% (7/13)	44.4% (8/18)	46.2% (12/26)	-	50.0% (3/6)	66.7% (2/3)	100% (1/1)	52.4% (22/42)	50.1% (173/345)	75.0% (3/4)	41.1% (267/650)	36.2% (17/47)
CD4 count (IQR)	48 (10-134)	217 (-)	64 (35-275)	21 (7-129)	75 (30-133)	46 (15-185)	566 (12-1121)	212 (134-347)	365 (42-688)	505 (-)	136 (58-340)	30 (9-70)	78 (21-180)	192 (56-426)	30 (12-63)
CD4 count known if HIV positive, % (n)	50.0% (22/44)	33.3% (1/3)	44.4% (4/9)	69.2% (9/13)	72.2% (13/18)	46.2% (12/26)	66.7% (2/3)	83.3% (5/6)	66.7% (2/3)	100% (1/1)	40.5% (17/42)	63.8% (220/345)	75% (3/4)	58.9% (383/650)	63.8% (30/47)
CSF Findings															
WCC pleocytosis (using age criteria)	21.8% (12/55)	14.3% (2/14)	69.2% (9/13)	15.3% (2/13)	23.7% (9/38)	31.0% (9/29)	30.0% (3/10)	66.6% (6/9)	80% (8/10)	100% (2/2)	49.2% (32/65)	26.5% (95/358)	31.8% (7/22)	7.9% (131/1657)	29.6% (16/54)
CSF Protein (g/dL), median (IQR)	0.58 (0.30-1.07)	0.39 (0.19-0.84)	0.93 (0.60-1.60)	0.58 (0.20-0.96)	0.33 (0.24-0.66)	1.01 (0.60-1.99)	0.31 (0.15-0.58)	1.43 (1.0-1.84)	1.99 (1.0-3.70)	1.83 (-)	1.55 (0.39-3.70)	0.51 (0.27-1.07)	2.13 (0.59-4.11)	0.42 (0.24-0.79)	0.42 (0.24-0.86)

CSF Glucose (mmol/L), median (IQR)	2.59 (1.73-3.40)	3.11 (2.65-4.05)	3.70 (2.80-4.40)	2.60 (0.78-2.92)	2.54 (2.10-3.90)	3.40 (2.33-4.07)	3.57 (2.65-4.15)	1.39 (0.89-3.49)	1.50 (1.24-2.32)	0.04 (-)	1.0 (0.04-1.83)	2.30 (1.50-3.17)	2.97 (0.60-4.01)	3.40 (2.83-4.13)	2.14 (1.73-2.97)
Cryptococcus co-detection (routine or enhanced diagnostics)	27.3% (14/55)	7.1% (1/14)	15.4% (2/13)	30.8% (4/13)	23.7% (9/38)	24.1% (7/29)	10.0% (1/10)	11.1% (1/9)	-	-	10.8% (7/65)	n/a	-	n/a	n/a
Outcome															
Discharged alive	50.9% (28/55)	64.3% (9/14)	61.5% (8/13)	38.5% (5/13)	76.3% (29/38)	55.2% (16/29)	70.0% (7/10)	100% (9/9)	80.0% (8/10)	100% (2/2)	60.0% (39/65)	58.1% (207/358)	68% (15/22)	71.0% (1177/1657)	46.3% (25/54)
In hospital mortality	27.3% (15/55)	21.4% (3/14)	15.4% (2/13)	46.2% (6/13)	18.4% (7/38)	27.5% (8/29)	10.0% (1/10)	-	10.0% (1/10)	-	24.6% (16/65)	31.8% (114/358)	23% (5/22)	12.1% (209/1657)	42.6% (23/54)
Unknown	21.8% (12/55)	14.3% (2/14)	23.1% (3/13)	15.4% (2/13)	5.3% (2/38)	17.2% (5/29)	20.0% (2/10)	-	10.0% (1/10)	-	15.4% (10/65)	10.1% (36/358)	9% (2/22)	16.4% (271/1657)	11.1% (6/54)

Table 7: Demographics, HIV details, CSF findings and outcomes of organisms detections on CSF

*Other microbiological diagnoses: *Coagulase negative staphylococcus (7), Klebsiella pneumoniae (4) and Acinetobacter baumannii, Candida spp, Citrobacter spp, Enterobacter spp, Enterococcus spp, Pseudomonas aeruginosa, Salmonella spp, Staphylococcus aureus, unidentified coliform, and an unidentified fungus (possible contamination), each with a count of 1*

**Co-detections were defined as the presence of 2 or more organism regardless of clinical relevance. Co-detection proportions were determined using the same denominator as other variables but did not contribute to the overall total

***Inflammatory CSF was defined as either the presence of an age-adjusted CSF white cell pleocytosis **or** CSF protein >1mg/mL **and** CSF glucose <2.2mmol/mL

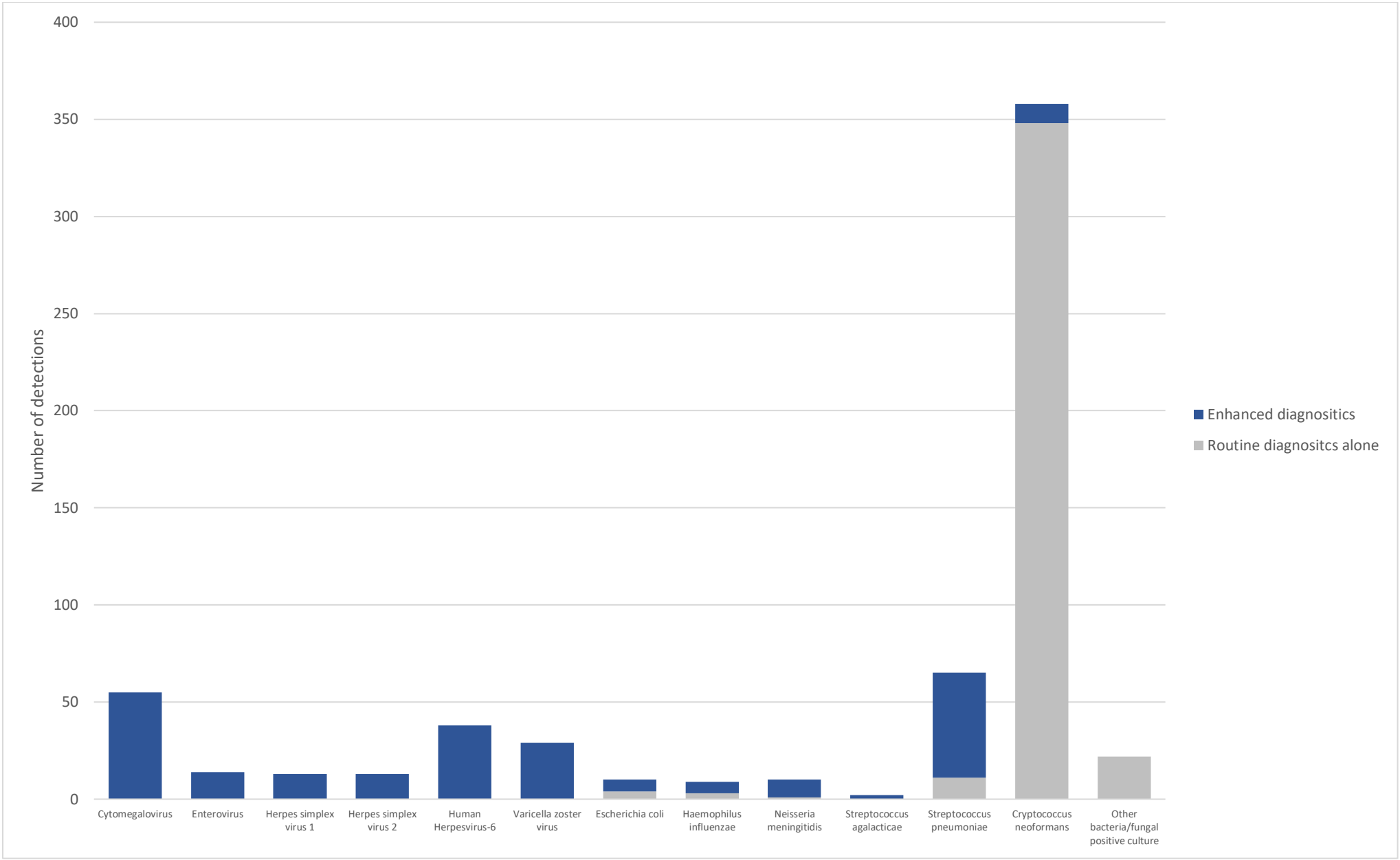

Figure 7a: Potential CNSI pathogens detected through routine and FilmArray-ME analysis

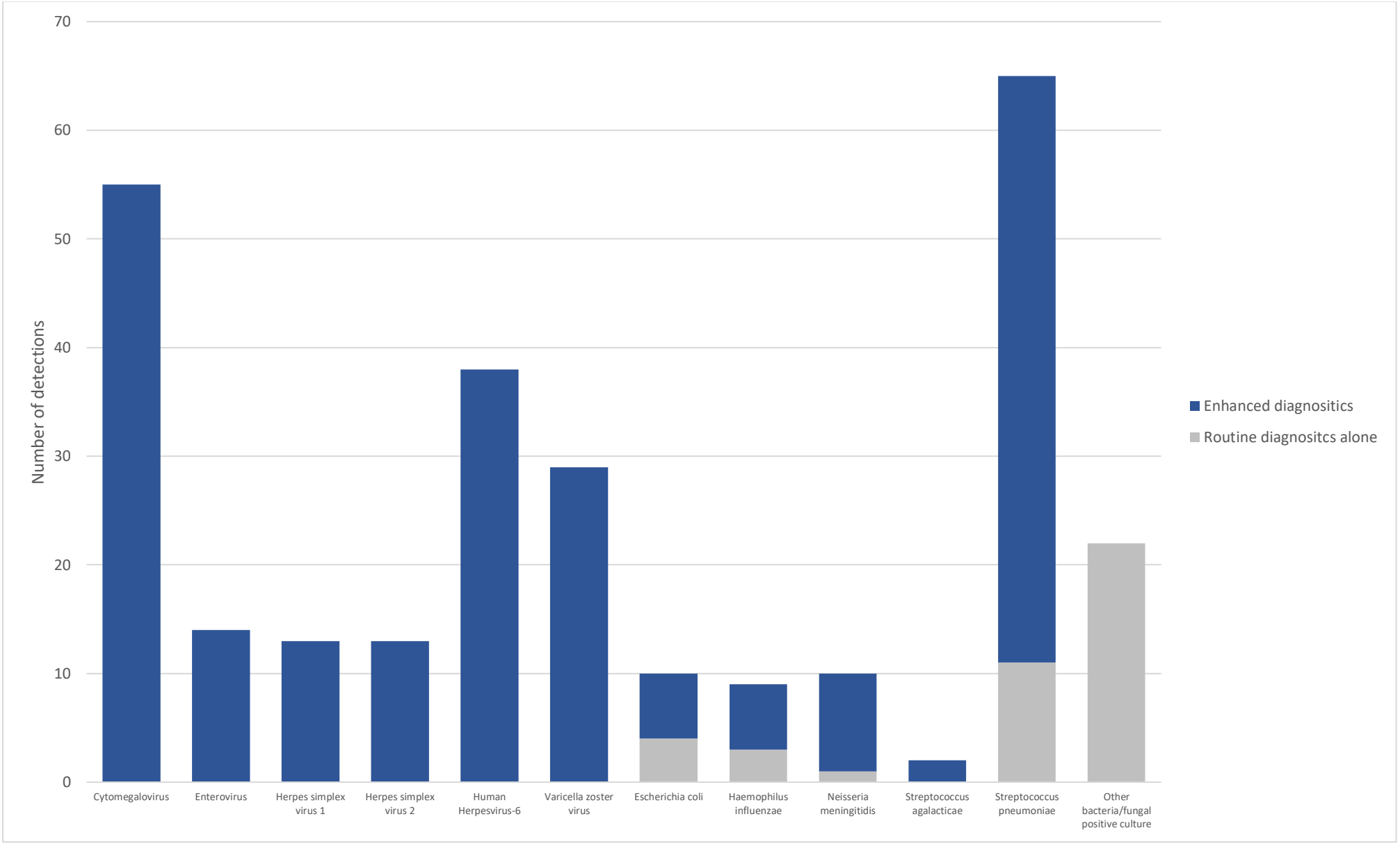

Figure 7b: *Potential CNSI pathogens detected through routine and FilmArray-ME analysis – EXCLUDING CRYPTOCOCCAL MENINGITIS DIAGNOSES*

Patient group	Number of patients	Diagnostic yield through routine testing alone	Diagnostic yield with addition of FilmArray-ME	Relative increase in diagnoses
Overall	2242	18.5% (414/2242)	26.4% (592/2242)	42.7%
Age category				
Neonates (0-28 days)	270	1.5% (4/270)	3.7% (10/270)	146.7%
Infants (1-23 months)	262	3.8% (10/262)	13.8% (36/262)	263.2%
Children (2-14 years)	131	6.1% (8/131)	15.3% (20/131)	150.3%
Adults (>14 years)	1525	25.7% (392/1525)	34.0% (524/1525)	32.3%
HIV status				
HIV negative adults (>14 years)	271	6.6% (18/271)	11.8% (32/271)	78.8%
HIV negative and UNinflammatory CSF	214	3.6% (8/224)	4.9% (11/224)	36.1%
HIV positive adults	1123	32.4% (364/1123)	42.1% (473/1123)	29.9%

HIV positive adults and negative CrAg	739	5.6% (41/739)	18.7% (138/739)	233.9%
HIV positive, negative CrAg and UNinflammatory CSF	536	3.3% (20/598)	14.7% (88/598)	345.5%
HIV positive, negative CrAg and inflammatory CSF	141	14.9% (21/141)	35.5% (50/141)	138.3%

Table 8: Diagnostic yield for routine diagnostics alone and combination of routine diagnostics and FilmArray-ME stratified by patient group. Relative increase in diagnostic yield determined at each level

Organism detected	In-hospital mortality	Organism type	Mortality
CMV	27.3% (15/55)	**Virus**	25.3% (41/162)
Enterovirus	21.4% (3/14)		
HSV-1	15.4% (2/13)		
HSV-2	41.2% (6/13)		
HHV-6	18.4% (7/38)		
VZV	27.5% (8/29)		
Escherichia coli	10% (1/10)	**Bacteria**	19.8% (23/116)
Haemophilus influenzae	0% (0/9)		
Neisseria meningitidis	10% (1/10)		
Streptococcus agalactiae	0% (0/2)		
Streptococcus pneumoniae	24.6% (16/65)		
Other bacteria	25.0% (5/20)		
Cryptococcus neoformans/gattii	31.8% (114/358)	**Fungi**	31.7% (114/360**)**
Other fungi	0% (0/2)		
No diagnosis	12.6% (209/1657)		
No diagnosis with UNinflammatory CSF	12.2% (181/1479)		
No diagnosis with inflammatory CSF	16.0% (28/175)		
Any diagnosis	26.9% (159/585)		

Table 9 Inpatient mortality by organism detected and organism kingdom

Co-detection	**Frequency**
Cryptococcus spp & CMV	14
Cryptococcus spp & HHV-6	9
Cryptococcus spp & *Streptococcus pneumoniae*	7
Cryptococcus spp & VZV	7
Cryptococcus spp & HSV-2	3
Streptococcus pneumoniae & CMV	3
Neisseria meningitidis & VZV	2
Coagulase negative *Staphylococcus* spp & HHV-6	1
Coagulase negative *Staphylococcus* spp & CMV	1
Cryptococcus spp & *Escherichia coli*	1
Cryptococcus spp & Enterovirus	1
Cryptococcus spp & *Haemophilus influenzae*	1
Cryptococcus spp & HSV-1	1
Cryptococcus spp & HSV-1 & HSV-2	1
Enterovirus & HSV-2	1
Proteus spp & CMV	1
Total	54

Table 10 Description of co-detections

3.4 Discussion

This analysis demonstrates that FilmArray-ME was successfully implemented in 4 countries within the WHO African region at sites with limited previous experience in molecular diagnostics. The use of FilmArray-ME provided a microbiological diagnosis for an additional 178 (7.9%) patients that would have otherwise not received a diagnosis. There were an additional 160 viruses, 57 bacteria and 10 fungi detected using FilmArray-ME that were not diagnosed through routine investigations. Furthermore, viral CNS pathogens including VZV and HSV were detected for the first-time in routine practice in some settings alongside increases in bacteria that were not detected through traditional culture and staining techniques[26].

Diagnostic yield varied between different study population. The highest diagnostic yield was seen in HIV positive adults where 41.7% of patients received a diagnosis through routine and enhanced diagnostics with a relative increase in diagnostic yield of 29.3% through the addition of FilmArray-ME. However, this relatively modest observed increase should be interpreted with caution given the high proportion of known CSF CrAg-positive samples tested due to the inclusion criteria of studies. If only CrAg negative samples from people with HIV were tested, then we observed a relative increase of 223.2% due to FilmArray-ME suggesting that some patient groups may have greater benefit from FilmArray-ME testing. However, whilst rationalising FilmArray-ME testing to those most at risk offers a potentially more cost-effective approach to wider implementation if testing had not been performed on CSF CrAg positive samples it would have led to 43 missed co-detections, 8.4% of all FilmArray-ME detections, although the majority of these detections would not have altered clinical management.

The detection of CMV and HHV-6 in the CSF is of uncertain direct clinical significance and therefore the added clinical benefit of their detection is unclear[26,27]. In children the detection of CMV and HHV-6 may represent self-limiting primary infection, but their detection would be unlikely to change

management. Antivirals are unlikely to be of benefit and cessation of empiric antibiotics should be performed cautiously as co-detection with other bacterial pathogens is reported. In adults, CMV and HHV-6 detection is observed principally in the context of immune-suppression however detection does not always correlate with clinical disease[27]. Their presence may be due to subclinical reactivation of latent infection in the context of advanced immunosuppression or intercurrent infection, or CNS inflammation allowing mixture of blood and CSF thereby increasing viral diversity in the CSF[28,29]. Whilst the detection of bacterial pathogens and *Cryptococcus neoformans/gattii* would likely prompt the immediate administration of antimicrobials and conversely a positive PCR for enterovirus would usually lead to the cessation of antimicrobials, management changes resulting from the detection of HHV-6 or CMV requires more nuanced clinical decision-making and additional information to determine their relevance[27,30]. Treatment of all positive CMV and HHV-6 CSF PCR results with antiviral agents would likely be unnecessary, expensive and have potentially serious side effects. Even with their exclusion FilmArray-ME still had a significant impact on the number of diagnoses made, providing an additional 116 (5.2%) individuals with a diagnosis.

Despite the ability to reliably detect a far wider range of potential CNS pathogens, cryptococcal meningitis remained the most common cause of CNSI in high HIV-prevalence African settings and most cases in our meta-analysis were diagnosed through CSF CrAg testing. CSF CrAg testing has a superior sensitivity to FilmArray-ME for the diagnosis of cryptococcal meningitis particularly at lower fungal burdens[15,20,31]. This is consistent with our data presented in this meta-analysis where FilmArray-ME detected 301 out of 358 cryptococcal meningitis cases. In 20 cases CSF CrAg was negative. This poses diagnostic challenges as CrAg testing is now often used as the only cryptococcal diagnostic for patients with suspected CNSI in high HIV-prevalence settings. Whilst false-negative CSF CrAg tests have been reported in cases of cryptococcal meningitis for several reasons including low fungal load, acapsular strains of *Cryptococcus* spp or high cryptococcal antigen titres leading to postzone effect all confirmed cryptococcal meningitis cases with negative CSF CrAg should be evaluated closely[32,33]. Monitoring for

increases in CrAg-negative cryptococcal meningitis cases due expansion of to acapsular or weakly-capsular *Cryptococcus* spp populations at sentinel sites could potentially be an important role for FilmArray-ME.

FilmArray-ME decreased the number of patients with a CSF pleocytosis but without a confirmed diagnosis from 234/309 to 174/309, a relative decrease in undiagnosed CNSI of 34%. Despite this improvement 56.4% of all patients with a CSF pleocytosis did not receive a diagnosis thereby limiting the ability to administer targeted antimicrobial therapy. There are a number of reasons why there are a significant proportion of patients without a diagnosis. Firstly, FilmArray-ME does not include common CNS infections in high HIV-prevalence settings such as *M.tuberculosis*, *T.gondii*, neurosyphilis or JC virus and expanded testing to include these analytes in the FilmArray-ME pouch would likely decrease the number of patients without a diagnosis in this highly immune-suppressed population. At present this suggests that in high HIV-prevalence settings FilmArray-ME should ideally be used in conjunction with other enhanced testing modalities, in particular Xpert MTB/RIF Ultra. Secondly there are several bacteria that were detected on culture that are not included on the FilmArray-ME panel. Given the limits of phenotypic techniques to identify bacteria in CSF particularly with the restricted laboratory facilities available it is likely that a significant proportion of bacterial infections not included in FilmArray-ME were also missed.

Mortality varied significantly between pathogens with the highest mortality being in patients with a positive HSV-2 PCR, 41.2% (6/13) and overall mortality from viral pathogens being higher than bacterial pathogens. Whilst not all viral pathogens and clinical syndromes resulting from viral infection warrant treatment with antivirals the limited availability of these medications for those that do will likely be driving the very high mortality. Previous data from high HIV-prevalence African settings also reported higher mortality from bacterial meningitis than described here[34]. This may be due to several reasons including better clinical recognition and improved access to CNS-penetrating antimicrobial

treatment. In addition, multiplex PCRs may be able to detect bacteria in CSF at lower concentrations than culture resulting in the early detection of potentially less severe cases of bacterial meningitis with lower bacterial burdens.

With the emergence of multiplex PCR testing, including with panels such as FilmArray-ME, co-detections in CSF have been increasingly described particularly in the context of advanced HIV [20,35,36]. In our study population 2.5% of patients (n=54) had more than one organism detected in CSF either on FilmArray-ME or through routine diagnostics. The most common co-detection was CMV and *Cryptococcus neoformans/gattii* which occurred in 14 cases followed by HHV-6 and *Cryptococcus neoformans/gattii* which occurred in 9 cases. The detection of more than one organism in CSF was significantly associated with an increased in-patient mortality. Whilst data on mortality in CSF co-detections is extremely limited and in our analysis we are unable to fully account for several important considerations including whether appropriate treatment was received and the higher rates of advanced immunosuppression amongst patients with more than one detection in CSF, co-prevalent extra-cranial infections including CMV viraemia are associated with increased mortality and therefore it is plausible that co-prevalent infections in the CSF will also carry a greater risk of mortality[37].

There are several limitations to this analysis. Firstly, in studies from Uganda the use of FilmArray-ME was not always random but rather targeted at those individuals where the investigators felt it would have the greatest yield. This likely introduced bias due to the inclusion of this pre-selected population that had already had other diagnoses including cryptococcal and tuberculous meningitis excluded. This population had a higher HIV prevalence and a higher pre-test probability for the detection of an organism and this was not controlled for. Formal risk of bias assessments were not used and therefore the significant heterogeneity in the included studies was not fully described or accounted for. Another limitation is the lack of data on any changes in management arising from FilmArray-ME, a key metric in determining the clinical impact of a diagnostic test. As outlined previously the majority of detections

of CMV and HHV-6 are likely not of direct clinical significance. However, in a minority of cases they may be true pathogens. As a result we may be underestimating the impact of FilmArray-ME in these cases.

In summary, we present individual patient data from the majority of published FilmArray-ME results from WHO African Region demonstrating an overall increase in number of patients receiving a confirmed microbiological diagnosis with significant variation in the incremental increase in diagnostic yield between key population groups.

3.5 References

1. Ward, K. N. *et al.* Herpes simplex serious neurological disease in young children: Incidence and long-term outcome. *Arch Dis Child* **97**, 162–165 (2012).
2. Petel, D. *et al.* Enteroviral and herpes simplex virus central nervous system infections in infants < 90 days old: A Paediatric Investigators' Collaborative Network on Infections in Canada (PICNIC) study. *BMC Pediatr* **20**, (2020).
3. Granerod, J. *et al.* Causes of encephalitis and diff erences in their clinical presentations in England: a multicentre, population-based prospective study. (2010) doi:10.1016/S1473.
4. McGill, F. *et al.* Incidence, aetiology, and sequelae of viral meningitis in UK adults: a multicentre prospective observational cohort study. *Lancet Infect Dis* **18**, 992–1003 (2018).
5. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: Findings from 4961 suspected cases. *BMC Infect Dis* **10**, (2010).
6. Tenforde, M. W. *et al.* Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey. *Journal of Infection* **79**, 212–219 (2019).
7. Tenforde, M. W. *et al.* Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana national meningitis survey: a prevalent cohort study. *Lancet Infect Dis* **19**, 740–749 (2019).
8. Kanegaye, J. T., Soliemanzadeh, P. & Bradley, J. S. Lumbar puncture in pediatric bacterial meningitis: Defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* **108**, 1169–1174 (2001).
9. Donovan, J. *et al.* Xpert MTB/RIF Ultra for the Diagnosis of Tuberculous Meningitis: A Small Step Forward. *Clinical Infectious Diseases* vol. 71 2002–2005 Preprint at https://doi.org/10.1093/cid/ciaa473 (2020).

10. Wunrow, H. Y. *et al.* Global, regional, and national burden of meningitis and its aetiologies, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* **22**, 685–711 (2023).

11. Nhantumbo, A. A. *et al.* Serotype distribution and antimicrobial resistance of Streptococcus pneumoniae in children with acute bacterial meningitis in Mozambique: Implications for a national immunization strategy. *BMC Microbiol* **16**, (2016).

12. Wall, E. C., Chan, J. M., Gil, E. & Heyderman, R. S. Acute bacterial meningitis. *Current Opinion in Neurology* vol. 35 386–395 Preprint at https://doi.org/10.1097/WCO.0000000000000934 (2021).

13. Leber, A. *et al.* Multi-Center Clinical Evaluation of a Multiplex Meningitis / Encephalitis PCR Panel for Simultaneous Detection of Bacteria, Yeast, and Viruses in Cerebrospinal Fluid Specimens. *J Clin Microbiol* **54**, 2251–2261 (2016).

14. Dack, K., Pankow, S., Ablah, E., Zackula, R. & Assi, M. Contribution of the BioFire® FilmArray® Meningitis/Encephalitis Panel: Assessing Antimicrobial Duration and Length of Stay. *Kans J Med* **12**, 1–3 (2019).

15. Van, T. T., Kim, T. H. & Butler-Wu, S. M. Evaluation of the Biofire FilmArray meningitis/encephalitis assay for the detection of Cryptococcus neoformans/gattii. *Clinical Microbiology and Infection* **26**, 1375–1379 (2020).

16. Tansarli, G. S. & Chapin, K. C. Diagnostic test accuracy of the BioFire® FilmArray® meningitis/encephalitis panel: a systematic review and meta-analysis. *Clinical Microbiology and Infection* **26**, 281–290 (2020).

17. Wall, E. C. *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* **58**, 137–145 (2014).

18. Rhein, J. *et al.* Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis* **84**, 268–273 (2016).

19. Ellis, J. *et al.* The Changing Epidemiology of HIV-Associated Adult Meningitis, Uganda 2015–2017. *Open Forum Infect Dis* **6**, 1–8 (2019).

20. Bridge, S. *et al.* Evaluation of the BioFire Ò FilmArray Ò Meningitis / Encephalitis panel in an adult and pediatric Ugandan population. *Journal of Medical Mycology* **31**, 101170 (2021).

21. Obaro, S. *et al.* Comparison of bacterial culture with BioFire® FilmArray® multiplex PCR screening of archived cerebrospinal fluid specimens from children with suspected bacterial meningitis in Nigeria. *BMC Infect Dis* **23**, (2023).

22. Williams, C. G. *et al.* Diagnostic Yield of Adding the BioFIRE FilmArray Meningitis/Encephalitis panel to routinely available diagnostics for Central Nervous System Infections in Botswana. in *ASTMH* (2021).

23. Milburn, J. *et al.* Botswana National Meningitis Survey: Implementing enhanced diagnostic platforms in low-resource, high HIV-prevalence settings to improve the diagnosis of central nervous system infections. in *ASTMH* (2022).

24. Bårnes, G. K. *et al.* New molecular tools for meningitis diagnostics in Ethiopia - A necessary step towards improving antimicrobial prescription. *BMC Infect Dis* **18**, 1–14 (2018).

25. Moher, D. *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Revista Espanola de Nutricion Humana y Dietetica* **20**, 148–160 (2016).

26. Michael, B. *et al.* Effect of delayed lumbar punctures on the diagnosis of acute bacterial meningitis in adults. *Emergency Medicine Journal* **27**, 433–438 (2010).

27. Green, D. A. *et al.* Clinical Significance of Human Herpesvirus 6 Positivity on the FilmArray Meningitis/Encephalitis Panel. *Clinical Infectious Diseases* **67**, 1125–1128 (2018).

28. Henderson, M. *et al.* Cerebrospinal fluid virology in people with HIV. *HIV Med* **24**, 838–844 (2023).

29. Smith, D. M. *et al.* Pleocytosis is associated with disruption of HIV compartmentalization between blood and cerebral spinal fluid viral populations. *Virology* **385**, 204–208 (2009).

30. Pandey, U. *et al.* Pathogen or bystander: Clinical significance of detecting human herpesvirus 6 in pediatric cerebrospinal fluid. *J Clin Microbiol* **58**, 1–9 (2020).

31. Liesman, R. M. *et al.* Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis. *J Clin Microbiol* **56**, 1–9 (2018).

32. Ssebambulidde, K. *et al.* Symptomatic Cryptococcal Antigenemia Presenting as Early Cryptococcal Meningitis with Negative Cerebral Spinal Fluid Analysis. *Clinical Infectious Diseases* **68**, 2094–2098 (2019).

33. Rutakingirwa, M. K., Kiiza, T. K. & Rhein, J. "False negative" CSF cryptococcal antigen with clinical meningitis: Case reports and review of literature. *Med Mycol Case Rep* **29**, 29–31 (2020).

34. Kelly, M. J. *et al.* Epstein-Barr Virus Coinfection in Cerebrospinal Fluid Is Associated With Increased Mortality in Malawian Adults With Bacterial Meningitis. **205**, (2012).

35. Siddiqi, O. K. *et al.* Molecular diagnosis of central nervous system opportunistic infections in HIV-infected zambian adults. *Clinical Infectious Diseases* **58**, 1771–1777 (2014).

36. Rajasingham, R. *et al.* Epidemiology of meningitis in an HIV-infected Ugandan cohort. *American Journal of Tropical Medicine and Hygiene* **92**, 274–279 (2015).

37. Ellis, J. *et al.* Baseline Cytomegalovirus Viremia at Cryptococcal Meningitis Diagnosis Is Associated With Long-term Increased Incident TB Disease and Mortality in a Prospective Cohort of Ugandan Adults With HIV. *Open Forum Infect Dis* **10**, (2023).

Chapter IV – Central nervous system infection diagnostics in routine care

4.1 Introduction

CNSI continue to carry significant mortality in high HIV-prevalence settings at least in part due to an absence of rapid, reliable diagnostics. Rapid diagnostics are available for the two most common causes of CNSI in high HIV-prevalence settings, cryptococcal antigen testing (CrAg) for cryptococcal meningitis and Xpert MTB/RIF for tuberculous meningitis (TBM). Both tests have been implemented in Botswana, although with varying levels of coverage and impact. This chapter comprised of two manuscripts, one published and one undergoing review, demonstrates the impact of widespread implementation of rapid CNSI diagnostics into routine care in a high HIV-prevalence resource-limited setting.

4.2 Manuscript - Tracking cryptococcal meningitis to monitor HIV program success during the Treat-All era: an analysis of national data in Botswana

Cryptococcal meningitis continues to be the most common cause of CNSI in Botswana and is associated with significant mortality. Cryptococcal meningitis can be diagnosed within 15 minutes using the highly-sensitive CrAg test and this has revolutionised the care of patients with cryptococcal meningitis. CSF CrAg testing was initially implemented to quickly and reliably diagnose cryptococcal meningitis in patients with advanced HIV disease. However, the impact of CrAg is such that it may now have the potential to serve as a more accessible indicator of advanced HIV disease and HIV programme success than other metrics such as CD4 testing that are not universally available in most high HIV-prevalence African settings.

A 2017 study from Botswana using data between 2000-2014 presented a comprehensive national analysis of temporal trends in cryptococcal meningitis following the scale-up of ART in Botswana which was published in *Clinical Infectious Diseases*. These data demonstrated that despite a decline in cryptococcal meningitis incidence a substantial burden of cryptococcal meningitis persisted despite improved antiretroviral coverage. Since this analysis, ART coverage has further increased with the introduction of universal treatment, "Treat All", where all patients with a positive HIV test are started on ART regardless of CD4 count. Botswana is a leader in HIV programming and is almost uniquely placed as a low-resource, high HIV-prevalence Southern African country to conduct national analyses of routinely collected data through electronic health records due to the ability to link patient electronic health record data through a national identification number.

The following manuscript uses 8 years of robust national meningitis surveillance data from Botswana to present cryptococcal meningitis incidence estimates between 2015 and 2022 to determine the impact of universal treatment of HIV on the burden of cryptococcal meningitis. These data demonstrate the potential utility of cryptococcal meningitis surveillance as an indicator of advanced

HIV disease and therefore HIV programmatic success. Furthermore, there is additional scope beyond what is presented here to provide valuable insights into key patient groups presenting with advanced HIV disease. If reliable data on HIV treatment status or date of HIV diagnosis were available this would allow characterisation of whether most advanced HIV disease presentations are new diagnoses or treatment failure.

During the study period a total of 1,744 episodes of cryptococcal meningitis were identified. The incidence declined from 15.0 (95% CI 13.4-16.7) cases/100,000 person years in 2015 to 7.4 (95% CI 6.4-8.6) cases/100,000 person years in 2022. The highest incidence was in males and individuals aged 40-44 with the majority of cases being diagnosed through the cheap, reliable and easy-to-use cryptococcal antigen test.

The manuscript has been published in Clinical Infectious Diseases.

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Student ID Number	2004168	**Title**	Dr
First Name(s)	James		
Surname/Family Name	Milburn		
Thesis Title	Strategies to improve diagnosis of central nervous system infections in high HIV-prevalence African settings		
Primary Supervisor	Prof J Jarvis		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Clinical Infectious Diseases		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised the project with guidance from Prof Jarvis following on from work by Dr Mark Tenforde. I accessed the data with support from the data warehouse team at the Botswana Ministry of Health and Wellness (primarily Ookeditse Ntwatagae and Tony Chebani). I merged and cleaned the dataset. I analysed the data with guidance from (Daniel Grint and Prof Jarvis), created the figures and tables and wrote the manuscript which was reviewed by Prof Jarvis and then subsequently all co-authors.

SECTION E

Student Signature	
Date	10/3/24

Supervisor Signature	
Date	15/3/24

Clinical Infectious Diseases

MAJOR ARTICLE

Tracking Cryptococcal Meningitis to Monitor Human Immunodeficiency Virus Program Success During the Treat All Era: An Analysis of National Data in Botswana

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Background. Cryptococcal meningitis (CM) causes substantial mortality in African countries with a high prevalence of human immunodeficiency virus (HIV), despite advances in disease management and increasing antiretroviral therapy (ART) coverage. Reliable diagnosis of CM is cheap and more accessible than other indicators of advanced HIV disease burden such as CD4 testing or investigation for disseminated tuberculosis; therefore, monitoring CM incidence has the potential to serve as a valuable metric of HIV programmatic success.

