UTILITY OF GENEXPERT IN EVALUATING PATIENTS WITH SUSPECTED TUBERCULOUS MENINGITIS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET KENYA

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A thesis submitted in part fulfilment of the requirement for the degree of Masters of Medicine in Internal Medicine, Moi University

Declaration

Student Declaration

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without prior written permission of the author and or Moi University.

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Dedication

To my loving wife,

Valentina,

Whose love and confidence is a constant source of inspiration and encouragement

To my mother and father,

Janet and Harry,

For sacrifices they made towards my academic success.

And to my daughter Kaylee,

For the joy you have brought into my life.

Abstract

Utility of GeneXpert in Evaluating Patients with Suspected Tuberculous Meningitis at Moi Teaching and Referral Hospital, Eldoret Kenya

Background: Tuberculous meningitis (TBM) is the most severe form of tuberculosis (TB) that is almost 100% fatal if not treated and case fatality rates remain high (15-40%) despite effective treatment. Early diagnosis and treatment can lead to reduction in morbidity and mortality caused by this disease. Liquid culture techniques, including the mycobacterial growth indicator tube (MGIT) has been the gold standard test for detecting *Mycobacterium tuberculosis* (MTB) in cerebrospinal fluid (CSF). CSF culture has a sensitivity of up to 77% but the clinical value is limited due to the long duration required to get results: 1 to 4 weeks. GeneXpert is a molecular real time polymerase chain reaction (PCR) test that is rapid and can give results for TB and rifampicin resistance in 2 hours. It has been approved by the World Health Organization for the diagnosis of pulmonary TB but there is paucity of data on its use in CSF.
Objective: The aim of this study was to compare the yields of GeneXpert and MGIT culture on CSF samples from patients presenting with suspected tuberculous meningitis.
Methods: This was a cross sectional study. Patients aged 18 years and above with suspected tuberculous meningitis based on a clinical entry criteria were recruited from the adult medical wards, Moi Teaching and Referral Hospital (MTRH) between December 2013 and July 2014. Recruited patients with no contraindications to lumbar puncture were consecutively enrolled after giving an informed consent until the desired sample size was obtained. They underwent a physical examination followed by a lumbar puncture. The CSF was analyzed for MTB using GeneXpert and MGIT culture. Part of the CSF was sent to MTRH laboratory for routine tests.
Results: 101 patients with suspected TBM were enrolled in the study. Of the 93 evaluable patients, 57 (61%) were female, median age was 36 years (27-46) and HIV positivity was 77 (83%). Using a consensus case definition, 10 (11%) had definite TBM, 27 (29%) had probable TBM, 49 (52%) had possible TBM and 7 (8%) had no TBM. 10 patients had a positive MGIT culture result while 8 patients had a positive GeneXpert test. The yield of GeneXpert compared to MGIT culture was 8/93 (0.086) vs 10/93 (0.1075) 95% C.I p=0.06199. The sensitivity and specificity of GeneXpert as compared to MGIT liquid culture was 80% and 100% respectively. One case of rifampicin resistance (1/8; 12.5%) was identified. Findings associated with a positive GeneXpert result include elevated proteins 233 (116.5-342.2) vs 77.0 (26.8-143.0) p=0.003, low glucose 1.5 (1.0-2.1) vs 2.6 (1.8-3.6) p=0.015 and high diagnostic score 11(10-11) vs 8 (7-11) p=0.024.
Conclusion: There was no statistical significant difference between the yield of GeneXpert and MGIT liquid culture in CSF samples of patients presenting with suspected TBM. The prevalence of Rifampicin resistance was 12.5% among patients with a positive GeneXpert test. Patients with high CSF protein count, low CSF glucose and a high diagnostic score were more likely to have a positive GeneXpert result.
Recommendations: GeneXpert should be used as the initial diagnostic test in place of MGIT liquid culture in patients with suspected tuberculous meningitis.

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List of Abbreviations

AAFB	-	Acid Alcohol Fast Bacilli
AIDS	-	Acquired immunodeficiency syndrome
ART	-	Anti-retroviral Therapy
CNS	-	Central Nervous System
CRL	-	Central Reference Laboratory
CSF	-	Central Nervous System
CT	-	Computerized tomography
DNA	-	Deoxyribonucleic acid
ELISA	-	Enzyme-linked immunosorbent assay
EPTB	-	Extra Pulmonary Tuberculosis
HBS	-	High burden countries
HIV	-	Human Immunodeficiency Virus
IREC	-	Institutional Research and Ethics Committee
LAM	-	Lipoarabinomannan
LP	-	Lumbar puncture
M/C/S	-	Microscopy, Culture and Sensitivity
MDR	-	Multi drug resistant
MRC	-	Medical Research Council
MRI	-	Magnetic Resonance Imaging
MTB	-	*Mycobacterium tuberculosis*
MTRH	-	Moi Teaching and Referral Hospital
NAAT	-	Nucleic acid amplification tests
NNRTI	-	Non-nucleoside reverse transcriptase inhibitors
PCR	-	Polymerase chain reaction
PI	-	Protease Inhibitors
RIF	-	Rifampicin
SR	-	Sample Reagent
TB	-	Tuberculosis
TBM	-	Tuberculous Meningitis
WHO	-	World Health Organization

Definition of Terms

MDR TB	-	*Mycobacterium tuberculosis* resistant to at least Isoniazid and Rifampicin

Chapter One: Introduction

1.1 Background

Tuberculosis is an infectious disease caused by the bacillus Mycobacterium tuberculosis. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB) (WHO., 2014). Tuberculosis is among the most widespread infectious diseases and is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent (WHO., 2014).

The causative organism of tuberculosis, the tubercle bacillus, was isolated and described by Robert Koch in 1882. It was subsequently included in the genus *Mycobacterium* and named *Mycobacterium tuberculosis.* M. tuberculosis (MTB) is a member of the *Mycobacterium tuberculosis* complex. Other members in this complex include M.*bovis*, M.*africanum,* M.*canetti,* M.*microti*, *M. pinnepedii*, and *M. caprae*.

Tubercle bacilli are aerobic, non-motile, non-sporing, usually slightly curved rods 2–4 μm in length and 0.3–0.5 μm in diameter.

Transmission of Tuberculosis is mainly through inhalation of respiratory droplets. Other modes of transmission include ingestion of infected animal products, through traumatic inoculation of the skin or rarely intra uterine infection resulting in congenital tuberculosis. Inhalation of the bacterium leads to immediate clearance, chronic/latent infection, rapidly progressive active disease (primary disease) or active disease many years after the infection (reactivation disease).

Both primary and reactivation disease can spread via the bloodstream to extra pulmonary sites leading to extra pulmonary TB. Extra pulmonary TB is more common among immunocompromised patients. Virtually any body organ can be affected

including lymph nodes, pleura, pericardium, meninges, liver, spleen, bones, spine, kidneys, bladder, skin, eyes and the gastro-intestinal system.

Tuberculous Meningitis is one of the most severe forms of tuberculosis and causes substantial morbidity and mortality (Garcia-Monco, 1999; Lakatos et al., 2011). TBM is almost 100% fatal if not treated and case fatality rates remain high (15-40%) despite effective treatment (John. M. Leonard, 2015). Central nervous system tuberculosis has been reported in 15 to 20 percent of patients with milliary TB(Gelb, Leffler, Brewin, Mascatello, & Lyons, 1973; Maartens, Willcox, & Benatar, 1990) and about 12% of all meningitis infections in Kenya (Jowi, Mativo, & Musoke, 2007; Lagat, 2010).

Diagnosis of TBM is based on history, physical examination and CSF studies. Definitive diagnosis of TBM is made by demonstrating *mycobacterium tuberculosis* in CSF. This can be done by smear microscopy, culture or by use of molecular probes to detect *mycobacterium tuberculosis* DNA. This is not always possible as some of the tests (e.g. smear microscopy) have low sensitivity and in the case of culture, results may take up 8-10 weeks to be available (Hooker et al., 2003; Venkataswamy, Rafi, Nagarathna, Ravi, & Chandramuki, 2007).

The definition of TBM when used in clinical research has varied from study to study (Bhigjee et al., 2007; Quan, Lu, Qiao, Xiao, & Li, 2006; Rafi, Venkataswamy, Nagarathna, Satishchandra, & Chandramuki, 2007). In most definitions, patients are given a definite, probable, or possible tuberculous meningitis status depending on clinical, laboratory, and radiological findings. Definite Tuberculous meningitis cases usually include patients with AFB on CSF microscopy or MTB cultured from CSF or another CNS source. Criteria for probable or possible tuberculous meningitis cases differ greatly between studies. This necessitated a uniform case definition for use in

clinical research reached by consensus by TBM experts worldwide in a conference in Cape Town, South Africa as described by Marais et al (Marais et al., 2010). This criteria classifies TBM as definite, probable, possible or absent based on a diagnostic scoring system.

In 2010, the WHO endorsed the use of Xpert MTB/RIF (GeneXpert), a rapid, fully automated Nucleic acid amplification tests (NAAT) for use on sputum specimens in resource-constrained TB endemic countries, at concessional pricing(WHO, 2010a). The main advantages of GeneXpert over pre-existing molecular assays are its simplicity of use – it requires minimal technical expertise and biosafety requirements and the low rates of cross-contamination (Banada et al., 2010; Van Rie, Page-Shipp, Scott, Sanne, & Stevens, 2010). Another key advantage is its ability to rapidly detect rifampicin resistance and hence predict multidrug resistant disease (MDR). GeneXpert has been extensively evaluated for MTB detection in sputum specimens and performs well on smear-positive samples (Steingart et al., 2013). Preliminary studies on a range of extrapulmonary TB samples have also been promising in smear-positive samples (Tortoli et al., 2012; Vadwai et al., 2011). For CSF samples specifically, further studies are required as the few that have been performed have small subject numbers, variable results and none has been performed in our setting (Armand, Vanhuls, Delcroix, Courcol, & Lemaître, 2011; Causse, Ruiz, Gutiérrez-Aroca, & Casal, 2011; Vinod B. Patel et al., 2013; Tortoli et al., 2012).

Multidrug resistant tuberculosis is mycobacteria resistant to at least isoniazid and rifampicin. As most rifampicin-resistant isolates are also resistant to isoniazid, rifampicin resistance can be used as a marker for MDR TB (Mokrousov, Otten, Vyshnevskiy, & Narvskaya, 2003; Prammananan et al., 2008; Traore, Fissette, Bastian,

Devleeschouwer, & Portaels, 2000). Multidrug-resistant (MDR) and rifampicin resistant pulmonary tuberculosis are well described in the literature (Cox et al., 2010; Ogwang et al., 2009; Theron et al., 2011; Visser et al., 2012). However, reports of MDR TBM are mostly limited to case reports and a single case series (V. B. Patel et al., 2004).

1.2 Problem Statement

Kenya is one of the 22 high-burden countries (HBCs) that collectively account for about 80% of the world's TB cases.(WHO, 2012) The double epidemic of tuberculosis and HIV/AIDS has led to an increase of extrapulmonary tuberculosis including tuberculous meningitis (Wiwatworapan & Anantasetagoon, 2008).

It is difficult to differentiate tuberculous meningitis from other causes of meningoencephalitis by use of clinical presentation alone as there are a number of CNS infections that can present similarly. Smear microscopy has a low sensitivity and culture takes a long time before results are available. This may lead to delays in initiating appropriate treatment which leads to a poorer outcome (Hosoglu et al., 2002; John. M. Leonard, 2015).

In MTRH, the prevalence of MDR TBM is unknown. Globally, 3.5% of new TB cases and 20.5% of previously treated cases are estimated to have MDR-TB (WHO., 2014). The prevalence of CNS infection caused by strains resistant to one or more first-line drugs is increasing (WHO., 2014). MDR TBM has a devastating effect on the outcome of TBM if treated with first line anti TB drugs (Guy E. Thwaites et al., 2005).

1.3 Justification

There is need for a rapid and accurate diagnostic tool for TBM. GeneXpert has the potential of providing a fast and reliable test for Tuberculous meningitis (Tortoli et al., 2012; WHO, 2013a). Only one study in Africa and no studies in Kenya have evaluated

its use in CSF samples (Vinod B. Patel et al., 2013). This study will also provide important information about MDR TBM in our setting.

1.4 Feasibility

The proposed study will be conducted at Moi Teaching and Referral Hospital based in Western Kenya-an area with a high prevalence of HIV AIDS and Tuberculosis. The hospital has a Mycobacterium reference laboratory based in AMPATH that has been certified to conduct liquid and solid cultures as well as two GeneXpert machines capable of running four samples each at a time. Given this setting, the proposed study is feasible.

1.5 Research Questions

What is the yield of GeneXpert compared to MGIT culture when used evaluate CSF from patients presenting with suspected TBM?

1.6 Study Objectives

1.6.1 Broad Objective:

To establish the yields of GeneXpert and MGIT culture on CSF samples from patients presenting with suspected TBM

1.6.2 Specific Objectives

1. To determine the yields of GeneXpert compared to MGIT culture on CSF samples from patients presenting with suspected TBM.

2. To determine the prevalence of Rifampicin resistance among patients diagnosed with TBM using GeneXpert.

3. To determine the clinical and laboratory parameters that are associated with GeneXpert positivity.

Chapter 2: Literature Review

2.1 Epidemiology of Tuberculosis and Tuberculous meningitis

In 2013 The World Health Organization estimates worldwide there were 9 million new TB infections and 1.5 million Tuberculosis deaths (WHO., 2014). Out of the 22 high burden TB countries worldwide, 9 are found in Africa (WHO., 2014). Kenya was ranked number 14 worldwide and 5th in Africa (WHO., 2014). In 2013, Kenya had 89,796 new TB cases notified with 14,478 of them being extra pulmonary cases (WHO, 2013c).

CNS Tuberculosis has been reported in approximately 1% of patients TB and 5-10% of all patients with extra pulmonary tuberculosis (Cherian & Thomas, 2011). Central nervous system (CNS) tuberculosis (TB) includes three clinical categories: tuberculous meningitis, intracranial tuberculoma, and spinal tuberculous arachnoiditis (John. M. Leonard, 2015). Tuberculous meningitis is the most common form of CNS tuberculosis and it is one of the most severe forms of Tuberculosis (John. M. Leonard, 2015).

Globally in 2013, an estimated 3.5% of new cases and 20.5% of previously treated cases had MDR-TB (WHO., 2014). In Kenya 2.6% of new cases and 13% of previously treated patients in 2013 had MDR-TB. Cases of MDR TBM are mostly limited to case reports and a single case series (Daikos, Cleary, Rodriguez, & Fischl, 2003; Duo et al., 2011).

Patel et al at Kwa Zulu Natal, South Africa found a prevalence of MDR TB of 8.6% among patients with culture positive TBM which was considerably higher than in pulmonary Tuberculosis among new patients (1-2%) or those previously treated for TB (7-8%) (V. B. Patel et al., 2004). There are no published studies or case reports about MDR TBM in Kenya.

2.2 Microbiology of M. Tuberculosis

Tubercle bacilli are aerobic, non-motile, non-sporing, usually slightly curved rods 2–4 μm in length and 0.3–0.5 μm in diameter. The mycobacterial cell envelope is composed of a core of three macromolecules covalently linked to each other (peptidoglycan, arabinogalactan, and mycolic acids) and a lipopolysaccharide, lipoarabinomannan (LAM). Mycolic acid, which is a beta-hydroxy fatty acid, is the major constituent of the cell envelope, accounting for more than 50 percent by weigh. The organism stains positive with Gram's stain. The mycolic acid structure confers the ability to resist de-staining by acid alcohol after being stained by certain aniline dyes, leading to the term acid fast bacillus (AFB).

2.3 Pathogenesis of Central Nervous System tuberculosis

Most tuberculous infections of the CNS are caused by MTB. Less frequently, other mycobacteria may be involved. The MTB bacilli reach the CNS by the haematogenous route secondary to disease elsewhere in the body. Rich and McCordock suggested that CNS tuberculosis develops in two stages (Rich AR, 1933). Initially small tuberculous lesions (Rich’s foci) develop in the CNS, either during the stage of bacteremia of the primary tuberculous infection or shortly thereafter. These initial tuberculous lesions may be in the meninges, the subpial or subependymal surface of the brain or the spinal cord, and may remain dormant for years after the initial infection. Later, rupture or growth of one or more of these small tuberculous lesions lead to the development of various types of CNS tuberculosis (Berger, 1994; Rich AR, 1933). The specific stimulus for rupture or growth of Rich’s foci is not known, although immunological mechanisms are believed to play an important role. Tuberculous meningitis develops if they rupture into the subarachnoid space or into the ventricular system. The type and extent of lesions that result from the discharge of tuberculous bacilli into the CSF

depend upon the number and virulence of the bacilli, and the immune response of the host. Less frequently, infection spreads to the CNS from a site of tuberculous otitis or calvarial osteitis. The pathogenesis of localized brain lesions is also thought to involve haematogenous spread from a primary focus in the lung. It has been suggested that with a sizeable inoculation or in the absence of an adequate cell-mediated immunity, the parenchymal cerebral tuberculous foci may develop into tuberculoma or tuberculous brain abscess (JR & RM, 1986).

2.4 Pathology of Tuberculous meningitis

In tuberculous meningitis there is a thick, gelatinous exudate around the sylvian fissures, basal cisterns, brainstem, and cerebellum. Hydrocephalus may occur as a consequence of obstruction of the basal cisterns, outflow of the fourth ventricle, or occlusion of the cerebral aqueduct. Hydrocephalus frequently develops in children and is associated with a poor prognosis. The brain tissue immediately underlying the tuberculous exudate shows various degrees of edema, perivascular infiltration, and a microglial reaction, a process known as ‘border zone reaction’. The basal exudates of tuberculosis are usually more severe in the vicinity of the circle of Willis, and produce a vasculitis-like syndrome. Inflammatory changes in the vessel wall may be seen, and the lumen of these vessels may be narrowed or occluded by thrombus formation. The vessels at the base of the brain are most severely affected, including the internal carotid artery, proximal middle cerebral artery, and perforating vessels of the basal ganglion. Cerebral infarctions are most common around the sylvian fissure and in the basal ganglion. In the majority of patients the location of infarction is in the distribution of medial striate and thalamo-perforating arteries. Hemorrhagic transformation of infarcted tissue is not unusual (Molavi & LeFrock, 1985)·(Dastur, Manghani, & Udani, 1995; J. M. Leonard & Des Prez, 1990; Newton, 1994).

2.5 Risk factors

Risk factors for CNS tuberculosis include age (children> adults,) HIV co-infection,(Rana et al., 2000) malnutrition, recent measles in children,(Yaramis et al., 1998) alcoholism, malignancies, the use of immunosuppressive agents in adults and disease prevalence in the community (Bidstrup, Andersen, Skinhoj, & Andersen, 2002; Phypers, Harris, & Power, 2006).

2.6 Diagnosis

TBM is diagnosed using a combination of clinical, laboratory and imaging studies.

2.6.1 Clinical presentation

In most patients with tuberculous meningitis, there is a history of vague ill health lasting 2–8 weeks prior to the development of meningeal irritation. These nonspecific symptoms include malaise, anorexia, fatigue, fever, myalgias, and headache. Adults with TBM can often present with the classic meningitis symptoms of fever, headache and stiff neck along with focal neurological deficits, behavioral changes, and alterations in consciousness (Sutlas, Unal, Forta, Senol, & Kirbas, 2003). A history of tuberculosis is elicited in only approximately 10% of patients with TBM (Sutlas et al., 2003). Patients co-infected with HIV do not seem to have an altered presentation of TBM (G. E. Thwaites et al., 2005). Cerebrovascular complications of tuberculous meningitis that occur typically as multiple or bilateral lesions in the territories of the middle cerebral artery perforating vessels are termed as tuberculous vasculopathy. Vessel pathology appears to be a consequence of its immersion in the local inflammatory exudate. Infiltrative, proliferative and necrotising vessel pathologies have been described, leading to luminal thrombosis. There is some evidence that vasospasm may mediate strokes early in the course of the disease and proliferative intimal disease may predispose to later strokes (Lammie, Hewlett, Schoeman, & Donald, 2009).

Severity of disease can be determined based on clinical signs based on modifications of the Medical Research Council staging system, which has been shown to have considerable prognostic value (Thwaites & Tran, 2005).