Methods. Botswana national meningitis surveillance data from 2015 to 2022 were obtained from electronic health records. All electronic laboratory records from cerebrospinal fluid samples analyzed within government healthcare facilities in Botswana were extracted from a central online repository. Adjustments for missing data were made through triangulation with prospective cohort study datasets. CM case frequency was enumerated using a case definition and incidence calculated using national census data.

Results. A total of 1744 episodes of CM were identified; incidence declined from 15.0 (95% confidence interval [CI], 13.4–16.7) cases/100 000 person-years in 2015 to 7.4 (95% CI, 6.4–8.6) cases/100 000 person-years in 2022. However, the rate of decline slowed following the introduction of universal treatment in 2016. The highest incidence was observed in men and individuals aged 40–44 years. The proportion of cases diagnosed through cryptococcal antigen testing increased from 35.5% to 86.3%.

Conclusions. CM incidence has decreased in Botswana following expansion of ART coverage but persists at a stubbornly high incidence. Most cases are now diagnosed through the cheap and easy-to-use cryptococcal antigen test, highlighting the potential of using CM as key metric of program success in the Treat All era.

Keywords. cryptococcal meningitis; advanced HIV disease; opportunistic infections; Botswana.

Cryptococcal meningitis remains the most common cause of meningitis in sub-Saharan Africa, typically affecting patients with advanced human immunodeficiency virus (HIV) disease (AHD) [1]. Despite widespread expansion of antiretroviral therapy (ART) programs, modeled estimates suggest that although there has been some reduction in global incidence of cryptococcal meningitis, it remains a major cause of mortality among people with HIV (PWH), accounting for 19% of all HIV-related deaths globally [1]. However, very few countries collect reliable statistics on cryptococcal meningitis incidence. Therefore, the impact of World Health Organization (WHO) universal HIV treatment (Treat All) guidelines introduced in 2016 and subsequent expansions in ART coverage on the incidence of opportunistic infections (OIs) such as cryptococcal meningitis is not known.

Botswana has been at the forefront of ART programming in Africa; it was the first African country to offer free ART to citizens in 2002 at a time when the national HIV prevalence was >25% [2], and a series of innovative HIV care models were implemented including the adoption of universal treatment in 2016 [3]. Under the Treat All strategy, any person who tested positive for HIV should be started on treatment, regardless of CD4 count or viral load, and in 2022 Botswana became one of the first countries globally to report reaching the Joint United Nations Programme on HIV/AIDS (UNAIDS) 95-95-95 targets. In the context of such extensive ART coverage, the incidence of AHD and the rates of OIs such as cryptococcal meningitis would be expected to decline. However, adult HIV prevalence rates remain high at 18.6% in those aged 15–49 years

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in 2021 [4], and presentations with AHD remain common through late diagnosis or disengagement with treatment [5–8]. Previous data from Botswana demonstrated that cryptococcal meningitis incidence initially fell following widespread ART rollout in the mid-2000s but plateaued between 2010 and 2014 [9], with the 2013–2014 cryptococcal meningitis incidence in Botswana comparable to pre-ART rates in neighboring South Africa. More recent data from South Africa demonstrated that the incidence of cryptococcal disease varies between regions, with some districts reporting an increase in incidence from 2018 to 2019 [10], highlighting that even in countries with high levels of ART coverage there remains a significant population of individuals developing AHD and associated OIs.

Cryptococcal meningitis is an important and potentially accessible metric to assess the performance of national HIV program success, although to date it has not been widely utilized due to the lack of established data collection systems. The majority of individuals who develop cryptococcal meningitis will present to healthcare facilities, and the disease can be easily and reliably diagnosed using cheap, highly sensitive, and easy-to-use cryptococcal antigen (CrAg) lateral flow assays. The IMMY CrAg lateral flow assay (IMMY, Norman, Oklahoma) is widely used in Botswana and can be performed in <15 minutes without significant laboratory infrastructure or training [11]. This is in marked contrast to many other indicators of AHD, such as CD4 count testing, which requires extensive laboratory infrastructure [12], or other indicator diseases such as disseminated tuberculosis or pneumocystis pneumonia (PCP), where there is a lack of sensitive diagnostics, often considerable diagnostic uncertainty clinically, and a large proportion of disease in the community rather than healthcare facilities, making accurate case ascertainment difficult [13, 14].

To explore the utility of cryptococcal meningitis surveillance in assessing the impact of national HIV programs, and to establish the impact of the Treat All strategy introduction in 2016 on cryptococcal meningitis incidence in the high-HIV-prevalence setting of Botswana, we analyzed 8 years of routine national laboratory data from the Botswana Ministry of Health and Wellness electronic medical record systems alongside data regarding ART coverage from the National ART Programme.

METHODS

Study Design

The Botswana National Meningitis Survey is an ongoing meningitis surveillance network utilizing routine national data to monitor trends in the etiology of central nervous system infections in Botswana [15]. Periodic review of national electronic laboratory records of cerebrospinal fluid samples (CSF) collected between 1 January 2015 and 31 December 2022 was undertaken. Between 2015 and 2022, CSF analysis was performed at government laboratories linked to 25 healthcare facilities: 2 referral hospitals, 7 district hospitals, and 16 primary hospitals. Universal healthcare, including CSF analysis and CrAg testing, is provided for free to Botswana citizens. Routine analysis of CSF samples in Botswana should consist of macroscopic examination and cell count with differential if white cell count is ≥10 cells/μL. The sample is centrifuged and Gram and India ink stained, and culture is performed on the sediment using Sabouraud dextrose, blood agar, and chocolate agar. CrAg testing is performed on uncentrifuged CSF samples from all adult patients ≥18 years of age and upon request for pediatric cases. However, these tests are reliant on receipt of sufficient volume of CSF and adequate supply of consumables. Therefore not all tests are performed on all samples. Laboratory records from laboratories performing CSF analysis are uploaded on to a national electronic health record system, the Integrated Patient Management System (IPMS). All CSF samples with results stored on IPMS were extracted from a centralized online repository in collaboration with the Botswana Ministry of Health and Wellness. There are 3 private hospitals that do not report to IPMS and therefore data from these hospitals were not captured. In 2014, based on a comprehensive nationwide surveillance study, the private sector accounted for 7.4% of all samples [16]. We have assumed that the proportional public/private workload has remained constant and applied this figure as an adjustment to our estimates. One hospital linked to a mining development did not report results to IPMS. This hospital had between 11 and 14 cases of cryptococcal meningitis annually from the same nationwide data; we applied an additional 2.5% uplift to incidence estimates to account for this.

Data capture in the electronic IPMS system is not 100% complete due to intermittent power outages, as well as poor internet connectivity or maintenance (known as "downtime"). In periods of IPMS downtime, results are disseminated locally on paper records. To correct for this incomplete coverage, data from the national referral hospital, Princess Marina Hospital (PMH), Gaborone, were used to triangulate the underestimation of cryptococcal meningitis cases due to periods of IPMS downtime. Comprehensive prospective data including all paper downtime records were collected from every patient with CSF submitted to PMH in 2022, which accounts for approximately 40% of all CSF samples analyzed in Botswana. In this prospective cohort, 34 cases of cryptococcal meningitis were diagnosed, 31 of which were identified on IPMS, an underestimation of 8.8%. As PMH is the national referral hospital in Botswana and has more robust information technology infrastructure than smaller regional or district hospitals where there will be a larger amount of CSF results reported on paper not captured in this study, cryptococcal meningitis case frequency and incidence rates were inflated by a conservative 10% to account for this underestimation.

Cryptococcal Meningitis Case Definition

A case of cryptococcal meningitis was defined as a positive CSF India ink stain, positive CSF culture for *Cryptococcus*

neoformans, and/or positive CSF CrAg. As CSF analysis can be repeated on patients with cryptococcal meningitis for management of raised intracranial pressure, when >1 CSF sample was analyzed for an individual patient, an episode was defined as a positive CSF sample >14 days apart from a previous positive sample. Positive samples ≤14 days from each other were considered part of the same episode.

Data Analysis

The number of cases were enumerated using the case definition. Patient age and sex were described using frequencies, percentages, or median and interquartile range (IQR) as appropriate. 2011 and 2021 census data were used to calculate cryptococcal meningitis incidence. A linear increase in population across intervening years was assumed to determine yearly cryptococcal meningitis incidence. Breakdown of population by sex is currently not available for the 2022 census; therefore, the same proportion of males and females from the 2011 census was assumed across all years. UNAIDS Spectrum model data were used to determine HIV prevalence and number of individuals receiving ART. The 95% confidence intervals (CIs) for incidence of cryptococcal meningitis were derived using the exact binomial method. Linear regression analysis was performed to assess the relationship between ART coverage among PWH and cryptococcal meningitis incidence.

The frequency of cryptococcal meningitis case diagnosis at the 2 national referral hospitals (PMH and Nyangabgwe Referral Hospital, Francistown) was compared before and after the introduction of Treat All in June 2016 using data shared from the previous national analysis [9]. This longitudinal analysis was restricted to the 2 referral hospitals as these were the only sites with comprehensive longitudinal data pre-2015.

Interrupted time series analysis was performed to assess the effect of Treat All as an intervention to decrease cryptococcal meningitis case frequency. Yearly cryptococcal meningitis case frequency data were smoothed using a moving average and Newey-West standard errors for coefficients were estimated by ordinary least squares regression. The intervention cut-point was set at 1 January 2017 to allow a lead-in time of 6 months for patients to be established on ART following the change in the national ART program strategy.

Ethical Approvals

The study was performed with the support of the Botswana Ministry of Health and Wellness. Institutional review board approval was in place from the Health Research Development Committee (Botswana Ministry of Health and Wellness), London School of Hygiene and Tropical Medicine, and University of Botswana, and local approval was obtained from the study's sentinel site (PMH).

RESULTS

A total of 1744 episodes of cryptococcal meningitis were identified occurring in 1440 individuals between 2015 and 2022 (Table 1). In patients diagnosed with cryptococcal meningitis, 84.5% (1217/1440) had a single episode of cryptococcal meningitis and 15.4% (223/1440) had 2 or more episodes. The median time between first and second episode was 48 days (IQR, 19–130 days). The median age at diagnosis was 38.9 years (IQR, 33.1–45.5 years) with more cases in males (938/1440 [65.1%]) than females; 44.2% (770/1744) of all cryptococcal meningitis cases were diagnosed in 1 of the 2 national referral hospitals and 33.2% (579/1744) were diagnosed in district hospitals. The remainder were diagnosed either in primary hospitals, clinics, or hospital linked to mining developments.

The estimated national incidence of cryptococcal meningitis in Botswana approximately halved between 2015 and 2022 (15.0 [95% CI, 13.4–16.7] cases/100 000 person-years and 7.4 [95% CI, 6.4–8.6] cases/100 000 person-years, respectively) (Figure 1*A*). In PWH, the incidence of cryptococcal meningitis decreased from 92.0 (95% CI, 82.2–102.6) cases/100 000 person-years in 2015 to 49.1 (95% CI, 42.2–57.0) cases/100 000 person-years in 2022 (Figure 1*B*). The incidence of cryptococcal meningitis decreased in both males and females between 2015 and 2022 (Figure 1*C*). Incidence remained nearly 3-fold higher

Table 1. Description of Basic Demographics and CD4 Count of Patients With Cryptococcal Meningitis and Cryptococcal Meningitis Diagnoses in Botswana by Month

Variable	No. (%) or Median (IQR)
Age at diagnosis[a] (n = 1439/1440)	
Median (IQR)	38.9 (33.1–45.5)
Sex[a] (n = 1440/1440)	
Male	938 (65.1)
Female	502 (34.9)
CD4 count at diagnosis, cells/μL (closest result within 6 mo of LP)[b] (n = 944/1744)	
Median (IQR)	48 (23–107)
Month of diagnosis[b] (n = 1744/1744)	
January	143 (8.2)
February	126 (7.2)
March	164 (9.4)
April	157 (9.0)
May	149 (8.5)
June	125 (7.2)
July	152 (8.7)
August	156 (8.9)
September	136 (7.8)
October	169 (9.7)
November	149 (8.5)
December	118 (6.8)

For CD4 testing the closest result to the date of cryptococcal meningitis diagnosis was reported if it was within a 6-month window of the diagnosis of cryptococcal meningitis.

Abbreviations: IQR, interquartile range; LP, lumbar puncture.

[a]De-duplicated to represent individual patients rather than episodes.

[b]Data from all episodes including relapses.

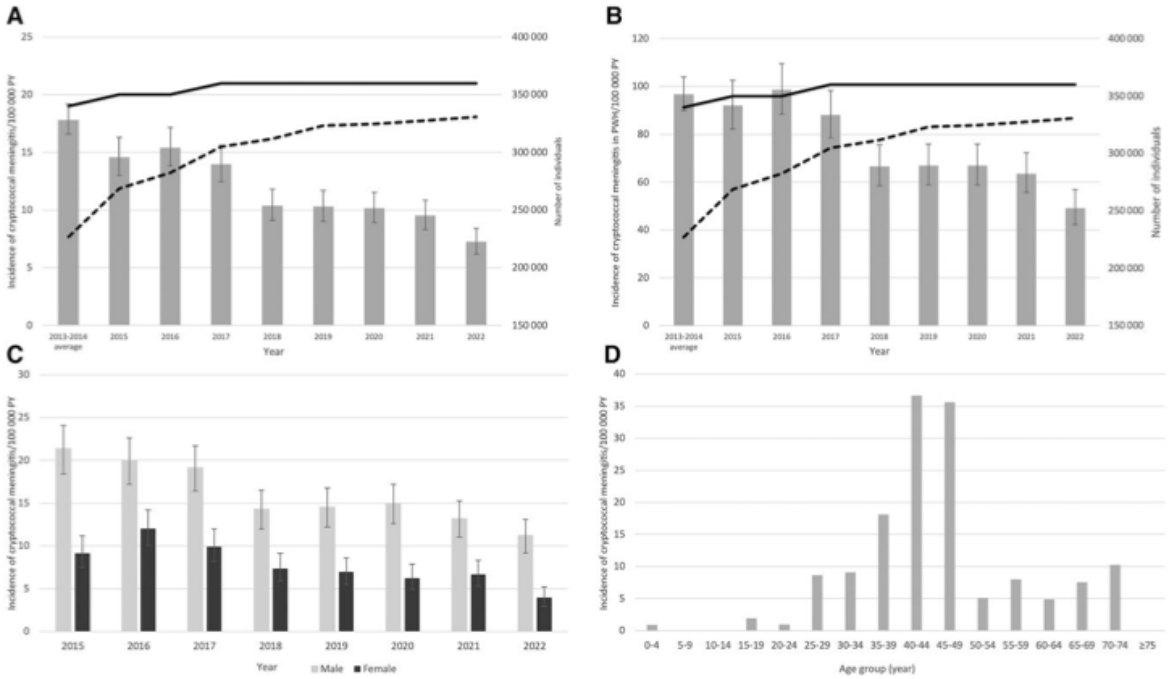

Figure 1. *A*, Incidence of cryptococcal meningitis in Botswana/100 000 person-years (PY) of observation from 2013 to 2022 (bar chart) with 95% confidence intervals (CIs). Joint United Nations Programme on HIV/AIDS (UNAIDS) estimate of total numbers of people with human immunodeficiency virus (PWH) (unbroken line) and number of people receiving antiretroviral therapy (dotted line). *B*, Incidence of cryptococcal meningitis/100 000 PY of observation in PWH between 2013 and 2022 with 95% CIs. UNAIDS estimate of total numbers of PWH (unbroken black line) and number of people receiving antiretroviral therapy (dotted line). *C*, Incidence of cryptococcal meningitis/100 000 PY of observation by sex between 2015 and 2022 with 95% CIs. *D*, Incidence of cryptococcal meningitis/100 000 PY of observation by age category in 2022.

in males in 2022, with 11.2 cases/100 000 person-years in males and 4.0 cases/100 000 person-years in females. Peak incidence by age was between 40 and 44 years (Figure 1*D*). Linear regression analysis of the association between cryptococcal meningitis incidence and proportion of PWH on ART showed that for every 5% increase in ART coverage, we observed a decrease in cryptococcal meningitis incidence of 2.5 cases/100 000 person-years.

The frequency of cryptococcal meningitis cases at the 2 national referral hospitals decreased between 2004 and 2022 (Figure 2). There was no significant decline in the case frequency of cryptococcal meningitis at the point of Treat All introduction, with a change in case frequency of 9.5 (95% CI, −1.4 to 20.3; $P = .082$) observed. The rate of decline in cryptococcal meningitis cases/year before the intervention date of 1 January 2017 was −13.0 cases/year (95% CI, −13.9 to −12.1; $P < .001$). The rate of decline after the intervention date was −6.2 cases/year (95% CI, −8.5 to −3.9; $P < .001$). The test for interaction between intervention period and time provided strong evidence that the rate of decline after the intervention date was lower than before ($P < .001$).

The proportions of cryptococcal meningitis diagnoses made through the 3 most common diagnostic modalities (India ink, culture, and CrAg testing) available in Botswana are displayed in Figure 3. CrAg testing became the most used modality, with the proportion of diagnoses made through CrAg testing increasing from 35.5% in 2015 to 86.3% in 2022.

DISCUSSION

Robust national cryptococcal meningitis incidence estimates from Botswana, an African country with high HIV prevalence, demonstrate that cryptococcal meningitis incidence has declined since 2015 with the narrowing ART treatment gap, but this rate of decline in cryptococcal meningitis incidence has slowed despite increasing ART coverage following the rollout of universal treatment in 2016. While individual patient–level data are lacking to make direct causal observations, our data demonstrated that the incidence of cryptococcal meningitis was significantly associated with the proportion of patients on ART. Patients presenting with cryptococcal meningitis in Botswana typically had a CD4 count <50 cells/μL, highlighting that a hard-to-reach population, with an overrepresentation of working age males, remains at risk of cryptococcal meningitis usually due to late diagnosis or cycling out of treatment services due to the failure of services to effectively engage and retain this group. Providing effective care for this group will require novel

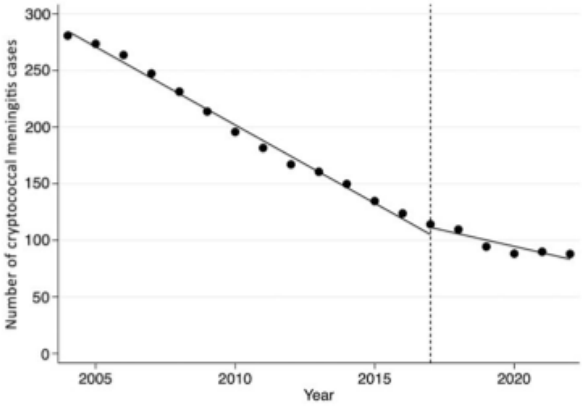

Figure 2. Frequency of cryptococcal meningitis cases at the 2 national referral hospitals between 2004 and 2023. Interrupted time series analysis was used to generate predicted trends in cryptococcal meningitis cases pre- and postintervention.

treatment strategies and enhanced OI screening and prevention to reduce presentations with AHD and associated OIs.

While the incidence of cryptococcal meningitis continued to decline after 2017, the rate of decline slowed compared to the period before 2017. In contrast, we might have expected to see the ongoing impact of universal HIV treatment to increase the rate of decline in cryptococcal meningitis incidence during this period. It is likely that during that period the coronavirus disease 2019 (COVID-19) pandemic adversely affected HIV care, as demonstrated in other countries where service utilization was decreased in a number of diseases [17, 18]. Botswana had one of the highest reported mortality rates from COVID-19 in Africa, and access to healthcare including HIV services was challenging due to COVID-19–related restrictions [19]. As such, it is possible that interruptions to HIV care during the peak of the COVID-19 pandemic prevented further decline in cryptococcal meningitis incidence, and continued monitoring is necessary to establish the true impact of universal treatment.

We have demonstrated the potential for cryptococcal meningitis to be a key metric for monitoring HIV program success in the Treat All era using national laboratory data from a high-HIV-prevalence country. Botswana is uniquely placed in the region to conduct nationwide surveillance of AHD presentations with cryptococcal meningitis due to a robust electronic health record system where patients are linked through a national identification number. These data show that continued presentations with AHD in Botswana are common even with widespread ART coverage. Patients presenting with AHD have increased mortality compared to those who do not present with AHD, and monitoring and addressing excess mortality from AHD is crucial to inform program success [20, 21]. However, capturing these data is often challenging. Accurate mortality data are lacking as very few high-HIV-prevalence countries have reliable systems in place to track mortality or cause of death. Furthermore, AHD is often not identified, as a reliance on WHO clinical staging alone misses a high proportion of patients with AHD [22], and since the advent of Treat All, routine preinitiation CD4 count testing has not been prioritized. CD4 testing requires significant laboratory infrastructure and regular supply of consumables, which, since donor funding for CD4 testing has been cut, are often not available. Therefore, other indicators of AHD are necessary. Screening for OIs such as disseminated tuberculosis or PCP is difficult as diagnoses cannot be made confidently on clinical findings alone and available diagnostics are often costly or insensitive. Cryptococcal meningitis can be diagnosed using the affordable, highly sensitive CrAg test and in contrast to other OIs, most cases of cryptococcal meningitis will be seen in healthcare facilities, making case number ascertainment easier. Our data confirm that the majority of cryptococcal meningitis cases are now diagnosed through CrAg testing, making cryptococcal meningitis a cheap and accessible metric for AHD surveillance and valuable indicator HIV program success.

There are some important limitations to the study. We derived incidence estimates solely from data stored on electronic health records, and not all results will be uploaded to this system due to interruptions in connection to the database or the testing facility reporting results to clinicians through a different modality. Although we used existing, reliable datasets to triangulate for this underestimation and account for this using conservative percentage uplifts in our estimates, this undercounting may not have been fully corrected for, and our corrections add an additional degree of uncertainty to our estimates. Some individuals will not seek medical care or die before being diagnosed with cryptococcal meningitis. Further underestimation may occur as some facilities are unable to reliably diagnosis cryptococcal meningitis due to a

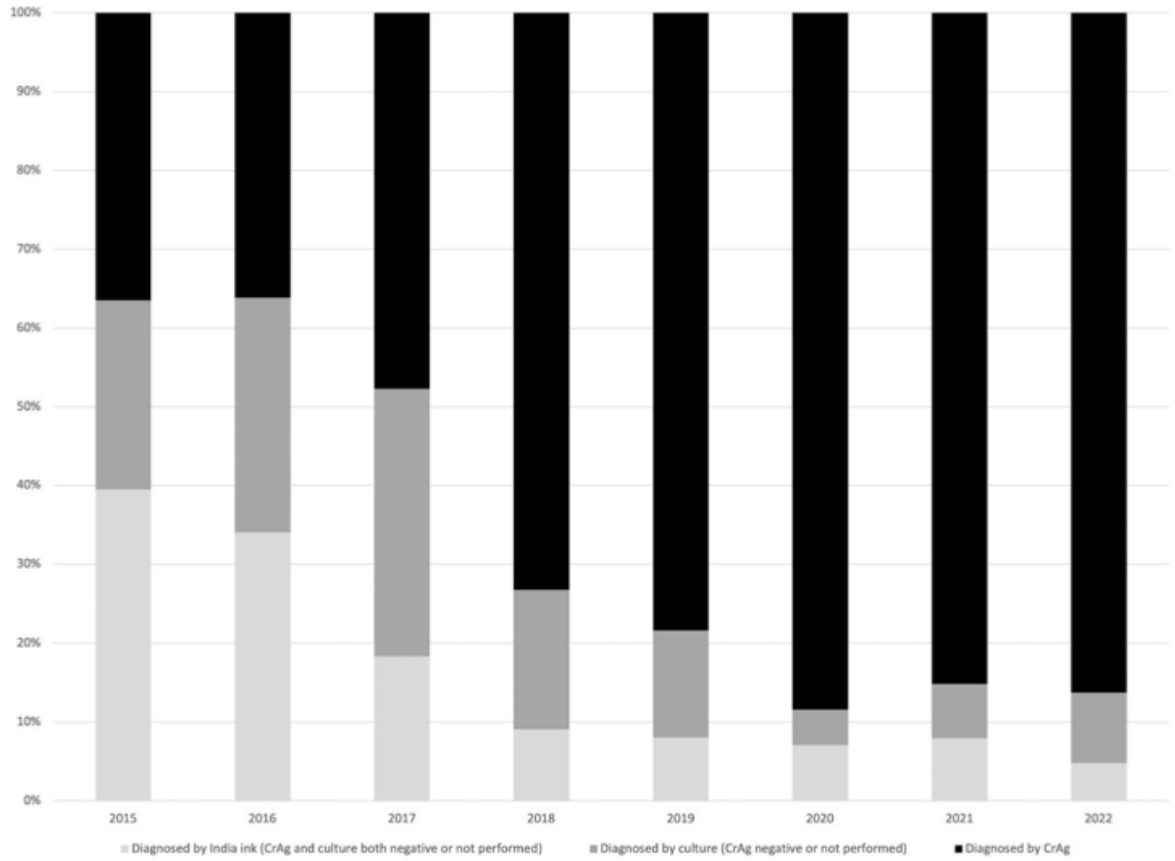

Figure 3. Yearly variation in cryptococcal meningitis diagnoses made through different diagnostic modalities. Group definitions are as follows: Diagnosed by cryptococcal antigen (CrAg): positive cerebrospinal fluid (CSF) CrAg; India ink testing and culture positivity not accounted for. Diagnosed by culture: positive CSF culture for *Cryptococcus neoformans*. CSF CrAg testing either negative or not performed; India ink testing not accounted for. Diagnosed by India ink: positive CSF India ink stain. CSF CrAg and culture both negative or not performed.

lack of clinical equipment such as lumbar puncture needles or laboratory reagents, including CrAg kits, to test for cryptococcal meningitis. As such, the estimates presented here are likely at the lowest range of cryptococcal meningitis incidence. ART coverage estimates were derived from UNAIDS Spectrum model data and we were unable to confirm the accuracy of these data as we did not capture ART status data. Cryptococcal meningitis case numbers were enumerated using a 2-week interval between lumbar punctures. While this cutoff has been used previously, CSF CrAg positivity can persist for >2 weeks, so in a small number of cases there may be some ambiguity as to whether a positive CSF CrAg represents a new infection. CSF CrAg testing is more sensitive than culture and India ink; the increased utilization of CSF CrAg to diagnose cryptococcal meningitis during the study period may have resulted in the detection of cases that would have otherwise been missed through India ink and culture alone. Therefore, lower estimates of cryptococcal meningitis incidence may have been observed in those years when the majority of cryptococcal meningitis cases were diagnosed through India ink or culture. While there was an association between ART coverage and cryptococcal meningitis incidence, the impact of other interventions such as CrAg screening programs have not been accounted for. CrAg screening was introduced in Botswana in 2016, but initially limited to small pilot programs in the capital city, with wider rollout not occurring until 2019; given that our data are restricted to CSF testing results, we were unable to account for the impact of blood CrAg screening programs, which may have also contributed to a decline in cryptococcal meningitis incidence, particularly in the last 2–3 years of observation. Analyses to determine the reach and impact of CrAg screening programs in Botswana are in progress.

Monitoring and understanding of mortality from AHD forms an important part of HIV programming, but mortality data are often lacking and many indicators of AHD are either inaccessible due to cost or infrastructure, insensitive, or used

on predominantly outpatient populations, making data collection more difficult. Cryptococcal meningitis incidence persists, disproportionately affecting key populations despite excellent ART coverage. Cryptococcal meningitis surveillance is therefore a potentially reliable and accessible metric that could be expanded to be a key monitoring marker to evaluate HIV program success.

Notes

Author Contributions. J. N. J., M. T. and J. M. conceived the project. J. M., O. N., R. S., K. N., C. N., J. P., M. K., I. M., S. E., A. A., D. R. and T. C. were involved in data extraction and data generation. J. M. performed the analysis with guidance from D. G. and J. N. J. All authors contributed to the manuscript writing, reviewing and approval of the final version.

Disclaimer. The views expressed in this publication are those of the author(s) and not necessarily those of the National Institute for Health and Care Research or the UK Department of Health and Social Care.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Rajasingham R, Govender NP, Jordan A, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet Infect Dis **2022**; 22:1748–55.
2. Stover J, Fidzani B, Molomo BC, Moeti T, Musuka G. Estimated HIV trends and program effects in Botswana. PLoS One **2008**; 3:e3729.
3. Vinikoor MJ, Hachaambwa L. Advanced HIV disease during the 'Treat All' era in Botswana. AIDS **2020**; 34:2321–3.
4. Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates Botswana. 2022. Available at: https://www.unaids.org/en/regionscountries/countries/botswana. Accessed 26 June 2023.
5. Jarvis JN, Lawrence DS, Meya DB, et al. Single-dose liposomal amphotericin B treatment for cryptococcal meningitis. N Engl J Med **2022**; 386:1109–20.
6. Lebelonyane R, Mills LA, Mogorosi C, et al. Advanced HIV disease in the Botswana combination prevention project: prevalence, risk factors, and outcomes. AIDS **2020**; 34:2223–30.
7. Leeme TB, Mine M, Lechiile K, et al. Utility of CD4 count testing in the era of universal ART: an analysis of routine laboratory data in Botswana. HIV Med **2021**; 22:1–10.
8. Tenforde MW, Milton T, Rulaganyang I, et al. Outcomes of reflex cryptococcal antigen (CrAg) screening in human immunodeficiency virus (HIV)-positive patients with CD4 counts of 100–200 cells/μL in Botswana. Clin Infect Dis **2021**; 72: 1635–8.
9. Tenforde MW, Mokomane M, Leeme T, et al. Advanced human immunodeficiency virus disease in Botswana following successful antiretroviral therapy rollout: incidence of and temporal trends in cryptococcal meningitis. Clin Infect Dis **2017**; 65:779–86.
10. Desanto DJ, Bangdiwala AS, Van Schalkwyk E, et al. Evaluation of the effectiveness of a South African laboratory cryptococcal antigen screening programme using a retrospective cohort and a cluster-randomised trial design. BMJ Open **2022**; 12:e054057.
11. Huang HR, Fan LC, Rajbanshi B, Xu JF. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: a meta-analysis and systematic review. PLoS One **2015**; 10: e0127117.
12. Pham MD, Agius PA, Romero L, et al. Acceptability and feasibility of point-of-care CD4 testing on HIV continuum of care in low and middle income countries: a systematic review. BMC Health Serv Res **2016**; 16:343.
13. Bateman M, Oladele R, Kolls JK. Diagnosing *Pneumocystis jirovecii* pneumonia: a review of current methods and novel approaches. Med Mycol **2020**; 58:1015–28.
14. Sharma SK, Mohan A, Kohli M. Extrapulmonary tuberculosis. Expert Rev Respir Med **2021**; 15:931–48.
15. Tenforde MW, Mokomane M, Leeme T, et al. Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: results from the Botswana national meningitis survey. J Infect **2019**; 79:212–9.
16. Tenforde MW, Mokomane M, Leeme TB, et al. Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana national meningitis survey: a prevalent cohort study. Lancet Infect Dis **2019**; 19:740–9.
17. Soko RN, Burke RM, Feasey HRA, et al. Effects of coronavirus disease pandemic on tuberculosis notifications, Malawi. Emerg Infect Dis **2021**; 27:1831–9.
18. McFall AM, Menezes NP, Srikrishnan AK, et al. Impact of the COVID-19 pandemic on HIV prevention and care services among key populations across 15 cities in India: a longitudinal assessment of clinic-based data. J Int AIDS Soc **2022**; 25:e25960.
19. Ensor S, Mechie I, Ryan R, et al. Measuring the impact of COVID-19 social distancing measures on sexual health behaviours and access to HIV and sexual and reproductive health services for people living with HIV in Botswana. Front Global Womens Health **2023**; 4:981478.
20. Walker AS, Prendergast AJ, Mugyenyi P, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clin Infect Dis **2012**; 55:1707–18.
21. Boyd AT, Oboho I, Paulin H, et al. Addressing advanced HIV disease and mortality in global HIV programming. AIDS Res Ther **2020**; 17:40.
22. Munthali C, Taegtmeyer M, Garner PG, et al. Diagnostic accuracy of the WHO clinical staging system for defining eligibility for ART in sub-Saharan Africa: a systematic review and meta-analysis. J Int AIDS Soc **2014**; 17:18932.

4.3 Manuscript – The impact of GeneXpert cerebrospinal fluid testing on tuberculous meningitis diagnosis in routine care in Botswana.

Tuberculous meningitis (TBM) disproportionately impacts individuals in high HIV-prevalence, resource-limited settings where the mortality of TBM remains unacceptably high. Diagnosis of TBM is challenged by the pauci-bacillary nature of the disease in cerebrospinal fluid and traditional diagnostic options such as culture requiring significant laboratory infrastructure. Furthermore, TB culture can take up to 6 weeks to yield a result meaning it cannot inform immediate management decisions.

Previous epidemiological data from Botswana on adult meningitis reported only 1% of microbiology confirmed diagnoses were attributed to TBM, diagnosed at sole TB culture facility in the country located in the capital Gaborone, up to 1000km from some healthcare facilities[1]. This is likely a significant underestimation resulting from limited testing for TBM with data from neighbouring South Africa TBM comprised 25% of diagnoses[2]. Easily accessible, decentralised, rapid and sensitive diagnostics are essential for improving outcomes from TBM and Xpert MTB/RIF has been demonstrated to be an effective option in high HIV-prevalence settings[3].

Routinely-available national data from Botswana a low-resource, high HIV-prevalence setting was used to evaluate the impact of Xpert MTB/RIF rollout on the number of CSF Xpert MTB/RIF examinations, the number of microbiologically confirmed MTB diagnosis and the characteristics of patients investigated for TBM.

Between January 1st 2016 – 31st December 2022 a total of 6,934 CSF samples were investigated of which 1114 (16.1%) were investigated using TB-specific investigations. The proportion of CSF samples receiving TB-specific investigation increased from 4.5% (58/1288) in 2016 to 29.0% (201/693) in 2022, primarily due to increased CSF analysis with Xpert MTB/RIF from 0.9% (11/1288) to 23.2% (161/693).

There was an overall decline in the annual number of CSF samples analysed but the proportion with microbiologically-confirmed TBM increased from 0.4% to 1.2%

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Student ID Number	2004168	**Title**	Dr
First Name(s)	James		
Surname/Family Name	Milburn		
Thesis Title	Strategies to improve diagnosis of central nervous system infections in high HIV-prevalence African settings		
Primary Supervisor	Prof J Jarvis		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Open Forum Infectious Diseases
Please list the paper's authors in the intended authorship order:	James Milburn, Ookeditse Ntwayagae, Kebatshabile Ngoni, Rachita Suresh, Neo Lemme, Cassie Northcott, James Penney, Matthew Kinsella, Imogen Mechie, Samuel Ensor, Tony Chebani, Daniel Grint, Mark W Tenforde, Ava Avalos, Dinah Ramaabya, Ronan Doyle, Margaret Mokomane,

	Madisa Mine, Katharina Kranzer and Joseph N Jarvis
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised the project with guidance from Prof Jarvis and Prof Kranzer. I accessed the data with support from the data warehouse team at the Botswana Ministry of Health and Wellness (primarily Ookeditse Ntwatagae and Tony Chebani). I merged and cleaned the dataset. I analysed the data with guidance from (Prof Kranzer and Prof Jarvis), created the figures and tables, with input from Dr Ronan Doyle for the size and frequncy geospatial plot of TBM diagnoses in Botswana. I wrote the manuscript which was reviewed by Prof Jarvis, Prof Kranzer and then subsequently all co-authors.