Contemporary criterion for staging TBM (Thwaites & Tran, 2005)

I Alert and oriented without focal neurological deficits

II Glasgow coma score of 14-11 or 15 with focal neurological deficits

III Glasgow coma score of 10 or less, with or without focal neurological deficits

Cranial nerve palsies occur in 20–30% of patients and may be the presenting manifestation of TBM. The sixth cranial nerve is most commonly affected (Berger, 1994). Vision loss due to optic nerve involvement may occasionally be a dominant presenting illness. Optochiasmatic arachnoiditis, third ventricular compression of optic chiasma (if hydrocephalus develops), optic nerve granuloma are possible factors for vision loss in these patients. Ophthalmoscopic examination may reveal papilloedema. Funduscopy may reveal choroid tubercles, yellow lesions with indistinct borders present either singly or in clusters. These choroid tubercles are more frequent with tuberculous meningitis associated with miliary tuberculosis and are virtually pathognomonic although they are present in only 10% of patients in whom the meningitis is not associated with miliary involvement (J. M. Leonard & Des Prez, 1990). Clinical manifestations of tuberculoma or tuberculous brain abscess depend largely on their location, and patients often present with headache, seizures, papilledema, or other signs of increased intracranial pressure. The presentation of brain

abscess is more sub-acute (1 week to 3 months) than tuberculoma but slower in onset than pyogenic brain abscesses (Kumar, Pandey, Bose, & Sahay, 2002).

The clinical spectrum of CNS tuberculosis with HIV infection includes meningitis, cerebral abscesses and tuberculomas. CNS involvement occurs in 10–20% patients with AIDS-related tuberculosis, and in these patients mortality is high (Berenguer et al., 1992). HIV-infected intravenous drug abusers are, in particular, at high risk of developing focal CNS tuberculosis (Farrar, Flanigan, Gordon, Gold, & Rich, 1997). Clinical features, including imaging characteristics, are similar to those seen in patients without HIV infection (Berenguer et al., 1992; Dube, Holtom, & Larsen, 1992; Farrar et al., 1997).

2.6.2 CSF Characteristics

The abnormalities found in CSF of untreated patients with tuberculous meningitis are well described. Usually, there is a predominant lymphocytic reaction (60–400 white cells per ml) with raised protein levels (0.8–4 g/l). In the early stages of infection, a significant number of polymorphonuclear cells may be observed, but over the course of several days to weeks they are typically replaced by lymphocytes. There is a gradual decrease in the sugar concentration of the CSF, which is usually less than 50% of serum glucose concentration, the values may range between 18–45 mg/dl (Molavi & LeFrock, 1985),(Berger, 1994; Newton, 1994). Definitive diagnosis of Tuberculous meningitis depends upon the detection of the tubercle bacilli in the CSF, either by smear examination or by bacterial culture. It has been claimed that if large volumes of CSF are carefully examined the organism can be found in over 90% of centrifuged CSF specimens, the highest detection rates being achieved in ventricular fluid. With repeated examinations of sequential CSF examinations Kennedy and Fallon reported tubercle bacilli in 87% of patients.(Kennedy & Fallon, 1979) In other series especially from

developing countries bacteriological confirmation of the diagnosis could be achieved in as few as 10% of the cases (Molavi & LeFrock, 1985). Cultures of the CSF for tubercle bacilli are not invariably positive. The sensitivity of CSF culture for MTB varies (60–70% in adults) (Hosoglu et al., 2002; Thwaites, Chau, & Farrar, 2004). It requires several weeks before culture is positive for mycobacterium bacilli. Because of frequent difficulty in detecting tubercle bacilli in smears or cultures of CSF, a number of tests have been developed to establish an early and definitive diagnosis (Daniel, 1987). In the past, radioactive bromide partition testing and identification of components of the mycobacterial cell wall (e.g. tuberculostearic acid) have been reported to have a sensitivity and specificity over 90%. However, the clinical utility of these has not been found to be satisfactory. Antibodies against tubercle bacilli can be detected with enzyme-linked immunosorbent assay (ELISA) with variable success. The latex particle agglutination test, which allows the rapid detection of tubercle bacillus antigen in CSF, has been reported to be a simple and specific test. The intradermal tuberculin skin test is helpful when positive. The test may, however, be falsely negative even in the absence of immunosuppression and in association with a positive reaction to common antigens used to determine anergy (eg, Candida and mumps).

2.6.3 Imaging

Computed tomography (CT) or magnetic resonance imaging (MRI) of the brain may reveal thickening and intense enhancement of meninges, especially in basilar regions. Ventricular enlargement is present in a majority of patients. The degree of hydrocephalus correlates with the duration of the disease. Infarcts are another characteristic imaging feature of Tuberculous meningitis (Ahuja, Mohan, Prasad, & Behari, 1994). The reported frequency of infarcts demonstrated by CT varies from 20.5% to 38%, however, in general, the incidence of infarction is significantly higher

on MRI than on CT. In addition, a large number of infarcts are seen to be haemorrhagic in nature on MRI, a finding not well documented on CT scan. The majority of infarcts are seen in thalamic, basal ganglion, and internal capsule regions (Berger, 1994). Thick basilar exudates appear as intensely enhancing areas in the basal cisterns (spider-leg appearance) and in the sylvian fissures (Ahuja et al., 1994). Tuberculomas are infrequently seen on CT or MRI of patients with tuberculous meningitis. Davis et al found tuberculomas in 16% of patients with culture-positive or presumptive tuberculous meningitis (Davis, Rastogi, Lambert, & Skipper, 1993). Multiple small intracranial tuberculoma are frequent when tuberculous meningitis is part of military tuberculosis (Eide, Gean, & So, 1993; Gee, Bazan, & Jinkins, 1992). The carotid or MR angiogram shows changes in vessels of the circle of Willis. These changes include uniform narrowing of large segments, small segmental narrowing, irregular beaded appearance and complete occlusion (Jinkins, Gupta, Chang, & Rodriguez-Carbajal, 1995). These vascular changes are due to either vasculitis or mechanical compression by the basilar exudate.

2.6.4 Nucleic acid amplification tests

The first NAATs for use on CSF specimens were described over two decades ago,(Kaneko, Onodera, Miyatake, & Tsuji, 1990)-(Shankar et al., 1991) and there have been numerous in house polymerase chain reaction (PCR) assays developed since. The design and performance of these are heterogeneous, however, which makes comparing them difficult. This issue was highlighted by a meta-analysis of the diagnostic accuracy of commercial and in-house NAATs for TBM diagnosis (Pai et al., 2003). Commercial assays were found to be insensitive for detecting MTB in CSF samples (sensitivity 56% and specificity 98%), whereas no useful comparative information could be obtained for in-house PCRs (Pai et al., 2003). Subsequently, a number of in house PCRs using a

variety of targets (IS6110, INS, protein b, rpoB, MPB64) and testing platforms have been described. They have an improved overall performance, especially with the use of multiplex PCRs, compared with commercial assays (sensitivity 71–94% and specificity 88–100%) (Bhigjee et al., 2007)·(Chaidir et al., 2012; Huang et al., 2009; Kusum et al., 2011; Rafi et al., 2007; Sharma et al., 2010). NAATs have a distinct advantage over microscopy and culture, in patients already on antituberculous therapy as the DNA remain detectable for up to a month after starting treatment (Donald, Victor, Jordaan, Schoeman, & van Helden, 1993; Thwaites, Caws, et al., 2004).

2.6.5 GeneXpert and Tuberculous meningitis

In 2010, the WHO endorsed the use of GeneXpert, a rapid fully automated NAAT for use on sputum specimens in resource-constrained TB endemic countries, at concessional pricing (WHO, 2010a). The main advantages of GeneXpert over pre-existing molecular assays are its simplicity of use – it requires minimal technical expertise and biosafety requirements and the low rates of cross-contamination. It also has the advantage of rapid rate of results which can be available in 2 hours. Another key advantage is its ability to rapidly detect rifampicin resistance and hence predict multidrug resistant disease (MDR). GeneXpert has been extensively evaluated for MTB detection in sputum specimens and performs well on smear-positive samples (sensitivity 98% compared with 68% in smear-negative samples; specificity 98%) (Steingart et al., 2013). Preliminary studies on a range of extrapulmonary TB samples have also been promising in smear-positive samples (sensitivity 96–100% vs. 37–90% in smear-negative specimens; specificity 98–100%) (Causse et al., 2011; Vadwai et al., 2011).

For CSF samples specifically, few studies have been performed with variable results. Even fewer studies have been done in countries with high TB burden or Africa (Nhu et

al., 2014; Vinod B. Patel et al., 2013; Tortoli et al., 2012). Patel et al in South Africa evaluated the incremental value of GeneXpert as compared to a clinical score, smear microscopy and culture. He found GeneXpert had a sensitivity and specificity of 82% and 95% respectively among centrifuged CSF samples when compared to MGIT culture (Vinod B. Patel et al., 2013). Tortoli et al in Italy compared GeneXpert, culture and a composite Gold standard consisting of culture and clinical diagnosis on extrapulmonary samples of TB suspects (Tortoli et al., 2012). TBM was diagnosed in 14 patients according to the composite Gold standard. GeneXpert was positive in 12 out of the 14 samples demonstrating a sensitivity of 85.7%. A study done in Vietnam by Nhu et al tested cerebrospinal fluid samples using Ziehl-Neelsen smear, mycobacterial growth indicator tube (MGIT) culture, and GeneXpert, Of the 121 patients who had a positive culture result, 103 tested positive using GeneXpert giving a sensitivity of 81.5%. During the study, he introduced a vortexing step after addition of sample reagent while processing the sample for GeneXpert analysis. This led to a 20.6% increase in sensitivity of GeneXpert for the diagnosis of definite TBM

In 2013 the World Health Organization (WHO) commissioned a systematic review to inform the recent update of the WHO policy on GeneXpert, aimed to assess the diagnostic accuracy of GeneXpert for TB detection in non-respiratory samples in adults and children(Denkinger et al., 2014; WHO, 2013a). Based on this meta-analysis, WHO recommends that GeneXpert should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis (WHO, 2013a). This is a strong recommendation given the urgency of rapid diagnosis in this condition but with very low quality evidence.

2.6.6 Consensus tuberculous meningitis diagnosis

The definition of TBM when used in clinical research has varied from study to study (Quan et al., 2006; Thwaites, Caws, et al., 2004). In most definitions, patients are given a definite, probable, or possible tuberculous meningitis status depending on clinical, laboratory, and radiological findings. Definite tuberculous meningitis cases usually include patients with AFB on CSF microscopy or M tuberculosis cultured from CSF or another CNS source. Criteria for probable or possible tuberculous meningitis cases differ greatly between studies. This necessitated a uniform case definition for use in clinical research reached by consensus by TBM experts worldwide in a conference in Cape Town, South Africa as described by Marais et al (Marais et al., 2010).

The uniform case definition scoring system has 20 parameters, which are divided in 4 categories (clinical, CSF, CNS imaging and evidence of TB elsewhere) with a maximum score of 20 (see appendix V). A definite diagnosis of TBM is made if there is evidence of Acid Fast Bacilli (AFB) in CSF smear, culture or on histopathology of brain or spinal cord. A probable diagnosis is made if the total score is >10 points if patients have no imaging, or >12 points if imaging was used. A possible diagnosis is made with scores between 6-9 without imaging or 6-11 with imaging. Based on the total scores assigned, the diagnosis of TBM is either definite, probable, possible or not present.

This consensus definition has been used successfully in research on TBM in various studies worldwide (Haldar et al., 2012; Ho Dang Trung et al., 2012; V. B. Patel et al., 2010; Pehlivanoglu, Kart Yasar, & Sengoz, 2012).

2.7 Rifampicin resistance and MDR TBM

Rifampicin resistance among cases of pulmonary tuberculosis in Kenya has been reported to be 0.81-1.4% (Ndung'u, Kariuki, Ng'ang'a, & Revathi, 2012; Ogaro et al., 2012). There is no reported prevalence of rifampicin resistance among TBM patients in the country. Multidrug resistant tuberculosis is mycobacteria resistant to at least isoniazid and rifampicin. In Kenya, 2.6% of new cases and 13% of previously treated patients in 2013 had MDR-TB (WHO, 2013c). As most Rifampicin-resistant isolates are also resistant to isoniazid, Rifampicin resistance can be used as a marker for MDR TB (Mokrousov et al., 2003; Prammananan et al., 2008; Traore et al., 2000). Patel et al in Kwa Zulu Natal, South Africa found a prevalence of MDR TB of 8.6% among patients with culture positive TBM which was considerably higher than in pulmonary Tuberculosis among new patients (1-2%) or those previously treated for TB (7-8%) (V. B. Patel et al., 2004). MDR tuberculous meningitis is a difficult-to-treat infection with a high fatality rate (Daikos et al., 2003; V. B. Patel et al., 2004).

GeneXpert can detect the presence of Rifampicin resistance which is used as a marker of MDR-TB. Initial validation studies reported 100% specificity for the detection of rifampicin resistance (Boehme et al., 2010). More recent studies have shown specificity to be much lower than this,(Carriquiry et al., 2012) prompting modifications in product software (Diagnostics, 2011). While GeneXpert technology has allowed increased decentralization of drug-resistance detection, all detected rifampicin resistant isolates should ideally be confirmed with conventional DST to detect false-positive results and in addition, concomitant isoniazid testing is required to avoid potential miss-assignment of MDR disease (WHO, 2011).

2.8 Treatment regimens

The World Health Organization (WHO) put CNS tuberculosis under TB treatment Category 1, and recommends initial phase therapy for 2 months with Streptomycin, isoniazid, rifampicin and pyrazinamide, followed by a 4-month continuation phase with isoniazid and rifampicin (Initiative, 2010). However some experts recommend 9–12 months of treatment for TB meningitis given the serious risk of disability and mortality (Blumberg et al., 2003; National Collaborating Centre for Chronic Conditions (UK)., Centre for Clinical Practice at NICE (UK). (2011))

All patients with TBM may receive adjunctive corticosteroids regardless of disease severity at presentation. Adults (>14 years) should start treatment with dexamethasone 0.4 mg/kg/24 hours with a tapering course over 6 to 8 weeks (Prasad & Singh, 2008).

CNS tuberculosis in HIV infected patients should be managed with the same anti-tuberculosis drug regimen as that recommended for HIV uninfected individuals. Adjunctive corticosteroids are recommended for those with TBM and HIV infection though data is more limited (WHO, 2010b). Starting anti-retroviral therapy depends upon balancing the risks of drug interactions and Immune Reconstitution Inflammatory Syndrome (IRIS) when started early and opportunistic diseases if delayed. WHO recommends early initiation of ARVs in all patients who have tuberculosis and HIV including TBM (WHO, 2013b).

2.9 Prognosis and sequelae

The single most important determinant of outcome, for both survival and sequelae, is the stage of tuberculous meningitis at which treatment has been started (Alvarez-Uria et al., 2012). If treatment is started in stage I, mortality and morbidity is very low, while in stage III almost 50% of patients die, and those who recover may have some form of

neurological deficit (Holdiness, 1990). About 20% to 30% of survivors manifest a variety of neurological sequelae, the most important of which are mental retardation, psychiatric disorders, seizures, blindness, deafness, ophthalmoplegia and hemiparesis. Endocrinopathies may become evident months or year after recovery. The endocrinopathies are most probably due to progressive damage of either the hypothalamus itself or adjacent basal cisterns. Obesity, hypogonadism, Frolich syndrome, sexual precoccity, diabetes insipidus, and growth retardation have been reported. Intracranial calcification develops in 20% to 48% of patients with tuberculous meningitis, usually becoming detectable 2 to 3 years after the onset of the disease (Molavi & LeFrock, 1985; Wallace et al., 1991).

Chapter Three: Materials and Methods

3.1 Study Site

Moi Teaching and Referral Hospital, Medical wards. MTRH is located in Eldoret, 350 km northwest of Nairobi and serves a network of 26 referring district hospitals encompassing over 10 million people from the provinces of North Rift Valley, Western and Nyanza.

The medical wards have two wards i.e. Umoja and Amani wards. Umoja wards admits male patients while Amani ward admits female patients. Each ward has a bed capacity of 42 to cater for the large number of in-patients referred to the facility

3.2 Study Population

Patients newly admitted with features of suspected tuberculous meningitis who have no contraindication to LP.

3.3 Study Design

Cross Sectional Study

3.4 Case Definition

Case definition as described by the consensus case definition for TBM which includes definite, probable, possible and not TBM.(Marais et al., 2010)

3.5 Inclusion Criteria

- Males or females older than 18 years in medical wards who have at least one of the following neurologic findings (headache, fever, Neck pain and rigidity, Confusion/altered mentation, convulsion, photophobia, cranial nerve palsies) plus one of the following:

- Symptom duration of more than 5 days
- Patients treated for bacterial meningitis for 1 week with no improvement.
- Systemic symptoms suggestive of tuberculosis: weight loss, night sweats, or persistent cough for more than 2 weeks
- History of recent (within past year) close contact with an individual with pulmonary tuberculosis.

– Informed consent from the patient or next of kin where the patient is unable to give consent.

3.6 Exclusion Criteria

– Contraindication to lumbar puncture
– Patients who have taken more than one dose of anti-tuberculous drugs (Rifampicin, Isoniazid, Ethambutol and Pyrazinamide).

3.7 Sample size determination

The total number of patients of patients with TBM in 2012 in MTRH is approximated as follows:

Total admissions in medical wards in 2012:	8898 patients
Prevalence of Meningitis among admitted patients in MTRH:	6.6 % (Lagat, 2010)
Prevalence of TBM among patients with meningitis in MTRH:	12% (Lagat, 2010)
Approximate number of patients with TBM in MTRH in 2012: (8898 *0.066*0.12)	71 patients

Assuming that the total number of patients with TBM at MTRH in 2013-2014 was similar to 2012, the normal approximation to the hypergeometric distribution formula which is used to determine sample size for small populations was used (Evan, 2008).

$$n = \frac{NZ^2pq}{E^2(N-1)+Z^2pq}$$

Where n=sample size; N= the population size, Z= the z value corresponding to 95% confidence (1.96); P = estimated sensitivity; E= the accuracy of sample proportions (5%).

Using an accuracy of 5% and estimated sensitivity of 85% (Tortoli et al., 2012) **a minimal sample of 56** patients was obtained. Patients were sequentially recruited until the desired sample size was achieved.

3.8 Study Procedure

1. 5-10 mls of CSF was collected and divided into 3 tubes: 1ml in the first two tubes for routine Microscopy/Culture/Sensitivity and biochemistry at MTRH laboratory, and the rest in a third tube for culture and GeneXpert
2. The specimen for GeneXpert and culture was transported to the AMPATH Mycobacteria Reference Laboratory in under one hour
3. The specimen was then centrifuged at 3000rpm for 20 min and the sediment re-suspended in 1 ml supernatant.
4. 0.5 ml of the re-suspended sediment was inoculated into a MGIT tube and processed according to Appendix V– BBL ™ MGIT™ procedure
5. The remaining 0.5 ml of re-suspended sediment was processed by GeneXpert according to the GeneXpert procedure (Appendix VI).