SECTION E

Student Signature	
Date	24/4/24

Supervisor Signature	
Date	25/4/24

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MAJOR ARTICLE

The Impact of GeneXpert Cerebrospinal Fluid Testing on Tuberculous Meningitis Diagnosis in Routine Care in Botswana

James Milburn,[1,2,©] Ookeditse Ntwayagae,[3] Kebatshabile Ngoni,[1] Rachita Suresh,[1] Neo Lemme,[4,5] Cassie Northcott,[1] James Penney,[1] Matthew Kinsella,[1] Imogen Mechie,[1] Samuel Ensor,[1] Tony Chebani,[6] Daniel Grint,[7,©] Mark W. Tenforde,[8] Ava Avalos,[1] Dinah Ramaabya,[6] Ronan Doyle,[2] Margaret Mokomane,[9] Madisa Mine,[4] Katharina Kranzer,[2,10,11,©] and Joseph N. Jarvis[1,2]

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Background. Tuberculous meningitis (TBM) disproportionately impacts high–HIV prevalence, resource-limited settings where diagnosis is challenging. The GeneXpert platform has utility in TBM diagnosis, but uptake remains limited. In Botswana, before the introduction of GeneXpert, tuberculosis (TB) testing was only available through mycobacterial culture at the National TB Reference Laboratory. Data describing routine use of Xpert MTB/RIF for cerebrospinal fluid (CSF) testing in resource-limited settings are scarce.

Methods. Electronic records for patients with CSF tested in government facilities in Botswana between 2016 and 2022 were obtained from a central online repository as part of ongoing national meningitis surveillance. Samples were excluded from 1 site where Xpert MTB/RIF is performed universally. The proportion receiving TB-specific investigation on CSF and the number positive for *Mycobacterium tuberculosis* following increased Xpert MTB/RIF capacity were determined.

Results. The proportion of CSF samples receiving TB-specific investigation increased from 4.5% (58/1288) in 2016 to 29.0% (201/693) in 2022, primarily due to increased analysis with Xpert MTB/RIF from 0.9% (11/1288) to 23.2% (161/693). There was an overall decline in the annual number of CSF samples analyzed, but the proportion with microbiologically confirmed TBM increased from 0.4% to 1.2%. The proportion of samples tested for TB that were collected from health care facilities >100 km from the National TB Reference Laboratory increased with Xpert MTB/RIF rollout from 65.9% (87/132) to 78.0% (494/633).

Conclusions. In Botswana, access to TB culture is challenging in remote populations; more accessible near-patient testing using Xpert MTB/RIF increased the number of patients receiving TB-specific testing on CSF and the number of confirmed TBM cases.

Keywords. TB meningitis; Ultra; Xpert MTB/RIF.

Globally, 1%–5% of people affected by tuberculosis (TB) have tuberculous meningitis (TBM). TBM is often associated with severe immunosuppression, specifically in the context of advanced HIV. Long-term sequelae are frequent, and the mortality of TBM is unacceptably high (up to 50% in adults), remaining unchanged for the past 2 decades [1, 2].

Diagnosis of TBM is difficult due to the paucibacillary nature of disease in cerebrospinal fluid (CSF). Limits of detection differ greatly between smear microscopy (10 000 colony-forming units [CFU]/mL), nuclear acid amplification testing (NAAT) including Xpert MTB/RIF and Xpert MTB/RIF Ultra (20–150 CFU/mL), and mycobacterial culture (1–10 CFU/mL) [3, 4], with sensitivity of smear microscopy, NAAT, and culture for diagnosis of TBM from CSF ranging between 9%–33%, 47%–76%, and 50%–70%, respectively [5, 6]. Time to result from the more sensitive tests is also highly variable. Results from Xpert MTB/RIF are available in 1–2 hours, whereas culture can take up to 6 weeks to yield a result, meaning it cannot inform immediate management decisions. In low-resource settings where the majority of TBM cases occur, diagnosis is further challenged by limited availability of culture and molecular diagnostics. As a result, patients are often empirically treated for TBM based on clinical presentation and CSF protein, glucose, and cell count, frequently resulting in inappropriate

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or unnecessary treatment [7]. Furthermore, in advanced HIV, TBM can present with atypical clinical and CSF findings, potentially delaying recognition of TBM and initiation of treatment [8]. Delayed TBM treatment increases morbidity, adverse neurological sequelae, and mortality.

Easily accessible, rapid, and sensitive diagnostics are essential for improving outcomes from central nervous system infections [9]. Xpert MTB/RIF run on the GeneXpert platform was endorsed by the World Health Organization (WHO) for the diagnosis of pulmonary TB in 2011 following a large multinational clinical validation study [10]. In the 2013 policy update, the WHO made a strong recommendation based on very low-quality evidence that Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TBM [11]. This recommendation was extended to Xpert MTB/RIF Ultra in 2017 [12]. Xpert MTB/RIF, and subsequently Xpert MTB/RIF Ultra, has been rolled out across many countries in Southern Africa including Botswana, where in many places it has replaced smear microscopy as the primary TB diagnostic in the context of pulmonary TB. However, data on its use for diagnosing extrapulmonary TB, especially TBM, are scarce [13–15].

We analyzed 6 years of national data from Botswana, a low-resource, high–HIV prevalence setting, to evaluate the impact of Xpert MTB/RIF rollout on the number of CSF Xpert MTB/RIF examinations, the number of microbiologically confirmed MTB diagnoses, and the characteristics of patients investigated for TBM.

METHODS

The Botswana National Meningitis Survey is an ongoing meningitis surveillance network monitoring trends in the etiology of central nervous system infections in Botswana. Botswana is an upper middle-income county in Southern Africa with an estimated HIV prevalence of 18.6% in adults aged 15–49 and an annual TB incidence of 235 per 100,000 in 2021 [16]. Compared with neighboring countries, Botswana has a relatively robust health care infrastructure, where 85% of the population live within 5 km of a health care facility [17]. Xpert MTB/RIF was initially introduced in Botswana between October 2012 and June 2013 at 13 centers as part of a research study using a stepped-wedge design [18]. Xpert MTB/RIF capacity was subsequently expanded following a donation from the World Bank. Currently 39 health facilities have GeneXpert platforms on site, including all 26 referral, district, and primary hospitals. In Botswana, the use of Xpert MTB/RIF on CSF has been advocated in national HIV/TB guidelines since 2016. Analysis of CSF using Xpert MTB/RIF and, since 2019, Xpert MTB/RIF Ultra was performed in 20 of these hospitals during the study period. Other tests advocated for use on CSF in the 2016 guidelines were microscopy, cell count and differential, CSF biochemistry, India ink stain, TB culture, and extended fungal culture. During the study period, manufacturer guidance for the use of Xpert MTB/RIF on CSF was followed. Data regarding whether the Xpert MTB/RIF or MTB/RIF Ultra was used during the transition period in 2019/2020 were not available; the terminology Xpert MTB/RIF is used throughout the manuscript to denote testing with either version. Culture for *Mycobacterium tuberculosis* is only performed at the National Tuberculosis Reference Laboratory (NTRL) in the capital, Gaborone.

Table 1. Laboratory Standard Procedures for Analysis of Cerebrospinal Fluid [19]

Tests performed on all samples:
• Macroscopic examination
• Total cell count using Neubauer counter
• Centrifugation of CSF at 3000 revolutions per minute for 3 min followed by gram stain, India ink stain, differential count (if CSF white cell count ≥10/mm^3), and culture using the sediment on:
◦ Sabouraud dextrose agar (incubation 10 d)
◦ Sheep blood agar (incubation 72 h)
◦ Chocolate agar (incubation 72 h)
Additional tests performed on all adult CSF samples
• Cryptococcal antigen testing (IMMY Lateral Flow Assay)
Additional tests performed on request:
• Acid-fast bacilli smear
• TB culture on Bactec 960 *Mycobacterium* Growth Indicator Tube (MGIT) automated culture system
• Xpert MTB/RIF Ultra

Abbreviations: CSF, cerebrospinal fluid; TB, tuberculosis.

Institutional review board approval was granted by the Health Research Development Council (HRDC reference number 6/14/1), London School of Hygiene and Tropical Medicine (LSHTM reference number 17322), and the University of Botswana (UB reference number UBR/RES/IRB/1631). This study used only retrospective, routine laboratory data; therefore, a waiver of informed patient consent was obtained.

Laboratory records from all laboratories performing CSF analysis are uploaded to a national electronic health record system termed the Integrated Patient Management System (IPMS). All CSF samples with results stored on IPMS from samples collected between January 1, 2016, and December 31, 2022, were extracted from an online repository in collaboration with the Botswana Ministry of Health and Wellness. The details of the CSF analysis are described in Table 1. Samples from patients admitted to the national referral hospital in Gaborone were excluded from the analysis because universal Xpert MTB/RIF Ultra testing and TB culture of CSF samples were introduced in 2021 as part of a research study. Data on standard CSF evaluation, TB-specific data, and HIV-related data were extracted as separate data sets from the online repository and merged through deterministic linkage of laboratory records using unique patient identifiers, either a 9-digit national identification number or a hospital identification number. Results were then de-duplicated before analysis.

Data were analyzed using STATA, version 16.0. Patient demographics, CSF test results, and HIV-related data were described using frequencies, percentages, or medians and interquartile ranges (IQRs), as appropriate. Clinical and CSF characteristics were compared for those patients who underwent investigation for TBM and those who did not using the Wilcoxon rank-sum test for medians and the chi-square test for proportions. Comparisons were also made between patients who underwent TB investigation before and after the scale-up of Xpert Ultra capacity. The cutoff chosen for this was 2020, when Xpert MTB/RIF consistently became the most commonly used modality for investigating for TBM in Botswana and the more sensitive Xpert MTB/RIF Ultra had replaced the original Xpert MTB/RIF. Maps were made using R, with geospatial data of TB analysis plotted using the tmap package.

Study outcomes were the number and proportion of CSF samples undergoing TB-specific investigations (microscopy, TB culture, or Xpert MTB/RIF) and the number and proportion of positive results from TB-specific investigations. All semiquantitative results from CSF analysis with Xpert MTB/RIF were considered positive.

RESULTS

Between January 1, 2016, and December 31, 2022, a total of 6934 CSF samples were investigated, of which 1114 (16.1%) were investigated using TB-specific investigations: 787 Xpert MTB/RIF, 340 smear microscopies, and 177 mycobacterial cultures (Table 2). Although there was an overall decline in the total number of CSF samples received for analysis during the study period from 1288 in 2016 to 693 in 2022, the number of patients receiving TB-specific analyses increased from 58/1288 (4.5%) in 2016 to 201/693 (29.0%) in 2022, largely due to an increase in Xpert MTB/RIF, which comprised 15.5% of all tests performed in 2016 and 78.1% in 2022 (Figure 1). Although the test positivity rate of mycobacterial culture was highest (8.0%) compared with Xpert MTB/RIF (6.7%) and smear microscopy (1.2%), more samples were tested using Xpert and more microbiologically confirmed TBM diagnoses were made by Xpert MTB/RIF: 53 positive Xpert MTB/RIF tests compared with 14 mycobacterial cultures and 4 positive smears (Table 2).

Patients whose CSF was investigated with a TB-specific investigation had a higher median age (39.1 vs 35.2 years) and higher HIV prevalence (61.3% vs 51.0%) compared with those who did not have TB-specific investigations. The group that had TB-specific investigations also had higher rates of CSF pleocytosis, raised CSF protein >1 mg/mL, and an extraneural sample positive for *M. tuberculosis*.

Laboratory characteristics from patients who underwent TB testing between 2016–2017 and 2020–2022 were compared to assess the impact of scaling up the use of Xpert MTB/RIF on CSF in Botswana. In the group that underwent testing between 2016 and 2017, the proportion of those tested who were HIV positive (70.5%) was higher than in 2020–2022 (58.6%). Among those patients who received testing between 2016 and 2017, there was a higher proportion of patients with a CSF pleocytosis >100 cells/mL and lymphocytic predominance in cellular CSF.

There was significant regional variability in the number of CSF samples submitted for analysis and the proportion that underwent testing with a TB-specific investigation (Figure 2). Hospitals from the 3 largest urban centers in Botswana outside the capital, Francistown, Maun, and Molepolole, submitted the highest proportion of CSF samples for TB-specific investigation, 21.3%–61.3%, 5.4% (43/796) of which were positive. Smaller hospitals tended to send fewer samples and to send a lower proportion of these samples for TB investigation; 8.4% of samples from smaller hospitals received TB testing, with 7.8% (25/320) of these being positive. Samples collected >500 km from NTRL were significantly less likely to receive TB culture than those collected in facilities <500 km from NTRL, 0.4% (4/917) compared with 2.9% (173/6017), and significantly more likely to be analyzed with Xpert MTB/RIF 21.8% (200/917) vs 9.8% (590/6017) (Table 3). The proportion of samples tested for TB that were collected from health care facilities >100 km from NTRL increased with the rollout of Xpert MTB/RIF, from 65.9% (87/132) in 2016–2017 to 78.0% (494/633) in 2020–2022 ($P < .01$) (Table 2).

DISCUSSION

This study, using robust national surveillance data from Botswana, demonstrates relatively low rates of investigation for TBM, even after the introduction of Xpert MTB/RIF testing. Over time, the rollout of Xpert MTB/RIF increased the proportion of CSF samples undergoing investigation with TB-specific tests, and this increased the overall number of microbiologically confirmed TBM diagnoses. Between 2016 and 2022, 16.1% of CSF samples had investigations for TBM, and 13.9% were tested using relatively sensitive cultures and/or Xpert MTB/RIF. In comparison, the cheap, easy-to-use, and widely available CSF cryptococcal antigen lateral flow (CrAg) assay was used on 63.9% of these CSF samples. The difference in cost might inform this discrepancy to some extent. CrAg costs ~$2 and Xpert MTB/RIF Ultra $7.97; therefore, CrAg testing is performed more indiscriminately on CSF samples. However, the major cause of limited TB-specific testing is likely to be due to the small numbers of clinicians initiating investigation for TB. This highlights the potential for expanding diagnostic coverage for TBM if a cheaper, more rapid, easy-to-use, and true point-of-care test for TB becomes available. There are extremely limited data describing the routine use of Xpert MTB/RIF in the context of TBM in resource-limited settings. Botswana is

Table 2. Results of TB-Specific Investigations Performed on CSF Samples in Botswana Between 2016 and 2022

		Microscopy			TB Culture			Successful Xpert MTB/RIF or Xpert MTB/RIF Ultra			Patients With Positive TB-Specific Investigation on CSF		
	Total No. of CSF Samples Analyzed	Total Performed, No.	Negative, No. (%)	Positive, No. (%)	Total Performed, No.	Negative, No. (%)	Positive, No. (%)	Total Performed, No.	Negative, No. (%)	Positive, No. (%)	Total Performed, No.	Negative, No. (%)	Positive, No. (%)
2016	1288	49	48 (98.0)	1 (2.0)	4	3 (75.0)	1 (25.0)	11	8 (72.7)	3 (27.3)	58	53 (91.4)	5 (8.6)
2017	1233	56	56 (100)	0	25	23 (92.0)	2 (8.0)	17	16 (94.1)	1 (5.9)	74	71 (96.0)	3 (4.1)
2018	1123	49	48 (98.0)	1 (2.0)	34	31 (91.2)	3 (8.8)	91	84 (92.3)	7 (7.7)	138	125 (91.9)	11 (8.1)
2019	1057	70	70 (100)	0	40	39 (97.5)	1 (2.5)	137	133 (97.1)	4 (2.9)	211	206 (97.6)	5 (2.4)
2020	869	44	43 (97.7)	1 (2.3)	32	28 (87.5)	4 (12.5)	206	188 (91.3)	18 (8.7)	247	225 (91.1)	22 (8.9)
2021	671	25	25 (100)	0	10	9 (90.0)	1 (10.0)	164	151 (92.1)	13 (7.9)	185	170 (93.4)	14 (7.6)
2022	693	41	40 (97.6)	1 (2.4)	31	29 (93.6)	2 (6.5)	161	154 (95.7)	7 (4.4)	201	193 (96.0)	8 (4.0)
Total	6934	334	330 (98.8)	4 (1.2)	176	162 (92.1)	14 (8.0)	787	734 (93.3)	53 (6.7)	1114	1043 (93.4)	68 (6.1)

Abbreviations: CSF, cerebrospinal fluid; TB, tuberculosis.

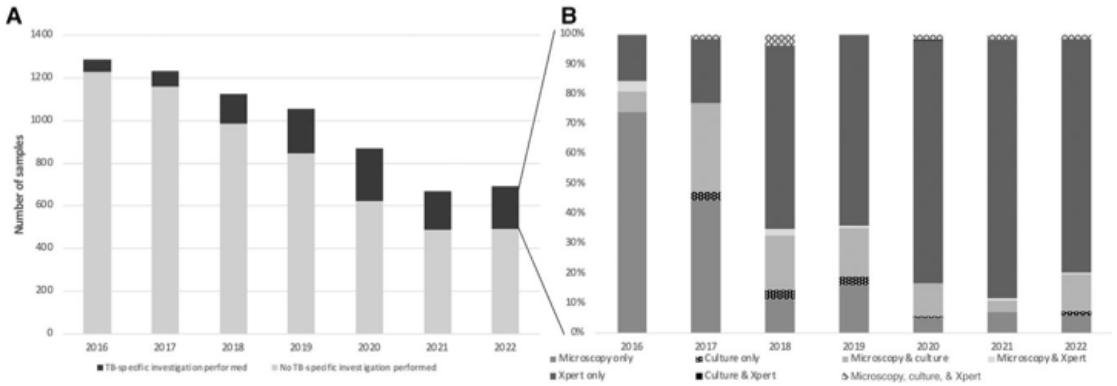

Figure 1. A, Number of CSF samples analyzed each year in Botswana, 2016–2022, with the proportion receiving TB-specific investigation represented in black. Samples from Princess Marina Hospital, Gaborone, were excluded as universal Xpert MTB/RIF Ultra was implemented since 2020. B, Yearly variation of the proportion of TB-specific investigations performed on CSF each year excluding Princess Marina Hospital, Gaborone, 2016–2022. Abbreviations: CSF, cerebrospinal fluid; TB, tuberculosis.

almost uniquely placed to generate these data as a low-resource, high–HIV prevalence Southern African country where routinely collected data are available through electronic health records and patients can be linked through a national identification number.

Previous epidemiological data on adult meningitis etiology in Botswana between 2004 and 2015 reported that cases of TBM only accounted for 1% of all microbiologically confirmed diagnoses nationwide. This contrasted with data from neighboring South Africa, where 25% of adult central nervous system infections were microbiologically confirmed as TBM [8, 20]. While TB incidence in South Africa is more than double that of Botswana, this is unlikely to explain a 25-fold difference in confirmed TBM diagnosis among people presenting with central nervous system infections. A more likely explanation is under investigation for TBM in Botswana as TB culture is only available in the capital, Gaborone, up to 1000 km away from some clinics, and Xpert MTB/RIF was not routinely performed on CSF in 2004–2015.

TB-specific testing was more likely to be performed when clinical features suggestive of TBM such as immunosuppression and pleocytosis were present. However, in the period between 2020 and 2022 there was a trend toward more widespread testing and an increase in test positivity from 6.1% to 7.0%. This suggests that the threshold for clinicians to order TB-specific tests was reduced, likely due to easier access through decentralization of TB testing and more widespread use of Xpert MTB/RIF Ultra, which, unlike culture, can deliver immediate results that influence treatment decisions. TB culture, which was the most sensitive test used for diagnosis of TBM pre–Xpert MTB/RIF, was only performed on 4 CSF samples collected >500 km from NTRL in the 7 years of observation, indicating access barriers. While we have focused on the impact of Xpert MTB/RIF, TB culture remains a key component of investigation for TB meningitis. Sensitivity estimates are higher than Xpert MTB/RIF Ultra, and culture can provide information on antimicrobial susceptibility. However, limited CSF volumes often restrict multiple analyses, and rapid tests that can immediately inform clinician decision-making are often prioritized.

The total number of CSF samples submitted for analysis annually declined during the study period, from 1288 in 2016 to 693 in 2022. A plausible explanation for this is improved antiretroviral therapy (ART) coverage, reducing the number of presentations with suspected central nervous system infections. Botswana is a leader in ART programming in Africa. It was the first African country to offer free ART to its citizens and has recently become one of the first countries globally to surpass the UNAIDS 95–95–95 targets, with recent data demonstrating a decline in the number of cryptococcal meningitis cases [21].

This study has several limitations. Detailed individual patient-level data were not available, including data on changes to management from the results of TB-specific investigations. Some patients may have already been treated empirically when results became available, and therefore the impact of test positivity on management is not known. While this study was not designed to report TBM incidence in Botswana but rather to describe the changes in TB-specific investigation following the rollout of Xpert MTB/RIF, the frequency of TBM diagnosis is likely underestimated in our study for several reasons. First, electronic data capture was not complete due to unreliable internet connectivity and power interruptions, and during these periods results were disseminated locally on paper. These paper records were not captured as part of this analysis, and triangulation against other reliable datasets

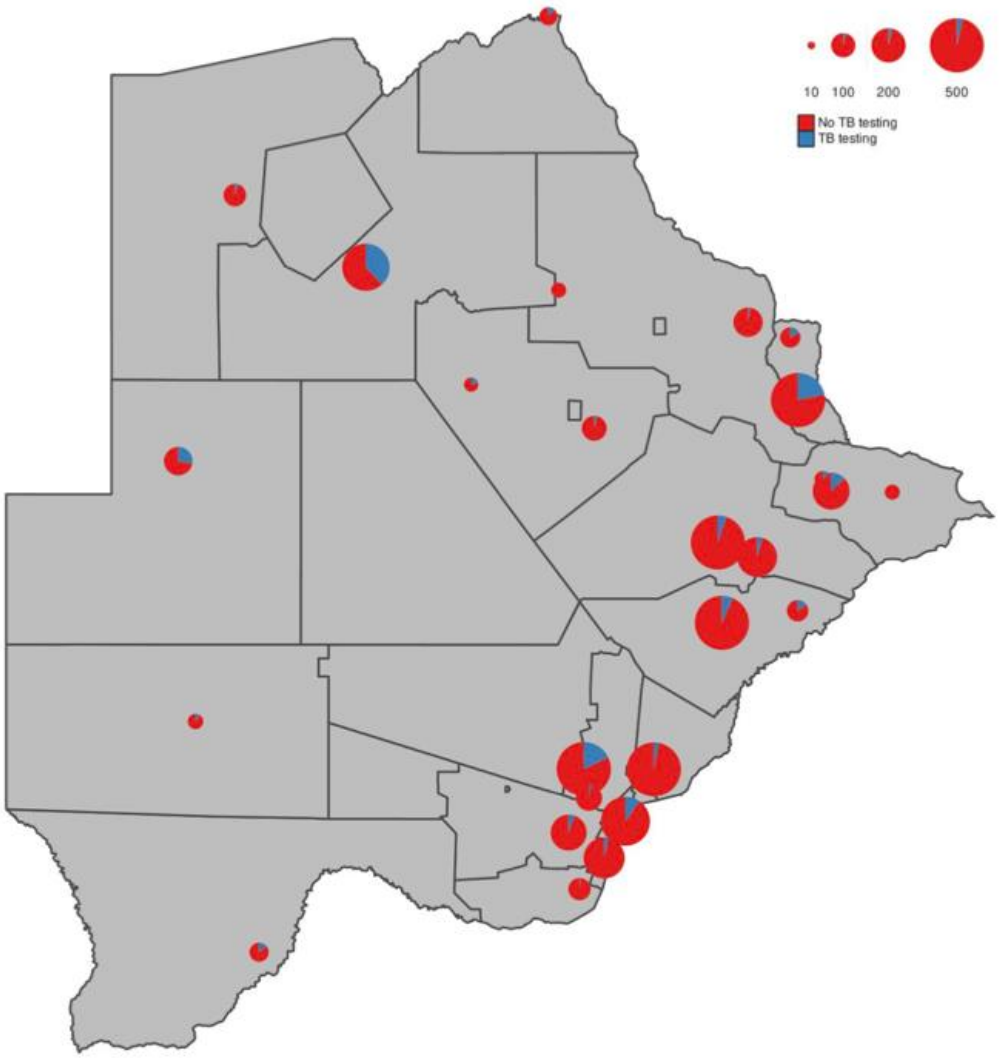

Figure 2. Geospatial size and frequency representation of the number of CSF samples analyzed in government health care facilities in Botswana and the proportion that undergo TB testing in Botswana. Abbreviations: CSF, cerebrospinal fluid; TB, tuberculosis.

demonstrated that they accounted for up to 10% of all laboratory records. Private hospitals and 1 hospital linked to a mining development do not upload data to the electronic IPMS, and data from these sites were not captured. We also are unable to comment on any intersite variation regarding pre-analytical processing of CSF that may have impacted the yield of Xpert MTB/RIF such as sample volume or prior centrifugation.

In addition to potential missed diagnoses resulting from our study methodology including only electronic records, all currently available TBM investigations have imperfect sensitivity, and furthermore a significant proportion of patients will never receive any TB-specific investigation for TBM. As such, TBM case frequency will be markedly under-reported, and due to limited clinical data in our data set, we were unable to reliably utilize uniform clinical case definitions for TBM to attempt to correct for this.

Despite some encouraging trends toward increased testing for TBM in patients presenting with suspected central nervous system infections, the rates of investigation remain comparatively low despite excellent Xpert MTB/RIF availability. This study demonstrates that the introduction of decentralized rapid molecular testing for TBM with relatively modest sensitivity increased the rate of TB-specific investigations and the number of

Table 3. (1) Comparison of Laboratory Data of Patients who Underwent Investigation for Suspected TBM With TB-Specific Investigations and Those who Did Not; (2) Comparison of Patients who Received TB Testing Before and After the Widespread Rollout of Xpert MTB/RIF and Xpert MTB/RIF Ultra

	Total (n = 6934), % (No.)	Did Not Receive TB Testing (n = 5820), % (No.)	Received TB Testing (n = 1114), % (No.)	*P* Value	Samples Analyzed With TB-Specific Investigations in Early Xpert MTB/RIF Rollout Period (n = 132), % (No.)	Samples Analyzed With TB-Specific Investigations in Late Xpert MTB/RIF Rollout Period (n = 633), % (No.)	*P* Value
Age, median (IQR), y	36.1 (19.6–46.0)	35.2 (15.6–45.6)	39.1 (29.3–48.3)	<.01	38.4 (31.7–45.1)	39.9 (29.1–47.8)	.63
Female	45.3 (3137)	45.3 (2634)	45.2 (503)	.94	44.7 (59)	42.3 (268)	.62
HIV-positive	52.7 (3651)	51.0 (2968)	61.3 (683)	<.01	70.5 (93)	58.6 (371)	.01
CD4, median (IQR),[a] cells/μL	204 (58–455)	207 (58–458)	196 (53–421)	.21	163 (47–400)	202 (53–456)	.33
CD4 <200 cells/μL (if CD4 known)	49.,4 (1515)	50.9 (1227)	50.6 (288)	.51	55.4 (46)	49.8 (149)	.37
CSF WCC >10 cells/mm^3 (if CSF WCC known)[b]	20.9 (1097)	20.1 (880)	24.8 (217)	<.01	29.4 (30)	24.4 (120)	.29
CSF WCC >100 cells/mm^3 (if CSF WCC known)[b]	9.5 (499)	9.2 (405)	10.7 (94)	.17	17.7 (19)	10.4 (51)	.04
Lymphocytic pleocytosis	36.8 (313)	37.2 (250)	35.6 (63)	.70	41.7 (150)	31.5 (79)	.01
CSF protein, median (IQR),[c] mg/mL	0.65 (0.31–1.59)	.63 (0.30–1.50)	0.75 (0.36–1.80)	<.01	1.31 (0.41–3.48)	0.81 (0.35–1.80)	.28
CSF protein >1 mg/mL (if CSF protein known)	35.9 (584)	34.4 (463)	43.4 (121)	<.01	53.9 (14)	41.9 (129)	.26
CSF glucose, median (IQR),[d] mmol/L	3.36 (2.42–4.23)	3.38 (2.46–4.24)	3.24 (2.35–4.13)	.14	3.09 (2.05–3.77)	3.37 (2.35–4.32)	.06
CSF glucose <2.2 mmol/L (if CSF glucose known)	20.8 (749)	20.7 (604)	21.6 (145)	.60	26.0 (19)	21.4 (82)	.38
Extraneural sample with *M.Tb* detected in last 3 mo[e]	1.7 (115)	1.5 (88)	2.4 (27)	.03	2.3 (3)	2.8 (18)	.72
Distance >100 km from sample collection site to TB culture facility (National TB Reference Laboratory)	66.2 (4590)	64.9 (3775)	73.2 (815)	<.01	65.9 (87)	78.0 (494)	<.01

Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range; TB, tuberculosis; TBM, tuberculous meningitis; WCC, white cell count.

[a]CD4 count was unknown in 55.7% (3865/6934) of patients overall, in 57.0% (3320/5820) of those who did not have TB testing, and in 48.9% (545/1114) of those who did (*P* < .01). Among those who had TB testing, CD4 count was unknown in 37.1% (49/132) of those who had TB testing in 2016–2017 and 52.7% (334/633) of those who had testing in 2020–2022 (*P* < .01).

[b]CSF WCC was unknown in 24.3% (1677/6934) of patients overall, in 24.7% (1439/5820) of those who did not have TB testing, and in 21.4% (238/1114) of those who did (*P* = .06). Among those who had TB testing, CSF WCC was unknown in 22.7% (30/132) of those who had TB testing in 2016–2017 and 22.4% (142/633) of those who had testing in 2020–2022 (*P* = .68).

[c]CSF protein was unknown in 76.6% (5308/6934) of patients overall, in 76.9% (4473/5820) of those who did not have TB testing, and in 75.0% (835/1114) of those who did (*P* = .42). Among those who had TB testing, CSF protein was unknown in 80.3% (106/132) of those who had TB testing in 2016–2017 and 76.9% (504/633) of those who had testing in 2020–2022 (*P* = .86).

[d]CSF glucose was unknown in 48.1% (3338/6934) of patients overall, in 49.8% (2896/5820) of those who did not have TB testing, and in 39.7% (442/1114) of those who did (*P* < .01). Among those who had TB testing, CSF glucose was unknown in 44.7% (59/132) of those who had TB testing in 2016–2017 and 39.3% (249/633) of those who had testing in 2020–2022 (*P* = .25).

[e]The source of the extraneural sample was sputum in 69.6% (80/115), gastric aspirate in 11.1% (13/115), pleural fluid in 1.7% (2/115), pus in 1.7% (2/115), and unknown in 11.3% (18/115).

microbiologically confirmed TBM diagnoses. Whether the increase in diagnoses translates into improved patient outcomes is currently unknown.

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References

1. Wilkinson RJ, Rohlwink U, Misra UK, et al. Tuberculous meningitis. Nat Rev Neurol **2017;** 13:581–98.
2. Stadelman AM, Ellis J, Samuels THA, et al. Treatment outcomes in adult tuberculous meningitis: a systematic review and meta-analysis. Open Forum Infect Dis **2020**; 7:ofaa257.
3. Chakravorty S, Simmons AM, Rowneki M, et al. The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. mBio **2017;** 8:e00812-17.
4. van Zyl-Smit RN, Binder A, Meldau R, et al. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. PLoS One **2011;** 6:e28815
5. Donovan J, Thwaites GE, Huynh J. Tuberculous meningitis: where to from here? Curr Opin Infect Dis **2020;** 33:259–66.
6. Ssebambulidde K, Gakuru J, Ellis J, Cresswell FV, Bahr NC. Improving technology to diagnose tuberculous meningitis: are we there yet? Front Neurol **2022;** 13:892224.
7. Ellis J, Bangdiwala AS, Cresswell FV, et al. The changing epidemiology of HIV-associated adult meningitis, Uganda 2015–2017. Open Forum Infect Dis **2019**; 6:ofz419.
8. 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.
9. Sheu J-J, Yuan R-Y, Yang C-C. Predictors for outcome and treatment delay in patients with tuberculous meningitis. Am J Med Sci **2009;** 338:134–9.
10. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med **2010;** 363:1005–15.
11. World Health Organization. *Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children*. World Health Organization; **2013**. Available at: www.who.int. Accessed 30 October 2023.
12. World Health Organization. *WHO Meeting Report of a Technical Expert Consultation: Non-Inferiority Analysis of Xpert MTB/RIF Ultra Compared to Xpert MTB/RIF*. World Health Organization; **2017**. Available at: http://apps.who.int/bookorders. Accessed 30 October 2023.
13. Cresswell FV, Tugume L, Bahr NC, et al. Xpert MTB/RIF ultra for the diagnosis of HIV-associated tuberculous meningitis: a prospective validation study. Lancet Infect Dis **2020;** 20:308–17.
14. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. Eur Respir J **2014;** 44:435–46.
15. Donovan J, Thu DDA, Phu NH, et al. Xpert MTB/RIF Ultra versus Xpert MTB/RIF for the diagnosis of tuberculous meningitis: a prospective, randomised, diagnostic accuracy study. Lancet Infect Dis **2020;** 20:299–307.
16. Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Botswana. Available at: https://www.unaids.org/en/regionscountries/countries/botswana. Accessed 6 November 2023.
17. UNICEF. UNICEF Country Profile. Available at: https://www.unicef.org/botswana/health-and-nutrition. Accessed 16 August 2023.
18. Auld AF, Agizew T, Pals S, et al. Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality. BMC Infect Dis **2016;** 16:606.
19. Tenforde MW, Mokomane M, Leeme TB, et al. Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana National Meningitis Survey: a prevalent cohort study. Lancet Infect Dis **2019;** 19:740–9.
20. Tenforde MW, Mokomane M, Leeme T, et al. Epidemiology of adult meningitis during antiretroviral therapy scale-up in Southern Africa: results from the Botswana National Meningitis Survey. J Infect **2019;** 79:212–9.
21. Milburn J, Ntwayagae O, Suresh R, et al. Tracking cryptococcal meningitis to monitor HIV program success during the treat-all era: an analysis of national data in Botswana. Clin Infect Dis **2024;** 79:462–8.

1. Tenforde, M. W. *et al.* Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey. *Journal of Infection* **79**, 212–219 (2019).
2. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: Findings from 4961 suspected cases. *BMC Infect Dis* **10**, (2010).
3. Cresswell, F. V. *et al.* Xpert MTB/RIF Ultra for the diagnosis of HIV-associated tuberculous meningitis: a prospective validation study. *Lancet Infect Dis* **20**, 308–317 (2020).

Chapter V – Impact of computed tomography on time to lumbar puncture, initiation of treatment and mortality in central nervous system infections in high HIV-prevalence settings

5.1 Introduction

Central nervous system infections (CNSI) continue to drive excess mortality in high HIV-prevalence African settings. Early diagnosis and treatment facilitated by prompt lumbar puncture (LP) is paramount to be able to improve outcomes. In resource-rich settings the role of computed tomography (CT) prior to LP in the investigation of suspected CNSI remains controversial however CT prior to LP has been shown to lead to delays in diagnosis and treatment. Guidelines exist on when to performed CT prior to LP to support clinician decision-making in resource-rich settings where CT is often available within hours. However, in resource-limited settings there are no reliable data to support clinician decision-making surrounding the role of CT prior to LP despite additional considerations found resource-limited settings. High HIV-prevalence broadens the differential diagnosis further challenging diagnostic workup whilst also potentially making the risk of LP delay or deferral greater. Access to CT is extremely limited with patients often waiting days or weeks for CT in urgent cases making clinical decisions to delay LP for CT even more impactful on patient outcomes. Understanding what impact CT has on time to LP, initiation of treatment and mortality is crucial to allow development of regionally- and context-specific guidelines on CT prior to LP.