The study procedure is summarized in the algorithm below

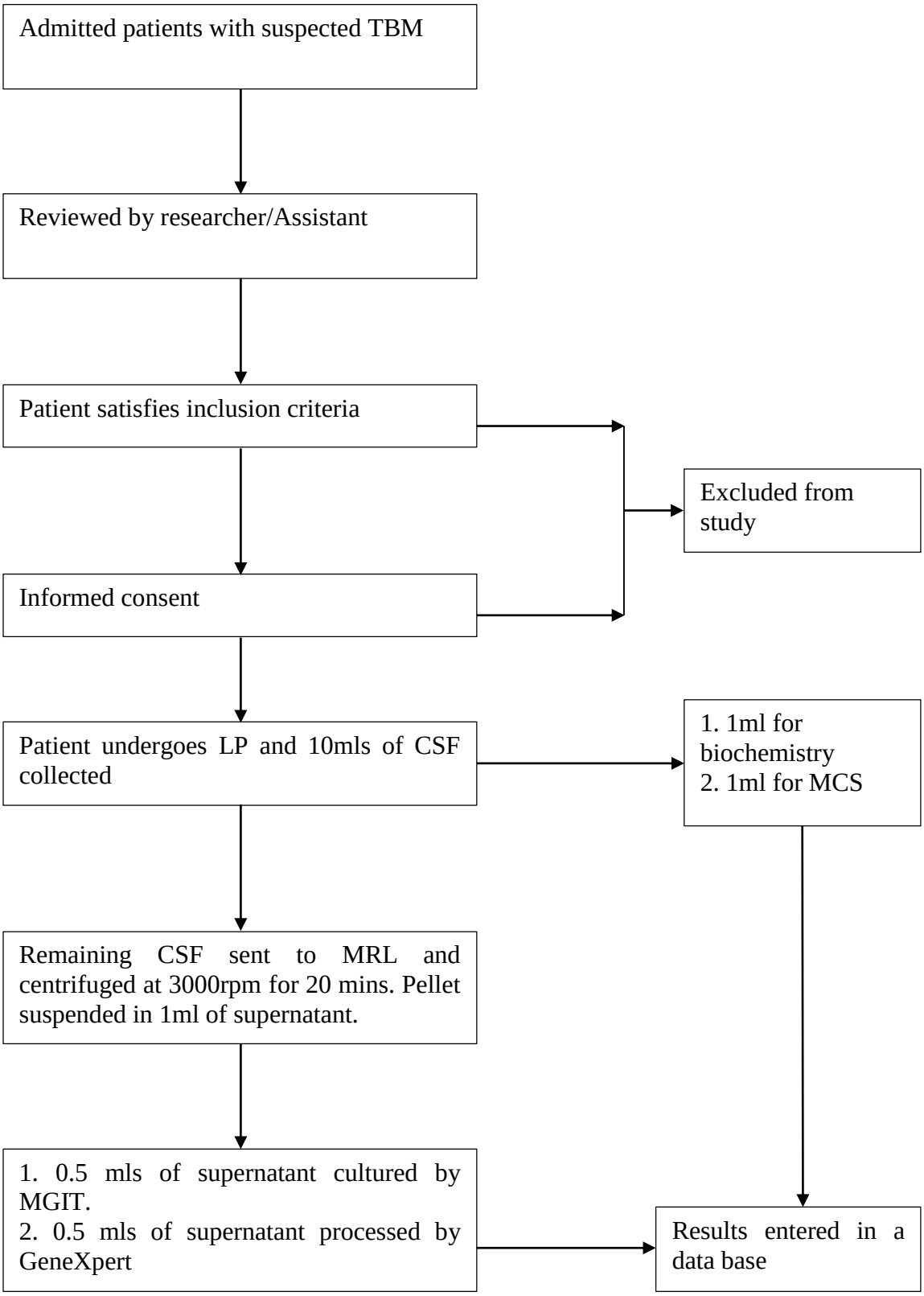

Figure 1: Algorithm of the study procedure

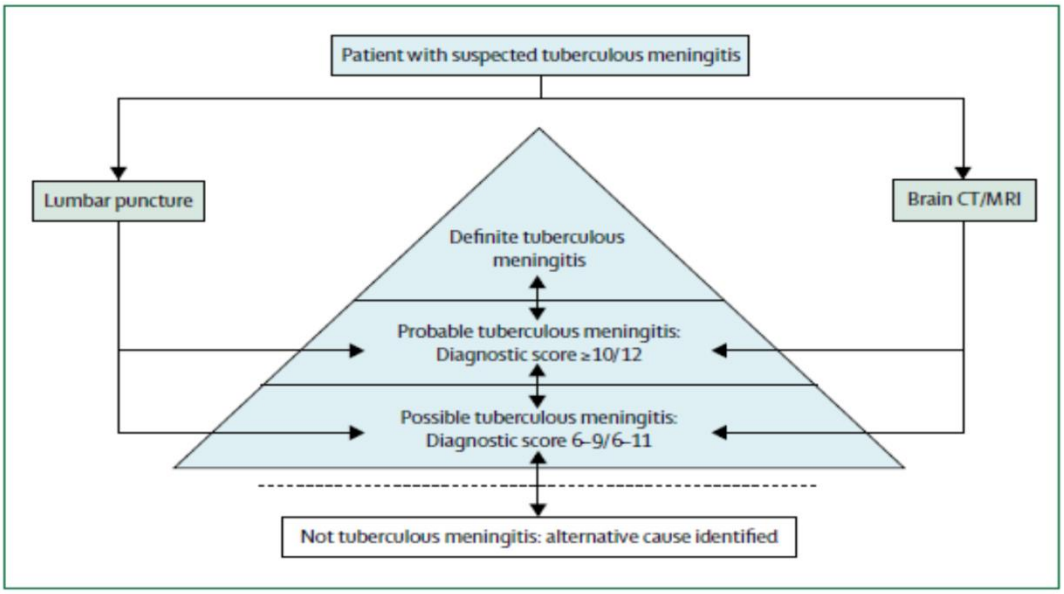

Figure 2: Categories for patients with suspected tuberculous meningitis

Patients suspected with tuberculous meningitis enter the study after lumbar puncture or brain imaging has been done in those meeting required criteria. Patients then move up or down the diagnostic pyramid as subsequent results become available and are classified into definite, probable, possible, or not tuberculous meningitis according to diagnostic criteria (Marais et al., 2010).

3.9 Data Collection and Analysis

Data analysis was done using STATA version 13 SE. Categorical variables were summarized as frequencies and corresponding percentages. Continuous variables that assumed Gaussian distribution were summarized as mean and the corresponding standard deviation (SD). Continuous variables that violated the Gaussian assumptions were summarized as median and the corresponding inter quartile range (IQR). Gaussian assumptions were assessed empirically as well as graphically using Shapiro-Wilk normality test, and normal probability plots respectively. Association between categorical variables was assessed using Pearson's Chi Square test while the association

between categorical and continuous variables was assessed using two sample Wilcoxon rank sum test. The variables that were associated with GeneXpert test outcome were incorporated into a logistic regression model to assess the magnitude and the direction of the effect. We reported the odds ratios (OR) and the corresponding 95% confidence limits.

3.10 Ethical Considerations

Approval was sought and obtained from the IREC and MTRH (Appendix 8.7 and 8.8). Informed verbal and written consent was sought from all patients before the procedure. For the illiterate patients, the researcher explained the contents of the consent form, and if the patient consents, a thumb print was used as an alternative to a written signature. Patients were informed about the outcome of result and counseled accordingly depending on the outcome. TBM positive patients were informed about the presence of infection and the same result was communicated to the attending clinician to initiate appropriate therapy. Costs for culture and GeneXpert and the writing material used to record results was paid for by the investigator. Patients were not given any inducements to participate in the study.

All patients participating in the study did so by free will and those who declined to participate were not discriminated against. They underwent the LP procedure for diagnostic purposes.

The GeneXpert testing procedure did not confer additional procedure risks other than those of LP itself for which the patient was referred. Instead, there was benefits from the results since it could change the management of the patient significantly.

All information from the procedure was handled confidentially. Only patient codes was used in the data entry form and no reference to their names was made. The referring

physician was informed of the results and the recommended way forward in the management of the patients.

3.11 Dissemination of results

The results of the study will be disseminated through a written thesis and an oral defense in a forum that shall be convened by the school of medicine. The results will also be shared with MTRH and published in a peer-reviewed journal.

Chapter Four: Results

4.1 Screening and enrolment.

A total of 132 patients who were admitted in the medical wards at MTRH were screened during the study period. Of all the patients screened: 31 patients did not meet the inclusion criteria (10 patients had taken more than one dose of anti TB drugs and 21 patients had various contra-indications to LP). 101 patients were enrolled in the study and had an LP done. 8 patients had a dry tap and were excluded from the study. The results of 93 patients were included in the final analysis.

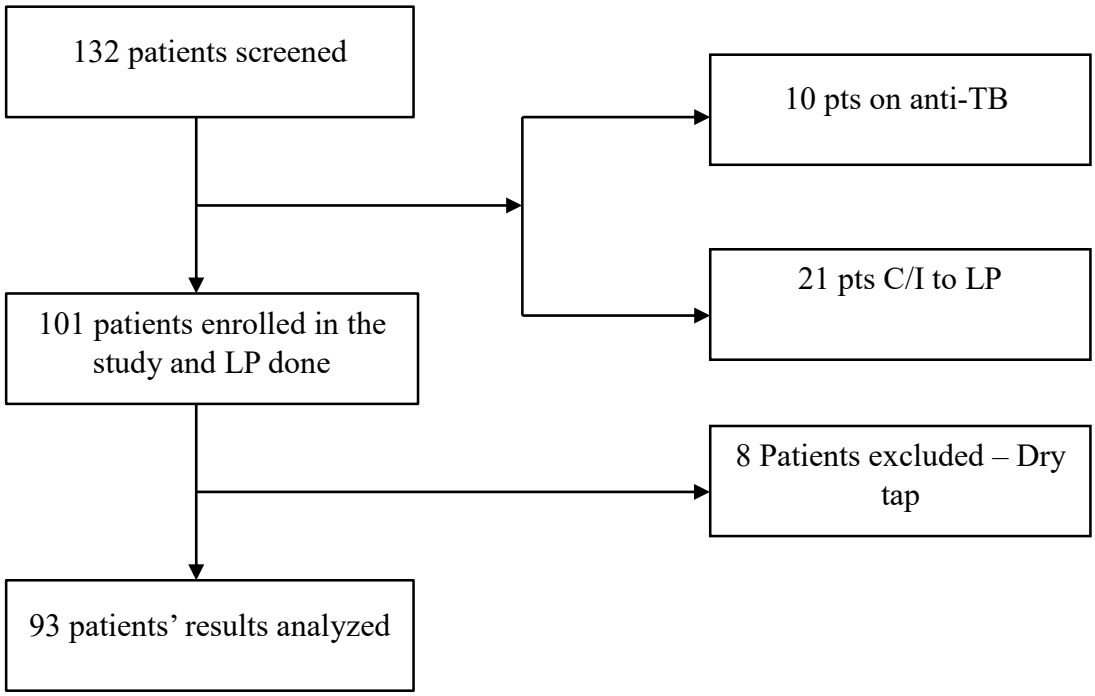

Figure 3: Recruitment Schema

4.2 Demographics and clinical characteristics

The complete socio-demographic and clinical picture of study participants is provided in Table 1. 57 (61%) were females and 77 (83%) were HIV positive. The median age was 38 (IQR: 26-46) years with a minimum and a maximum of 18 and 74 years respectively. The median symptoms duration was 2 (IQR: 1-3) weeks with a minimum and a maximum of 1 and 160 weeks respectively.

There were 23 (25%), 63 (68%), and 6 (7%) participants who were in MRC stages 1, 2, and 3 respectively. Sixty seven (72%), had a history of weight loss, 29 (31%) had night sweats, and 14 (15%) had persistent cough. There were 3 (3%) who had history of contact with someone with TB in the past one year. Focal neurological deficits were present in 9 (10%) participants. 74 (80%) participants had altered mental status/confusion.

4.3 CSF characteristics

There were 88 (95%) participants whose CSF had clear appearance while the rest had cloudy appearance. The median protein level was 85.3 mg/l (IQR: 29.0-164.0) while the median glucose levels was 2.3 mmol/l (IQR: 1.6-3.5). There were 8 (9%) participants who had a positive CSF GeneXpert result (MTB detected). Rifampicin resistance was detected in one of these participants 12.5% (95% CL: 3.2%-52.7%)). Ten (11%) of participants had a positive TB CSF culture. (Table 2).

Table 1: Demographic and clinical characteristics

Variable	Median (IQR) or n (%)
Male	36 (39)
HIV-infected	77 (83)
Median Age	38 (26-46)
Symptoms/Signs	
Weight loss	67 (72)
Night sweats	29 (31)
Persistent cough	14 (15)
Focal neuro-deficiency	2 (2)
Cranial palsy	7 (8)
Altered mentation/confusion	74 (80)
History of contact a person with TB in the last 1 year	3 (3)
Median symptom duration (weeks)	2 (1-3)
MRC stage	
Stage 1	23 (25)
Stage 2	63 (68)
Stage 3	6 (7)

4.4 Evidence of Tuberculosis elsewhere

Out of the 92 patients who had a chest x-ray, 37 (40%) had a chest X-ray suggestive of PTB and 2 (2%) had evidence of milliary tuberculosis on X-ray. Sputum AFB smear was positive in 4 (4%). (Table 3).

Table 2: CSF characteristics

Variable	**Number**	**Median (IQR) or n (%)**
Clear	93	88 (95)
Proteins (mg/l)	93	85.3 (29.0-164.0)
Glucose (mmol/l)	93	2.3 (1.6-3.5)
GeneXpert Positivity	93	8 (9)
Rifampicin resistance	8	1 (12.5)
MGIT liquid culture	93	10 (11)
Cryptococcus	93	4 (4)
Toxoplasmosis	93	1 (1)

Table 3: Evidence of TB elsewhere

Variable	**Sample size**	**N (%)**
Chest Xray suggestive of PTB	92	37 (40)
Milliary TB on Chest Xray	92	2 (2)
CT scan head (Tuberculoma)	2	2 (100)
Positive Sputum AFB smear	92	4 (4)

4.5 Consensus case definition for tuberculous meningitis

10 (11%) participants had a definite diagnosis for TBM using CSF mycobacterial culture as the gold standard. There were 27 (29%), and 49 (53%) who had probable and possible TBM respectively. Only 7 (8%) had no evidence for TBM. The median diagnostic score based on the standardized scoring system was 8 (IQR 7-11).

Table 4: Consensus case definition for TBM

Type of TB	93	Definite TB	10 (11%)
		Probable TB	27 (29%)
		Possible TB	49 (52%)
		No TB	7 (8%)
Diagnostic score	93		8 (7-11)

4.6 Yield of GeneXpert compared to MGIT liquid culture

Among 93 patients with suspected TBM, GeneXpert was positive in 8 (0.086) while MGIT liquid culture was positive in 10 (0.1163). 86 patients had TBM using the consensus case definition. In this category, 8 (0.093) patients had a positive GeneXpert result while 10 (0.1163) patients had a positive MGIT liquid culture. There was no statistical difference between the positivity rates of GeneXpert and MGIT liquid culture among patients with suspected TBM and also in patients with TBM as per the consensus case definition.

4.7 Measures of accuracy of GeneXpert with MGIT culture as the Gold standard

The sensitivity of GeneXpert was found to be 80%. There was a false negative rate of 20%. The specificity of GeneXpert was established to be 100% since all those who had

a CSF GeneXpert positive for MTB also had positive mycobacterial cultures. The positive predictive and negative predictive values of GeneXpert were 100%, and 98%, respectively.

Table 5 Yield of GeneXpert compared to MGIT liquid culture

Test Population	N	GeneXpert +ve (95% C.I)	MGIT culture +ve (95% C.I)	p value
Suspected TBM patients	93	8 (0.086) (0.029-0.143)	10 (0.1075) (0.0445-0.1705)	0.06199
TBM CCCD	86	8 (0.093) (0.0316-0.1544)	10 (0.1163) (0.0485-0.1841)	0.06177

Table 6: Measures of accuracy of GeneXpert

	Liquid Culture		
GeneXpert	**MTB Present**	**MTB not present**	**Total**
MTB positive	8 (80%)	0	8
MTB negative	2 (20%)	83 (100%)	85
Total	10	83	93

4.7 Factors associated with GeneXpert positivity

There was no association between GeneXpert positivity and any of the following demographical and clinical, laboratory or imaging characteristics: Age (P = 0.983), sex (P = 0.640), symptoms duration (P = 0.780), HIV positivity (P = 0.632), MRC stage (P = 1.000), weight loss (P = 0.340), night sweats (P = 0.459), persistent cough (P = 0.239), history of contact with someone with TB (P = 0.751), Focal neurological deficit (P = 0.173), cranial palsy (P = 0.495), altered mentation/confusion (P = 0.490), CSF

appearance (P = 0.062), Milliary chest X-ray (P = 0.825), CT/MRI/US suggestive of TB (P = 0.825), and sputum AFB smear results(P = 0.749)

The level of proteins among the participants who screened positive for GeneXpert was significantly higher than that of GeneXpert negative participants, median: 233.0 (IQR: 116.5-342.2) vs. 77.0 (IQR: 26.8-143.0), P = 0.003.

The glucose level was significantly lower among those who were positive for GeneXpert compared to those who were negative for GeneXpert, median: 1.5 (IQR: 1.0-2.1) vs. 2.6 (IQR: 1.8-3.6), P = 0.015.

A significantly higher proportion of those who were GeneXpert positive compared to those who were negative had chest X-ray with features suggestive of TB, 6 (75%) vs. 31 (37%), P = 0.044.

The diagnostic score was significantly higher among those who had a positive GeneXpert result compared to those who had a negative GeneXpert result, median: 11 (IQR: 10-11) vs. 8 (IQR: 7-11), P = 0.024.

The variables that were significant in the univariate analysis were then included in the logistic regression model to assess the direction and the magnitude of the effect. These variables included protein levels, glucose, chest X-ray suggestive of PTB, and diagnostic score. Three continuous variables (Elevated CSF proteins, low CSF glucose and diagnostic score) were significant in the model but none of the variables was independent of the others. Due to small numbers, chest X-ray for suggestive of PTB was not statistically significant in the model. (Table 7)

Table 7: Logistic regression model

Variable	**Sample size**	**Odds Ratio (95% CL)**
Elevated Proteins	93	1.64 (1.09, 2.46)
Decreased Glucose	93	2.56 (1.03, 6.67)
Chest Xray suggestive of PTB	92	5.13(0.97, 26.99)
Diagnostic score	93	1.54(1.03, 2.30)

Chapter Five: Discussion

5.1 Yields of GeneXpert Vs MGIT Liquid culture

The diagnostic yield of GeneXpert was comparable to that of MGIT liquid culture (8/93 (0.086) vs 10/93 (0.1075) 95% C.I) p=0.06199. Van Zyl-Smit et al in 2011 compared the detection threshold of GeneXpert and MGIT liquid culture using serial dilutions of Mycobacterium bovis and Mycobacterium tuberculosis H37RV (van Zyl-Smit et al., 2011). He found the detection threshold of GeneXpert to be between 80 and 100 organisms per sample as compared to MGIT culture, where the detection threshold was as low as 1–10 organisms per milliliter. From this study MGIT culture is expected to have a higher yield compared to GeneXpert. However, various studies done have showed using high CSF volumes and centrifugation of the sample improved sensitivity of GeneXpert and this may have contributed to the comparable yields between the two diagnostic tests (Denkinger et al., 2014; Nhu et al., 2014; Vinod B. Patel et al., 2013).

5.2 Sensitivity and specificity of GeneXpert with MGIT culture as Gold standard

In this study, we found GeneXpert had a sensitivity of 80% and a specificity of 100% as compared MGIT culture. The sub optimal sensitivity of GeneXpert as compared to MGIT culture can be attributed to the different levels of detection where (van Zyl-Smit et al., 2011). Our results were comparable to several studies. Patel et al in South Africa found a sensitivity and specificity of 82% and 95% respectively among centrifuged CSF samples from HIV positive patients (Vinod B. Patel et al., 2013). Tortoli et al in Italy had a sensitivity and specificity of 84.6% and 98% respectively (Tortoli et al., 2012). Nhu et al in Vietnam found a sensitivity and specificity of 85.1% and 98% respectively among CSF samples (Nhu et al., 2014). He added a brief vortexing step after addition

of sample reagent during preparation for GeneXpert analysis. This increased the sensitivity of GeneXpert in the diagnosis of definite TBM by an additional 20.6% (P = 0.04). The similarity in results between our study and these studies may be due to similar preparation methods where CSF was centrifuged before it was processed for GeneXpert analysis and high volumes of CSF were used in the analysis. The meta-analysis commissioned by WHO and done by Denkinger et al analyzed 13 studies (839 samples; 10 with >10 samples, 159 culture positive) that evaluated Xpert in CSF against culture (Denkinger et al., 2014). Sensitivity varied widely (0–100%), with a pooled sensitivity of 80.5% (95% CI 59.0–92.2%) (Table 8)

Table 8: GeneXpert sensitivity and specificity for tuberculosis detection in cerebrospinal fluid with culture as the reference standard

Study	N	Sensitivity	Specificity
Nhu (Vietnam 2014)	379	.85	.98
Tortoli (Italy 2012)	133	.85	.98
Nyukuri* (Kenya 2015)	**93**	**.80**	**1.0**
Causse (Spain 2011)	50	.83	1.0
Patel[x] (S. Africa 2013)	46	.82	.95
Zeka (Turkey 2011)	31	1.0	1.0
Vadwai (India 2011)	19	0	0
Malbruny (France 2011)	15	1.0	1.0
Moure (Spain 2012)	14	1.0	1.0
Hanif (Kuwait 2011)	5	1.0	1.0

* Study under review, [x] Centrifuged samples

5.3 Sensitivity and specificity of GeneXpert with consensus case definition as gold standard

When GeneXpert was compared to the consensus case definition the sensitivity was 9%. The reduced sensitivity was also apparent when MGIT culture was compared to the consensus case definition (11.6%). This reduced sensitivity was greater than that observed by Patel et al who demonstrated a sensitivity of 65% when comparing GeneXpert to a clinical score. The difference can be attributed to the scoring system that was used to define probable, possible and no TBM. In our study we used the consensus case definition developed by Marais et al (Marais et al., 2010). Patel et al developed a clinical score for their study using factors significantly associated with definite TBM in HIV-infected individuals from a multiple logistic regression model, and the scores were proportionally weighted to the level of significance assigned. They chose a cut point with a high specificity, so as to give a good rule-in test so that the performance of the clinical assessment was directly comparable to the performance of the diagnostic assays under study. This may have disproportionately selected patients more likely to have a positive GeneXpert and culture result as compared to the consensus case definition. Tortoli et al demonstrated a sensitivity of 85% and specificity of 100% when comparing GeneXpert and a composite reference standard as the gold standard. In Tortoli's study, culture negative patients with radiological and/or histological signs suggesting TB were considered TB cases only if there was documented clinical improvement after anti-TB treatment was initiated. Patients not treated for TB, or those who demonstrated no improvement after treatment, were not considered TB cases. This was also a highly selected group that was more likely to be GeneXpert and/or culture positive as compared to the consensus case definition. Nhu et al used the standard case definition and found GeneXpert had a sensitivity of 59.3%.