Using data from the Botswana National Meningitis Survey: Protocol 2 these results represent a relatively unique dataset describing delays to diagnostic LP and initiation of appropriate therapy resulting from CT prior to LP in resource-limited, high HIV-prevalence settings. In this cohort of 711 patients underwent an LP for suspected CNSI, 73% were HIV positive and 191 had a CT prior to LP. Sensitivity, specificity, and negative and positive predictive values were determined for the detection of abnormality on CT for international and local guidelines. Estimates for were derived from variables

reporting the presence or absence of radiological abnormalities on CT and the proportion of patients that met clinical criteria for each guideline surrounding the need for CT prior to LP. This analysis highlighted that whilst IDSA guidelines were the most sensitivity detecting the majority of patients with CT abnormality they would be entirely impractical to implement due to resource-constraints. Time from admission to LP was 2.8 hours longer and time from admission to appropriate treatment 12.7 hours longer in patients who had a CT prior to LP compared to those that did not. Importantly there was no difference in mortality between those who had a CT and those who did not CT, with no increase in mortality in patients with a clinical indication for CT who did not have a CT, therefore highlighting the limited utility in the investigation of patients with suspected CNSI in high HIV-prevalence African settings.

There are significant limitations to the data presented. Most importantly that through the study design only patients who had an LP and CSF were recruited, therefore missing patients who had an LP deferred due to abnormal findings on CT. There was limited scope to alter the design of the study to recruit patients that had suspected CNSI but no LP due to abnormalities on CT as the indications for performing CT were poorly recorded on CT requests and if the patient was not admitted then the patient often left with their CT images and report thereby restricting the ability to capture these data. Another limitation is that patients who had a CT prior to LP were not chosen at random but rather selected by treating clinicians leading to potential confounding by indication. This is of particular relevance in the mortality analysis where more unwell patients are more likely to have a CT and are therefore more likely to have a higher mortality. Whilst efforts were made to adjust for this using a weighted scoring system that includes physiological characteristics it is likely that important criteria were not fully accounted for. Furthermore, other scoring systems such as the Malawi Adult Meningitis Score (MAMS) may have been more suitable. However, these are significant results that add to an important yet considerably under-investigated topic.

The paper has been published in Open Forum Infectious Diseases.

5.2 Manuscript – Computed tomography of the head before lumbar puncture in adults with suspected meningitis in high HIV-prevalence settings

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SECTION A – Student Details

Student ID Number	2004168	**Title**	Dr
First Name(s)	James		
Surname/Family Name	Milburn		
Thesis Title	Strategies to improve diagnosis of central nervous system infections in high HIV-prevalence African settings		
Primary Supervisor	Prof J Jarvis		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Clinical Infectious Diseases
Please list the paper's authors in the intended authorship order:	James Milburn, Christopher G Williams, Kwana Lechiile, Keatlaretse Siamisang, Leah Owen, Ezekiel Gwakuba, Thandi Milton, Tichaona Machiya, Tshepo Leeme, Hannah E Barton, Ponego Ponatshego, Kaelo Seatla, Gerald Boitshepo, Rachita Suresh, Ikanyeng Rulaganyang, William

	Hurt, Samuel Ensor, Kebatshabile Ngoni1, Ronan Doyle, Daniel Grint, Wallace T Miller Jr, Mark W Tenforde, Madisa Mine, David M Goldfarb, Margaret Mokomane, and Joseph N Jarvis
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised the project with guidance from Prof Jarvis using data from Botswana National Meningitis Survey: Protocol 2. I analysed the data with guidance from (Daniel Grint and Prof Jarvis), created the figures and tables, I wrote the manuscript which was reviewed by Prof Jarvis and then subsequently all co-authors.

SECTION E

Student Signature	
Date	24/4/24

Supervisor Signature	
Date	25/4/24

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MAJOR ARTICLE

Computed Tomography of the Head Before Lumbar Puncture in Adults With Suspected Meningitis in High–HIV Prevalence Settings

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Background. The role of computed tomography (CT) before lumbar puncture (LP) is unclear, with limited evidence for a causal link between LP and cerebral herniation or for the ability of CT to identify individuals at risk of herniation. The risks of LP delay or deferral are potentially greater in high–HIV prevalence, resource-limited settings; we analyzed data from such a setting to determine the impact of CT on time to LP and treatment, as well as mortality.

Methods. Adults with suspected central nervous system (CNS) infection were enrolled prospectively into the Botswana National Meningitis Survey between 2016 and 2019. Inpatient mortality and clinical data including time of treatment initiation and CT were captured from medical records. Associations between preceding CT and outcomes were assessed using logistic regression.

Results. LPs were performed in 711 patients with suspected CNS infection; 27% had a CT before LP, and 73% were HIV positive. Time from admission to LP and time from admission to appropriate treatment were significantly longer in patients who had a CT before LP compared with those who did not (2.8 hours and 13.2 hours, respectively). There was some evidence for treatment delays being associated with increased mortality; however, there was no significant difference in mortality between those who had or did not have CT.

Conclusions. Patients who had a CT had delays to diagnostic LP and initiation of appropriate treatment; although treatment delays were associated with increased mortality, our observational study could not demonstrate a causal association between delays in diagnosis and treatment introduced by CT and mortality.

Keywords. central nervous system infection; computed tomography; HIV; meningitis; neuroimaging.

Central nervous system (CNS) infections are a major cause of morbidity and mortality globally, with the highest disease burdens in resource-limited settings [1]. Rapid diagnosis and initiation of treatment are essential to improve CNS infection outcomes [2]. Lumbar puncture (LP) is the key investigation for guiding management. However, clinicians are often reluctant to perform LPs due to concerns about precipitating cerebral herniation in patients with increased intracranial pressure (ICP) [3–5].

Computed tomography (CT) is often requested before performing LPs to identify imaging appearances that may contraindicate LP. Evidence supporting the association between LP and cerebral herniation is limited, and while a temporal association has been demonstrated, no clear causal relationship has ever been established. There is no evidence that CT performed before LP improves outcomes in patients with suspected CNS infection [6, 7], and it is unclear whether CT accurately predicts those at risk of herniation or raised ICP; normal CT findings have been seen in up to 80% of bacterial meningitis cases with brainstem herniation [3, 8–10]. Furthermore, CT before LP has been shown to delay diagnostic LP, often resulting in administration of antimicrobials before LP and a subsequent reduction in yield from cerebrospinal fluid (CSF) culture [6, 11, 12].

Despite lack of evidence for benefit, in resource-rich countries most patients have CT before LP; 94% of UK patients had CT before LP, with only 17% having an indication based on national guidelines [13]. This results in large amounts of potentially unnecessary imaging, adding delays, and cost to

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management. In 2009, Sweden introduced guidelines limiting the number of indications for CT in patients with suspected CNS infection; a series of studies subsequently demonstrated that prompt LP without preceding CT resulted in earlier treatment initiation and more favorable outcomes in bacterial meningitis [7, 14].

Several guidelines recommend when to perform CT in patients with suspected CNS infection based on clinical features predicting the presence of radiological abnormalities [15–17]. However, most originate from countries with rapid access to CT and do not consider the distinct challenges in resource-limited settings where the burden of meningitis is greater and mortality higher [1]; the need for prompt diagnosis and appropriate antimicrobial therapy is therefore crucial [18, 19]. High HIV prevalence in many resource-limited regions widens the differential diagnosis for suspected CNS infection, making diagnostic LP essential for guiding treatment, but also raises the likelihood of pathology that may contraindicate LP. CT availability is extremely constrained in resource-limited settings, often only available in specialized hospitals, and even then, with limited access. These factors mean the risk–benefit balance of delaying LPs to perform CT is potentially very different than in resource-rich settings. As such, adoption of guidelines from resource-rich countries may not be appropriate or feasible and could result in unnecessary delay or deferral of LP, leading to poorer outcomes [20].

Data from resource-limited high–HIV prevalence settings describing the impact of CT on treatment delay and mortality in suspected CNS infection are limited. We analyzed data from a prospective cohort of adults presenting with suspected CNS infection to a national referral hospital in Botswana, where HIV prevalence in adults aged 15–49 is 18.6% [21], describing the use of CT and determining the impact of CT on time to LP, treatment initiation, and in-hospital mortality.

METHODS

Study Setting

The Botswana National Meningitis Survey is an ongoing meningitis surveillance study [22]. Clinical and laboratory data are collected prospectively from all patients with CSF submitted to the microbiology laboratory at the national public referral hospital, Princess Marina Hospital (PMH) in Gaborone. We analyzed data collected between November 2016 and September 2019. Botswana has a relatively well-resourced health care infrastructure compared with other low- and middle-income countries in Sub-Saharan Africa, with CT available and provided free of charge to citizens at PMH.

Study Methodology

All adults aged 18 years or older with CSF submitted to the microbiology laboratory were included. Repeat LPs during the same or a subsequent admission and LPs performed for reasons other than suspected CNS infection were excluded. LPs were performed by treating clinicians, and CSF analysis was performed as previously described [23]. Patients were followed by the study team during admission. Laboratory data including timing of CSF collection were captured from laboratory records. Clinical data were recorded from the patients' medical notes and drug administration charts. Final diagnoses were assigned by 2 members of the study team (J.M./C.W.) based on standardized case definitions (Supplementary Table 1), with arbitration by J.N.J. in cases of disagreement. Appropriate CNS infection treatment was defined as antibiotics (third-generation cephalosporins or other accepted bacterial meningitis treatment regimens) in confirmed or probable bacterial meningitis, antifungal treatment with amphotericin B–based therapy in confirmed cryptococcal meningitis, antituberculous therapy in possible, probable, or definite tuberculous meningitis (TBM) cases [24], and antiviral therapy in confirmed herpes simplex virus (HSV)–1, HSV-2, or varicella zoster virus CNS infection when there was clinical suspicion of encephalitis.

CT was performed upon the request of treating clinicians with no input from the study team. CT timing was determined from scan image time stamps, CT scanner electronic log, or patients' medical notes. Published consensus guidelines for performing CT before LP have been developed for PMH (Supplementary Table 2) [25]. Reporting of CT scans was performed by consultant physicians or by radiologists upon clinician request. Potential contraindications to LP were defined as lateral shift of midline structures, hydrocephalus, any intracranial lesion regardless of size or mass effect, cerebral edema, or intracranial bleed.

Patients were followed up to hospital discharge. The study team had no role in the management of recruited patients. All data were anonymized, linked through unique study ID numbers, and uploaded onto a secure electronic data capture system [26, 27].

Clinical and CSF characteristics were compared for patients who received CT before LP and those who did not; continuous variables were compared using Wilcoxon rank-sum testing and categorical variables using the chi-square test for proportions. Time to appropriate CNS infection antimicrobial initiation was dichotomized at clinically meaningful time points depending on the pathogen. Multivariate regression analysis was performed to determine associations between the key exposure of interest (CT before LP) and outcome variables (time from admission to LP and to appropriate CNS infection–targeted antimicrobial initiation, inpatient mortality). Sensitivity, specificity, and positive and negative predictive values for the detection of potential radiological contraindication to LP on CT were calculated for 3 guidelines. To adjust for differences in the severity of presentations, a composite value based on a standardized scoring system that utilizes abnormalities in physiological

parameters was included in adjusted analyses (Supplementary Table 3) [28]. Fundoscopy was infrequently performed, and therefore papilloedema was not included in adjusted Infectious Diseases Society of America (IDSA) guidelines for this analysis.

Ethics

Institutional review board approval was in place from the Health Research Development Council (Botswana Ministry of Health and Wellness), London School of Hygiene and Tropical Medicine, University of Botswana, and PMH.

RESULTS

Between November 2016 and September 2019, 861 LPs were performed on adults at PMH; 144 repeat LPs and 6 LPs performed for reasons other than suspected CNS infection were excluded. Data from the remaining 711 adults with suspected CNS infection were included for analysis (Figure 1).

Study Population

The median age (interquartile range [IQR]) was 39 (32–48) years; 378/711 (53%) were male. HIV status was known in 669/711 (94.1%) patients, and 77% (519/669) of participants with known HIV status were HIV positive (Table 1).

CT before LP was performed in 191/711 (26.9%) patients. Those with CT before LP were more likely to have a reduced Glasgow Coma Scale score, recent seizures, and focal neurological deficits (Table 1). Age and sex were similar in those who did and did not have CT, but those who had CT had a lower HIV prevalence (66.9% [123/184] vs 81.7% [396/485]; $P < .001$). There was no significant difference in the proportion of patients with pleocytosis between those who did and did not have CT, and similar proportions received treatment for confirmed CNS infection or empiric antibiotics in both groups.

Based on local guidelines (Supplementary Table 2), an indication for CT before LP was present in 83.3% (159/191) of those who had CT before LP and 50.4% (262/520) of those who did not have CT; a smaller proportion of HIV-positive individuals had an indication for CT before LP than patients who were not HIV positive (58.4% [303/519] vs 71.3% [107/150]; $P = .004$).

Impact of CT on Time to LP and Treatment

CT before LP was associated with a longer median (IQR) duration from admission to LP (11.8 [7.0–44.2] hours compared with 9.0 [4.6–25.0] hours; $P = .004$) (Table 2). Overall, there was a longer delay from admission to appropriate CNS infection treatment (IQR) in patients who had CT before LP (32.1 [16.7–96.3] hours vs 18.9 [11.1–44.1] hours; $P = .05$) (Figure 2), and a greater proportion of patients received appropriate CNS infection–targeted antimicrobial treatment within the first 24 hours if they did not undergo preceding CT (58.5% vs 40.0%; $P = .04$) (Table 2).

TBM treatment was given in 54/71 patients with possible, probable, or definite TBM based on a uniform case definition [24] and no confirmed alternative CNS infection, no treatment was given in 17 patients, and treatment status was unknown in 1 patient.

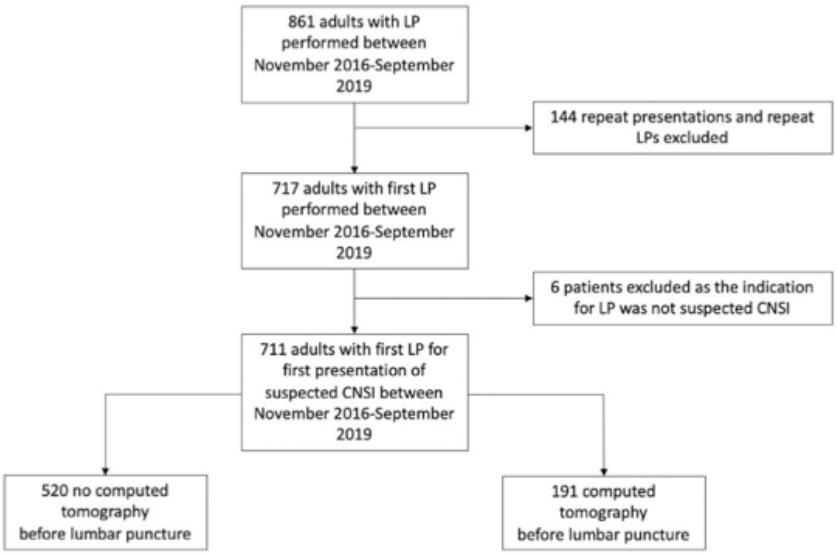

Figure 1. Screening, exclusion and analysis populations. Abbreviations: CNSI, central nervous system infection; LP, lumbar puncture.

Table 1. Clinical and Laboratory Characteristics of Patients With Suspected CNS Infection and Proportion of Patients who Received Appropriate Treatment During Admission

	All Patients (n = 711)	No Preceding CT Before LP (n = 520)	CT Performed Before LP (n = 191)	*P* Value
Median age (IQR), y	39 (32–48)	39 (32–47)	40 (32–54)	.16
Male gender, No. (%)	378 (53.2)	276 (53.1)	102 (53.4)	.94
HIV positive, No. (%)[a]	519 (77.6)	396 (81.7)	123 (66.9)	**<.001**
Median CD4 (IQR),[b] cells/mm^3	190 (55–412)	172 (49–390)	237 (80–480)	.05
GCS				
Normal GCS (GCS 15)	362 (50.9)	301 (57.9)	61 (31.9)	**<.001**
Minimally impaired GCS (GCS 13–14)	195 (27.4)	127 (24.4)	68 (35.6)	**.003**
Moderately impaired GCS (GCS 9–12)	103 (14.5)	62 (11.9)	41 (21.5)	**.001**
Severely impaired GCS (GCS ≤8)	7.2 (51)	30 (5.8)	21 (11.0)	**.02**
History of seizure, No. (%)	113 (15.9)	59 (11.4)	54 (28.3)	**<.001**
Focal neurology, No. (%)	95 (13.4)	45 (8.7)	50 (26.2)	**<.001**
Indication for imaging before LP based on local guidelines, No. (%)[c] (GCS <15 or history of seizure or focal neurology)	421 (59.2)	262 (50.4)	159 (83.3)	**<.001**
CSF pleocytosis, No. (%)	201 (28.3)	156 (30.0)	45 (23.6)	.08
First review by clinician outside of routine working hours (1900–0900), No. (%)	321 (45.2)	217 (41.7)	104 (54.5)	.17

P values compare patients with no computed tomography performed before LP and patients with preceding computed tomography, calculated using Wilcoxon rank-sum for medians and the chi-square test for proportions. *P* values <.05 are displayed in bold.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; GCS, Glasgow Coma Scale; IQR, interquartile range; LP, lumbar puncture.

[a]HIV status known in 669 patients; 485 with no CT before LP and 184 with CT before LP. One hundred nine new diagnoses of HIV were made on presentation to Princess Marina Hospital, 21.2% (84/109) in patients with no CT before LP and 20.3% (25/109) in those with CT before LP.

[b]CD4 known in 385/519 HIV-positive patients.

[c]Based on consensus guidelines from Princess Marina Hospital, Gaborone [25].

Empiric CNS infection–targeted antibiotics were given in 81.1% of all cases regardless of final diagnosis, most commonly third-generation cephalosporins (76.8% of all antibiotic prescriptions) started at a median (IQR) of 8 (3.2–17.1) hours after admission (before LP in 58.1% of patients), and time to appropriate antimicrobial treatment (IQR) was not significantly longer in patients with confirmed or suspected bacterial meningitis who had preceding CT compared with those who did not (6.4 [1.2–8.2] hours vs 5.5 [1.9–8.9] hours; $P = .87$). Appropriate treatment was initiated later in patients who underwent CT with cryptococcal meningitis (32.7 [22.2–68.0] hours vs 22.1 [15.4–36.0] hours; $P = .024$) and TBM (128.1 [37.0–198.0] hours vs 42.7 [17.7–112.3] hours; $P = .09$).

Impact of Treatment Delay on Mortality

Outcome was known for 687/711 (96.7%) patients; all-cause in-hospital mortality was 27% (184/687). Mortality in patients with a final diagnosis of CNS infection (Supplementary Table 1) and a known outcome was 34.4% (56/163); mortality was 27.5% (11/40) in those who received appropriate treatment within 12 hours of admission and 35.8% (45/123) in those receiving treatment >12 hours after admission; however, this did not reach statistical significance (adjusted OR, 1.66; 95% CI, 0.66–4.18) (Table 3). Limiting analysis to individuals with confirmed or probable bacterial meningitis, mortality in patients who received antibiotics within 12 hours after admission was 30.4% (7/23), and it was 40.0% (2/5) in those receiving treatment >12 hours after admission (adjusted OR, 2.22; 95% CI, 0.17–29.98); among those with cryptococcal meningitis, mortality was 18.2% (2/11) in those treated within 12 hours of admission and 32.4% (24/74) in those receiving treatment >12 hours after admission (adjusted OR, 1.41; 95% CI, 0.24–8.31), although these associations were not statistically significant. Delays >24 hours from admission in starting antituberculous treatment were significantly associated with increased mortality in patients with possible, probable, or definite TBM, 48.4% (15/31) vs 23.5% (4/17; adjusted OR, 6.89; 95% CI, 1.01–46.99).

Impact of Preceding CT on Inpatient Mortality

Limiting the analysis to those with a final diagnosis of CNS infection, including those with CSF pleocytosis suggestive of CNS infection but without definitive microbiology, inpatient mortality for patients without an indication for preceding CT was 20% (21/105). In those patients without an indication for CT, mortality was 18.2% (2/11) in patients who had CT compared with 20.2% (19/94) in those without (adjusted OR, 0.92; 95% CI, 0.17–4.81). For patients with an indication for CT before LP, mortality was 41.2%; it was 41.7% (25/73) in those who had CT compared with 41.0% (48/117) in those who did not (adjusted OR, 1.01; 95% CI, 0.50–2.08).

Detection of Contraindications to Lumbar Puncture on CT

Potential radiological contraindications were identified on 24.5% (40/163) of CT scans performed before LP among those patients with an available report. Among patients who had an

Table 2. Comparison of Time to Treatment Among Patients who Did and Did Not Undergo CT Before LP

	All Patients (n = 711)	No Preceding CT Before LP (n = 520)	CT Performed Before LP (n = 191)	*P* Value
Median time from admission to LP (IQR), h	9.8 (5.6–29.9) n = 484	9.0 (4.6–25.0) n = 327	11.8 (7.0–44.2) n = 157	**.004**
Median time to appropriate treatment (IQR), h	22.6 (13.1–49.6) n = 168	18.9 (11.1–44.1) n = 123	32.1 (16.7–96.3) n = 45	.05
Median time from admission to antibiotics in patients with confirmed bacterial meningitis or neutrophilic CSF pleocytosis (IQR), h	5.9 (1.4–8.3) n = 29	5.5 (1.9–8.9) n = 19	6.4 (1.2–8.2) n = 10	.87
Median time from admission to cryptococcal meningitis treatment (IQR), h	24.1 (16.3–38.5) n = 88	22.1 (15.4–36.0) n = 66	32.7 (22.2–68.0) n = 22	**.02**
Median time from admission to antituberculous treatment in patients with "possible," "probable," or "definite" tuberculous meningitis and no alternative CNS infection diagnosis (IQR), h	48.7 (18.4–129.2) n = 50	42.7 (18.0–112.3) n = 37	128.1 (37.0–198.0) n = 13	.09
Appropriate treatment started during admission, n/N (%)[a]	189/240 (78.8)	141/185 (76.2)	48/55 (87.3)	.60
Appropriate treatment administered in first 24 h of admission, % (n/N)[b]	53.6 (90/168)	58.5 (72/123)	40.0 (18/45)	**.04**

P values <.05 are displayed in bold.

Abbreviations: CNS, central nervous system; CT, computed tomography; HSV, herpes simplex virus; IQR, interquartile range; LP, lumbar puncture; VZV, varicella zoster virus.

[a]Appropriate treatment by organism: Cryptococcal meningitis treatment was given in 101/119 patients with cryptococcal meningitis, no treatment given in 9 patients, and treatment status unknown in 9 patients. Antibiotics were given in 31/31 in patients with confirmed or probable bacterial meningitis. Antiviral treatment was given in 3/3 patients with viral CNS infection due to HSV-1, HSV-2, or VZV. There was 1 VZV/cryptococcal meningitis coinfection that received both cryptococcal meningitis treatment and antiviral treatment. The first treatment given was cryptococcal meningitis treatment, and this was used for all analyses.

[b]Only 1 patient with viral meningitis had time to treatment data available. This was included in the time to appropriate treatment analysis but not presented separately.

indication for imaging based on local guidelines, 25.7% (35/136) had a potential radiographic contraindication to LP compared with 18.5% (5/27) who did not have an indication for imaging ($P = .43$). There was a trend toward higher inpatient mortality in the group with potential radiological contraindications when compared with the group without, 39.5% and 26.0%, respectively; however, this did not reach statistical significance (adjusted OR, 2.02; 95% CI, 0.93–4.44) (Table 4).

Using data from all individuals undergoing CT, the sensitivity and specificity and positive and negative predictive values of different guidelines in identifying potential radiological contraindications are described in Supplementary Table 4.

DISCUSSION

CT before LP in a high–HIV prevalence African setting led to significant delays to diagnostic LP and initiation of appropriate treatment. There was some evidence that delays in treatment were associated with increased mortality in patients with confirmed CNS infection; however, we were unable to determine a direct link between imaging and mortality, potentially due to the observational nature of the study, with imaging requested at the discretion of responsible clinicians, and significant confounding by indication. Clinical criteria to determine the need for CT before LP had poor sensitivity and specificity for subsequent detection of radiological criteria that would potentially contraindicate LP.

Our data are consistent with studies from high-income settings showing that performing CT in individuals with suspected CNS infection delays LP and definitive diagnosis. However, differences in CNS infection epidemiology in high-income settings, with a predominance of bacterial and self-limiting viral meningitis and a high frequency of empiric antibiotic administration, mean that delays in LP are less likely to delay effective treatment [10, 11, 14, 29], in contrast to settings where the spectrum of common CNS infections is broader, many requiring specific treatments including TB therapy and antifungals. In high–HIV prevalence settings in Sub-Saharan Africa, cryptococcal and TB meningitis account for 70%–90% of CNS infection, with bacterial meningitis accounting for only 8%–30% [22, 30, 31], highlighting why context-specific guidance regarding CT before LP is needed.

In our study, LPs were performed nearly 3 hours later in patients who had CT, with considerable knock-on delays in the subsequent initiation of targeted antimicrobial therapy. The impact of CT on treatment initiation time varied by condition, with a degree of delay seen for all major pathologies. Empiric antibiotics were administered to most patients, limiting the impact of imaging on time to appropriate antimicrobials with confirmed or probable bacterial meningitis; longer delays were seen with antituberculous, antiviral, and cryptococcal meningitis treatment, which are more complex and typically only started once diagnostic information from LPs is available. The delays to treatment initiation associated with CT in these

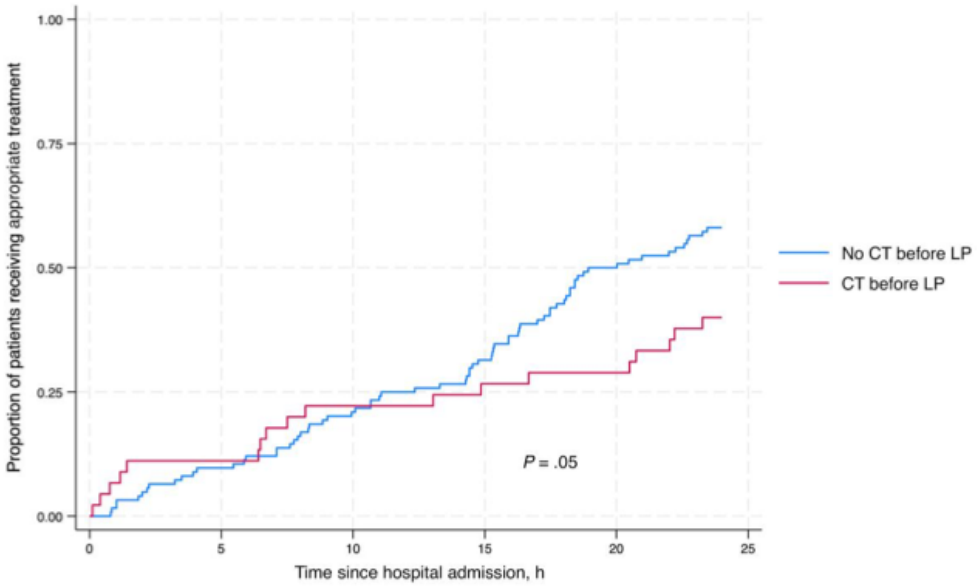

Figure 2. Kaplan-Meier analysis comparing receipt of appropriate antimicrobial treatment among patients with and without CT before LP in the first 24 hours after hospital admission in patients with microbiologically confirmed CNS infection or TB meningitis diagnosed through a uniform case definition. Abbreviations: CNS, central nervous system; CT, computed tomography; LP, lumbar puncture; TB, tuberculosis.

cases were considerably longer than could be accounted for by simply the delays to LP. This may be due to hospital workflows where treatment decisions are made on daily ward rounds; if diagnostic information is unavailable at the initial postintake rounds, then treatment decisions are deferred until the next medical review, often the next day.

Previous studies have demonstrated that delay to the administration of appropriate treatment is associated with increased mortality [32–36]; antibiotic delays in bacterial meningitis significantly increase unfavorable outcomes, and TBM treatment delays beyond 3 days lead to a 70% mortality increase [35, 36]. Our data corroborated this, with delays of >24 hours to treatment initiation in TBM being associated with a significant increase in mortality on multivariate regression analysis. Increased mortality with delays to initiation of therapy was seen in other conditions, but our sample size was too small to demonstrate significant effects.

We were unable to determine whether there was a causal link between CT-induced delays in LP and treatment and increased mortality due to limitations in our study design. We only included patients who had an LP; therefore patients with suspected CNS infection who did not have an LP due to CT findings would not be captured, meaning we cannot comment on overall percentages of individuals with imaging abnormalities in the population presenting with a clinical syndrome compatible with a CNS infection. The selection of patients who received CT was not random and was chosen by the treating physicians based on clinical features; those who underwent CT are likely to have presented with more severe illness; although we tried to account for differences in disease severity based on recorded variables and adjust for these in our analyses, it is inevitable that several markers of severity will not have been captured and fully accounted for, leaving residual confounding by indication.

Guidelines on the use of CT in CNS infection management in resource-limited settings are not widely available. Although consensus guidelines have been published for our study site, adherence was poor, with over half of those without imaging having a guideline indication for CT. Furthermore, these guidelines were poorly predictive of radiologic contraindications. Most patients with an indication for CT who received CT had no radiographic contraindication to immediate LP, and 18.5% of patients with no indication for imaging who had a CT scan had a potential contraindication to LP. The sensitivity of IDSA guidelines for detecting abnormalities in this cohort would have been 95%, the highest among guidelines tested [37]. However, using immunosuppression as an indication for CT, as in the IDSA guidance, would be impractical to implement in Botswana, where 73% (519/711) of our study population was known to be HIV positive. If IDSA guidelines were followed appropriately, this would have meant an additional 446 patients would have had CT before LP in this cohort (Supplementary Table 4) in a center where radiology is already

Table 3. Table Describing Mortality in Patients who Received Appropriate Treatment for CNS Infection Within 12 Hours After Admission, Amphotericin B–Based Treatment for Cryptococcal Meningitis Within 12 Hours After Admission, and Appropriate Tuberculous Meningitis Treatment Within 24 Hours; Logistic Regressions Analysis Adjusting for Age, Sex, Glasgow Coma Score, and Composite NEWS

Time From Admission to Therapy	Mortality, % (No.)	Crude Odds Ratio for Mortality (95% CI, *P* Value)	Adjusted Odds Ratio for Mortality (95% CI, *P* Value)
Appropriate therapy for all CNS infection			
Under 12 h	27.5 (11/40)	-	-
After 12 h	35.8 (45/123)	1.52 (0.69–3.35, .29)	1.66 (0.66–4.18, .28)
Antibiotics in confirmed bacterial meningitis or probable bacterial meningitis with neutrophilic pleocytosis and no confirmed CNS infection			
Under 12 h	30.4 (7/23)	…	…
Over 12 h	40.0 (2/5)	1.52 (0.20–11.72, .68)	2.22 (0.17–29.98, .55)
Amphotericin B–based cryptococcal meningitis treatment			
Under 12 h	18.2 (2/11)	-	-
Over 12 h	32.4 (24/74)	2.16 (0.43–10.98, .34)	1.41 (0.24–8.31, .71)
Antituberculous treatment in patients with possible, probable, or definite tuberculous meningitis			
Under 24 h	23.5 (4/17)	-	-
Over 24 h	48.4 (15/31)	3.05 (0.77–12.10, .10)	6.89 (1.01–46.99, .05)

Abbreviation: CNS, central nervous system.

Table 4. Associations Between Clinical and Laboratory Features Included in National or International Guidelines, CSF Analysis, Final Diagnosis and Mortality, and the Presence or Absence of Potential Radiological Contraindications to LP; Radiological CI Defined as Presence of Hydrocephalus, Intracranial Lesion, Cerebral Edema, Midline Shift or Intracranial Bleed; Patients Without a CT Report Were Not Included in the Analysis

	No Radiological CI to LP Identified on CT Head (n = 123)	Potential Radiological CI to LP Identified on CT Head (n = 40)	*P* Value
Final diagnosis of:			
Cryptococcal meningitis, No. (%)	16 (13.0)	5 (12.5)	.93
Possible/probable/definite tuberculous meningitis, No. (%)	9 (7.3)	9 (22.5)	.008
Viral meningitis, No. (%)	1 (0.8)	2 (5)	.09
Confirmed or probable bacterial meningitis, No. (%)	7 (5.7)	2 (5)	.87
Mortality	32/123 (26.0)	15/38 (39.5)	
	…	Crude OR for mortality, 1.86 (95% CI, 0.86–4.02)	.11
	…	Adjusted OR[a] for mortality, 2.03 (95% CI, 0.93–4.44)	.08

Outcome data were missing for 2 patients.

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; CT, computed tomography; LP, lumbar puncture; OR, odds ratio.

[a]Adjusted for age and sex.

operating at maximum capacity. Increased CT scanning in patients with suspected CNS infection would also have significant resource implications alongside potentially unnecessary delays to LP and treatment.

People with HIV (PWH) were significantly less likely to have an indication for CT based on local guidelines (or to undergo CT), in contrast to most guidelines where immunosuppression is an indication for CT. The relatively low level of CT being performed among PWH might be explained by the high rates of cryptococcal meningitis in this group [38]. Clinicians in high–HIV prevalence settings recognize that prompt LPs are a crucial, time-sensitive therapeutic intervention in cryptococcal meningitis to reduce ICP and may be less likely to request imaging when this is clinically suspected.

Our study had several further limitations. The standard of reporting of CTs was variable and not always by radiologists; as such, contraindications might have been under-recognized. The radiological contraindications to LP used in this study were pragmatically chosen to be easily identifiable by clinicians. Due to the real-world nature of the data with imaging requested at clinician discretion, and often not in line with local guidelines, we cannot determine whether CT before LP could prevent adverse consequences of LP; notably, it was observed that mortality was no different between the groups in our cohort with an

indication for CT who did and did not undergo CT before LP. The outcomes of individuals with suspected CNS infection who had contraindications identified on CT and did not then undergo LP are not known. Finally, this study was performed in a relatively well-resourced hospital that has been involved in several clinical trials for CNS infection; therefore, these findings may not be generalizable to settings with additional resource limitations.

The risk–benefit balance of CT before LP in suspected CNS infections remains contentious. Our data demonstrate that performing CT before LP in a low-resource, high–HIV prevalence setting significantly increased time to diagnostic LP and time to appropriate treatment, with no evidence that CT before LP was associated with improved outcomes in individuals for whom current guidelines would recommend imaging. These findings need to be taken into consideration when developing context-appropriate guidelines to support clinician decision-making.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Disclaimer. The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR or the UK Department of Health and Social Care.

Patient consent. As this study used only routinely collected programmatic samples and data, a waiver of informed patient consent was issued in the ethical approvals from Botswana Health Research and Development Committee, the University of Pennsylvania, and the London School of Hygiene and Tropical Medicine.

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References

1. GBD 2019 Meningitis Antimicrobial Resistance Collaborators. Global, regional, and national burden of meningitis and its aetiologies, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol **2023;** 22: 685–711.
2. Van De Beek D, Brouwer M, Hasbun R, Koedel U, Whitney CG, Wijdicks E. Community-acquired bacterial meningitis. Nat Rev Dis Primers **2016;** 2:16074.
3. April MD, Long B, Koyfman A. Emergency medicine myths: computed tomography of the head prior to lumbar puncture in adults with suspected bacterial meningitis—due diligence or antiquated practice? J Emerg Med **2017;** 53: 313–21.
4. Elafros MA, Belessiotis-Richards C, Birbeck GL, Bond V, Sikazwe I, Kvalsund MP. A qualitative study of patient, caregiver, doctor and nurse views of factors influencing lumbar puncture uptake in Zambia. Trans R Soc Trop Med Hyg **2022;** 116:322–7.
5. Saylor D, Elafros M, Bearden D, et al. Patient, provider, and health systems factors leading to lumbar puncture nonperformance in Zambia: a qualitative investigation of the "Tap Gap." Am J Trop Med Hyg **2023;** 108:1052–62.
6. Salazar L, Hasbun R. Cranial imaging before lumbar puncture in adults with community-acquired meningitis: clinical utility and adherence to the Infectious Diseases Society of America guidelines. Clin Infect Dis **2017;** 64:1657–62.
7. Glimåker M, Sjölin J, Åkesson S, Naucler P. Lumbar puncture performed promptly or after neuroimaging in acute bacterial meningitis in adults: a prospective national cohort study evaluating different guidelines. Clin Infect Dis **2018;** 66:321–8.
8. Kastenbauer S, Winkler F, Pfister H-W. Cranial CT before lumbar puncture in suspected meningitis. N Engl J Med **2002;** 346:1248–51.
9. Larsen L, Poulsen FR, Nielsen TH, Nordström C-H, Schulz MK, Andersen ÅB. Use of intracranial pressure monitoring in bacterial meningitis: a 10-year follow up on outcome and intracranial pressure versus head CT scans. Infect Dis (Lond) **2017;** 49:356–64.
10. Costerus JM, Brouwer MC, Sprengers MES, Roosendaal SD, van der Ende A, van de Beek D. Cranial computed tomography, lumbar puncture, and clinical deterioration in bacterial meningitis: a nationwide cohort study. Clin Infect Dis **2018;** 67:920–6.
11. Michael B, Menezes BF, Cunniffe J, et al. Effect of delayed lumbar punctures on the diagnosis of acute bacterial meningitis in adults. Emerg Med J **2010;** 27:433–8.
12. Costerus JM, Brouwer MC, Bijlsma MW, Tanck MW, van der Ende A, van de Beek D. Impact of an evidence-based guideline on the management of community-acquired bacterial meningitis: a prospective cohort study. Clin Microbiol Infect **2016;** 22:928–33.
13. Ellis J, Harvey D, Defres S, et al. Clinical management of community-acquired meningitis in adults in the UK and Ireland in 2017: a retrospective cohort study on behalf of the National Infection Trainees Collaborative for Audit and Research (NITCAR). BMJ Open **2022;** 12:e062698.
14. Glimaker M, Johansson B, Grindborg O, Bottai M, Lindquist L, Sjolin J. Adult bacterial meningitis: earlier treatment and improved outcome following guideline revision promoting prompt lumbar puncture. Clin Infect Dis **2015;** 60:1162–9.
15. Hasbun R, Abrahams J, Jekel J, Quagliarello VJ. Computed tomography of the head before lumbar puncture in adults with suspected meningitis. Infect Dis Clin Pract **2002;** 11:35–6.
16. McGill F, Heyderman RS, Michael BD, et al. The UK joint specialist societies guideline on the diagnosis and management of acute meningitis and meningococcal sepsis in immunocompetent adults. J Infect **2016;** 72:405–38.
17. van de Beek D, Cabellos C, Dzupova O, et al. ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. Clin Microbiol Infect **2016;** 22:S37–62.
18. GBD 2016 Meningitis Collaborators. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol **2018;** 17:1061–82.
19. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis **2017;** 17:873–81.
20. Scarborough M, Thwaites GE. The diagnosis and management of acute bacterial meningitis in resource-poor settings. Lancet Neurol **2008;** 7:637–48.
21. Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Botswana. Available at: https://www.unaids.org/en/regionscountries/countries/botswana. Accessed 11 June 2023.
22. Tenforde MW, Mokomane M, Leeme T, et al. Epidemiology of adult meningitis during antiretroviral therapy scale-up in Southern Africa: results from the Botswana National Meningitis Survey. J Infect **2019;** 79:212–9.
23. Tenforde MW, Mokomane M, Leeme TB, et al. Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana National Meningitis Survey: a prevalent cohort study. Lancet Infect Dis **2019;** 19:740–9.
24. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis **2010;** 10:803–12.
25. Kestler A, Caruso N, Chandra A, Goldfarb D, Haas M. CT before lumbar puncture in suspected meningitis in Botswana: how established guidelines may not apply. Afr J Emerg Med **2014;** 4:76–82.
26. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform **2009;** 42:377–81.
27. Harris PA, Taylor R, Minor BL, et al. The REDCap Consortium: building an international community of software platform partners. J Biomed Inform **2019;** 95: 103208.
28. Smith GB, Prytherch DR, Meredith P, Schmidt PE, Featherstone PI. The ability of the National Early Warning Score (NEWS) to discriminate patients at risk of early cardiac arrest, unanticipated intensive care unit admission, and death. Resuscitation **2013;** 84:465–70.
29. Bodilsen J, Dalager-Pedersen M, Schønheyder HC, Nielsen H. Time to antibiotic therapy and outcome in bacterial meningitis: a Danish population-based cohort study. BMC Infect Dis **2016;** 16:392.
30. 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.

31. Wall EC, Everett DB, Mukaka M, 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;** 58:137–45.
32. Sheu J-J, Yuan R-Y, Yang C-C. Predictors for outcome and treatment delay in patients with tuberculous meningitis. Am J Med Sci **2009;** 338:134–9.
33. Hovmand N, Lundbo LF, Kronborg G, Darsø P, Benfield T. Factors associated with treatment delay and outcome in community acquired bacterial meningitis. IJID Reg **2023;** 7:176–81.
34. Thwaites G, Fisher M, Hemingway C, et al. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. J Infect **2009;** 59:167–87.
35. Køster-Rasmussen R, Korshin A, Meyer CN. Antibiotic treatment delay and outcome in acute bacterial meningitis. J Infect **2008;** 57:449–54.
36. Soria J, Chiappe A, Gallardo J, Zunt JR, Lescano AG. Tuberculous meningitis: impact of timing of treatment initiation on mortality. Open Forum Infect Dis **2021;** 8:ofab345.
37. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis **2004;** 39:1267–84.
38. Rajasingham R, Govender NP, Jordan A, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet Infect Dis **2022;** 22:1748–55.

Supplementary material

Viral CNS infection – Patient with symptoms consistent with CNS infection OR clinically suspected CNS infection – AND positive CSF PCR for a HSV-1, HSV-2 or VZV
Confirmed bacterial meningitis – Patient with symptoms consistent with meningitis OR clinically suspected meningitis – AND detection of an appropriate pathogen in CSF by PCR, culture, or Gram stain, OR detection of an appropriate pathogen in blood by PCR, culture, or Gram stain, with CSF pleocytosis.
Probable bacterial meningitis – Patient with symptoms consistent with meningitis OR clinically suspected meningitis – AND neutrophilic pleocytosis with no other cause of CNS infection identified
Cryptococcal Meningitis – Patient with symptoms consistent with meningitis OR clinically suspected meningitis – AND identification of *Cryptococcus neoformans/gattii* in CSF by culture, CrAg, India Ink or PCR
Definite Tuberculous meningitis – Patient with symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy – AND acid-fast bacilli seen in the CSF, OR identification of *Mycobacterium tuberculosis* in the CSF by culture or nucleic acid amplification test.
Probable Tuberculous meningitis – Patient with symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy. – AND, using Marais' criteria, a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available). At least 2 points should either come from CSF or cerebral imaging criteria. – AND alternative diagnoses excluded.
Possible Tuberculous meningitis – Patient with symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy. – AND, using Marais' criteria, a total diagnostic score of 6-9 points (when cerebral imaging is not available) or 6-11 points (when cerebral imaging is available). – AND alternative diagnoses excluded.

Supplementary table 1 Meningitis case definitions for final diagnosis of central nervous system infection

GCS ≤8 and/or posturing
New onset seizures
Unexplained altered mental status not clearly caused by alternative diagnosis (e.g. hypoxia, hypotension, hypoglycaemia)
Focal neurologic findings

Supplementary table 2 *Summary of consensus guidelines for preceding CT*

	3	2	1	0	1	2	3
HR	≤40		41-50	51-90	91-110	111-130	≥131
Systolic BP	≤90	91-100	101-110	111-219			≥220
RR	≤8		9-11	12-20		21-25	≥25
Temp					>37.5		
O2 Sats		<88	88-92	>92			

Supplementary table 3 *Composite scoring system based on routinely collected physiological parameters. Missing values were scored as zero.*

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Percentage of patients that would meet criteria to require scan if guidelines followed
Adjusted IDSA guidelines *(GCS<15, HIV-positive, history of seizure or focal neurology)*	95.0% (95% CI 83-99%)	6.5% (95% CI 3-12%)	24.8% (95% CI 18-33%)	80% (95% 44-98%)	89.6% (637/711)
Princess Marina Hospital guidelines *(GCS<15, history of seizure or focal neurology)*	87.5% (95% CI 73-96%)	17.9% (95% CI 12-26%)	25.7% (95% CI 19-34%)	81.5% (95% 62-94%)	59.2% (421/711)
Adjusted Swedish guidelines *(GCS<6 or focal neurology)*	37.5% (95% CI 23-54%)	74.0% (95% CI 65-82%)	31.9% (95% CI 19-47%)	78.4% (95% CI 70-86%)	14.6% (104/711)

Supplementary table 4 *Sensitvity, specificity, and positive and negative predictive value for the detection of potential radiological contraindications to lumbar puncture estimates for IDSA, PMH and Swedish guidelines. Fundoscopy was infrequently performed and therefore papilloedema was not included in the adjusted IDSA guidelines for this analysis*

Chapter VI – Botswana National Meningitis Survey: Protocol 3 and Harare Meningitis Aetiology Survey

6.1 Introduction

Central nervous system infections (CNSI) are a major cause of mortality in high HIV-prevalence African settings[1,2]. Despite improved access to antiretroviral therapy Botswana and Zimbabwe both still have high HIV prevalence of 16.4% and 11.0% in adults aged 15-49 in 2022, respectively[3,4]. HIV has markedly altered the epidemiology of CNSI and cryptococcal, tuberculous and pneumococcal meningitis are the most commonly diagnosed causes of CNSI[5–9]. Conventional diagnostics have significant limitations and if used in isolation result in large numbers of patients not receiving a diagnosis[5,7]. Patients with inflammatory CSF and no confirmed diagnosis have a high mortality indicating the presence of severe pathology that needs appropriate diagnosis and treatment to improve outcomes[6].

Molecular diagnostics implemented in trial settings have been shown to increase diagnostic yield and reduce the proportion of patients without a diagnosis[10–17]. However, despite their introduction there remains a significant proportion of patients without a diagnosis and therefore the optimal combination of enhanced diagnostics to be used in the region is yet to be determined. Furthermore, a number of trials using molecular diagnostics for CNSI diagnosis in high HIV-prevalence African settings have implemented these platforms in a stepwise algorithm which is stopped once a diagnosis is made and targeted only at HIV-positive adults where the diagnostic yield is presumed to be greatest[12–14]. Whilst this is potentially where the optimal utility of molecular diagnostics lies this approach will not detect co-detections or make diagnoses outside of the population of interest and therefore these data cannot be used to determine which patients would not derive benefit from enhanced testing.

BioFIRE FilmArray-Meningitis/Encephalitis (FilmArray-ME) is a rapid multiplex PCR platform that tests for 14 common central nervous system pathogens and is widely used in high-resource settings. The majority of more recent trials in high HIV-prevalence settings use FilmArray-ME alongside conventional diagnostics[14,16]. Whilst there is robust evidence supporting the use of FilmArray-ME in the diagnosis of CNSI in the Global North where it was developed the relevance of FilmArray-ME in high HIV-prevalence settings, where the aetiology of CNSI is very different, is less clearly established[18]. A key limitation is that FilmArray-ME used in isolation does not test for CNSI pathogens highly relevant to immune-suppressed populations, such as *Mycobacterium tuberculosis* and *Toxoplasma gondii*.

Determining how best to implement molecular diagnostics in resource-limited requires careful consideration. Financial constraints mean extensive adoption will be challenging but targeted use may represent a cost-effective solution. At present data is lacking to support targeted testing of key population groups as most previous studies have presumptively excluded HIV-negative patients or patients with another CNSI. Indiscriminate testing of all samples from all patients with suspected CNSI will potentially identify which groups can be safely excluded from enhanced testing if they meet key criteria.

To determine the impact of enhanced diagnostics for CNSI in low-resource, high HIV-prevalence settings on overall diagnostic yield, reduction in the number of patients without a diagnosis and determining the variation in diagnostic yield between key population groups a package of enhanced diagnostics was integrated into routine CSF analysis at Princess Marina Hospital, Gaborone and Parirenyatwa Hospital, Harare alongside additional retrospective CSF analysis.

6.2 Methods

Study population

Patients were recruited from Princess Marina Hospital, Gaborone, Botswana from April 2022 to January 2024 and Parirenyatwa Hospital, Harare, Zimbabwe from September 2022 to January 2024 as part of the Botswana National Meningitis Survey: Protocol 3 and the Harare Meningitis Aetiology Survey, respectively.

All adults aged 18 years or older who had CSF submitted to the microbiology laboratory at either Princess Marina Hospital or Parirenyatwa Hospital were included. BioFIRE FilmArray-ME and Xpert MTB/RIF Ultra were integrated into routine CSF analysis in the microbiology departments of both hospitals. Patients were excluded if CSF was collected for any reason other than suspected CNSI, if a CNS device was in situ, or if a successful FilmArray-ME run was not performed on the sample. Lumbar punctures performed on subsequent presentations and repeat lumbar punctures from the same admission were also excluded from the analysis.

CSF analysis

CSF was divided between different platforms as described chapter III with a portion of CSF stored for additional retrospective analyses. If CSF volume limited all analyses being performed, then mandated investigations or those requested by the treating clinician were prioritised.

Conventional testing

Standard operating procedures for CSF examination are in place at both hospitals these include macroscopic examination followed by a total cell count using a Neubaeur counter performed prior to centrifugation at 3000 revolutions/minute for 3 minutes. The sediment is then used for Gram stain, India ink stain, differential (if CSF white cell count is over 10 cells/mm^3) and culture. At Princess Marina Hospital CSF culture was performed using Sabouraud agar for 10 days incubation and sheep blood agar and chocolate agar for 72 hours incubation. At Parirenytwa hospital culture is performed for 48

hours on sheep blood agar, Sabouraud agar and chocolate agar. Cryptococcal antigen testing using IMMY lateral flow assay (IMMY, Normal. OK) was performed routinely on adult samples.

Enhanced diagnostics – routine care

FilmArray-ME and Xpert MTB/RIF Ultra were integrated into routine investigation of CSF samples at both hospitals. Laboratory staff at both sites underwent training on the use of FilmArray-ME by the manufacturers. All staff had been trained previously on the use of GeneXpert platform. Analysis was performed in line with manufacturer guidelines with the addition of a centrifugation step for Xpert MTB/RIF Ultra for all samples over 2ml in volume. Results from both FilmArray-ME and Xpert MTB/RIF Ultra were released to clinical teams alongside results from conventional testing using existing systems for result dissemination.

Quality assurance and validation of both BioFIRE FilmArray instruments was performed using Zeptometrix ME molecular QC panels to calibrate each instrument prior to initiation of the study. In the case of failure or servicing the machine was recalibrated. Each batch of FilmArray-ME panel kits was validated using Maine molecular QC panel. Validation and quality assurance of GeneXpert platforms and every batch of Xpert MTB/Kits was performed in line with local and manufacturer guidelines at both sites.

Enhanced diagnostics – retrospective testing

Retrospective analyses were dependent on sufficient volume being available. All HIV positive patients with enough residual CSF were tested analysed using a monoplex *Toxoplasma gondii* PCR and all patients with residual CSF were investigated for neurosyphilis using a combination of treponemal and non-treponemal tests.

Neat CSF was analysed with Rapid Plasma Reagin (RPR) and Treponema Pallidum Particle Assay (TPPA). Nucleic acid was subsequently extracted from stored CSF stored at -80°C. *Toxoplasma gondii* PCR was performed on CSF samples collected from people living with HIV using a commercially-available PCR kit. Metagenomic sequencing was performed using GridION (Oxford Nanopore Technology, UK) at Botswana Harvard Health Institute, Gaborone, UK. Metagenomic sequencing was performed in two separate analyses the first in January 2023 and second in October 2023. Samples sequenced were a mixture of samples with inflammatory CSF findings suggestive of CNSI but no diagnosis and known positive and negative controls. Samples with inflammatory CSF but without a confirmed microbiological diagnosis were defined by either the presence of an age-appropriate pleocytosis alone or both CSF glucose <2.2mmol/mL and CSF protein >1mg/mL and no confirmed microbiological-diagnosis made through FilmArray-ME, Xpert MTB/RIF Ultra, RPR/TPPA or *Toxoplasma gondii* PCR. Purified RNA was reverse transcribed into cDNA. DNA and cDNA were quantified and quality checked before being prepared for sequencing using ONT Rapid Barcoding Sequencing kits. For the first analysis twelve sample sequencing libraries were multiplexed on a single run on a flow cell, the flow cell was washed after the first run and a second run loaded. The manufacturer updated their library preparation kits shortly after this analysis and therefore in the second run twenty-four samples were multiplexed on a single run on a flow cell which was only run once. During sequencing the overriding abundance of human reads was depleted using on-the-fly reference alignment to the human genome and reversal of the pore current[19]. Resulting sequenced reads were analysed for possible de novo pathogens using krakenuniq metagenomic read classifier[20]. The presence of known pathogens was confirmed by read mapping to representative pathogen reference genomes using minimap2[21]. Analysis with RPR, TPHA, *Toxoplasma gondii* PCR and metagenomic sequencing were performed retrospectively, often several months after collection of CSF, and were not shared with treating clinical teams.

Data collection

Patients were identified from laboratory records of CSF samples submitted for analysis. Clinical data was collected from patients' medical records and entered on an online data capture system, REDcap[22]. Clinical data focused on clinical presentation including the presence or absence of features suggestive of CNS infection such as headache, fever, neck stiffness or seizure, HIV status including level of immunosuppression, virological suppression, current ART status and other co-morbidities. Data on treatment including antimicrobials and adjunctive therapies, such as steroids in TB meningitis, alongside outcome data on discharge diagnosis and mortality during admission were captured from the patients' medical records. Data on LP timing and timing of receipt of specimen was captured from the laboratory reporting system. Results from microbiological samples that are not CSF samples, such as blood cultures or sputum Xpert MTB/RIF testing, laboratory data from initial blood results including haemoglobin, peripheral white cell count, creatinine and electrolytes were collected from laboratory records. Plasma glucose was rarely performed, therefore capillary blood was captured from the patient medical records. Patients were followed to discharge and outcome at discharge was recorded.

Data analysis

Primary outcomes were the increase in diagnostic yield resulting from additional CSF analysis with enhanced diagnostics stratified by key population groups and the decrease in number of patients with inflammatory CSF and no confirmed diagnosis. Population groups of interest were HIV positive and HIV negative patients, patients with and without inflammatory CSF, and patients with a positive or negative CSF CrAg or combinations of these criteria.

Data were analysed using STATA version 18.0. Patient demographics, CSF test results and HIV-related data were described using frequencies, percentages, or median and interquartile range (IQR) as appropriate. Inflammatory CSF was defined as CSF WCC over 5 cells/ml or CSF protein over 1mg/mL and CSF glucose under 2.2mmol/mL. Clinically appropriate antiviral administration was defined as antivirals given in patients with HSV-1, HSV-2 and VZV detected on CSF.

Ethics

Institutional review board approval was in place from the London School of Hygiene and Tropical Medicine for both projects. For the Botswana National Meningitis Survey: Protocol 3 institutional review board approval was granted by the Health Research Development Council (HRDC reference number 6/14/1), London School of Hygiene and Tropical Medicine (LSHTM reference number 17322), and the University of Botswana (UB reference number UBR/RES/IRB/1631). Approvals for the Harare Meningitis Aetiology Survey were obtained from the Research Council of Zimbabwe (MRCZ/A/2896), the Medical Research Council of Zimbabwe (MRCZ/A/2896) and the Joint Research and Ethics Committee (Parirenyatwa Group of Hospitals, reference number220/2022).

6.3 Results

Study population

Between April 2022 and December 2023 at Princess Marina Hospital 502 adults had CSF analysed with FilmArray-ME and between September 2022 and December 2023 481 adults had CSF analysed with FilmArray-ME at Parirenyatwa Hospital. In total, 983 adults had CSF analysed with FilmArray-ME, 117 were excluded. Exclusions were due to repeat lumbar punctures in 92 cases, failed FilmArray-ME runs in 17, the presence of CNS devices in 4 and CSF collected for reasons other than suspected CNSI in 4 (figure 1). CSF was successfully analysed with FilmArray-ME from 866 adult patients, 437 in Botswana and 429 in Zimbabwe.

Due to limitations in sample volume and disruptions to supply of consumables not all patients had all available tests. All 866 patients had FilmArray-ME, 762 had FilmArray-ME and Xpert MTB/RIF Ultra,

308 had FilmArray-ME, Xpert MTB/RIF and PCR for *Toxoplasma gondii* and 186 additional syphilis testing with CSF RPR and TPPA. There were 96 patients that received FilmArray-ME, Xpert MTB/RIF Ultra and CSF RPR/TPHA testing.

Patient demographics and HIV

The median age of the study population was 41.0 (IQR 33.2-49.9) and the majority were male 54% (468/866), (table 1). Amongst the included patients 77.1% (586/866) were HIV positive with a median CD4 of 107 cells/mm^3 (IQR 40-263). A new diagnosis of HIV was made on presentation with suspected CNSI in 94 patients. Amongst people with HIV 47.7% (279/586) were either ART naïve (183/279) or had cycled out of care (97/279), compared to 45.9% (269/586) currently taking ART. First-line antiretroviral therapy was the most common regime for patients taking ART at the time of admission with 75.8% of patients taking combination antiretroviral therapy of tenofovir, lamivudine and dolutegravir.

Conventional diagnostics

Conventional diagnostics alone detected a CNS pathogen in 17.1% (148/866) of patients, the most common diagnosis was cryptococcal meningitis identified in 89.4% (126/148) of patients with a diagnosis on routine testing alone. Excluding cryptococcal meningitis, 2.5% (22/866) of patients received a diagnosis through routine diagnostics alone, 15 from positive CSF cultures and 7 from CSF gram stain. Through the use of routine diagnostics alone 69.1% (141/204) of patients with inflammatory CSF suggestive of CNSI did not have a diagnosis.

Enhanced diagnostics

Enhanced diagnostics approximately doubled the number of patients with a potential CSF pathogen detected in CSF. The proportion of patients with a pathogen detected in CSF with the addition of FilmArray-ME to conventional diagnostics was 26.1% (226/866). Due to limitations of sample volume not all tests were performed on all patients (figure 8). The proportion of patients with any pathogen detected after analysis with Xpert MTB/RIF was 24.9% (190/762), after analysis with Toxoplasma gondii PCR 26.9% (88/327) and RPR/TPHA 22.0% (41/186). The combination of all enhanced diagnostics (FilmArray-ME, Xpert MTB/RIF, *Toxoplasma gondii* PCR and RPR/TPHA) alongside conventional diagnostics detected a pathogen in 33.1% (287/866) of patients. Cryptococcal meningitis remained the most common diagnosis in 45.6% (131/287) of patients. There were an additional 5 cases that were diagnosed on FilmArray-ME only. TB meningitis was diagnosed in 6.8% (53/762) of patients that had Xpert MTB/RIF performed on CSF.

The proportion of patients with inflammatory CSF but no diagnosis reduced from 69.1% (141/204) to 35.3% (72/204) with the addition of enhanced diagnostics. The median CSF white cell count in patients with inflammatory CSF but no diagnosis was 15 cells/mm^3 (8-40 cells/mm3), amongst those patients that had a differential performed the majority were lymphocytic, 77.8% (21/27). Amongst patients with inflammatory CSF but no diagnosis after enhanced diagnostics and with available treatment data 65.6% (42/64) received treatment with either a third-generation cephalosporin or carbapenem at CNSI dose, 36.5% (23/63) received empiric anti-tuberculous treatment, 12.7% received empiric antiviral treatment and 1 patient was treated with anti-cryptococcal meningitis treatment on the basis of a positive serum CrAg alone. Findings from non-CSF specimens potentially aided diagnosis in 5 patients; 4 positive blood cultures (2 *Staphylococcus aureus*, 1 *Streptococcus pneumoniae*, 1 *Candida tropicalis*) and 1 positive serum RPR without confirmatory CSF testing in an HIV positive patient.

The relative increase in yield of enhanced diagnostics above routine diagnostics was 93.6% overall. Stratified by HIV positivity, CSF analysis and CSF CrAg positivity the relative increase varied by

population group (table 2). The highest diagnostic yield through a combination of routine and enhanced diagnostics was in the HIV positive inflammatory CSF group, 68.7%. The largest relative increase in diagnostic yield was seen in HIV negative patients with inflammatory CSF where the addition of enhanced diagnostics increased the diagnostic yield from 10% to 55%, a relative increase of 450%. Testing only patients with a negative CSF CrAg would have missed 18 co-detections, most commonly CMV (4/18) and *T.gondii* (4/18).

Not all patients had all available tests, amongst those that received all available coverage of enhanced diagnoses 42.1% (16/38) had inflammatory CSF with no microbiological diagnosis.

Tuberculous meningitis

Tuberculous meningitis prevalence was 6.8% (53/762) amongst all patients who had a successful Xpert MTB/RIF Ultra performed on CSF. TB meningitis prevalence varied by CSF abnormalities with 2.8% (16/571) of patients with uninflammatory CSF having a positive Xpert MTB/RIF compared to 19.4% (37/191) of those with inflammatory CSF. TB meningitis was more common with increasing CSF white cell count with 18.1% (32/177), 23.4% (25/107), 25.0% (18/72) and 29.8% (17/57) of patients having confirmed TB meningitis in known CSF white cell counts of >5 cells/mm^3, >20 cells/mm^3, >50 cells/$mm^3$and >100 cells/mm^3, respectively. A further 15 patients had probable TBM and 207 had possible TBM based on a uniform case definition[23]. Amongst patients with possible or probable TBM 20.7% (46/222) were either treated as presumed TB meningitis by the clinical team or had a final diagnosis of presumed TBM.

HIV positivity was 68.6% in patients with TB meningitis, but the proportion of patients with confirmed TB meningitis was higher in known HIV negative patients than in known HIV positive patients, 7.4% (11/138) versus 6.7% (35/521). Amongst those with an unknown HIV status 10.4% (5/48) of patients

had a positive Xpert MTB/RIF Ultra. CD4 cell count was available in 24 patients with TBM and the median CD4 was 151 (IQR 44-258). Amongst HIV positive patients with TBM 54.3% (19/35) were receiving ART and 45.7% (16/35) had defaulted or cycled out of treatment. Median time on ART was 3.5 months (IQR 2.1-72.4).

The median volume used for analysis with Xpert MTB/RIF Ultra was 5ml (IQR 3.5-5.5). Xpert MTB/RIF positivity was higher in patients with 5ml or more used solely for Xpert analysis than amongst those with less than 5ml used, 5.4% (15/278) and 7.9% (38/484) respectively, however this did not reach statistical significance, (p=0.20). Resistance to rifampicin was detected on in 3.8% (2/53) of patients, 1 in Gaborone and 1 in Harare, a further 32.1% (17/53) of CSF samples had indeterminate resistance to rifampicin. The proportions of patients who had previously received TB treatment with no resistance to rifampicin, indeterminate resistance to rifampicin and rifampicin resistance were 6.3% (2/32), 12.5% (2/16) and 50% (1/2).

Details on treatment were available in 49 patients. Amongst those with treatment data available 20.4% (10/49) did not receive treatment; in 8 patients this was because they died before Xpert MTB/RIF Ultra results became available, in 1 patient they died before anti-tuberculous medication became available, and 1 patient was discharged back to their local hospital before starting treatment as the Xpert MTB/RIF Ultra result was not available prior to discharge, they started treatment at the other facility. Amongst the 39 patients who received anti-tuberculous treatment 5 were missing either Xpert MTB/RIF Ultra result release time or anti-tuberculous treatment start time. A positive Xpert MTB/RIF Ultra triggered initiation of treatment in 61.8% (21/34) of those patients with a positive Xpert MTB/RIF Ultra with complete data on treatment times. All patients without resistance to rifampicin or indeterminate resistance to rifampicin received standard anti-tuberculous therapy. Amongst those who received treatment 74.4% (29/39) also received steroids; 51.7% (15/29) received oral prednisolone, 44.8% (13/29) received intravenous dexamethasone and 3.4% (1/29) received

intravenous hydrocortisone. Inpatient mortality was known for 49/53 patients. Mortality for patients with a positive Xpert MTB/RIF Ultra was 52.8% (28/53) compared to 25.7% (182/709) in those patients with a negative Xpert MTB/RIF Ultra. Mortality was higher in HIV negative patients compared to HIV positive patients, 72.7% (8/11) and 45.7% (16/35) respectively.

Toxoplasma gondii

Amongst patients with HIV who had residual CSF stored for further analyses 4.9% (16/327) had *Toxoplasma gondii* detected on PCR. The median CD4 was 88 cells/mm^3 (IQR 81-216). ART status was known in 15/16 patients and 25% (4/16) were on ART whilst 68.8% (11/16) were either ART naïve or had cycled out of treatment. Headache and fever or history of fever were the most common presenting complaints seen in 68.8% (11/16) patients, 56.3% (9/16) patients had a history of altered mental state and 1 patient presented with a seizure. No patients had any focal neurological abnormalities on examination. Computed tomography of the head was performed in 5 patients, 4 scans were performed without contrast and were reported as normal. One contrast-enhanced scan was reported as having a space-occupying lesion. Co-detections were seen in 37.5% (6/16) patients, 4 patients with cryptococcal meningitis, 1 with *Streptococcus pneumoniae* and 1 with CMV.

Clinicians were unaware of the results as they were performed retrospectively on stored samples often up to a year after sample collection and therefore did not influence management decisions. Empiric treatment with high dose co-trimoxazole was prescribed in 12.5% (2/16) patients and 50% (8/16) were receiving prophylactic dose co-trimoxazole due to having a CD4 count under 200 cells/mm^3. Inpatient mortality amongst patients with *T.gondii* detected on CSF was 12.5% (2/16), amongst patients that died during the admission one patient had intercurrent cryptococcal meningitis and in the other *T.gondii* was the only pathogen detected.

Metagenomic sequencing

Metagenomic sequencing was performed on 168 CSF samples, 45 of which had inflammatory CSF and no diagnosis after investigation with enhanced diagnostics. Overall, sequencing detected 65 potentially pathogenic organisms in 53/168 patients, however the clinical relevance of some organisms is uncertain.

Focussing on the added value of metagenomics above the panel of enhanced diagnostics patients with inflammatory CSF metagenomic sequencing identified potential CNS pathogens in a further 15.9% of cases (7/44); 3 protozoa (*2 Plasmodium falciparum* and 1 *Plasmodium malariae*), 2 bacteria (1 *Staphylococcus aureus,* which had an accompanying blood culture positive for *S.aureus* and 1 *Enterococcus faecalis*) and 2 viruses (HIV & Human Herpesvirus 7). Amongst the 2 patients with *Plasmodium falciparum* detected one had a positive blood film for malaria parasites and a positive malaria antigen test with >500 red blood cells/ml on CSF red cell count, they were treated with intravenous artemisinin combination therapy. The other patient had a negative blood film for malaria parasites and negative malaria antigen test. There were an additional 4 viruses detected in patients with a clinical syndrome compatible with CSNI but with uninflammatory CSF and no diagnosis (2 Human adenovirus species C, 1 Parvovirus B19 and 1 JC virus). There were 4 further bacteria identified in patients with uninflammatory CSF however given the absence of laboratory features of CSF inflammation these results should be interpreted with caution and may represent potential contamination (*2 Pseudomonas aeruginosa, 1 Citrobacter koseri and 1 Bordatella trematum*).

Amongst those patients who CSF was sequenced 69.8% were HIV positive and 75% (15/20) of those with recent viral load testing had an undetectable viral load. HIV was detected in the CSF of 10 patients overall; plasma HIV viral load was only available for one patient and was 215572 copies/ml. Median

CD4 in those patients with HIV detected in CSF viral escape was 55 (IQR 20-196) cells/mm^3 and 60% (6/10) were ARV naïve.

Treatment

Antibiotic therapy was administered in 85.6% (680/794) of patients, anti-tuberculous treatment in 13.9% (109/786) and antivirals in 11.3% (98/794). Amongst those patients with confirmed bacterial meningitis and available treatment data 94.0% (47/50) received antibiotics and 84.4% (38/45) with timing data available received antibiotics prior to release of FilmArray-ME results. In patients with a viral CNSI that would warrant appropriate antiviral treatment, defined as detection of HSV-1, HSV-2 and VZV in CSF, 72.4% (21/29) received antivirals. Amongst those with treatment timing data available antivirals were administered in 5.3% (1/19) of patients prior to FilmArray-ME results, in the remainder of patients antivirals were only administered after detection of HSV-1, HSV-2 or VZV in CSF. Amongst the 98 patients who received antivirals 62.2% (61/98) had no virus of any type detected on CSF.

Mortality

Mortality was 27.9% (242/866) in the entire study population. The median age of patients that died in hospital was 44 (IQR 36-53) years. The highest mortality was seen in patients with TB meningitis at 51.0% (25/49), mortality was 40.7% (11/27) in patients with *Streptococcus pneumoniae,* 35.1% (39/111) in patients with cryptococcal meningitis and 10% (1/10) in patients with *Toxoplasma gondii*. Mortality in patients with uninflammatory CSF and no confirmed diagnosis was 22.9% (116/507), stratified by HIV status mortality was higher in patients living with HIV than in HIV negative patients, 25.3% (77/305) and 20.2% (25/124) respectively. In patients with inflammatory CSF mortality was 25.0% (18/72), 25.5% (12/42) in patients living with HIV and 27.8% (5/18) in HIV negative patients.

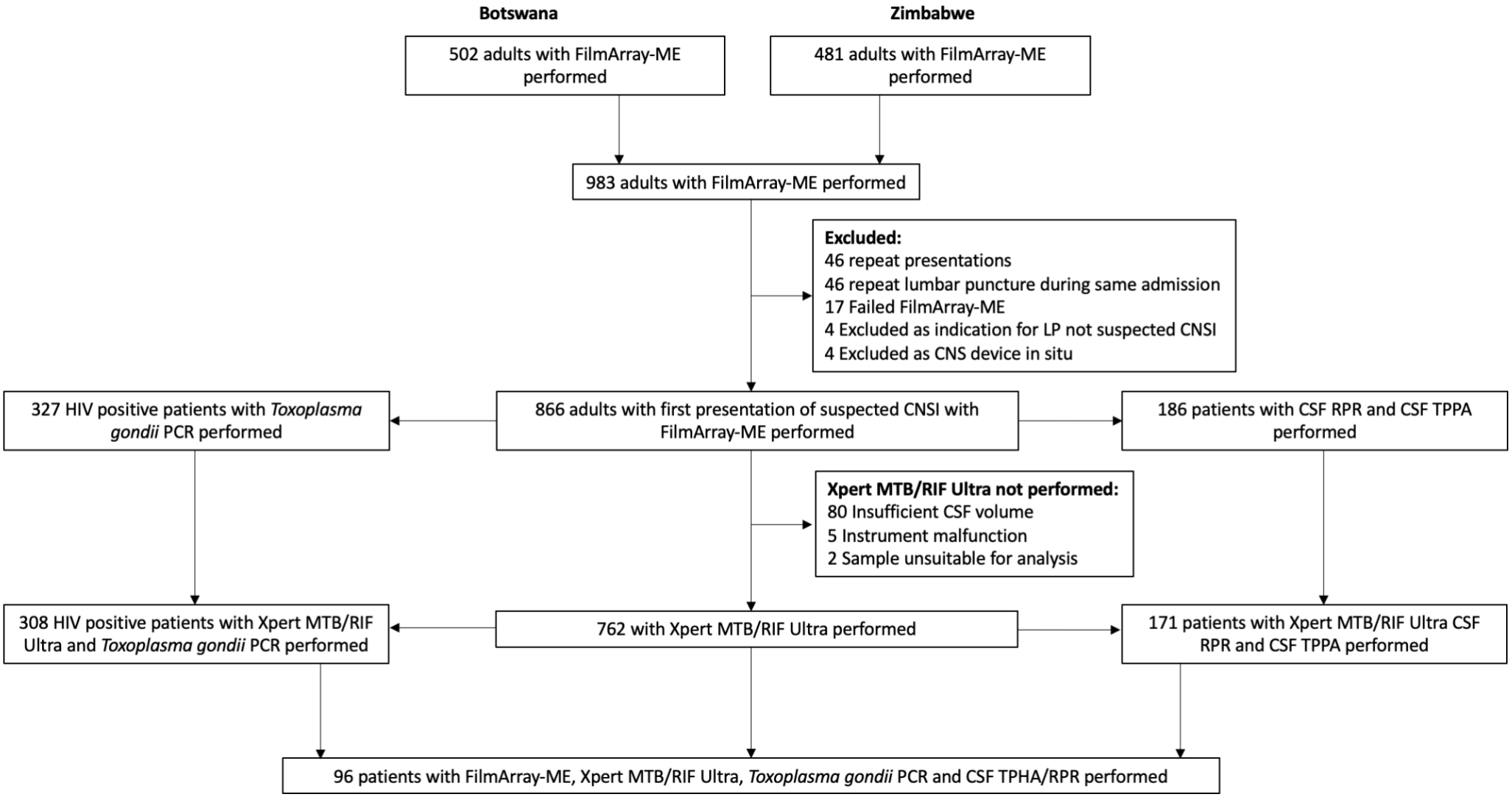

Figure 8 Screening, exclusion and analysis populations

	Total (n=866)	Botswana (n=437)	Zimbabwe (n=429)
Demographics			
Median age, years (IQR)	41.0 (33.2-49.9)	41.1 (33.9-51.6)	40.7 (32.1-48.7)
Female sex, % (n)	46.0% (398)	46.9% (205)	45.0% (193)
Clinical details			
Fever of history of fever	38.0% (329)	31.6% (138)	44.5% (191)
Neck stiffness	39.8% (345)	35.5% (155)	44.3% (190)
Altered mental status	48.7% (422)	46.9% (205)	50.6% (217)
At least one of fever, neck stiffness or altered mental status	74.9% (649)	72.1% (315)	77.9% (334)
Fever, neck stiffness AND altered mental status	11.9% (103)	8.7% (38)	15.2% (65)
Median duration of symptoms prior to presentation, days (IQR)	10 (4-23)	8 (3-21)	12 (7-28)
Previous history of TB	11.7% (102)	10.9% (48)	12.6% (54)
Co-morbidities (excluding TB) *None* *1* *2 or more*	 79.8% (691) 15.7% (136) 4.5% (39)	 73.2% (320) 20.1% (88) 6.6% (29)	 86.5% (371) 11.2% (48) 2.3% (10)
Abnormal neurology	16.0% (124)	24.0% (92)	8.2% (32)
GCS *Under 8* *Under 15*	 4.4% (38) 47.7% (413)	 5.7% (25) 47.6% (208)	 3.0% (13) 47.8% (205)
Adult HIV details			
HIV positive, % (n)*	77.1% (586)	71.3% (288)	83.7% (298)

HIV diagnosed on admission, % (n)	16.0% (94)	15.3% (44)	16.8% (50)
Median CD4 If HIV positive, cells/mm^3 (IQR)**	107 (40-263)	122 (47-350)	76 (34-217)
Proportion of adults living with HIV with CD4 <200 cells/mm^3, % (n)	43.9% (257)	47.6% (137)	40.3% (120)
Currently on ART, % (n)	45.9% (269)	37.5% (108)	54.0% (161)
CSF Analysis			
Median CSF volume submitted for analysis, ml (IQR)	10 (6.5-12)	8.8 (4-11)	10 (9-13)
WCC pleocytosis >5 cells/mm^3, % (n)	21.6% (187)	26.8% (117)	16.3% (70)
CSF Protein >1 mg/mL, % (n)	12.2% (106)	9.6% (42)	14.9% (64)
CSF Glucose <2.2mmol/mL, % (n)	12.6% (109)	12.6% (55)	12.5% (54)
Inflammatory CSF, % (n)	23.9% (207)	27.7% (121)	20.1% (86)
Diagnoses with routine analysis alone, % (n)	17.1% (148)	16.5% (72)	17.7% (76)
Diagnoses with routine analysis alone excluding cryptococcal meningitis, % (n)	2.5% (22)	3.0% (13)	2.1% (9)
Diagnoses with routine and enhanced – excluding metagenomic sequencing, % (n)	33.1% (287)	29.3% (128)	37.1% (159)
Inflammatory CSF* with no diagnosis (routine only), % (n/N)**	69.1% (141)	69.9% (86)	67.9% (55)
Inflammatory CSF* with no diagnosis (routine and enhanced – excluding MGS), % (n/N)**	35.3% (72)	43.1% (53)	23.5% (19)
Outcome			
Discharged alive	61.2% (530)	59.0% (258)	63.4% (272)

Died in hospital	27.9% (242)	28.2% (123)	27.7% (119)
Unknown	10.8% (94)	12.8% (56)	8.9% (38)

Table 11 Study population demographics, clinical features on presentation CSF analysis and in-hospital mortality

*HIV status was unknown in 6.7% (55) patients overall, 3.3% (14) in Botswana and 10.3% (41) in Zimbabwe
** CD4 count was unknown in 35.3% (207) of HIV positive patients overall, 26.7% (77) in Botswana and 43.6% (130) in Zimbabwe
** ART treatment status was unknown in 6.5% (38) of HIV positive patients overall, 8.7% (25) in Botswana and 4.4% (13) in Zimbabwe

	Routine, % (n/N)	Enhanced, % (n/N)	Relative increase, %
Overall	17.1 (148/866)	33.1 (287/866)	93.6
Uninflammatory CSF	12.8 (85/662)	23.4 (155/662)	82.8
Inflammatory CSF	30.9 (63/204)	64.7 (132/204)	109.4
HIV negative	5.2 (9/174)	18.4 (32/174)	253.8
HIV negative uninflammatory CSF	3.7 (5/134)	7.5 (10/134)	102.7
HIV negative Inflammatory CSF	10 (4/40)	55 (22/40)	450.0
HIV positive adult	22.5 (132/586)	39.9 (234/586)	77.3
HIV positive uninflammatory CSF	17 (74/436)	30.1 (131/436)	77.1
HIV positive inflammatory CSF	38.7 (58/150)	68.7 (103/150)	77.5
HIV positive with negative CSF CrAg	5.2 (24/461)	26.7 (123/461)	413.5

Table 12 Diagnostic yield stratified by population group with both routine and enhanced diagnostics and the relative increase of enhanced diagnostics above routine diagnostics alone

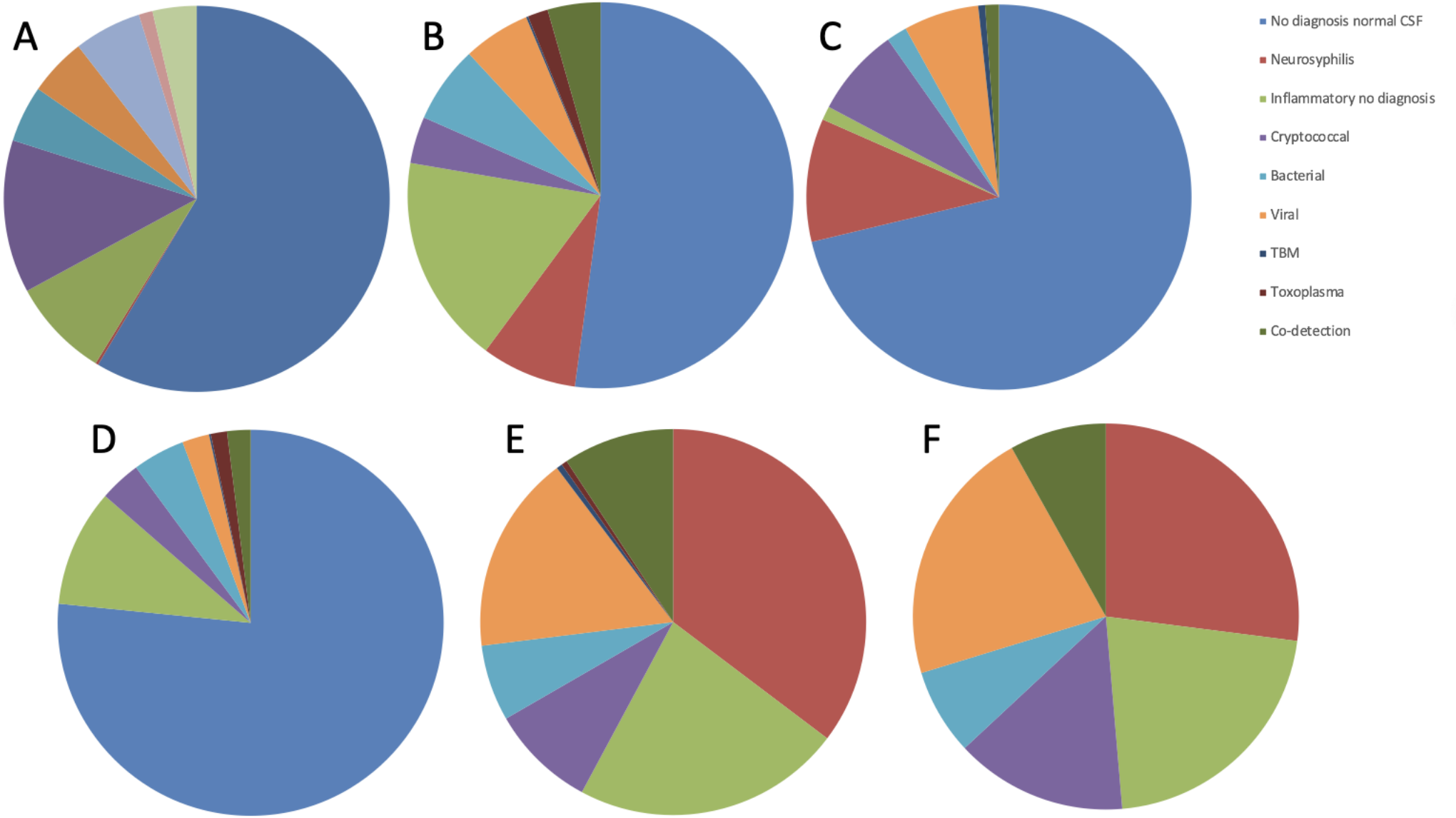

Figure 9 Diagnoses made through routine and enhanced diagnosis. (A) All adults (B) HIV positive (C) HIV negative (D) Uninflammatory CSF, (E) Inflammatory CSF and (F) CSF WCC >20 cells/mm^3

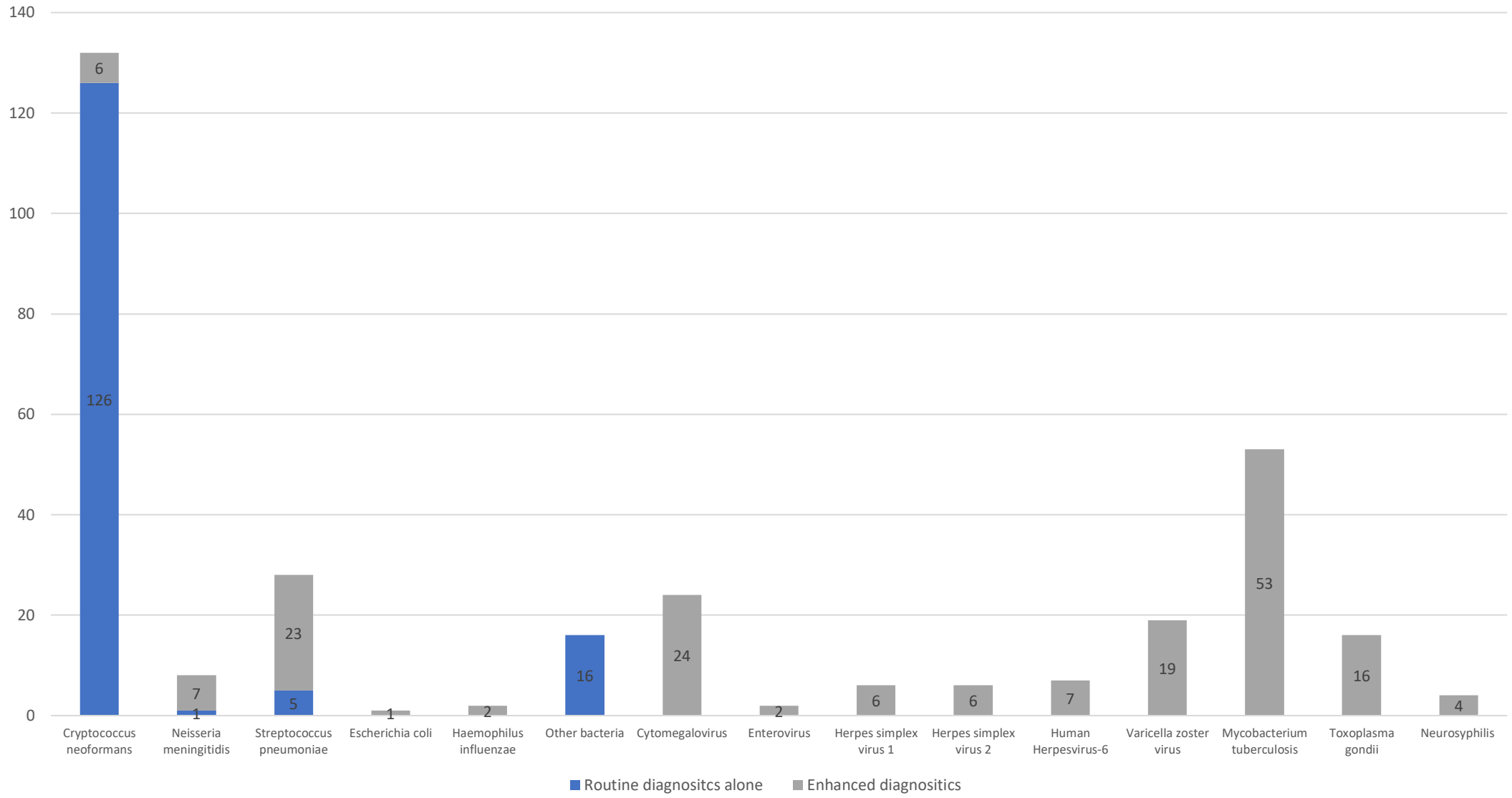

Figure 10 Pathogens detected through combination of routine and enhanced diagnostics

Final diagnosis	Died in hospital	Discharged alive	Unknown
Overall	27.9% (242)	61.4% (532)	10.6% (92)
HIV negative	25.9% (45)	67.8% (118)	6.3% (11)
HIV positive	30.0% (176)	64.2% (376)	5.8% (34)
Uninflammatory CSF and no diagnosis	22.9% (116)	64.9% (329)	12.2% (62)
Inflammatory CSF and no diagnosis	25.0% (18)	61.1% (44)	13.9% (10)
Cryptococcal meningitis	35.1% (39)	55.9% (62)	9.0% (10)
TB meningitis	51.0% (25)	40.8% (20)	8.2% (4)
Bacterial CNSI*	37.5% (15)	57.5% (23)	5.0% (2)
Viral CNSI**	28.6% (12)	66.7% (28)	4.8% (2)
Toxoplasma gondii	10.0% (1)	90.0% (9)	-
Neurosyphilis	-	100% (2)	-
Co-detection	46.9% (15)	46.9% (15)	6.3% (2)

Table 13 Mortality by final diagnosis

*22 *Streptococcus pneumoniae,* 6 *Neisseria meningitidis*, 2 *Haemophilus influenzae*, 2 *Klebsiella pneumoniae*, 1 *Staphylococcus aureus,* 1 *Acinetobacter baumanii*, 1 *Enterobacter* spp., 1 *Pseudomonas aeruginosa*, 1 *Salmonella* spp., 2 unidentified gram positive organism and 1 unidentified gram negative organism

**15 Cytomegalovirus, 15 Varicella zoster virus, 4 Herpes Simplex Virus-1, 4 Herpes Simplex Virus -2, 3 Human Herpesvirus-6 and 1 Enterovirus

6.4 Discussion

Enhanced diagnostics almost doubled the overall number of confirmed diagnoses in high HIV-prevalence African settings. The diagnostic yield from routine diagnostics alone was low, detecting a pathogen in 17.5% of patients and only 2.5% of patients overall received a diagnosis other than cryptococcal meningitis. The vast majority of cryptococcal meningitis cases were diagnosed using the highly sensitive and easy-to-use cryptococcal antigen test. The addition of enhanced diagnostics to routine testing increased the diagnostic yield to 33.1% overall, cryptococcal meningitis remained the most commonly diagnosed CNSI.

The increase in diagnostic yield was not uniform across population groups. The greatest relative increase in diagnostic yield was seen in HIV negative patients with inflammatory CSF (18 additional cases, 450% relative increase). This represents a relatively small subset of our study population that is most closely aligned with the Global North where the majority of CNSI diagnostics are made. The most commonly diagnosed CNSI in this group was meningococcal meningitis, a condition typically challenging to diagnose with conventional testing due to rapid sterilisation of CSF after administration of antimicrobials[24]. Enhanced diagnostics in patients with HIV with a negative CSF CrAg had a similarly large relative increase in diagnostic yield with an additional 99 diagnoses and relative increase of 413.5%. Targeting testing solely to higher yield groups represents a potentially cost-effective approach to CNSI investigation in resource-limited settings where financial constraints are likely the major barrier to widespread use of molecular diagnostics. Based on these data excluding patients with HIV with a positive CSF CrAg would have resulted in a greater than four-fold increase in diagnostic yield but would have missed 18 co-detections, most commonly CMV and *Toxoplasma gondii* each with 4 detections. Although co-detections in this group had limited impact on changes to clinical management, with additional treatment being prescribed by the treating clinician in 3/18 patients, the mortality in patients with co-detections was higher than all single pathogens apart from TB.

The proportion of patients with CSF findings suggestive of CSNI but no confirmed diagnosis decreased from 69.1% to 35.3% through the addition of enhanced diagnostics. Microbiologically-confirmed TB meningitis prevalence diagnosed using Xpert MTB/RIF Ultra was very high in our cohort reaching 19.4% in patients with inflammatory CSF, comparable to previous studies from the region using TB culture[25]. Despite the large number of TBM diagnoses this still likely represents an underestimation due to a combination of imperfect diagnostics and 12% of the study population not receiving TB testing due to limited CSF volume submitted for analysis or invalid Xpert MTB/RIF Ultra runs. More diagnoses were made through Xpert MTB/RIF Ultra than through conventional diagnostics, excluding those from CrAg testing. Furthermore, a positive Xpert MTB/RIF Ultra triggered the initiation of anti-tuberculous treatment in 61% of patients. Whilst we cannot comment on the counterfactual and some of these patients may have received treatment at a later stage regardless of CSF Xpert MTB/RIF Ultra results, Xpert MTB/RIF Ultra will have at least expedited treatment in number of patients in a condition where treatment delays carry significant mortality. These data create a strong argument for the integration of Xpert MTB/RIF Ultra testing into routine CSF analysis in high HIV- and TB-prevalence settings.

This study had several important limitations. Due to challenges with obtaining consumables and limited amounts of CSF available for some analyses not all tests could be performed on all samples. Therefore, accurate prevalence estimates of pathogens which had incomplete testing with the appropriate diagnostic test was not possible. Routine CSF analysis outcomes varied between laboratories. Although both laboratories had standard operating procedures in place for CSF analysis with similar laboratory infrastructure and laboratory technician skillset CSF pleocytosis was reported significantly less frequently in Zimbabwe than in Botswana. In addition, Zimbabwe had more patients with other markers of CSF inflammation such as elevated CSF protein and there were a similar number

of diagnoses between both sites suggesting that CSF pleocytosis in Zimbabwe might be underreported.

In this study enhanced diagnostics significantly increased the number of CNSI diagnoses made, however financial constraints may limit widespread adoption of these platforms in resource-limited settings. Understanding how best these data can be used to inform clinical practice is therefore crucial. There are several key points. Firstly, the prevalence of TBM was very high, nearly a quarter of patients with pleocytic CSF were diagnosed with TBM using a test with imperfect sensitivity and inpatient mortality in confirmed TBM cases was over 50%. There were more confirmed cases of TBM than all confirmed cases of bacterial CNSI combined however empiric TBM treatment was far less common than empiric bacterial CNSI treatment. These data suggest early empiric TBM treatment may be appropriate in high HIV-prevalence settings, particularly if TB diagnostics are not available. Consideration should also be given to prioritising TB treatment above antibiotic therapy when regionally-important diagnoses such as cryptococcal meningitis have been excluded and no diagnosis has been made in pleocytic CSF samples. However the potential side effects of anti-tuberculous therapy need to be considered and may preclude empiric TB treatment. These data demonstrate that a positive result from Xpert MTB/RIF Ultra triggered the initiation of anti-TB treatment in nearly two-thirds of patients who had microbiologically confirmed TBM, suggesting these patients would have either had delays to starting treatment or have never received treatment at all. Incorporating Xpert MTB/RIF Ultra into routine CSF testing in high HIV-prevalence settings would almost certainly increase the number of confirmed TBM diagnoses. In addition, it would likely increase the number of patients with TBM who receive treatment, reduce the time to anti-tuberculous treatment initiation and provide drug susceptibility data in a disease with an unacceptably high mortality. Conventional diagnostics such as culture, Gram and India ink stains have significant limitations and in resource-limited settings where laboratory infrastructure is challenged by a lack of consumables and technical experience the sensitivity of some tests can be reduced further. The introduction of PCR for common

CNS bacterial pathogens tripled the number of bacterial pathogens detected further highlighting the limitations of conventional diagnostics and emphasising the need to improve testing capacity even for organisms that are comparatively straightforward to diagnose. Viral pathogens and *Toxoplasma gondii* are not diagnosed in CSF in routine care in Botswana or Zimbabwe. Viral PCR testing revealed that 7% of adults with suspected CNSI had a potential viral CNS pathogen and approximately 5% of HIV positive patients had *Toxoplasma gondii* detected in CSF. Whilst the clinical significance of all viral results was not clearly defined expanded testing capacity is important primarily to improve diagnosis but may also serve to reduce antibiotic use and aid antimicrobial stewardship. Finally, variation in diagnostic yield between population groups suggests targeted use of these enhanced diagnostic platforms may be possible and potentially preferable in resource-constrained areas where universal testing would not be possible.

In conclusion, enhanced diagnostics were successfully implemented in two referral hospitals in high HIV-prevalence Southern Africa leading to a significant increase in the number of microbiologically-confirmed diagnoses made and demonstrating previously undiagnosed high TBM and viral CNSI prevalence.

References

1. Wunrow, H. Y. *et al.* Global, regional, and national burden of meningitis and its aetiologies, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* **22**, 685–711 (2023).
2. Tenforde, M. W. *et al.* Mortality from HIV-associated meningitis in sub-Saharan Africa: a systematic review and meta-analysis. *J Int AIDS Soc* **23**, e25416 (2020).
3. Joint United Nations Programme on HIV/AIDS (UNAIDS). Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Zimbabwe. Accessed 6/11/23. (2022).
4. Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and AIDS estimates—Botswana. Available at: https://www.unaids.org/en/regionscountries/countries/botswana.
5. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: Findings from 4961 suspected cases. *BMC Infect Dis* **10**, (2010).
6. Tenforde, M. W. *et al.* Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana national meningitis survey: a prevalent cohort study. *Lancet Infect Dis* **19**, 740–749 (2019).
7. Tenforde, M. W. *et al.* Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey. *Journal of Infection* **79**, 212–219 (2019).
8. Wall, E. C. *et al.* High Mortality amongst Adolescents and Adults with Bacterial Meningitis in Sub-Saharan Africa: An Analysis of 715 Cases from Malawi. *PLoS One* **8**, (2013).
9. Hakim, J. G. *et al. Impact of HIV Infection on Meningitis in Harare, Zimbabwe: A Prospective Study of 406 Predominantly Adult Patients*. *Lippincott Williams & Wilkins AIDS* vol. 14 http://journals.lww.com/aidsonline (2000).
10. Siddiqi, O. K. *et al.* Molecular diagnosis of central nervous system opportunistic infections in HIV-infected zambian adults. *Clinical Infectious Diseases* **58**, 1771–1777 (2014).

11. Rajasingham, R. *et al.* Epidemiology of meningitis in an HIV-infected Ugandan cohort. *American Journal of Tropical Medicine and Hygiene* **92**, 274–279 (2015).

12. Rhein, J. *et al.* Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis* **84**, 268–273 (2016).

13. Ellis, J. *et al.* The Changing Epidemiology of HIV-Associated Adult Meningitis, Uganda 2015-2017. *Open Forum Infect Dis* **6**, 1–8 (2019).

14. Bridge, S. *et al.* Evaluation of the BioFire Ò FilmArray Ò Meningitis / Encephalitis panel in an adult and pediatric Ugandan population. *Journal of Medical Mycology* **31**, 101170 (2021).

15. Bårnes, G. K. *et al.* New molecular tools for meningitis diagnostics in Ethiopia - A necessary step towards improving antimicrobial prescription. *BMC Infect Dis* **18**, 1–14 (2018).

16. Obaro, S. *et al.* Comparison of bacterial culture with BioFire® FilmArray® multiplex PCR screening of archived cerebrospinal fluid specimens from children with suspected bacterial meningitis in Nigeria. *BMC Infect Dis* **23**, (2023).

17. Benjamin, L. A. *et al.* Detection of herpes viruses in the cerebrospinal fluid of adults with suspected viral meningitis in Malawi. *Infection* **41**, 27–31 (2013).

18. Leber, A. *et al.* Multi-Center Clinical Evaluation of a Multiplex Meningitis / Encephalitis PCR Panel for Simultaneous Detection of Bacteria, Yeast, and Viruses in Cerebrospinal Fluid Specimens. *J Clin Microbiol* **54**, 2251–2261 (2016).

19. Payne, A. *et al.* of gigabase-sized genomes. *Nat Biotechnol* **39**, (2021).

20. Breitwieser, F. P., Baker, D. N. & Salzberg, S. L. KrakenUniq: Confident and fast metagenomics classification using unique k-mer counts. *Genome Biol* **19**, (2018).

21. Li, H. Sequence analysis Minimap2 : pairwise alignment for nucleotide sequences. **34**, 3094–3100 (2018).

22. Harris, P. A. *et al.* The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* **95**, 103208 (2019).

23. Marais, S. *et al.* Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis* **10**, 803–812 (2010).

24. Michael, B. *et al.* Effect of delayed lumbar punctures on the diagnosis of acute bacterial meningitis in adults. *Emergency Medicine Journal* **27**, 433–438 (2010).

25. Siddiqi, O. K. *et al.* Prospective cohort study on performance of cerebrospinal fluid (CSF) Xpert MTB/RIF, CSF lipoarabinomannan (LAM) lateral flow assay (LFA), and urine LAM LFA for diagnosis of tuberculous meningitis in Zambia. *J Clin Microbiol* **57**, (2019).

Chapter VII – Conclusion

Introduction

This thesis presents detailed epidemiological data on central nervous system infections in high HIV-prevalence African settings and addresses knowledge gaps surrounding the aetiology of CNSI and the impact of improved CNSI diagnostics in routine care in high HIV-prevalence African settings. This chapter recaps and summarises the key findings from this thesis mapping results to prespecified objectives and outlines key conclusions and their wider implications, and potential areas for future research arising from this work.

Summary of key findings

Objective 1 *– Define the current epidemiology of central nervous system infections in high HIV-prevalence African settings including the impact of an enhanced diagnostic package on diagnostic yield and the proportion of patients with inflammatory CSF suggestive of CNSI but no diagnosis.*

This objective was addressed using prospective data from two consecutive CNSI cohorts in Chapter VI and through analyses of national meningitis surveillance data in Chapter IV.

The introduction of enhanced diagnostics into routine care in two low-resource, high HIV-prevalence Southern African countries detected large amounts of previously undiagnosed TB meningitis and viral CNSI. The prevalence of tuberculous meningitis was 6.8%, greater than all bacterial pathogens combined, and viruses were detected in 7.3% of adults. Furthermore, the number of confirmed bacterial meningitis cases tripled with the addition of FilmArray-ME and *Toxoplasma gondii* was detected in the CSF of 4.9% of HIV-positive adults. The proportion of patients with a diagnosis

increased from 17.1% with routine diagnostics alone to 33.1% with the addition of enhanced diagnostics and the proportion of patients with inflammatory CSF suggestive of CNSI but no diagnosis decreased from 69.1% to 35.3% with the addition of enhanced diagnostics.

Cryptococcal meningitis remained the most common cause of CNSI in both Botswana and Harare in the prospective data collected as part of BNMS and HarMenAeS. This is consistent with national meningitis surveillance data that demonstrated that the incidence of cryptococcal meningitis in Botswana in 2022 remained high at 7.4 cases/100,000 person-years despite excellent ART coverage. Prospective data from Botswana and Zimbabwe described in Chapter VI demonstrated high TB meningitis prevalence particularly in pleocytic CSF samples following the introduction of Xpert MTB/RIF testing on all CSF samples. This contrasts with nationwide data from Botswana which highlighted that TB meningitis was infrequently diagnosed in routine care. In 2022 outside of the capital Gaborone there were a total of 8 diagnoses of TB meningitis made in Botswana from 201 samples analysed with TB-specific tests, highlighting the potential for upscaling of CSF TB testing in Botswana.

Objective 2 – *Determine the increase in diagnostic yield of CNSI due to the addition of BioFIRE FilmArray-ME to routine diagnostics in the diagnosis of CNSI in high HIV-prevalence African settings*

This was addressed by performing an individual patient level data systematic review and meta-analysis of all available FilmArray-ME data in the WHO African region, from study sites in Botswana, Ethiopia, Uganda and Zimbabwe. The use of FilmArray-ME provided a microbiological diagnosis for an additional 178 (7.9%) patients that would have otherwise not received a diagnosis. There were an additional 160 viruses, 57 bacteria and 10 fungi detected using FilmArray-ME that were not diagnosed through routine investigations. The proportion of patients receiving a microbiologically-confirmed diagnosis increased from 18.5% with conventional diagnostics alone to 26.4% with the addition of

FilmArray-ME. However, over half of patients with a CSF pleocytosis did not receive a diagnosis suggesting in high HIV-prevalence areas FilmArray-ME should not be used in isolation. This is likely because FilmArray-ME does not include a number of regionally-important CNS pathogens, such as TB and *Toxoplasma gondii*, and so ideally should be used in combination with platforms that test for these, such as Xpert MTB/RIF or monoplex PCR for *T. gondii*.

Objective 3 – *Describe changes in the detection of the most common CNSIs in Southern Africa, cryptococcal and tuberculous meningitis, over time following expansion of CNSI diagnostic capacity in routine care (CrAg and Xpert MTB/RIF Ultra) and increased ART coverage in Botswana.*

National meningitis surveillance data were extracted in collaboration with the Botswana Ministry of Health and Wellness to address this objective. Using laboratory data between 2015 and 2022 there were 1,744 episodes of cryptococcal meningitis identified. The incidence of cryptococcal meningitis declined from 15.0 (95% CI 13.4-16.7) cases/100,000 person-years in 2015 to 7.4 (95% CI 6.4-8.6) cases/100,000 person-years in 2022 with increasing national ART coverage. Although it was not possible to make a causal link between ART coverage and cryptococcal meningitis incidence linear regression modelling demonstrated that for every 5% increase in ART coverage there was a decrease in cryptococcal meningitis incidence of 2.5 cases/100,000 person-years. Importantly, there was a major shift in the way cryptococcal meningitis was diagnosed during this period. In 2015 only 35.5% of cases were diagnosed with the cheap and easy-to-use cryptococcal antigen test and this increased to 86.3% in 2022 highlighting the potential to use cryptococcal meningitis as an accessible metric to monitor HIV programmatic success.

The same national meningitis surveillance data, excluding Princess Marina Hospital, Gaborone, were used to describe the impact of increased availability of decentralised Xpert MTB/RIF Ultra capacity. Although there were some trends suggesting TB-specific testing was increasing with 4.5% of CSF

samples tested in 2016 and 29.0% testing in 2022 the rates of investigation remain comparatively low despite Xpert MTB/RIF Ultra being available at all major hospitals in Botswana. Between 2016-2022 13.9% of CSF samples were tested with Xpert MTB/RIF Ultra compared to 63.9% of the same samples being tested with CrAg demonstrating the potential to expand diagnostic coverage if there was a cheaper and easier to use platform available. These limitations in access and use of Xpert MTB/RIF alongside a modest sensitivity when compared to CrAg testing precluded a similar analysis to determine national TBM incidence estimates as was performed for cryptococcal meningitis.

Objective 4 – *Determine how computed tomography is used in routine clinical practice in high HIV-prevalence settings and characterise the effect of computed tomography performed prior to lumbar puncture on patients with suspected CNSI on delay to diagnostic lumbar puncture and treatment initiation.*

Detailed prospective clinical, treatment and outcome data from 711 adults included in the Botswana National Meningitis Survey: Protocol 2 were used to describe the use of CT and determine the impact of CT on time to LP, time to treatment initiation, and in-hospital mortality. These data showed adherence to local consensus guidelines was poor with over half of those without imaging having a guideline indication for CT. CT before LP was associated with significant delays to diagnostic LP and initiation of appropriate CNSI treatment. We did not demonstrate a direct association between imaging and mortality but this may be potentially due to a number of limitations with our data namely that those patients that received imaging was not random but based on the request of the treating clinician and also significant confounding by indication.

Implications of findings

These results reiterate that the diagnostic yield of conventional diagnostics in patients with suspected CNSI in low-resource, high HIV-prevalence settings remains poor. Excluding diagnoses made with cryptococcal antigen testing only 2.5% of patients received a diagnosis through conventional diagnostics alone. Enhanced diagnostics significantly increased the number of diagnoses made in patients with suspected CNSI. However, it is important to recognise that not all detections had a direct impact on clinical management and understanding how enhanced diagnostics influenced clinician decision-making is crucial to understand whether large scale investment in these platforms in resource-constrained settings is appropriate.

Through the introduction of FilmArray-ME the number of patients with confirmed bacterial CNSI was approximately tripled. However, 84% of patients with confirmed bacterial CNSI were already receiving appropriate antibiotic therapy prior to the release of FilmArray-ME results and therefore the results did not change immediate management, although whether the planned duration of antibiotics was extended following FilmArray-ME results is not known. FilmArray-ME allowed the rapid detection of viruses in routine care but not all viruses included on the FilmArray-ME panel are of direct clinical relevance. Despite a limited evidence base for their use most clinicians would consider it appropriate to treat patients with antivirals following the detection of HSV-1, HSV-2 and VZV in CSF, particularly in the context of immune suppression. Therefore, these detections are relevant and directly influence clinical management. The detection of enterovirus and parechovirus should usually prompt cessation of antibiotics and so should also be considered a clinically relevant result that impacts patient management. The detection of the two other viruses found on the FilmArray-ME panel, CMV and HHV-6, are of less certain clinical relevance and would not change clinical management in the majority of cases. CMV and HHV-6 made up 15.1% (93/616) of all FilmArray-ME detections in the IPD meta-analysis in Chapter III and 9.8% (31/316) of all diagnoses in BNMS and HarMenAeS in Chapter VI. The vast majority of these detections would not have directly influenced clinical management as they do not usually represent acute infection. The clinical significance of their detection and the contribution

of CMV and HHV-6 to disease in this population is therefore unclear. Furthermore, negative FilmArray-ME results did not always lead to cessation of antiviral therapy despite a lack of indication. Antiviral therapy was continued despite a negative FilmArray-ME result in 62% of patients who received antivirals in BNMS/HarMenAeS. These data suggest that additional education regarding the interpretation of FilmArray-ME results is needed to optimise antimicrobial stewardship efforts. As such, whilst FilmArray-ME increased the number of patients with a confirmed microbiological diagnosis the true clinical impact of FilmArray-ME does not mirror the absolute number of detections of CNS pathogens in CSF and this should be considered during subsequent health economic and implementation analyses.

Conversely the impact of Xpert MTB/RIF was far more clearly defined. The prevalence of TBM was very high in patients with inflammatory CSF, 19% in those with a CSF pleocytosis or abnormal CSF biochemistry and rising with increased CSF white cell count to 30% in those with CSF white cell counts over 100 cells/mm^3. These results contrast with national data from Botswana presented in Chapter IV showing comparatively low rates of TB diagnosis made in routine care. Amongst patients with a positive Xpert MTB/RIF result from CSF analysis 39 patients received anti-tuberculous treatment and in 62% of these patients treatment initiation was triggered by a positive CSF Xpert MTB/RIF result. Over half of patients with a positive Xpert MTB/RIF died in hospital in the prospective cohorts despite prompt universal TB testing being performed on all CSF samples from patients with suspected CNSI. Mortality data is not available for the national data however outside of BNMS/HarMenAeS trial settings where TB testing is only performed upon clinician request, diagnosis and appropriate treatment is likely to have been significantly more delayed inevitably leading to increased mortality. These data suggest that the introduction of TB testing into routine care would increase the number of TB cases diagnosed. Alternatively, TB testing could be performed only on samples with a negative CSF CrAg test. This would likely represent a more clinically- and cost-effective approach given the high burden of cryptococcal meningitis and low prevalence of cryptococcal and tuberculous co-infection.

In resource-limited settings with high TBM prevalence Xpert MTB/RIF likely represents the most practical investigation for suspected TBM as the turnaround time of mycobacterial culture is too slow to influence management and microscopy techniques are usually very insensitive. However, even when universal testing was performed in BNMS/HarMenAeS additional interventions are still needed to reduce the very mortality. One approach could be that given the high TB prevalence and that TB treatment was often only initiated after the confirmation of TBM diagnosis early, empiric anti-tuberculous treatment should be considered alongside or even in place of antibiotic therapy whilst a diagnosis is sought. Furthermore, the imperfect sensitivity of Xpert MTB/RIF likely missed a significant proportion of TBM cases with an additional 15 patients having probable TBM based on a uniform case definition highlighting the need for additional TBM diagnostics that can reliable diagnose TBM and reiterating the low threshold clinicians should have to start anti-tuberculous treatment.

Toxoplasma gondii PCR and syphilis testing were performed retrospectively and therefore their impact on clinical decision-making was not assessed. Amongst those tested 5% of patients had *T. gondii* detected in CSF but very few patients were treated empirically suggesting Toxoplasmic encephalitis was rarely considered in differential diagnoses despite these data suggesting a relatively high prevalence. In-hospital mortality was low in patients with a positive *T.gondii* PCR despite the majority not receiving targeted treatment. However, mortality data beyond hospital discharge is lacking and therefore the impact of *T.gondii* CSF detection on longer term prognosis is not understood. Given the high prevalence improving access to *T.gondii* testing or integrating it as an analyte on multiplex platforms for use on CSF would allow a greater epidemiological understanding of the burden and significance of *T.gondii* CNSI.

Despite the limitations described enhanced diagnostics nearly doubled the number of patients with a potential CNS pathogen detected on CSF and in a large proportion of patients this will have led to improvements in management. There is also an argument for their use beyond the individual patient.

Epidemiological data on CNSI in Southern Africa remains limited and continued surveillance of CNSI epidemiology with robust diagnostics is necessary to monitor for progress in vaccination strategies and allocation of limited resources. They will also serve as a platform for clinician and microbiological scientist development. Clinicians and scientists often learn experientially through the confirmation or exclusion of diagnoses from results of investigations. For clinicians managing complex patients in the face of constant diagnostic uncertainty is challenging and often disheartening. Whilst these data suggest education in interpretation of results could be improved, the increase in confirmation of suspected diagnoses will have reinforced diagnostic skills amongst doctors and microbiological scientists. Ultimately, establishing whether implementing a package of enhanced diagnostics in Southern Africa is feasible will come down to cost however these data show that is sufficient funds are allocated then adoption of these platforms would improve diagnostics approaches.

Computed tomography prior to LP was associated with an increased time to diagnostic LP and initiation of appropriate treatment. Local guidelines on when to perform a CT were poorly adhered to and international guidelines from low HIV-prevalence settings would not be practical to implement. An association with CT prior to LP and mortality was not demonstrated but there was no increase in mortality amongst patients with an indication for CT based on local guidelines who did not have a CT prior to LP. These data suggest that CT had a limited role in the investigation of patients with suspected CNSI. Most hospitals in Southern Africa do not have a CT meaning patients often need to be transferred to referral hospitals adding further costs and delays to management. These data whilst having significant limitations may be able to contribute to more regionally- and context-specific guidelines on when to perform CT prior to LP that would aid clinician decision-making in low-resource, high HIV-prevalence settings.

Although these data show a clear improvement in the diagnosis of CNSI following expansion of diagnostic capacity it is important to recognise that additional considerations will be needed to

improve outcomes in patients with CNSI in resource-limited, high HIV-prevalence settings. The failure to accurately and rapidly diagnose patients with CNSI is not solely the result of limited diagnostic capability and whilst outside the scope of this thesis additional factors both in diagnostic pathway and in the management of patients need to be addressed to improve diagnosis and ultimately outcomes. Delayed presentation to hospital can occur due to poor transport infrastructure, financial constraints, societal pressures requiring senior family pressures to guide individual healthcare decisions or limited health literacy and subsequent failure to seek timely medical advice. All these need tailored interventions to improve access to healthcare beyond improvement in diagnostics. Furthermore, improvements in diagnostics need to be performed in conjunction with improved access to treatment. Whilst molecular diagnostics allowed the detection of organisms that would not have been detected with conventional diagnostics alone appropriate treatments, such as intravenous antivirals, were not always readily available. In addition, healthcare workers were often unfamiliar with these treatment options would therefore need appropriate education to allow prompt intervention as a result of improved diagnostics. In addition, supportive care in hospitals in Botswana and Zimbabwe is very limited. Basic nursing needs are often not fully met due to staffing constraints and capacity in higher dependency wards for deteriorating patients is extremely limited. Therefore to have a meaningful and sustainable impact on outcomes of CNSI all of these factors will need to be addressed.

Implications for future research

Whilst these data demonstrate the benefit of introducing enhanced diagnostics into the investigation of CNSI in high HIV-prevalence settings, identifying how best to integrate them into care will require robust health economic and implementation science data to support widespread uptake of these platforms.

The optimal clinical choice for implementation would be to have widespread access to molecular platforms however this is unlikely to be feasible due to financial constraints. Therefore, modelling other options will be needed to guide use in routine care. These options for implementation could include only using molecular diagnostics on a subset of patients with other diagnoses excluded or employing them as part of a surveillance network at sentinel sites to provide epidemiological data to guide empiric treatment elsewhere.

Molecular diagnostic platforms are expensive, but their introduction may have cost benefits elsewhere that may make their implementation more economically viable and identifying these may facilitate their introduction. Empiric antimicrobial use, including expensive antivirals, was relatively common in BNMS/HarMenAeS. It may be possible to offset the cost of consumables required for diagnosis against reduced antimicrobial use when negative PCR results are available and analyses from high-resource settings have supported this approach. Data from Ethiopia demonstrated a reduction in the use of antimicrobials with the implementation of BioFIRE although robust health economic data were not presented. However, in BNMS and HarMenAeS even after introduction of FilmArray-ME 62% of patients who received antivirals did not have a virus detected on CSF, suggesting additional education regarding indications for antivirals would be needed before implementing this approach. Another approach to reduce cost of implementation may be replacing components of conventional CSF analysis with FilmArray-ME. CSF culture requires significant laboratory infrastructure and technical skill for a comparatively low diagnostic yield and could potentially be replaced with a multiplex PCR such as FilmArray-ME. If FilmArray-ME was performed in place of CSF culture in BNMS/HarMenAeS then 9 cases of bacterial meningitis would have been missed but 33 more cases of bacterial CNSI would have been diagnosed compared to conventional diagnostics alone. Amongst the patients with culture positive bacterial CNSI that would not have been detected on FilmArray-ME 7/9 were on empiric antibiotics prior to CSF analysis and so this intervention would not have altered their management. Bacterial susceptibility data would have been captured and this may be an important

limitation to this approach. Given the variations in diagnostic yield between populations demonstrated in the prospective cohorts perhaps the most clinically- and cost-effective approach would be to direct interventions at higher yield groups through the use of a targeted diagnostic algorithm. In this case cheaper, more-accessible tests for common CNSI in the region such as CrAg for cryptococcal meningitis and Xpert RIF/MTB for TBM would be used to exclude these conditions before more expensive tests such as FilmArray-ME were introduced. Whilst this may increase the diagnostic yield and decrease cost of implementation our cohort had a number of co-detections that would have been missed if this approach had been applied. Data generated through the two prospective cohorts alongside local healthcare costing data could be used to model the associated costs, cost-consequences and cost-effectiveness of varied implementation strategies. These data could be presented to key policymakers to demonstrate the nominal costs of implementing diagnostic strategies for CNSI in high HIV-prevalence settings. This health economic analysis is planned to start using data generated from BNMS/HarMenAeS and existing costing data from other meningitis studies in Central/Southern Africa.

Robust implementation science data is also essential to support health economic data. This work is currently underway and uses a composite of data sources including quantitative data already collected such as sample turnaround time and impact on clinical decision-making and qualitative data from interviews with laboratory staff, clinicians and other relevant stakeholders to define the barriers and facilitators to more widespread adoption. Interviews, tailored to individual disciplines, are being used to learn from experiences of those who worked with molecular diagnostics in routine clinical practice and also to gain a greater understanding of how healthcare staff used and interpreted the data generated by these platforms. For example, whilst most doctors place sufficient trust in a positive result on BioFIRE FilmArray-ME to alter clinical management the majority interviewed do not feel that they are able to reliably exclude a diagnosis with a negative result. This is evidenced by the BNMS and HarMenAeS data where there were persistent failures to stop antiviral treatment with negative

BioFIRE FilmArray-ME results. These insights into how clinicians perceive new diagnostics and therefore the potential training needs required in conjunction with implementation are crucial to maximise the impact of the platforms. Qualitative interviews have been performed with doctors, laboratory staff and managers at both sites and transcription of these data and subsequent analysis of these data is due to begin.

Current practice in routine care in the majority of Southern Africa is often syndromic management of CNSI based on a number of clinical and laboratory parameters. This approach is necessitated by a lack of available resources to accurately diagnose CNSI. If the costs of enhanced diagnostics were considered too high for widespread implementation comparison of diagnostic-driven approaches compared to syndromic-based management may be warranted. Learning from the experiences of syndromic-based management used in the treatment of sexually-transmitted infections would be crucial. In particular, establishing surveillance systems to monitor for emerging antimicrobial resistance would be essential to prevent the development of resistant strains that would be extremely challenging to treat in settings where access to basic antimicrobials is often limited.

This work was primarily focussed on enhancing laboratory analysis of CSF to improve the diagnosis of CNSI. Other microbiological samples, including blood cultures or sputum, can also support the diagnosis of CNSI. There is significant overlap between conventional techniques used in analysis of CSF and non-CSF samples and therefore the same limitations apply to analysis of all sample types with diagnostic yield from any culture being limited and highly operator dependent, and phenotypic identification of organisms often being inaccurate. Similarly, the expansion of molecular diagnostic capacity for non-CSF samples would likely have comparable benefits to those seen following the introduction of enhanced diagnostics in BNMS and HarMenAeS. Molecular platforms exist for use on non-CSF samples such as BioFIRE Blood Culture Identification Panel or automated platforms, such as VITEK 2, that can simultaneously provide information on identification and sensitivity of pathogens

cultured from any sample are rarely available in resource-limited settings. These platforms can be used on a range of sample types, but blood would likely be the most suitable and data from high resource-settings has demonstrated the relatively high diagnostic yield of blood cultures in CNSI. Blood culture samples are typically collected more promptly than CSF culture and therefore less likely to be affected by prior antimicrobial administration. Whilst this approach may afford increased sensitivity not all positive blood cultures in patients with a syndrome consistent with CNSI will be causative and this will need to be correlated clinically. However, the main advantage of using these platforms above CSF-specific tests is that if the platforms can be used on both CSF and non-CSF samples to provide a diagnosis for CNSI they represent a more cost-effective approach than targeted CSF testing. Studies evaluating the impact of molecular platforms on non-CSF samples to improve CNSI diagnosis could provide valuable insights in to whether these platforms should be prioritised for integration into routine care above targeted CSF analysis.

Although molecular diagnostic platforms can effectively diagnose a broad range of pathogens they have some important limitations. They are costly, need a reliable electricity supply and often require significant laboratory experience. The development of an affordable, true point of care, rapid diagnostic test ideally able to detect multiple potential CNS pathogens simultaneously is urgently needed to have a greater impact in low-resource settings. Additional analyses of bio-banked samples containing known pathogens is crucial to facilitate research into rapid diagnostic tests. Samples collected from the Botswana National Meningitis Survey and Harare Meningitis Aetiology Study have been stored and could be used to contribute towards development as a part of a wider collaboration.

Conclusions

Despite extensive ART coverage in high HIV-prevalence Southern Africa there remains a significant burden of CNSI predominantly affecting people with HIV that are challenging to diagnose and carry

significant mortality. These data clearly demonstrate that platforms are available to improve the diagnosis of CNSI in high HIV-prevalence settings. In particular, integration of TB testing into routine care was a highly effective intervention revealing a significant prevalence of microbiologically-confirmed TB meningitis in the region that has not been previously described. Based on these data, considerations should be made to integrate TB testing into routine investigation of CSF in high HIV-prevalence settings and advocating for early, empiric treatment of suspected TBM when diagnostics are not available.

Nationwide surveillance of cryptococcal meningitis as a metric to identify the burden of advanced HIV disease in Botswana identified a hard-to-reach population of working-age males with advanced HIV disease, a population that will need novel strategies to ensure optimal engagement with HIV care cascades. These data further highlight the importance of ongoing development of CNSI diagnostics that have the potential to generate robust surveillance data that can have benefit beyond those patients undergoing investigation for CNSI.

Despite the increase in patients with a confirmed diagnosis resulting from the introduction of enhanced diagnostic platforms there remains a significant proportion of patients with presumed CNSI without a diagnosis. There is an ongoing need for further development and implementation of novel diagnostic platforms in high HIV-prevalence settings to reduce mortality from CNSI.

Appendix I – Protocol Botswana National Meningitis Survey

Study Protocol

Study: The Botswana National Meningitis Survey. Protocol 2: Prospective Enhanced Meningitis Surveillance

Version: Study Protocol V5.2 (11th March 2022)

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Summary:
The Botswana National Meningitis Survey (Protocol 2) aims to establish a prospective surveillance system in Botswana to capturing laboratory data from all cerebrospinal fluid specimens examined in public sector microbiology laboratories in Botswana. An enhanced meningitis surveillance network will be established at sentinel sites in Botswana with enhanced molecular diagnostic testing, plus pathogen detection and deep sequencing studies, coupled with clinical outcome data, to definitively determine the aetiology and outcomes of meningitis in Botswana. These data will be used to monitor the incidence of meningitis, assess the impact of interventions such as expanded ART roll out with the "test and treat" strategy on cryptococcal meningitis incidence, and determine the effectiveness of vaccination programmes (e.g. pneumococcal and HiB vaccination). The network will also serve as a platform for laboratory validation studies of affordable and practical novel meningitis diagnostics for use in Botswana with the potential for near patient or point-of-care use.

1. Aim

To establish an enhanced prospective meningitis surveillance network in Botswana (a) providing detailed epidemiological and aetiological information to guide clinicians in meningitis management, (b) providing public health specialists with an invaluable tool to assess the efficacy of public health interventions aimed at reducing meningitis incidence and guide effective resource allocation, and (c) creating a uniquely powerful platform to validate novel diagnostic assays with the potential to transform meningitis diagnosis in Botswana.

2. Objectives

1. To establish a nationwide meningitis surveillance system capturing laboratory data from *all* cerebrospinal fluid specimens examined in public sector microbiology laboratories in Botswana.
2. To establish an *enhanced meningitis surveillance network* at sentinel sites in Botswana with enhanced molecular diagnostic testing, plus pathogen detection and deep sequencing studies, coupled with clinical outcome data, to definitively determine the aetiology and outcomes of meningitis in Botswana.
3. To perform laboratory validation studies of affordable and practical novel meningitis diagnostics with the potential for near patient or point-of-care use.

3. Background

The Botswana National Health Laboratory (NHL) is expanding its current remit to become a National Public Health Laboratory. A key component of this new mandate is to perform national disease surveillance. Such surveillance is essential for rational health care planning and to guide appropriate allocation of human and material resources. Effective infectious disease surveillance also enables rapid recognition and response to outbreaks and epidemics, allows emergence of drug resistance to be detected and contained, and provides real-time data to assess the effectiveness of national infectious disease control and treatment programmes.

Meningitis is one of the most severe infectious diseases, associated with very high mortality rates and serious sequelae[1-3]. Treatment is complex, often requiring prolonged hospital stays, intensive supportive treatments, and intravenous antimicrobial agents, placing a large burden on health care services. With the advent of the HIV epidemic in southern Africa meningitis has become one of the most common opportunistic infections in HIV-infected individuals, and a leading cause of death in HIV-infected cohorts, accounting for up to 30% of all mortality[4,5]. The aetiology of meningitis has also changed markedly with the HIV-epidemic. Cryptococcal meningitis accounts for the majority of laboratory confirmed cases, the proportion of bacterial meningitis due to *Streptococcus pneumoniae* has increased, and the incidence of TB meningitis has risen [3,6]. Mortality rates from all types of meningitis in the context of HIV co-infection are high, with acute mortality rates approaching 50% in cryptococcal, pneumococcal, and TB-meningitis in the southern African region[1-3].

This unacceptably high mortality is in large part due to delays in diagnosis and the initiation of effective treatment. One of the key reasons for these delays is the inadequacy of current diagnostic tests. With standard diagnostics between 50-70% of meningitis cases do not receive a definitive microbiological diagnosis, making effective management very difficult[6,7]. And in the absence of robust epidemiological data about meningitis aetiology, evidence-based decisions regarding empiric treatment are impossible.

We have recently undertaken a 15-year nationwide retrospective meningitis surveillance study (The Botswana National Meningitis Survey, protocol 1 ETHICS). Data from almost 30,000 cerebrospinal fluid (CSF) samples have been collected, and are currently being analysed. Preliminary data shows that cryptococcal meningitis remains the most frequently diagnosed cause of adult meningitis in Botswana; and in keeping with prior studies, approximately 30-50% of patients with laboratory evidence of meningitis (on the basis of raised white cell counts and abnormal protein and glucose levels) do not receive a confirmed microbiological diagnosis.

Following on from this, in collaboration with the NHL, we are planning to establish a prospective meningitis surveillance network in Botswana. Utilising the data collection systems put in place during the retrospective study we will collate all CSF study results processed in the public sector laboratory network. These data will be used to monitor the incidence of meningitis, assess the impact of interventions such as expanded ART roll out with the "test and treat" strategy on cryptococcal meningitis incidence, and determine the effectiveness of vaccination programmes (e.g. pneumococcal and HiB vaccination). In additional, at sentinel sites we plan to put in place an enhanced surveillance system, incorporating in-country detailed molecular testing to ascertain meningitis aetiology in culture negative cases. Sample bio banking will enable detailed pathogen discovery testing in cases with no confirmed diagnosis to provide a comprehensive description of the aetiology of meningitis in this setting, and will also enable validation studies of novel diagnostic technologies aimed at improving meningitis diagnosis in Botswana.

Overall, the enhanced prospective surveillance network will provide detailed epidemiological and aetiological information to guide clinicians in meningitis management; provide public health specialists with an invaluable tool to assess the efficacy of public health interventions aimed at reducing meningitis incidence and guide effective resource allocation; and create a uniquely powerful platform to validate novel diagnostic assays with the potential to transform meningitis diagnosis in Botswana.

4. Methods

Nationwide meningitis surveillance: There are currently 30 public sector laboratories, operating under NHL supervision, that process all CSF samples in Botswana (aside from small numbers processed in three private laboratories – see appendix 1). Since early 2015, all of the NHL laboratories have entered data directly into the electronic medical records system, IPMS. A system of querying the IPMS database has been established in the Monitoring and Evaluation (M&E) Department of the Ministry of Health and Wellness (MoHW), enabling central reporting of all CSF data for the retrospective Botswana National Meningitis Survey (BNMS) protocol 1. This reporting system will continue to be utilized for the prospective meningitis surveillance, with weekly collation of meningitis statistics at the M&E department of the MoHW. In addition, sites will be requested to provide a monthly paper return to the NHL listing any CSF samples not captured on IPMS due to internet down-time. Data collected will be the same as that collected for the BNMS protocol 1, and is listed in appendix 2. To comply with the Ministry of Health (MoHW) Data Management Policy 2014, any personal identifying variables from IPMS will not be removed from Ministry premises for analysis. They will be used only to facilitate linkage of records. These personal identifiers will be removed and all data fully anonymised prior to the database being permitted to leave the MoHW for further analysis. As with the retrospective BNMS protocol 1, we will triangulate IPMS data with the death registry at the Department of Civil and National Registration, Ministry of Labour and Home Affairs, to determine mortality rates.

Enhanced prospective surveillance: The enhanced prospective surveillance is planned for seven sites (the two tertiary referral centres, and five secondary level hospitals, see appendix 1) that between them process over 80% of all CSF samples in Botswana. The enhanced surveillance will consist of four elements.

1. *Extended real-time molecular diagnostics.* A panel of enhanced diagnostics will be put in place for all CSF samples processed at the sentinel sites, consisting of cryptococcal antigen screening, TB gene-Xpert and culture, PCR for common bacteria and viruses and a TB-LAM assay (Fujifilm SILVAMP TB-LAM). These will all be standard diagnostic tests approved for use on CSF. Where possible, these will be performed on site, but due to resource limitations, certain tests such as TB culture and PCR will be performed centrally at the NHL in Gaborone. Results will be reported to the clinicians providing care to the patients. The full panel of tests is dependent on receipt of a sufficient volume of CSF, thus all tests may not be performed an all patients. In such cases, the routine tests mandated by the NHL Standard Operating Procedures will be prioritized; the patient will also be approached to seek consent to perform an additional lumbar puncture if a diagnosis has not been made through routine investigation. The utility of the available tests is dependent on sufficient volumes of CSF. In particular, the sensitivity of TB gene-Xpert has been shown to increase with larger volumes of CSF[8,9]. As such the additional CSF collected will be used both to perform additional tests described below as well as increasing the potential diagnostic yield of those investigations available locally that may directly inform decision-making surrounding patient care. CSF samples and bacterial and fungal isolates will be archived for repeat testing if required and quality assurance monitoring. To fully maximise the clinical utility of the multiplex PCR we will collect blood samples from the small number of patients with HHV-6 detected in the CSF to exclude the condition of chromosomally integrated HHV-6. From the previous BioFIRE analyses we detected HHV-6 in 13 patients and would anticipate similar numbers.

   To help support the longer-term use of these diagnostics in Botswana we will also collect qualitative data through in-depth interviews or surveys surrounding meningitis diagnostics from key stakeholders, including medical doctors, laboratory staff and leadership teams, in the management and healthcare provision of patients with meningitis. We hope this will identify important barriers and facilitators to implementation of improved meningitis diagnostics and provide data to support more widespread use outside of clinical trials.

2. Pathogen detection and deep sequencing analysis. In samples where a definitive diagnosis has not been reached using the above methodology, residual CSF will be archived at -80°C for microarray testing and deep sequencing to attempt to identify the causative organism. These may be known pathogens that are missed by standard molecular testing due to low pathogen burdens or prior antibiotic exposure, or unusual or novel pathogens not usually associated with meningitis. Such analyses can currently only be performed at a very limited number of specialised centres worldwide. Samples will therefore be transported to one of these centres for analysis (either at the University of Pennsylvania, or University College London). It is hoped that over the next few years the capacity to perform these type of analyses will be developed within the region (initially in either South Africa or at a Wellcome Trust funded regional sequencing hub in Malawi), however such capacity is unlikely to be available in Botswana in the near future. To ensure local capacity development, local investigators will be involved in these analyses and trained in the relevant techniques at the specialised centres. Appropriate Material Transfer Agreements will be put in place in all instances where shipment to overseas institutions is required.

3. Novel diagnostic evaluation. Laboratory evaluations of promising novel meningitis diagnostics that have the potential for near-patient or point-of-care use, and are likely to be affordable and practical in

the southern African setting, will be performed using residual CSF collected at the sentinel surveillance sites. These new tests will be evaluated against the current standard diagnostics, and the molecular assays described in elements 1) and 2), using standard laboratory diagnostic evaluation techniques. These analyses will be performed in the laboratory in Gaborone. All patient management decisions will be made on the basis of the currently approved diagnostic tests. Currently, we propose to test two candidate diagnostics with potential to markedly improve meningitis diagnostics in Botswana (see appendix 3). Details of all further tests to be evaluated will be submitted to the IRB as amendments for approval prior to diagnostic evaluation.

4. Clinical outcome assessment. The final element of the enhanced surveillance will be collection of data regarding the clinical management and outcome of meningitis cases. Routinely collected clinical data only will be retrieved from the patient notes to ascertain clinical diagnosis, treatment given, and clinical outcomes of the patients (whether they are discharged home alive or die in the hospital). In addition, where additional details surrounding the clinical presentation are required we would seek consent to consult with the patient to gather any outstanding information. Data will be abstracted by study data capturers and entered into a fully anonymised database, linked to CSF results by unique sample identifiers.

5. Study Population, Sample Size, and Statistical Analysis

The Nationwide meningitis surveillance study will include all patients undergoing lumbar puncture (LP) with CSF analysis at any health facility in Botswana from January 2016 onwards. There are no exclusion criteria. We do not have a pre-selected or known sample size but rather intend to include all patients meeting the above criteria. Based upon historical data, we estimate approximately 2500 CSF samples will be processed annually. The enhanced prospective surveillance study will include all patients undergoing lumbar puncture (LP) with CSF analysis at the seven sentinel surveillance sites. Data will be analysed using simple descriptive statistics as indicated (median and interquartile range, percentile, etc.), and categorised by site and causative organism. For the diagnostic evaluations, anticipating a 95% sensitivity of the novel assays when tested against the gold reference standards (combined culture plus molecular diagnostic), at a
95% significance level with a precision of +/- 2.5%, a sample including 292 confirmed cases would be required for each evaluation. Based on historical data, we expect to achieve these numbers over 2 to 3 years of prospective surveillance.

6. Ethical Issues and Protection of Human Subjects.

Where feasible, this study uses only routinely collected programmatic samples and data and the surveillance activities fall under the standard remit of a National Surveillance Laboratory. However, to maximise the utility of the available investigations larger amounts of CSF than what has been routinely collected may be required. In instances where the volume of CSF collected as part of standard care is insufficient to perform all relevant analysis, patients will be approached to seek consent for additional lumbar punctures. All individual patient data will be de-identified for analysis or publication, and data maintained in a secure, password-protected database only accessible to authorized study personnel. Full confidentiality will be maintained, with no analysis or reporting of identifiable patient data. This study poses minimal risk to research participants.

7. Timeline

Data collection started in January 2016. The surveillance activities are open ended. Continuing approval will be sought annually.

8. Funding

The nationwide surveillance activities are based on routine work performed and funded by the National Health Laboratory and Ministry of Health. The diagnostics study is funded in part through an NIH CFAR pilot award to Dr Jarvis, and through additional discretionary research funds held by Dr Jarvis through the Botswana-UPenn Partnership. Additional funding for ongoing surveillance activities has been obtained through a personal fellowship award to Dr Jarvis and will receive support in kind from the NHL.

References

1. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis* 2014; 58(5): 736-45.
2. Scarborough M, Thwaites GE. The diagnosis and management of acute bacterial meningitis in resource-poor settings. *The Lancet Neurology* 2008; 7(7): 637-48.
3. Thwaites GE, Duc Bang N, Huy Dung N, et al. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with Tuberculous meningitis. *The Journal of infectious diseases* 2005; 192(12): 2134-41.
4. Walker AS, Prendergast AJ, Mugyenyi P, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. *Clin Infect Dis* 2012; 55(12): 1707-18.
5. Lawn S, Harries A, Anglaret X, Myer L, Wood R. Early Mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS (London, England)* 2008; 22(15): 1897-908.
6. 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(1): 67.
7. Bisson GP, Lukes J, Thakur R, Mtoni I, MacGregor RR. Cryptococcus and lymphocytic meningitis in Botswana. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* 2008; 98(9): 724-5.
8. Bahr N, Tugume L, Rajasingham R, et al. Improved Diagnostic Sensitivity for TB Meningitis with Xpert MTB / Rif of centrifuged CSF : a prospective study. *Int J Tuberc Lung Dis*. 2015;19(10):1209-1215. doi:10.5588/ijtld.15.0253.
9. Bahr NC, Nuwagira E, Evans EE, 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. doi:10.1016/S1473-3099(17)30474-7

Appendix II – Protocol Harare Meningitis Aetiology Study

Study: Harare Meningitis Aetiology Study

Version: Study protocol v1.8

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Ms Kathryn Boyd, Laboratory scientist. University of Zimbabwe and London School of Hygiene and Tropical Medicine.
Dr James Milburn, Clinical Research Fellow. Botswana Harvard AIDS Institute Partnership and London School of Hygiene and Tropical Medicine.

Summary:
The Harare Meningitis Aetiology Study aims to establish a prospective meningitis surveillance system at Parirenyatwa Hospital capturing clinical and laboratory data from routinely-collected cerebrospinal fluid specimens examined in microbiology laboratories in addition to data from enhanced diagnostics performed on a smaller subset of samples. An enhanced meningitis surveillance system will be established in the Internal Medicine Unit, UZ-Faculty of Medicine & Health Sciences consisting of enhanced molecular diagnostic testing, plus pathogen detection and deep-sequencing studies coupled with clinical outcome data, to definitively determine the aetiology and outcomes of meningitis in the region. These data will be used to monitor the incidence of meningitis, assess the impact of interventions such as expanded ART roll out with the "test and treat" strategy on cryptococcal meningitis incidence, and determine the effectiveness of vaccination programmes (e.g. pneumococcal and HiB vaccination). The surveillance system will also serve as a platform for laboratory validation studies of affordable and practical novel meningitis diagnostics for use in Zimbabwe with the potential for near patient or point-of-care use.

1. Aim

To establish an enhanced prospective meningitis surveillance system at a large referral hospital in Harare (a) providing detailed epidemiological and aetiological information to guide clinicians in meningitis management, (b) providing public health specialists with an invaluable tool to assess the efficacy of public health interventions aimed at reducing meningitis incidence

and guide effective resource allocation, and (c) creating a platform to validate novel diagnostic assays with the potential to transform meningitis diagnosis in Zimbabwe.

2. Objectives

1. To establish a *meningitis surveillance system* capturing routinely-collected clinical data from adult patients with suspected central nervous system (CNS) infections and laboratory data from *all* cerebrospinal fluid specimens from adult patients examined in the public sector microbiology laboratories at Parirenyatwa Hospital.
2. To create an *enhanced meningitis surveillance system* in the Internal Medicine Unit at Parirenyatwa Hospital through the development of an enhanced diagnostic algorithm for CSF analysis in those patients with suspected CNS infection. Through a combination of routine investigation, enhanced molecular diagnostic testing, plus pathogen detection and deep sequencing studies, coupled with clinical outcome data, we aim to definitively determine the aetiology and outcomes of meningitis in the region.
3. To perform laboratory validation studies of affordable and practical novel meningitis diagnostics with the potential for near patient or point-of-care use.

3. Background

Meningitis is one of the most severe infectious diseases, associated with very high mortality rates and serious sequelae[1–3]. Treatment is complex, often requiring prolonged hospital stays, intensive supportive treatments, and intravenous antimicrobial agents, placing a large burden on health care services. With the advent of the HIV epidemic in southern Africa meningitis has become one of the most common opportunistic infections in HIV-infected individuals, and a leading cause of death in HIV-infected cohorts, accounting for up to 30% of all mortality[4,5]. The aetiology of meningitis has also changed markedly with the HIV-epidemic. Cryptococcal meningitis accounts for the majority of laboratory confirmed cases, the proportion of bacterial meningitis due to *Streptococcus pneumoniae* has increased, and the incidence of TB meningitis has risen[3,6,7]. Mortality rates from all types of meningitis in the context of HIV co-infection are high, with acute mortality rates approaching 50% in cryptococcal, pneumococcal, and TB-meningitis in the southern African region[1–3].

This unacceptably high mortality is in large part due to delays in diagnosis and the initiation of effective treatment. One of the main reasons for these delays is the inadequacy of current diagnostic tests. A key finding from a national retrospective surveillance study conducted in neighbouring Botswana from 2000-2014, was that of 8759 patients with abnormal cerebrospinal fluid (CSF) findings, 3750 (43%) did not have any microbiological diagnosis on standard laboratory testing[8]. Unpublished data from a study of 1355 CSF samples completed in 2019 also from Botswana demonstrated a similar pattern of diagnostic uncertainty. 283 patients had a CSF pleocytosis however 110 of these patients with a CSF pleocytosis did not have confirmed microbiological diagnosis despite multiplex PCR for common CNS pathogens being performed on 743 of these samples and Xpert® MTB/Rif Ultra on 165 alongside routine CSF analysis. The absence of a definitive microbiological diagnosis makes effective

management of this patient group very difficult[6,9]. In the absence of robust epidemiological data about meningitis aetiology, evidence-based decisions regarding empiric treatment are impossible. These challenges in patient management are reflected in the mortality rates of these individuals without a confirmed microbiological diagnosis which is over 40% at 10 weeks indicating the presence of serious underlying pathology which needs appropriate diagnosis and treatment to improve outcomes[8].

In both Botswana studies described above, most of the patients with cellular CSF had a lymphocytic predominance and we strongly suspect that the majority of these were due to TB meningitis (TBM). Despite this, very few patients had microbiologically confirmed TBM (3 out of 165 patients tested positive on Xpert® MTB/Rif Ultra) but much larger numbers are treated empirically. The relatively low diagnostic yield for TBM was felt to be in a large part due to the small volumes of CSF analysed, typically 0.5-1.0mls. Promising findings from Uganda on the sensitivity of Xpert® MTB/Rif Ultra for the diagnosis of TB meningitis (TBM) suggest that larger volumes of CSF increase the diagnostic yield for TBM[10]. However, clinicians frequently collect very limited amounts CSF thereby restricting the utility of this test. Furthermore, local evidence supporting the analysis of larger volumes of CSF for Xpert® MTB/Rif Ultra will likely influence this practice and improve the diagnosis of TBM in the region.

Developing novel diagnostic modalities for TB meningitis remains an important area of research to improve outcomes. Lateral flow lipoaribomannan assays (LAM) are well established for use in the urine to detect a constituent part of the cell wall of the TB bacilli in patients with TB. A recent meta-analysis demonstrated that a newly developed TB-LAM assay, SILVAMP TB-LAM (FujiLAM), has a superior sensitivity compared to Alere Determine TB LAM (AlereLAM), currently the most widely used assay, when used to diagnose pulmonary and extrapulmonary TB in HIV-positive adults[11]. Encouraging data from Uganda investigating the use of FujiLAM on CSF to diagnose TB meningitis demonstrated a sensitivity comparable to Xpert® MTB/Rif Ultra, 52% and 55% respectively[12]. FujiLAM has several advantages over Xpert® MTB/Rif Ultra including a faster turnaround time, no need for additional materials and limited training needed to perform the test. This represents an interesting development that warrants further investigation before more widespread use.

The introduction of multiplex PCR panels, such as BioFire FilmArray ME (Biomerieiux, France), that are able to detect a broad variety of common CNS pathogens has been performed in clinical trial settings in other African countries including Ethiopia, Uganda and Botswana[7,13,14] and has demonstrated and incremental diagnostic yield above routine investigations. Despite the clinical benefit of molecular diagnostics in detecting pathology that would otherwise have been missed by routine CSF analysis, widespread implementation will be challenging in sub-Saharan Africa due to financial constraints and different implementation strategies may need to be explored. Furthermore, defining how FilmArray ME should fit in diagnostic algorithms in resource-limited settings to meet the needs of clinicians, laboratory staff and policy makers is yet to be determined.

Overall, this enhanced prospective meningitis surveillance system will provide detailed epidemiological and aetiological information to guide clinicians in meningitis management; provide public health specialists with an invaluable tool to assess the efficacy of public health interventions aimed at reducing meningitis incidence and guide effective resource allocation; and create a uniquely powerful platform to validate novel diagnostic assays with the potential to transform meningitis diagnosis in Zimbabwe.

4. Methods

Prospective meningitis surveillance

All CSF specimens submitted for analysis from patients aged over 18 years old will be identified from laboratory records at Parirenyatwa Hospital.
Data from routine CSF analysis requested by the treating physician and performed as standard of care for patients with suspected CNS infection will be collected. This data is readily available from patient notes and electronic record systems and we are requesting a waiver of consent to collect these routine and anonymised data (see ethics section below).
Laboratory data will include microscopy and culture, cell count and differential, gram stain and india ink in addition to biochemical testing such as CSF protein and glucose. Where enhanced molecular testing has been already been performed by the public sector laboratory as part of routine care (such as cryptococcal antigen screening and Xpert® MTB/Rif Ultra) this data will also be collected.
Routinely collected clinical data only will be retrieved from the patient notes to ascertain clinical diagnosis, treatment given, and clinical outcomes of the patients (whether they are discharged home alive or die in the hospital).

Enhanced prospective meningitis surveillance

1. Extended real-time molecular diagnostics. An algorithm of enhanced diagnostics will be developed for patients with suspected CNS infections. This will consist of initial cryptococcal antigen screening (if not performed as part of routine care), Xpert® MTB/Rif Ultra and TB culture, SILVAMP TB-LAM (FujiLAM) and additional antibody/antigen tests and PCR for common bacteria and viruses. These will all be standard diagnostic tests approved for use on CSF. Results will be reported to the clinicians providing care to the patients. The full panel of tests is dependent on receipt of a sufficient volume of CSF, thus all tests may not be performed an all patients. In such cases, the routine tests requested by the treating physician and/or mandated by the public sector laboratory will be prioritized; the patient will also be approached to seek consent to perform an additional lumbar puncture if a diagnosis has not been made through routine investigation. The utility of the available tests is dependent on sufficient volumes of CSF. In particular, the sensitivity of TB Gene-Xpert has been shown to increase with larger volumes of CSF[10,15]. As such the additional CSF collected will be used both to perform additional tests described below as well as increasing the potential diagnostic yield of

those investigations available locally that may directly inform decision-making surrounding patient care. CSF samples and bacterial and fungal isolates will be archived for repeat testing if required and quality assurance monitoring.

2. Pathogen detection and deep sequencing analysis. In a subset of samples including those where a definitive diagnosis has not been reached alongside a number of positive controls, residual CSF will be archived at -80°C for microarray testing and deep sequencing to attempt to identify the causative organism. As part of this project, a novel long read metagenomics sequencing assay will also be developed and optimised using a MinION nanopore sequencer to be used on selected samples to confirm diagnoses or test for causative pathogens in samples in which we do not gain a diagnosis using the techniques described above. These may be known pathogens that are missed by standard molecular testing due to low pathogen burdens or prior antibiotic exposure, or unusual or novel pathogens not usually associated with meningitis. Such analyses can currently only be performed at a limited number of specialised centres worldwide i.e. outside Zimbabwe. Fully anonymised samples will therefore be transported to the London School of Hygiene and Tropical Medicine for analysis. It is hoped that over the next few years the capacity to perform these types of analyses will be developed within the region. To ensure local capacity development, the local investigator, Kathy Boyd, will be involved in these analyses and trained in the relevant techniques in London, and is obtaining a PhD through this and related studies. Appropriate Material Transfer Agreements will be put in place in all instances where shipment to overseas institutions is required.

3. Novel diagnostic evaluation. Laboratory evaluations of promising novel meningitis diagnostics that have the potential for near-patient or point-of-care use, and are likely to be affordable and practical in the southern African setting, will be performed using residual CSF collected at the sentinel surveillance sites. These new tests will be evaluated against the current standard diagnostics, and the molecular assays described in elements 1) and 2), using standard laboratory diagnostic evaluation techniques. All patient management decisions will be made on the basis of the currently approved diagnostic tests.

4. Clinical outcome assessment. The final element of the enhanced surveillance will be collection of data regarding the clinical management and outcome of meningitis cases. Routinely collected clinical data will be retrieved from the patient notes to ascertain clinical diagnosis, details of cranial or extra-cranial imaging performed, treatment given, and clinical outcomes of the patients (whether they are discharged home alive or die in the hospital). In addition, where additional details surrounding the clinical presentation are required we would seek consent to consult with the patient to gather any outstanding information. Data will be abstracted by study data capturers and entered into a fully anonymised database, linked to CSF results by unique sample identifiers.

<u>Evaluation of meningitis diagnostic practices</u>

Feasibility and acceptability of the implementation of molecular diagnostics will be assessed using multiple data sources. We will focus on key domains relevant to this work within the Consolidated Framework for Implementation Research (CFIR). Acceptability of implementation and approaches to meningitis diagnosis amongst key stakeholders, such as laboratory staff, clinicians and policy makers from within the hospital and the Ministry of

Health, will be assessed using interviewer-administered questionnaires. These questionnaires will be tailored to each discipline being interviewed and will consist of numeric scales to assess the interviewee's level of agreement with each statement alongside less structured areas to allow the interviewee to expand on each statement.

Data on objective metrics including run success rate and timings of specimen collection and result release will be captured to calculate turnaround time. Any sample that is not processed due to insufficient CSF volume for analysis or if it is heavily blood stained will be recorded. Data on time to clinician awareness and subsequent change in management will also captured.

A micro-costing approach will be used to estimate the current costs of conducting routine CSF analysis at Parirenyatwa Hospital to estimate an average cost of CSF analysis per patient. Costs will be gathered from the provider perspective Ministry of Health. Recurrent costs (i.e. staff time, consumables), capital costs (i.e. major equipment, building infrastructure) together with time and motion studies will be included. Using the data gathered, we will then estimate the normative costs of implementing molecular diagnostics at Parirenyatwa Hospital (including commissioning costs).

5. Study Population, Sample Size, and Statistical Analysis

This meningitis surveillance study will include all patients aged over 18 years old undergoing lumbar puncture with CSF analysis at Parirenyatwa Hospital. Apart from age there are no other exclusion criteria. We do not have a pre-selected or known sample size but rather intend to include all patients meeting the above criteria. Not all patients will be included in the enhanced surveillance if a diagnosis is obtained from routine CSF analysis and there is sufficient clinical information in the patient notes.

Data will be analysed using simple descriptive statistics as indicated (median and interquartile range, percentile, etc.), and categorised by causative organism. For the diagnostic evaluations, anticipating a 95% sensitivity of the novel assays when tested against the gold reference standards (combined culture plus molecular diagnostic), at a 95% significance level with a precision of +/- 2.5%, a sample including 292 confirmed cases would be required for each evaluation.

6. Ethical Issues and Protection of Human Subjects.

Where feasible, this study uses only routinely collected clinical information and data from CSF analysis. For the portions of the research study that will involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, and use of validated and approved tests, being performed and run for their standard indication providing a clinical diagnostic service, a waiver of consent is requested. The information will be recorded by the investigators in such a manner that subjects cannot be identified, directly or through identifiers linked to the subject, meeting standard criteria for consent waivers as laid out in GCP and other comparable regulation, for example the Health and Human Services criteria for waiver of consent (HHS.gov, 45 CFR 46.101(b)):

- The research involves no more than minimal risk to the subjects;
- The waiver or alteration will not adversely affect the rights and welfare of the subjects;
- The research could not practicably be carried out without the waiver or alteration; and
- Whenever appropriate, the subjects will be provided with additional pertinent information after participation (45 CFR 46.116(d)).

However, to maximise the utility of the available investigations larger amounts of CSF than what has been routinely collected may be required. In instances where the volume of CSF collected as part of standard care is insufficient to perform all relevant analysis, patients will be approached to seek consent for additional lumbar punctures. If additional clinical detail is required beyond what is recorded in the patient's notes consent will also be sought for further consultation with the patient.

All individual patient data will be de-identified for analysis or publication, and data maintained in a secure, password-protected database only accessible to authorized study personnel. Full confidentiality will be maintained, with no analysis or reporting of identifiable patient data. This study poses minimal risk to research participants.

1. Jarvis, J. N. *et al.* Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: Implications for improving outcomes. *Clin. Infect. Dis.* (2014) doi:10.1093/cid/cit794.
2. Scarborough, M. & Thwaites, G. E. The diagnosis and management of acute bacterial meningitis in resource-poor settings. *Lancet Neurol.* **7**, 637–648 (2008).
3. Thwaites, G. E. *et al.* The influence of HIV infection on clinical presentation, response to treatment and outcome in adults with tuberculous meningitis. *J. Infect. Dis.* **192**, 2134–2141 (2005).
4. Walker, A. S. *et al.* Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. *Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am.* **55**, 1707–1718 (2012).
5. Lawn, S. D., Harries, A. D., Anglaret, X., Myer, L. & Wood, R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS (London, England)* vol. 22 1897–1908 (2008).
6. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: Findings from 4961 suspected cases. *BMC Infect. Dis.* **10**, (2010).
7. Ellis, J. *et al.* The Changing Epidemiology of HIV-Associated Adult Meningitis, Uganda 2015-2017. *Open Forum Infect. Dis.* **6**, 1–8 (2019).
8. Tenforde, M. W. *et al.* Mortality in adult patients with culture-positive and culture-negative meningitis in the Botswana national meningitis survey: a prevalent cohort study. *Lancet Infect. Dis.* **19**, 740–749 (2019).
9. Bisson, G. P., Lukes, J., Thakur, R., Mtoni, I. & MacGregor, R. R. Cryptococcus and lymphocytic meningitis in Botswana. *S. Afr. Med. J.* **98**, 724–725 (2008).
10. Cresswell, F. V. *et al.* Xpert MTB/RIF Ultra for the diagnosis of HIV-associated tuberculous meningitis: a prospective validation study. *Lancet Infect. Dis.* **20**, 308–317 (2020).
11. Broger, T. *et al.* Diagnostic accuracy of a novel tuberculosis point-of-care urine lipoarabinomannan assay for people living with HIV: A meta-analysis of individual in-A nd outpatient data. *PLoS Med.* **17**, 1–16 (2020).
12. Quinn, C. M. *et al.* Fujifilm SILVAMP TB LAM Assay on Cerebrospinal Fluid for the Detection of Tuberculous Meningitis in Adults With Human Immunodeficiency Virus. *Clin. Infect. Dis.* **73**, e3428–e3434 (2021).
13. Bridge, S. *et al.* MENINGITIS / ENCEPHALITIS PANEL IN AND ADULT AND. *J. Med. Mycol.* 101170 (2021) doi:10.1016/j.mycmed.2021.101170.
14. Bårnes, G. K. *et al.* New molecular tools for meningitis diagnostics in Ethiopia - A necessary step towards improving antimicrobial prescription. *BMC Infect. Dis.* **18**, 1–14 (2018).
15. Bahr, N. C. *et al.* Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect. Dis.* **18**, 68–75 (2018).

Information for Participants

Study title: *The Botswana National Meningitis Survey. Protocol 2: Prospective Enhanced Meningitis Surveillance*

Why am I being asked to volunteer?
You are being invited to take part in a research study. Your participation is voluntary which means you can choose whether or not to participate. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?
The Botswana national meningitis survey is a project aiming to find out the cause of meningitis in Botswana. Meningitis is a potentially life-threatening disease caused by infection of the of the membranes and/or cerebrospinal fluid (CSF) surrounding the brain and spinal cord from a wide range of germs. It can affect anyone of any age and can cause long-lasting effects and the treatment depends on what germ is causing the meningitis. Meningitis is diagnosed by a lumbar puncture (or spinal tap) which is a procedure where a needle is inserted in to your back below the level of your spinal cord to collect some fluid which can be analysed in the laboratory. To process the samples effectively, or perform additional tests, occasionally we need to perform an additional lumbar puncture.

2. Why have I been chosen?
Your doctor is concerned you have meningitis but we have not identified the germ that is causing the infection. To perform additional tests we need another sample of CSF. The extra CSF would be used to perform any available, relevants tests that have not already been done. This may allow us to get an accurate diagnosis and alter your treatment. Some of the CSF taken during the same proceedure would also be sent to another laboratory for more detailed testing to identify the germ causing the meningitis. This testing would look for genes that we know belong to certain germs through a type of testing caused metagenomics. This test takes much longer and it is unlikely that any results from this test would affect your treatment. We hope that the information from this type of testing will help patients in the future.

3. Do I have to take part?
It is up to you to decide to join the study. Participation is voluntary. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

4. What will happen to me if I take part? What am I being asked to do?
We are asking to perform a lumbar puncture (or spinal tap) to take an extra sample of CSF to perform additional tests. Some of these tests may give information that may change management however some of them may not. We hope this information will benefit future patients with the same condition.

The doctor will perform a lumbar puncture and they will go through the details of how the procedure will be performed with you. It is a commonly performed procedure with very few serious complications. Potential risks are described later on.

5. What will happen to any samples I give?

The CSF sample you give will be stored at the Botswana Harvard AIDS Institute Partnership in Gaborone, Botswana. We will then perform tests locally to look for common germs including certain bacteria, Tuberculosis and a fungus called Cryptococcus. If we do not get a positive result from these tests which we are able to do in Gaborone we will send some of the CSF for further tests to look for the genes of germs causing the meningitis. In line with international guidelines, samples will be stored for up to fifteen years. Samples may be analysed outside of this country. Any such samples will have only an identification number and the results will be anonymous. There will be no way that you or your personal details can be identified from these samples.

6. Expenses and payments

There will be no payment for your contribution today.

7. What are the possible disadvantages and risks of taking part?

Lumbar punctures are commonly performed proceedures with very few serious complications. There are however some risks associated and these are described below:

- Headache – between 10-30% of patients will have a headache after a lumbar puncture that is worse on standing up. This usually resolves with simple analgesia (such as paracetamol), good hydration and drinking liquids with caffeine in (such as Coffee, Coca-Cola and certain Teas but not Rooibos). However if it does not settle you should seek advice from your doctor.
- Infection – this is a rare complication after lumbar puncture. Your doctor will clean the area thoroughly and use sterile equipment to reduce any chance of infection
- Bleeding – this is a rare complication after a lumbar puncture. Your doctor will check your blood to ensure that you are not at an increased risk of bleeding and will not proceed if this is the case
- Back pain – pain around the site of lumbar puncture is common. It usually resolves with simple analgesia and rest. However if it does not settle you should seek advice from your doctor.

8. What are the possible benefits of taking part?

We may be able to diagnose the cause of your meningitis and this may alter your treatment depending on the result we find. We hope that your contribution will help improve the health of patients in the future.

9. Will my taking part in the study be kept confidential?

Yes. All information collected about you during the course of the research will be kept strictly confidential.

Any information about you which leaves the clinic will have your name and address removed so that you cannot be recognised from it.

10. What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. This will not affect the care you receive at PMH. Any stored CSF samples that can still be identified as yours will be destroyed if you wish (please ask the study nurse or doctor).

11. What will happen to the results of the research study?

The results of the study will be used to develop new ways to prevent, diagnose and treat meningitis. These results will be discussed with doctors, health policy makers and patient groups, and published in medical journals. Participants will not be identified in any reports or publications.

12. Who is funding the research?

The research is being funded by the National Institute for Health Research in the UK.

13. Who has reviewed the study?
This study was given a favourable ethical opinion by the Health Research and Development Council Research Ethics Committee.

14. Contact Details
For additional information about this study and for questions regarding your participation you can contact Dr Tshepo Leeme 7298 5111 or Miss Tumalano Sekoto 3903540 ext119The ethical oversight of clinical studies in Botswana is performed by the Health Research and Development Council (HRDC) of the Ministry of Health. They can be contacted at:

HRDC, Head of Health Research Unit
Ministry of Health
Private Bag 0038
Botswana
Tel: (+267) 3914467
Fax: (+267) 3914697

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this sheet.
INFORMED CONSENT FORM

Full Title of Project: *The Botswana National Meningitis Survey. Protocol 2: Prospective Enhanced Meningitis Surveillance*

Name of Principal Investigator: Prof J. N. Jarvis

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated 12/3/20 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I agree to have an additional lumbar puncture. I undertand why it is being performed and have had the relevant risks explained to me	
4. I agree for my samples to be transported abroad for additional testing if required	
5. I understand that any relevant sections of my medical notes, and data collected during the study may be looked at by responsible individuals from the Sponsor or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
6. I agree to take part in the above study.	

Name of Participant	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date
Principal Investigator	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness *(if required)*	Signature	Date

1 copy for participant; 1 copy for Principal Investigator

Information for Participants

Study title: *The Botswana National Meningitis Survey. Protocol 2: Prospective Enhanced Meningitis Surveillance*

Why am I being asked to volunteer?
You are being invited to take part in a research study. Your participation is voluntary which means you can choose whether or not to participate. If you choose not to participate, this will not affect your future care. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?
The Botswana national meningitis survey is a project aiming to find out the cause of meningitis in Botswana. Meningitis is a potentially life-threatening disease caused by infection of the of the membranes and/or cerebrospinal fluid (CSF) surrounding the brain and spinal cord from a wide variety of germs. It can affect anyone of any age and can cause long-lasting effects and the treatment depends on what germ is causing the meningitis. Meningitis is diagnosed by a lumbar puncture (or spinal tap) which is a procedure when a needle is inserted in to your back below the level of your spinal cord to collect some fluid which can be analysed in the laboratory.

2. Why have I been chosen?
Your doctor is concerned you have meningitis. We would like to discuss your case further with you to gather more detail on your symptoms, background and past medical history and also examine you if required.

3. Do I have to take part?
It is up to you to decide to join the study. Participation is voluntary. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

4. What will happen to me if I take part? What am I being asked to do?
A member of the research team will complete a questionnaire with you that will go into detail about your case and how you became unwell. They may also need to examine you which will be similar to the assessment the admitting doctor carried out when you came into hospital. This information will be analysed alongside similar information from other patients to investigate the cause of meningitis in Botswana. It will be anonymous and people will not be able to tell that the information came from you.

5. Expenses and payments
There will be no payment for your contribution today.

6. What are the possible disadvantages and risks of taking part?
The duration of the assessment will vary from patient to patient but it may take some time to go through your case in detail. If you are feeling unwell this may be difficult to you. However you are able to stop at anytime and this will not affect your care.
There are no significant risks associated with this additional consultation.

7. What are the possible benefits of taking part?
There will be no direct benefit to you but we hope that your contribution will help improve the health of patients in the future.

8. Will my taking part in the study be kept confidential?

Yes. All information collected about you during the course of the research will be kept strictly confidential.

Any information about you which leaves the clinic/ward will have your name and address removed so that you cannot be recognised from it.

9. What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. This will not affect the care you receive at PMH.

10. What will happen to the results of the research study?

The results of the study will be used to develop new ways to prevent, diagnose and treat meningitis. These results will be discussed with doctors, health policy makers and patient groups, and published in medical journals. Participants will not be identified in any reports or publications.

11. Who is funding the research?

The research is being funded by the National Institute for Health Research in the UK.

12. Who has reviewed the study?

This study was given a favourable ethical opinion by the Health Research and Development Council Research Ethics Committee.

13. Contact Details

For additional information about this study and for questions regarding your participation you can contact Dr Tshepo Leeme 7298 5111 or Miss Tumalano Sekoto 3903540 ext119. The ethical oversight of clinical studies in Botswana is performed by the Health Research and Development Council (HRDC) of the Ministry of Health. They can be contacted at:

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Ministry of Health
Private Bag 0038
Botswana
Tel: (+267) 3914467
Fax: (+267) 3914697

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this sheet.
INFORMED CONSENT FORM

Full Title of Project: *The Botswana National Meningitis Survey. Protocol 2: Prospective Enhanced Meningitis Surveillance*

Name of Principal Investigator: Prof J. N. Jarvis

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated 12/3/20 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I agree to discuss my case with a member of the study team and for the information gathered evaluated anonymously as part of the study	
4. I understand that any relevant sections of my medical notes, and data collected during the study may be looked at by responsible individuals from the Sponsor or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
5. I agree to take part in the above study.	

Name of Participant	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date
Principal Investigator	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness	Signature	Date

(if required)

1 copy for participant; 1 copy for Principal Investigator

P. O. Box A 178
Avondale
HARARE, Zimbabwe

INTERNAL MEDICINE UNIT
Telephone: 263-4-791631
Fax: 263-4-251017
Telegrams: UNIVERSITY
Unit Coordinator: Dr G.W. Ngwende, MMed (Medicine) UZ, FCP (ECSA), PD Clin Neurology (UCL) Email:medicine@medsch.uz.ac.zw

FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITY OF ZIMBABWE

Information for Participants

Study title:
Harare Meningitis Aetiology Survey

Chief Investigator:
Professor Joseph Jarvis, NIHR Global Health Professor. Botswana Harvard AIDS Institute Partnership and London School of Hygiene and Tropical Medicine.

Co-Principal Investigators:
Dr Gift Ngwende, Specialist Physician and Neurologist, University of Zimbabwe Faculty of Medicine and Health Sciences.
Professor Chiratidzo Ndhlovu, Specialist Physician and Nephrologist, University of Zimbabwe Faculty of Medicine and Health Sciences.

Co-Investigators:
Ms Kathryn Boyd, Laboratory scientist. University of Zimbabwe and London School of Hygiene and Tropical Medicine.
Dr James Milburn, Clinical Research Fellow. Botswana Harvard AIDS Institute Partnership and London School of Hygiene and Tropical Medicine.
Dr Lenon Gwaunza, Neurologist, Neurodiagnostic Centre, Harare Zimbabwe

Why am I being asked to volunteer?
You are being invited to take part in a research study. Your participation is voluntary which means you can choose whether or not to participate. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

The Harare Meningitis Aetiology Survey is a project aiming to find out the cause of meningitis in Zimbabwe. Meningitis is a potentially life-threatening disease caused by infection of the linings of the brain and/or cerebrospinal fluid (CSF) surrounding the brain and spinal cord from a wide range of germs. It can affect anyone of any age and can cause long-lasting effects and the treatment depends on what germ is causing the meningitis. Meningitis is diagnosed by a lumbar puncture where a needle is inserted into your back below the level of your spinal cord to collect some fluid which can be analysed in the laboratory. To process the samples or to perform additional tests we may to perform an extra lumbar puncture.

2. Why have I been chosen?

Your doctor is concerned you have meningitis. We would like to perform additional tests that may either identify the germ causing meningitis in you or allow further analysis of CSF that will benefit patients with meningitis in the future. Some of these tests require larger amounts of CSF than others to be able to give accurate results and we currently do not have enough of your CSF to run all available tests. The extra CSF would be firstly be used to perform any available, relevant tests locally. These tests may allow us to get an accurate diagnosis if it is not known already and alter your treatment. Some of the CSF taken during the same LP will also be sent to another laboratory for more detailed testing to identify the germ causing your meningitis. This further testing will look for genes that we know belong to certain germs through a type of testing caused metagenomics. This test takes much longer and any results from this test will not affect your treatment. However we hope that the information from this type of testing will help other patients in the future.

3. Do I have to take part?

It is up to you to decide to join the study. Participation is voluntary. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign this consent form. You are free to withdraw from this study at any time, without giving a reason.

4. What will happen to me if I take part? What am I being asked to do?

We are asking to perform a lumbar puncture (LP) to take an extra sample of CSF to perform additional tests. We will take between 10-15mls of CSF, this is a safe volume as your body makes approximately 22mls of CSF each hour. Some of these tests may give information that may change your management however some of them may not. We hope this information will benefit future patients with the same condition.

The doctor will perform a lumbar puncture and they will go through the details of how the procedure will be performed with you. It is a commonly performed procedure with very few serious complications. Potential risks are described later on.

5. What will happen to any samples I give?

The CSF sample you give will be stored at the University of Zimbabwe, Faculty of Medicine and Health Sciences, Harare, Zimbabwe. We will then perform tests locally to look for common germs including certain bacteria, Tuberculosis and a fungus called Cryptococcus. If we do not get a result from these tests which we are able to do in Harare, we will send some of the CSF outside the country for further tests to look for the genes of germs causing the meningitis. In line with international guidelines, samples will be stored for up to fifteen years. Samples may be analysed outside of this country. Any such samples will only have an identification number and the results will not show your name. There will be no way that you or your personal details can be identified from these samples.

6. Expenses and payments

There will be no payment for your contribution today.

7. What are the possible disadvantages and risks of taking part?

Lumbar punctures are commonly performed procedures with very few serious complications. There are however some risks associated and these are described below:

Headache – between 10-30% of patients will have a headache after a lumbar puncture that is worse on standing up. This usually resolves with simple analgesia (such as paracetamol), good hydration and drinking liquids with caffeine in (such as Coffee, Coca-Cola and certain Teas but not Rooibos). However if this does not settle you should seek advice from your doctor.

Infection – this is a rare complication after lumbar puncture. Your doctor will clean the area thoroughly and use sterile equipment to reduce any chance of infection

Bleeding – this is a rare complication after a lumbar puncture. Your doctor will check your blood to ensure that you are not at an increased risk of bleeding and will not proceed if this is the case

Back pain – pain around the site of lumbar puncture is common. It usually resolves with simple analgesia and rest. However if it does not settle you should seek advice from your doctor.

8. What are the possible benefits of taking part?

We may be able to explain the cause of your meningitis and this may alter your treatment depending on the result we find. We hope that your contribution will help improve the health of patients in the future.

9. Will my taking part in the study be kept confidential?

Yes. All information collected about you during the course of the research will be kept strictly confidential. The additional tests performed at Parirenyatwa Hospital will be shared with your doctors to allow them to make any changes to your medications if needed.

Any information about you which leaves the clinic will have your name and address removed and you will be allocated a participant ID number so that you cannot be recognised from it.

10. What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. This will not affect the care you receive. Any stored CSF samples that can still be identified as yours will be destroyed if you wish (please ask the study nurse or doctor).

11. What will happen to the results of the research study?

The results of the study will be used to develop new ways to prevent, diagnose and treat meningitis. These results will be discussed with doctors, health policy makers e.g. Ministry of Health and Child Care and patient groups, and published in medical journals. Participants will not be identified in any reports or publications.

12. Who is funding the research?

The research is being funded by the National Institute for Health Research in the UK.

13. Who has reviewed the study?

This study has been reviewed by Joint Research Ethics Committee at the University of Zimbabwe and Parirenyatwa Hospital (JREC), Medical Research Council of Zimbabwe (MRCZ) and the Research Council of Zimbabwe (RCZ)

14. Contact Details

For additional information about this study and for questions regarding your participation you can contact Professor Chiratidzo Ndhlovu (0772 412701 or 0712206313), Dr Gift Ngwende (0773303709) or Dr James Milburn (+267 76622296).

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this sheet.
INFORMED CONSENT FORM

Full Title of Project: *Harare Meningitis Aetiology Survey*

Name of Principal Investigators: Prof J. N. Jarvis
Co-Principal Investigators: Prof Chiratidzo Ndhlovu and Dr Gift Ngwende
Lead study doctor: Dr James Milburn

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated 1/12/21 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I agree to have an additional lumbar puncture. I understand why it is being performed and have had the relevant risks explained to me	
4. I agree for my samples to be transported abroad for additional testing if required	
5. I understand that any relevant sections of my medical notes, and data collected during the study may be looked at by responsible individuals from the Sponsor or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
6. I agree to take part in the above study.	

Name of Participant	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness *(if required)*	Signature	Date

1 copy for participant; 1 copy

YOU WILL BE OFFERED A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research participant or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe (MRCZ) on telephone (0242)791792 or (0242) 791193 and cell phone lines 0784 956 128. The MRCZ Offices are located at the National Institute of Health Research premises at Corner Josiah Tongogara and Mazowe Avenue in Harare.

P.O. Box A 178
Avondale
HARARE,
Zimbabwe

INTERNAL MEDICINE UNIT
Telephone: 2634-791631
Fax: 263-4-251017
Telegrams: UNIVERSITY
Unit Coordinator: Dr G.W. Ngwende, MMed (Medicine) UZ, FCP (ECSA), PD Clin Neurology (UCL) Email:medicine@medsch.uz.ac.zw

FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITY OF ZIMBABWE

Information for Participants

Study title:
Harare Meningitis Aetiology Survey

Chief Investigator:
Professor Joseph Jarvis, NIHR Global Health Professor. Botswana Harvard AIDS Institute Partnership and London School of Hygiene and Tropical Medicine.

Co-Principal Investigators:
Dr Gift Ngwende, Specialist Physician and Neurologist, University of Zimbabwe Faculty of Medicine and Health Sciences.
Professor Chiratidzo Ndhlovu, Specialist Physician and Nephrologist, University of Zimbabwe Faculty of Medicine and Health Sciences.

Co-Investigators:
Ms Kathryn Boyd, Laboratory scientist. University of Zimbabwe and London School of Hygiene and Tropical Medicine.
Dr James Milburn, Clinical Research Fellow. Botswana Harvard AIDS Institute Partnership and London School of Hygiene and Tropical Medicine.
Dr Lenon Gwaunza, Neurologist, Neurodiagnostic Centre, Harare Zimbabwe

Why am I being asked to volunteer?
You are being invited to take part in a research study. Your participation is voluntary which means you can choose whether or not to participate. If you choose not to participate, this will not affect your future care. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?
The Harare Meningitis Aetiology Survey is a project aiming to find out the cause of meningitis in Zimbabwe. Meningitis is a potentially life-threatening disease caused by infection of the linings of the brain and/or cerebrospinal fluid (CSF) surrounding the brain

and spinal cord from a wide variety of germs. It can affect anyone of any age and can cause long-lasting effects and the treatment depends on what germ is causing the meningitis. Meningitis is diagnosed by a lumbar puncture (or spinal tap) which is a procedure when a needle is inserted in to your back below the level of your spinal cord to collect some fluid which can be analysed in the laboratory.

2. Why have I been chosen?

Your doctor is concerned you have meningitis. We would like to discuss your case further with you to gather more detail on your symptoms, background and past medical history and also examine you if required.

3. Do I have to take part?

It is up to you to decide to join the study. Participation is voluntary. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

4. What will happen to me if I take part? What am I being asked to do?

A member of the research team will complete a questionnaire with you that will go into detail about your case and how you became unwell. They may also need to examine you which will be similar to the assessment the admitting doctor carried out when you came into hospital. This information will be analysed alongside similar information from other patients to investigate the cause of meningitis in Zimbabwe. It will be anonymous and people will not be able to tell that the information came from you.

5. Expenses and payments

There will be no payment for your contribution today.

6. What are the possible disadvantages and risks of taking part?

The duration of the assessment will vary from patient to patient but it may take some time to go through your case in detail. If you are feeling unwell this may be difficult to you. However you are able to stop at anytime and this will not affect your care.
There are no significant risks associated with this additional consultation.

7. What are the possible benefits of taking part?

There will be no direct benefit to you but we hope that your contribution will help improve the health of patients in the future.

8. Will my taking part in the study be kept confidential?

Yes. All information collected about you during the course of the research will be kept strictly confidential.
Any information about you which leaves the hospital laboratory for the study purposes will have your name and address removed and you will be allocated a participant ID number so that you cannot be recognised from it.

9. What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. This will not affect the care you receive.

10. What will happen to the results of the research study?

The results of the study will be used to develop new ways to prevent, diagnose and treat meningitis. These results will be discussed with doctors, health policy makers and patient groups, and published in medical journals. Participants will not be identified in any reports or publications.

11. Who is funding the research?

The research is being funded by the National Institute for Health Research in the UK.

12. Who has reviewed the study?

This study was given a favourable ethical opinion by the Medical Research Council of Zimbabwe

13. Contact Details

For additional information about this study and for questions regarding your participation you can contact Professor Chiratidzo Ndhlovu on 0712206313/0772412701. The ethical oversight of clinical studies in Zimbabwe is performed by the Medical Reseach Council of Zimbabwe. They can be contacted at 20 Cambridge Road, Avondale, Harare. Tel: +242 791792/791193/792747.

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this sheet.
INFORMED CONSENT FORM

Full Title of Project: *Harare Meningitis Aetiology Survey*

Name of Principal Investigators: Prof J. N. Jarvis, Dr Lenon Gwaunza, Prof Chiratidzo Ndhlovu and Dr Gift Ngwende

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated(…….) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I agree to discuss my case with a member of the study team and for the information gathered evaluated anonymously as part of the study	
4. I understand that any relevant sections of my medical notes, and data collected during the study may be looked at by responsible individuals from the Sponsor or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
5. I agree to take part in the above study.	

Name of Participant	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date
Principal Investigator	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness *(if required)*	Signature	Date

1 copy for participant; 1 copy for Principal Investigator