However, he did a more extensive work up on patients with probable/possible TBM and he was able to come up with alternative diagnosis in this patient group. We were limited in our study by lack of finance, laboratory support and imaging needed to do a more comprehensive work up. In the meta-analysis by Denkinger et al five studies (711 samples) that assessed Xpert in CSF samples versus a composite reference standard (CRS) found variable sensitivity (20–86%). Pooled sensitivity was 62.8% (95% CI 47.7–75.8%) and pooled specificity was 98.8 (95% CI 95.7–100%) (Table 9).

Table 9: GeneXpert sensitivity and specificity for tuberculosis detection in cerebrospinal fluid compared with a composite reference standard

Study	**N**	**Sensitivity**	**Specificity**
Nhu (Vietnam 2014)	379	.72	1.0
Tortoli (Italy 2013)	133	.86	.99
Nyukuri (Kenya 2015)	**93**	**.09**	**1.0**
Patel (S. Africa 2013)	53	.65	.95
Zeka (Turkey 2011)	31	0.6	1.0
Vadwai (India 2011)	19	0.20	1.0

5.4 Rifampicin resistance

Rifampicin resistance was detected in one patient by GeneXpert in this study. As per national TB program guidelines, the patient was started on MDR TB treatment and she had marked improvement with resolution of symptoms early on during her treatment. Unfortunately she passed away at month 5 of treatment after deterioration for two weeks. The exact cause of deterioration and death is unknown since a post mortem was not carried out. Nhu et al detected 4 Rifampicin resistant cases out of 108 GeneXpert positive samples (7.4%). He did not draw any robust conclusions about the sensitivity

of Xpert for the diagnosis of MDR TBM given the low prevalence of MDR TBM in their study. The overall low prevalence of MDR TB in our setting may explain our findings.

5.5 Factors associated with GeneXpert positivity

Low CSF glucose, high CSF proteins and a high overall score by the consensus case definition were associated with a higher odds of turning positive for GeneXpert and culture. However, our study was not powered to make associations that were statistically significant with other potential predictors. In the study conducted by Patel et al Cryptococcal latex agglutination test, CSF:plasma glucose ratio, CD4 count, Lymphocyte count and Hydrocephalus were identified as factors predictive for TBM in HIV infected patients. He did not specifically look for factors that were likely to predict a positive GeneXpert test.

5.6 WHO recommendations

In 2013 the World Health Organization (WHO) commissioned a systemic review to inform the recent update of the WHO policy on GeneXpert, aimed to assess the diagnostic accuracy of GeneXpert for TB detection in non-respiratory samples in adults and children(Denkinger et al., 2014; WHO, 2013a). Based on this meta-analysis, WHO recommends that GeneXpert should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis (WHO, 2013a). This was despite the low quality of evidence available at that time. The recommendation was made because of the urgency for a rapid diagnosis in this disease. The clear advantage of GeneXpert is that results are available within 2 hours, allowing definitive management to be initiated sooner and with more confidence.

This review was done after this study had commenced and data collection was on going but our results support the recommendation made by WHO.

5.7 Limitations

Exclusion of patients with contra-indications to LP may have reduced the number of potential patients with a positive GeneXpert and MGIT culture results as they may have been more likely to have TBM.

Patients were recruited based on a clinical criteria which may have excluded some patients with TBM.

Due to logistical constrains, we performed a limited number of tests on the CSF samples to evaluate for other conditions. This likely contributed to the large number of patients with probable or possible TBM who may have had an alternative diagnosis.

Chapter Six: Conclusion and Recommendations

6.1 Conclusion

There was no statistical significant difference between the yield of GeneXpert and MGIT liquid culture in CSF samples of patients presenting with suspected TBM.

GeneXpert has a good sensitivity (80%) and an excellent specificity when compared to MGIT liquid culture.

Prevalence of Rifampicin resistance was 12.5% among patients with a positive GeneXpert test

Patients with high CSF protein count, low CSF glucose and a high diagnostic score were more likely to have a positive GeneXpert result.

6.2 Recommendations

We concur with the WHO recommendations that GeneXpert should be used as the initial diagnostic test in place of culture in CSF samples.

Given that there is no perfect clinical criteria that can predict which patient with meningitis has TBM, a larger study that includes GeneXpert testing for all patients with suspected meningitis might be useful in further defining the population in which this limited resource should be utilized.

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Appendices

Appendix I Data Entry Form

1. Patient Number ☐

   Age ☐ Gender ☐

   Telephone No ☐ Address ☐

2. Has the patient been on any of the following medications for more

   Rifampicin ☐ Ethambutol ☐ Pyrazinamide ☐

   Isoniazid ☐ Fluoroquinolones ☐ Don't know ☐

3. Does the patient have any of these symptoms? (Tick where appropriate)

☐ Headache ☐ Neck pains ☐ Confusion/Altered mentation

☐ Convulsions ☐ Photophobia ☐ Cranial nerve palsies

Plus any of these (Tick where appropriate)

☐ Symptoms > 5 ☐ Systemic symptoms of TB

☐ Patient already in ward who is now suspected to have TBM

☐ History of close contact with individual with TB in last 1 year

4. Clinical Characteristics

   HIV Status Positive ☐ Negative ☐

   MRC Stage 1 ☐ 2 ☐ 3 ☐

5. CSF characteristics

☐ Clear ☐ Cloudy ☐ Blood stained

☐ Traumatic tap ☐ Others

6. GeneXpert results MTB detected Rif Resistant

Yes ☐ Yes ☐ No ☐

No ☐

7. Culture results

Positive ☐

Negative ☐

8. Biochemistry results

Proteins ☐ Glucose ☐

Crag ☐

9. Microscopy and culture ……..

……..

10. Cell count

Lymphocytes ……

Granulocytes ……

RBC ……

11. Imaging results

CT scan

……

MRI

……

Others

……

Appendix II Consent Form: English version

Patient Name

I am Dr. Nyukuri Duncan Wekesa; a student in the department of medicine, Moi university. I am carrying out a study to determine the utility of GeneXpert among patients admitted in medical wards at Moi Teaching and Referral Hospital with suspected TBM. TBM is an infection of the meninges (cover on top of the brain) that is caused by *Mycobacterium tuberculosis*-the same organism that causes tuberculosis of the pulmonary system. If diagnosed late or untreated it can lead to disability and death

The study will be done on the CSF which would have been taken for routine examination for meningitis. It involves a collection of your personal details like name, age and address and thereafter a lumbar puncture will be performed. Part of the CSF will go to our laboratory at MTRH for routine investigations and the rest will be taken for tests using GeneXpert and culture. You will not be charged any additional fee for the test. You will be informed of the test results as soon as they are available. The risks faced are the same as if you were undergoing LP for routine testing. You are free to withdraw from the study at any time before or after the procedure and your result from the study will be treated with confidence. Your withdrawing from the study does not interfere with you undergoing the LP.

I agree to participate in the study,

NAME_____ SIGN_____

(Patient/Guardian)

WITNESS_____ SIGN_____

Appendix III Consent form: Swahili version

Jina la mgonjwa

Mimi ni Daktari Nyukuri Duncan Wekesa; mwanafunzi katika idara ya Utabibu, Chuo kikuu cha Moi. Ninafanya utafiti kuona matumizi ya GeneXpert kati ya wagonjwa waliolazwa wakishukiwa kuwa na ugonjwa wa Kifua kikuu kilichoenea hadi kwa kichwa. Kifua kikuu kilichoenea hadi kwa kichwa kinasababishwa na viumbe vinavyosababisha kifua kikuu kifuani. Kama kutakua kuchelewa au kutotibiwa kwa ugonjwa huu inaweza kusababisha ulemavu na kifo.

Utafiti utafanyika kwa maji itakayotolewa kutoka kwa uti wa mgongo kwa vipimo vya kawaida unaposhukiwa una ugonjwa wa menengi. Inahusisha ukusanyaji wa maelezo yako ya kibinafsi kama umri, jina, anwani na baada ya hapo maji hiyo itatolewa. Sehemu ya maji hii itapelekwa kwenye maabara yetu ya MTRH kwa ajili ya uchunguzi wa kawaida na nyingine zitachukuliwa kwa ajili ya vipimo kwa kutumia GeneXpert. Hautatakiwa kulipa ada yoyote ya ziada kwa ajili ya kipimo hiki. Utaarifiwa juu ya matokeo ya vipimo hivi wakati majibu itakua tayari. Madhara yeyote yanayoweza kutokea ni sawa na ile ingekua kama unafanyiwa vipimo hivi kwa kawaida.

Una uhuru wa kujiondoa kutoka kwa utafiti huu wakati wowote kabla au baada ya kupimwa na matokeo yako yatawekwa vyema. Kujiondoa kwako kutoka utafiti haitazuia wewe kufanyiwa vipimo vya kawaida.

Mimi nimekubali kushiriki katika utafiti huu.

Jina SIGN_____

(Mgonjwa/Mchungaji)

Shahidi_____ SIGN_____

Appendix IV – Consensus tuberculous meningitis diagnosis

Clinical entry criteria

Symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiff ness, convulsions, focal neurological deficits, altered consciousness, or lethargy.

Tuberculous meningitis classification

Definite tuberculous meningitis

Patients should fulfill criterion A or B:

A) Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; *Mycobacterium tuberculosis* cultured from the CSF; or a CSF positive commercial nucleic acid amplification test.

B) Acid-fast bacilli seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

Probable tuberculous meningitis

Clinical entry criteria plus 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) plus exclusion of alternative diagnoses. At least 2 points should either come from CSF or cerebral imaging criteria.

Possible tuberculous meningitis

Clinical entry criteria plus a total diagnostic score of 6–9 points (when cerebral imaging is not available) or 6–11 points (when cerebral imaging is available) plus exclusion of

alternative diagnoses. Possible tuberculosis cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.

Not tuberculous meningitis

Alternative diagnosis established, without a definitive diagnosis of Tuberculous meningitis or other convincing signs of dual disease.

Table 10: Diagnostic criteria for classification of TBM

	Diagnostic score
Clinical criteria	(Maximum category score=6)
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for more than 2 weeks	2
History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
Altered consciousness	1
CSF criteria	(Maximum category score=4)
Clear appearance	1
Cells: 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2·2 mmol/L	1
Cerebral imaging criteria	(Maximum category score=6)
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere	(Maximum category score=4)
Chest radiograph suggestive of active tuberculosis: signs of tuberculosis=2; miliary tuberculosis=4	2/4
CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
AFB identified or *Mycobacterium tuberculosis* cultured from another source—ie, sputum, lymph node, gastric washing, urine, blood culture	4
Positive commercial *M tuberculosis* NAAT from extra-neural specimen	4
Exclusion of alternative diagnoses	
An alternative diagnosis must be confirmed microbiologically (by stain, culture, or NAAT when appropriate), serologically (eg, syphilis), or histopathologically (eg, lymphoma). The list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include: pyogenic bacterial meningitis, cryptococcal meningitis, syphilitic meningitis, viral meningo-encephalitis, cerebral malaria, parasitic or eosinophilic meningitis (*Angiostrongylus cantonesis*, *Gnathostoma spinigerum*, toxocariasis, cysticercosis), cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging) and malignancy (eg, lymphoma)	

TST=tuberculin skin test. IGRA=interferon-gamma release assay. NAAT=nucleic acid amplification test. AFB=acid-fast bacilli. The individual points for each criterion (one, two, or four points) were determined by consensus and by considering their quantified diagnostic value as defined in studies.

Table: **Diagnostic criteria for classification of definite, probable, possible, and not tuberculous meningitis**

Appendix V BBL ™ MGIT™ procedure

Concentration of specimen

If the specimen volume is more than 10 ml, concentrate by centrifugation at 3,000 x g for 15 min. Pour off supernatant fluid. Inoculate MGIT tube with sediment.

Inoculation of MGIT Tubes:

1. Label the MGIT tube with specimen number.
2. Unscrew the cap and aseptically add 0.5 ml of MGIT OADC.
3. Aseptically add 0.1 ml of reconstituted MGIT PANTA antibiotic mixture. For best results, the addition of OADC enrichment and PANTA antibiotic mixture should be made just prior to specimen inoculation.
4. Add 0.5 ml of the concentrated specimen suspension prepared above. Also add a drop (0.1ml) of specimen to a 7H10 agar plate or other mycobacterial solid agar or egg-based medium. NOTE: Specimen volumes greater than 0.5 ml can increase contamination or otherwise adversely affect the performance of the tubes.
5. Tightly recap the tube and mix well.
6. Tubes should be incubated at 37°C.
7. Read tubes daily starting on the second day of incubation following the procedure “Reading the Tubes” below.

Preparation of Interpretive Negative and Positive Control Tubes

Use of the Positive and Negative Control tubes is only for the interpretation of fluorescence and is not intended as a control for the performance of the media. They will be run once for each lot of MGIT tubes.

Positive Control Tube:

1. Empty broth from an uninoculated MGIT tube.

2. Label tube as a Positive Control and record the date.

3. Prepare 0.4% sodium sulfite solution (0.4 g in 100 ml sterile distilled or deionized water). Discard unused portion.

4. Add 5 ml of sodium sulfite solution to the tube, replace the cap, tighten and allow the tube to stand for a minimum of 1 h at room temperature before use.

5. Positive Control tubes can be used many times. Each Positive Control tube can be used for up to four weeks when stored at room temperature.

Negative Control Tube

An unopened, uninoculated MGIT tube is used as a control.

Reading the Tubes:

1. A Positive Control and a Negative Control are important for correctly interpreting results.

2. Remove tubes from the incubator. Place tubes on the UV light next to a Positive Control tube and an uninoculated tube (Negative Control). It is recommended that one rack at a time of tubes (4 by 10 tubes) be placed on the UV light. NOTE: Wear UV protective glasses when observing fluorescence. Normal room light is preferred. Avoid reading tubes in a sunlit room or in a darkened room.

3. Visually locate MGIT tubes that show bright fluorescence. Fluorescence is detected as a bright orange color in the bottom of the tube and also an orange reflection on the meniscus. The MGIT tube should then be taken out of the rack and compared to Positive Control and Negative Control tubes. The Positive Control should show a high amount of fluorescence (very bright orange color). The Negative Control should have very little or no fluorescence. If fluorescence in the MGIT tube looks more like the Positive Control, it is a positive tube. If it looks more like the Negative Control, it is a negative tube. Growth can also be detected by the presence of a non-homogeneous turbidity, small grains or flakes in the culture medium.

4. Positive tubes should be stained for acid-fast bacilli. Smear-negative tubes should be checked for bacterial contamination. Subcultures for identification and drug susceptibility testing may be performed using fluid from the BBL MGIT tube.

5. Negative tubes should continue to be read daily for eight weeks or longer depending on the type of specimen and the past experience of the laboratory. Alternative reading schedules may be established. Failure to read the tubes for several days, such as during weekends or holidays, may delay the detection of positive tubes, but will not otherwise adversely affect the performance of the media. Tubes should be visually checked for the presence of turbidity and small grains or granules before discarding. Negative MGIT tubes cannot be reused. If mycobacterial growth is suspected, follow the "Processing a Positive MGIT Tube" procedure as stated below.

Processing a Positive MGIT Tube:

NOTE: All steps should be performed in a biological safety cabinet.

1. Remove MGIT tube from test rack.

2. using a sterile transfer pipet, remove an aliquot from the bottom of the tube (approx. 0.1 ml) for stain preparations (AFB and Gram stains).

3. Inspect smear and preparations. Report preliminary results only after acid-fast stain evaluation.

If AFB positive, subculture to solid media and report as: Growth positive, AFB smear positive, ID pending.

If microorganisms other than AFB are present, report as: Growth positive, AFB smear negative, Contaminated.

If no microorganisms are present, no reportable result. Subculture broth to blood agar plate and mycobacterial culture medium; repeat smear using the addition of protein to ensure the inoculum has been adequately fixed to the slide.

Appendix VI GeneXpert procedure

Liquefaction of the resuspended sediment

1. Label each Xpert MTB/RIF cartridge with the sample ID.
2. Transfer at least 0.5 mL of the total resuspension pellet to a conical, screw-capped tube for the Xpert MTB/RIF using a sterile transfer pipette.
3. Store re-suspended sediments at 2–8 °C if they are not immediately processed for Xpert MTB/RIF. Do not store for more than 12 hours.
4. Add 1.5 mL of Xpert MTB/RIF Sample Reagent (SR) to 0.5 mL of resuspended sediment sample using a sterile transfer pipette and shake vigorously 10 – 20 times. Note: One back-and-forth movement is a single shake.
5. Incubate the specimen for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation, again shake the specimen vigorously 10 – 20 times. Samples should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample.

Preparing the Cartridge

Important: Start the test within 30 minutes of adding the sample to the cartridge.

1. Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Do not process the sample further if there is insufficient volume.
2. Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. Dispense slowly to minimize the risk of aerosol formation.
3. Close the cartridge lid. Make sure the lid snaps firmly into place. Remaining liquefied sample may be kept for up to 12 hours at 2 – 8 ºC should repeat testing be required.

Important: Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of preparing the cartridge.

Starting the Test

Important: Before you start the test, ensure that the system is equipped with the GX2.1 software, and the Xpert MTB/RIF assay is imported into the software.

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.

2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.

3. Log on to the GeneXpert Dx System software using your user name and password.

4. In the GeneXpert Dx System window, click Create Test. The Scan Cartridge Barcode dialog box appears.

5. Scan the barcode on the Xpert MTB/RIF cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

6. In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the “View Results” window and all the reports.

7. Click Start Test. In the dialog box that appears, type your password.

8. Open the instrument module door with the blinking green light and load the cartridge.

9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

10. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.

11. Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

Interpretation of Results

MTB Detected: MTB target DNA is detected.

• MTB Detected—The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample. Table 1 lists the Ct value ranges for the displayed MTB results.

Table 11: MTB result name and Ct value range

MTB	**Ct range**
High	<16
Medium	16-22
Low	22-28
Very Low	>28

• Rif Resistance DETECTED, Rif Resistance NOT DETECTED, or Rif Resistance INDETERMINATE will be displayed only in MTB DETECTED results and will be on a separate line from the MTB DETECTED result.

• Rif Resistance DETECTED; a mutation in the rpoB gene has been detected that falls within the valid delta Ct setting.

• Rif Resistance INDETERMINATE; the MTB concentration was very low and resistance could not be determined.

• Rif Resistance NOT DETECTED; no mutation in the rpoB gene has been detected.

• SPC— NA (not applicable); SPC signal is not required since MTB amplification may complete with this control.

• Probe Check—PASS; all probe check results pass.

MTB Not Detected: MTB target DNA is not detected, SPC meets acceptance criteria.

• MTB not detected—MTB target DNA is not detected

• SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.

• Probe Check—PASS; all probe check results pass.

RIF Not Detected: RIF target DNA is not detected, SPC meets acceptance criteria.

• RIF not detected—RIF target DNA is not detected

• SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.

• Probe Check—PASS; all probe check results pass.

INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

• MTB INVALID—Presence or absence of MTB DNA cannot be determined.

• SPC—FAIL; MTB target result is negative and the SPC Ct is not within valid range.

• Probe Check—PASS; all probe check results pass.

ERROR

• MTB—NO RESULT

• SPC—NO RESULT

• Probe Check—FAIL*; one or more of the probe check results fail.

*If the probe check passed, the error is caused by a system component failure.

NO RESULT

• MTB—NO RESULT

• SPC—NO RESULT

• Probe Check—NA (not applicable)

Reasons to Repeat the Assay

Repeat the test using a new cartridge or initiate alternate procedures if one of the following test results occurs:

• An INVALID result indicates that the SPC failed. The sample was not properly processed or PCR was inhibited.

• An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded or there was a GeneXpert module failure.

• A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Appendix VIII IREC approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2013/127
Approval Number: 0001060

12th September, 2013

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
12 SEP 2013
P.O. Box 4606 ELDORET

Dr. Duncan Wekesa Nyukuri,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Nyukuri,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Utility of GeneXpert in Evaluating Patients with Suspected Tuberculous Meningitis at Moi Teaching and Referral Hospital, Eldoret".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1060*** on 12th September, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 11th September, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director - MTRH Dean - SOM Dean - SON
Principal - CHS Dean - SPH Dean - SOD

Approval MTRH

MOI TEACHING AND REFERRAL HOSPITAL

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P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

12th September, 2013

Dr. Duncan Wekesa Nyukuri,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Utility of GeneXpert in Evaluating Patients with Suspected Tuberculous Meningitis at Moi Teaching and Referral Hospital, Eldoret".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM