PREVALENCE OF DISSEMINATED TUBERCULOSIS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED PERSONS ADMITTED WITH SEPSIS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, MOI UNIVERSITY.

DECLARATION

DECLARATION BY CANDIDATE

This thesis has been prepared as partial fulfillment of requirements for award of master of medicine in internal medicine by the Moi university school of medicine.

I declare that this thesis is my original work and it has not been presented for an award of a degree or an academic credit in any university. No part of this thesis may be reproduced without the permission of the author and or Moi University.

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DEDICATION

I dedicate this thesis to God and my family. It is only by God's grace that I was able to carry this thesis to completion. To my family I appreciate every one of you, your prayers, words of encouragement, patience and endurance as I toiled with this thesis has not been in vain. Thank you for your continued support and love.

ABSTRACT

Background: Disseminated Tuberculosis (TB) has been identified as one of most common causes of death in adult HIV-infected persons. It has a non-specific clinical presentation mimicking that of bacterial sepsis requiring a high index of suspicion to make a diagnosis. The gold standard of diagnosis is blood cultures that have a long incubation period hence delay in initiation of treatment thus an associated high mortality rate. The current burden of disseminated TB in adult HIV-infected persons is not known in Western Kenya. Urinary LAM antigen assay detects the presence of lipoarabinomannan (LAM), a lipopolysaccharide on the TB cell wall. Its sensitivity increases with decreasing CD_4 counts and in in-patient, PLHIV regardless of their CD_4 counts with a pooled sensitivity of 61% and specificity of 89%.

Broad Objective: To determine the prevalence of disseminated tuberculosis and describe the clinical characteristics of PLHIV admitted with sepsis at MTRH.

Specific objectives: To determine the prevalence of disseminated tuberculosis in PLHIV admitted with sepsis at MTRH. To describe the clinical characteristics of participants with disseminated tuberculosis in PLHIV admitted with sepsis at MTRH.

Methods: A cross sectional study of HIV-infected persons admitted with sepsis conducted at the MTRH adult medical wards, 300 participants recruited. Interviewer administered structured questionnaires used to collect data that included demographics (age and sex); admission vital signs (pulse rate, axillary temperature, blood pressure, and respiratory rate); HIV associated variables (use of antiretroviral therapy). Blood samples taken for a full blood count, serum creatinine and urea levels. A urine sample taken for LAM antigen assay. Data entered into EPIDATA version 13 and analyzed using STATA version 15. Independent variables were summarized using frequencies (percent), mean (SD) and median (IQR); dependent variables were analyzed using Student t-test, Chi square and Wilcoxon rank sum test. A significant p value was less than 0.05.

Results: Two hundred and ninety-eight (298) participants were analyzed; fifty eight percent (173) were female. Overall mean age was 41.68 (SD 11.72) with no difference in age between the LAM positive and negative. Prevalence of disseminated TB 26% (95% CI 21.2-31.4). All participants had anemia with the LAM positive group having a lower hemoglobin level of 9.71g/dl versus 10.47g/dl in the LAM negative group (p0.04). One hundred and seventy eight (178) participants had lymphopenia. Out of the seventy-seven participants with LAM antigen assay positive, 55 had lymphopenia, only two had lymphocytosis and 20 had a normal lymphocyte count (p 0.04). Out of the seventy-seven participants with LAM antigen assay positive, fifty-eight were not on HAART with only nineteen being on HAART (p0.02).Thirty seven percent in the LAM positive group died 10 days from admission and a further twenty seven percent died within one month ($p < 0.001$).

Conclusion: The prevalence of disseminated TB was 26%. Possible factors associated with LAM antigen positivity included anemia, lymphopenia and not on antiretroviral therapy. There was higher mortality seen in the LAM positive group within 1 month of admission.

Recommendations: LAM antigen assay should be a routine diagnostic test in HIV-infected persons admitted with sepsis and anti TB medication started promptly due to the high associated mortality.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB	Acid Fast Bacillus
AIDS	Acquired Immune Deficiency Syndrome
AMPATH	Academic Model providing access to Healthcare
ANTI-TB	Anti-tuberculosis
BPM	Beats per Minute
CD 8	Cluster of Differentiation antigen 8
CD4	Cluster of Differentiation antigen 4
EDTA	Ethylene Diamine Tetra-acetic Acid
EPTB	Extra Pulmonary Tuberculosis
HAART	Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IREC	Institution Research and Ethics Committee
LAM	Lipoarabinomannan
LF LAM	Lateral Flow Lipoarabinomannan antigen assay
MDRTB	Multi Drug Resistant Tuberculosis
MTB	Mycobacterium Tuberculosis
MTRH	Moi Teaching and Referral Hospital
MUSOM	Moi University School of Medicine
NAAT	Nucleic Acid Amplification Test
PLHIV	People Living with HIV
PTB	Pulmonary Tuberculosis
RTP	Room Temperature and Pressure
TB	Tuberculosis
WHO	World Health Organization

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To my fellow registrars in the department of internal medicine please know that the efforts you took helping me recruit the participants has been highly appreciated. Were it not for your keen eyes majority of these participants I would not have caught them in time.

To the staff at Nyayo (Moi teaching and referral hospital medical wards) wards from the nurses, to the subordinate stuff who did not discard my urine samples, the phlebotomist who agreed to share their workspace with me, I acknowledge you too. I say special regards to each one of you for the various roles that you have played to make this study a success.

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CHAPTER ONE: INTRODUCTION

1.1 Background

Tuberculosis (TB) has been identified as a major global health burden for over a millennium now. It was identified as one of the top ten leading cause of death from a single infectious agent worldwide in the year 2017. (WHO, 2018). In the same year, 10 million cases were diagnosed, with 9% occurring in PLHIV whereby 72% of them occurred in the African region. (WHO, 2018)

About 1.3 million deaths were attributed to TB in the HIV uninfected persons in the same year with an additional 300,000 deaths reported in persons who were PLHIV. Thus World health organization (WHO) recognizes that with early detection and appropriate treatment a majority of these deaths would have been avoided (WHO, 2018)

In the year 2015, WHO revised the high TB burden country list, and categorized the countries into three groups:

High TB burden

High TB/HIV burden

High Multi drug resistance TB burden

Kenya is one of the 14 countries that has high TB burden in all of the afro-mentioned three categories (WHO, 2017).

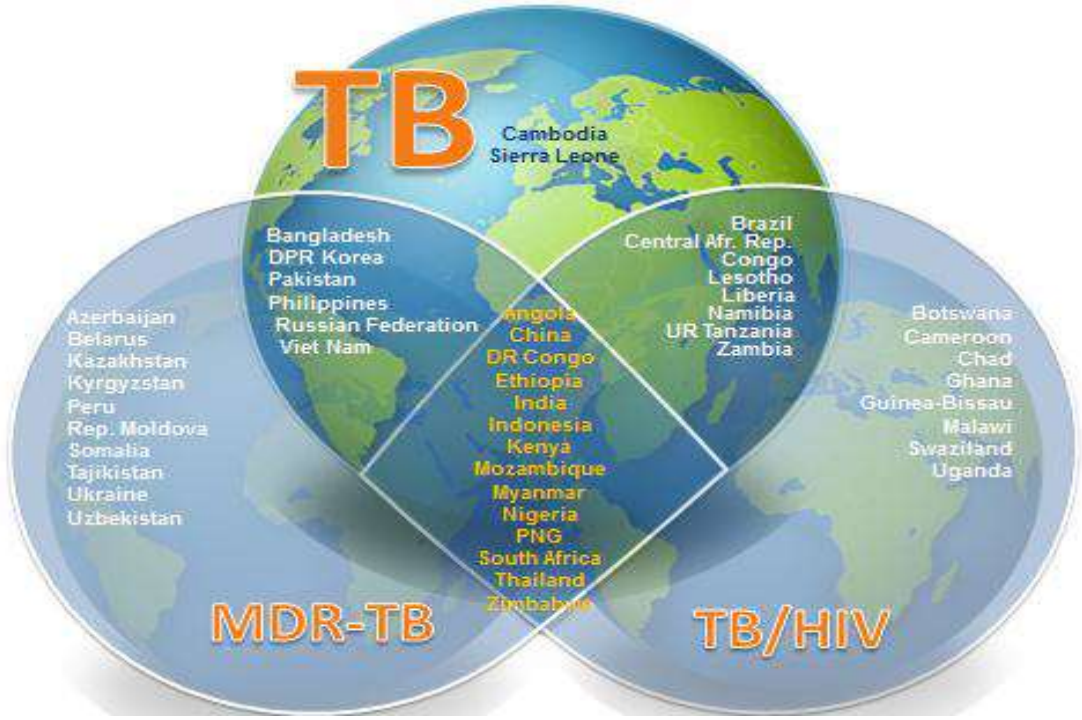


Figure 1: 30 High TB burden countries list

In Kenya, TB is the fourth leading cause of death mainly affecting the young persons between the ages of 15-34 years (Kenya, 2016). A survey conducted by the ministry of health in Kenya, found that the prevalence of bacteriologically confirmed pulmonary TB is at 558 per 100,000(Kenya, 2016). Eighty three percent (83%) of these cases occurred in the HIV negative males of 25-34 years. They estimated an annual incidence of 138,105 in comparison to WHO figures for the same year (82,000 cases) meaning that approximately 40% of the cases are undetected annually (Kenya, 2016).

It was recognized from this survey, that twenty six percent (26%) of persons with TB were missed because they did not present with the classical sign and symptoms of fever, weight loss, night sweats and cough for more than 2 weeks. Thus, maybe broader diagnostic criteria are required to cover asymptomatic persons. (Kenya, 2016)

Those that presented with classical signs and symptoms of pulmonary TB and sought medical care, eighteen percent (18%) were missed at the initial visit due to the medical facility personnel basing their diagnosis on smear microscopy. Due to the paucibacillary nature of Mycobacteria Tuberculosis (MTB) in PLHIV most patients actually have smear negative TB, thus smear microscopy may miss a majority of the cases. (Kenya, 2016)

Sixty seven percent (67%) of the participants did not seek any medical care despite having classical symptoms of TB and their reasons varied from financial constraints to the participants feeling they were not severely sick. Thus, more screening of the participants and community awareness is required (Kenya, 2016).

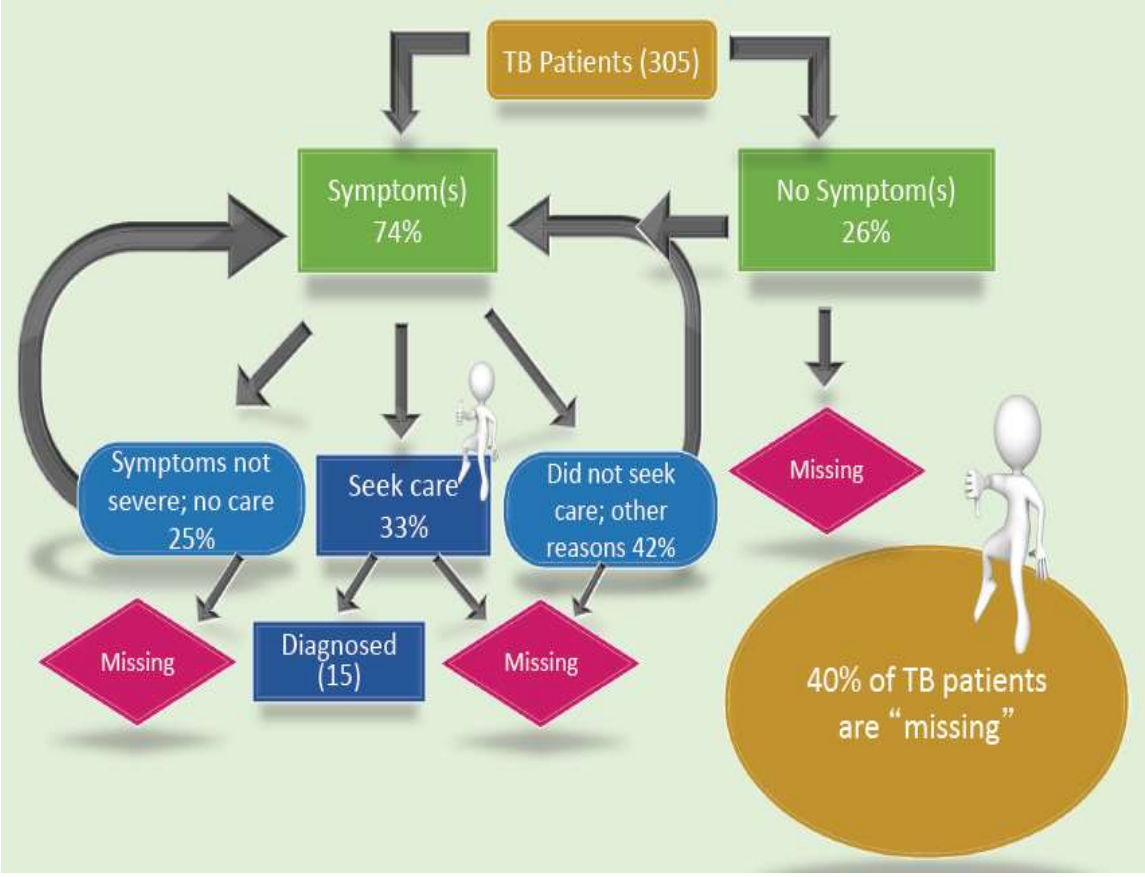


Figure 2: Health seeking behavior in Kenya TB survey

The TB epidemic globally has been majorly influenced by several factors that include HIV, poverty, malnutrition and smoking cigarettes (WHO, 2017). The combination of HIV and TB has been described as a synergistic relationship with both diseases having a high prevalence in resource-limited countries that lack finances for diagnosis and treatment (Bartlett et al., 2007).

Tuberculosis is defined to be disseminated when MTB is isolated from blood or bone marrow, or from two or more noncontiguous sites (Jolobe et al., 2017). In the setting of HIV disseminated TB has a nonspecific clinical presentation mimicking that of sepsis, thus a high index of suspicion is required to make a diagnosis (Wang et al., 2007).

MTB blood culture is the gold standard for diagnosing disseminated TB. Despite MTB blood cultures being routinely available in most resource-limited countries, they are coupled by the fact that they have long incubation period, approximately 2 weeks hence delay in initiating treatment.

Most cases of disseminated TB are thus diagnosed posthumously. A study carried out at MTRH, which aimed to identify the cause of death in PLHIV who were already on HAART at the time of death; they found the prevalence of TB at autopsy to be at 36%. They identified TB (pulmonary, extra-pulmonary and disseminated) as the leading cause of death in PLHIV (Siika et al., 2012).

A global meta-analysis that was conducted by Gupta et al, reported the prevalence of TB in PLHIV cadavers to be 40% with a majority of these cases (43%) being from Africa. Ninety one percent (91%) of these cases TB was the primary cause of death. This goes to show that TB in PLHIV not only is it a marker of

immunosuppression but also it greatly contributes to the high mortality rate (Gupta et al., 2015).

Most patients suspected of having disseminated TB are started on empiric anti-tuberculosis treatment as per the WHO algorithm (Organization, 2007).However a high mortality is still seen in these patients, due to delay in initiation of treatment, since empiric treatments are often initiated after other alternatives treatments have failed.

Thus there is need to reduce the time to make a definitive diagnosis and initiate treatment. Lipoarabinomannan (LAM) is a lipopolysaccharide present on the mycobacterial cell wall. It has immune modulatory propensity that aids MTB to bypass the host defense mechanism. LAM has been shown to be secreted by infected alveolar macrophages with high tissue concentrations being seen around the primary site of infection (Strohmeier et al., 1999).

The LAM antigen is then disseminated into the blood stream. After dissemination into the bloodstream, the LAM antigen via simple ultrafiltration mechanism is then filtered into urine. (Lawn et al., 2012). LAM antigen can be detected in the urine of persons with pulmonary TB; however, the utility of urine LAM antigen assay in the detection of pulmonary TB has been found to be questionable. A study conducted at MTRH that aimed to evaluate the utility of LAM antigen assay in the diagnosis of MTB infections in PLHIV found a sensitivity of 20% (Tonui et al., 2012)

The current hypothesis is that the presence of LAM antigen in urine is due to the presence of the whole MTB bacilli and not just antigenuria. The sensitivity of LAM

antigen assay has been shown to be high in PLHIV with disseminated TB in comparison to PLHIV with other forms of TB (Lawn et al., 2012).

The WHO reports that the urinary LAM antigen assay has a pooled sensitivity of 44% and specificity of 92% in persons who are PLHIV. Its sensitivity increases in inpatient PLHIV with low CD_4 counts who have a high risk of disseminated TB with a pooled sensitivity of 61% and specificity of 89%. Thus the WHIO recommends its use to aid diagnosis of TB in PLHIV with low CD_4 counts ($\leq$100cells/mm^3) or who are seriously ill regardless of their CD_4 counts or with unknown CD_4counts (WHO, 2015).

1.2 Problem Statement

The clinical presentation of disseminated TB is nonspecific and requires a high index of suspicion for the clinician to make a diagnosis. (Wang et al, 2007). A study conducted in Tanzania that aimed to identify the etiology of fever in persons admitted in two northern Tanzania hospitals, identified MTB bacteremia in 29 of the 508 participants. The clinical diagnosis of these patients at the time of admission was malaria and a majority of these patients had not undergone routine TB testing (Crump et al., 2013).

Another study conducted in Uganda that aimed to identify the prevalence of MTB bacteremia in PLHIV admitted with sepsis, reported that 1 out of 4 persons had MTB bacteremia. Out of the 23.4% that had disseminated TB, only 26% had a sputum smear microscopy, 57% had a chest radiograph and 21% had both chest radiography and sputum smear for microscopy (Jacob et al., 2013).

Both the Uganda and Tanzania studies reported a high 30-day mortality of approximately 50% (Crump et al., 2012: Jacob et al., 2013). The high mortality rate seen in this group of participants could be due to missed diagnosis due to low clinical index of suspicion, thus they are managed for other conditions like malaria and sepsis hence a delay in initiation of TB treatment.

Majority of disseminated tuberculosis cases are thus diagnosed posthumously as earlier mentioned. The global meta-analysis conducted by Gupta et al found that out of the 40% TB cases diagnosed at autopsy in PLHIV cadavers, approximately 85% of them had disseminated TB and 45.8% had not been diagnosed at the time of death. There is a crucial need for improvement in diagnosis and management of HIV associated TB (Gupta et al., 2015).

Disseminated TB in the setting of advanced HIV is more of a haematogenous spread thus the gold standard of diagnosis is MTB blood cultures. MTB is differentiated from other bacteria on culture by the production of niacin. However, culture media like Lowenstein-Jensen, which is an egg-based agar, are limited by the fact that they have a long incubation period (approximately median of 3 weeks). Hence, there is delay in initiation of treatment. Thus, an alternative diagnostic tool that hastens the diagnosis and allows an early initiation of treatment is paramount.

The sensitivity of blood cultures is further limited by the need to have bacilli present in the blood for it to grow. In persons living with HIV, this is a limitation because of the pauci-bacillary nature.

There are new automated cultures like MB/BacT, BACTEC 9000, Mycobacterial Growth Indicator Tube (MGIT) and the Microscopic Observation Drug Susceptibility assay (MODS) culture. These new culture media growth rely on detection of

metabolism as the bacilli multiply which occurs earlier at approximately 9-16 days. There are even faster culture based tests that utilize bacteriophages and produce results in about 2 days. Though these cultures are faster, they are not routinely available especially in resource-limited settings.

1.3 Justification

Lipoarabinomannan (LAM) is a lipopolysaccharide that is present on the MTB bacteria wall. Metabolically active or degenerating bacteria release it thus can only be present in active TB disease (WHO, 2015).

Urinary LAM antigen assay detects the presence of LAM antigen in urine. The fact that LAM antigen assay uses urine instead of sputum makes it a very attractive test since urine is easy to collect and store and it requires minimal bio safety requirements in comparison to sputum (WHO, 2015).

Most patients with disseminated TB may not be able to expectorate sputum either due to the fact they may have reduced levels of consciousness or they have no pulmonary involvement. Even in instances that they are able to expectorate sputum routine tests like sputum for microscopy and gene Xpert MTB/RIF may be falsely negative in these patients due to the paucibacillary nature of the disease in those with advanced HIV. LAM antigen assay is a useful diagnostic test as it overcomes many of these challenges (WHO, 2015).

The gold standard for diagnosing disseminated TB is blood cultures. However, blood cultures take approximately 2 weeks and waiting for results delay in initiation of treatment. In contrast urinary LAM antigen assay is a point of care diagnostic test and results are available within 25minutes (WHO, 2015).

Blood cultures also require technical expertise that may not be routinely available in most resource-limited settings.

Urinary LF LAM antigen assay has several attributes that makes it a good point of care test:

Inexpensive

Requires minimal biosafety equipment

Results are rapid within 25 minutes.

It is easy to perform (WHO, 2015)

Thus in 2015, WHO recommended the use of LAM antigen assay to aid in the diagnosis of TB in the following target groups:

PLHIV who present both in the inpatient and outpatient hospital facility and have signs and symptoms of TB (both PTB and EPTB) and have CD_4 counts <100cells/mm^3

PLHIV who present in the inpatient facility and are seriously ill regardless of their CD_4 counts. They (WHO) defined seriously ill based on 4 danger signs that are;

Respiratory rate >30breaths/min,

Heart rate >120beats/min

Temperature >39ºC

Inability to walk unaided (WHO, 2015)

Currently Kenyan Government also recommends LAM antigen assay as a point of care diagnostic test in PLHIV with advanced HIV based on WHO clinical staging3/4 or CD_4<200cells/mm^3 with presumed TB or PLHIV with any danger sign of severely illness or currently admitted in hospital (Kenya Ministry of Health, 2018).

A study that was carried out in 4 Sub Saharan African countries; where HIV infected persons who were severely ill requiring hospital admission were randomly grouped into two groups. In one groups the decision to initiate TB treatment was based on LAM antigen assay positivity, while the second group was based on routine TB tests that included chest radiographs, MTB cultures, Gene Xpert MTB/RIF and empirical treatment. The group that was initiated TB treatment based on LAM antigen assay positivity, had a shorter time to treatment initiation of 0-3 days in comparison to the group that was initiated treatment based on routine tests. Approximately 52% of the LAM antigen assay group was initiated TB treatment, in comparison to only 47% in the group that relied on routine tests. (P value 0.024). Hence, by relying on LAM antigen assay, more participants were started on TB treatment and this occurred earlier in comparison to participants who did not have a LAM antigen assay done. This is the first TB diagnostic test study that has been shown to have a mortality benefit. In comparison to the Gene Xpert MTB/RIF, study that was shown to reduce time to treatment initiation but did not have a morbidity or mortality, benefit (Peter et al, 2016).

Thus, by relying on urine, LAM antigen assay positivity to initiate TB treatment will lead to a rapid and lifesaving treatment initiation in HIV infected persons who are most susceptible at a low cost.

1.4 Research Questions

1. What is the prevalence of disseminated tuberculosis in PLHIV admitted with sepsis at MTRH?
2. What are the clinical characteristics of PLHIV with disseminated TB admitted with sepsis at MTRH?

1.5 Objectives

1.5.1 Broad Objective

To determine the prevalence of disseminated tuberculosis and describe the clinical characteristics of PLHIV admitted with sepsis at MTRH.

1.5.2 Specific Objectives

1. To determine the prevalence of disseminated tuberculosis in PLHIV admitted with sepsis at MTRH.
2. To describe the clinical characteristics of participants with disseminated tuberculosis in PLHIV admitted with sepsis at MTRH.

CHAPTER TWO: LITERATURE REVIEW

2.1 Cell biology of mycobacterium tuberculosis complex.

A German physician and microbiologist Dr.Robert Koch, first identified mycobacterium Tuberculosis (MTB), on March 24th 1882. The MTB genome was then sequenced in 1998. Humans are the natural reservoirs for mycobacterium tuberculosis.

Mycobacterium tuberculosis is an obligate aerobe found to have high affinity of the well-aerated regions of the lungs especially the upper lobes. It is a large non-motile rod shaped bacteria ranging approximately 2-4 micrometers in length and 0.2-0.5 μm in width. MTB in contrast to other bacteria has a slow generation time dividing every 15-20 hours while many other bacteria divide every minute.

The Mycobacterium tuberculosis cell wall is highly rich with lipids that constitute approximately 60% of the cell wall. These lipids include mycolic acids, cord factor and wax D. The high lipid content of the mycobacterial wall makes it impermeable to stains and dyes.

The lipid cell wall is covalently bound to the underlying peptidoglycan bound polysaccharide arabinogalactan. That confers the mycobacterium resistance to host defense mechanism and resistance to antibiotics. Lipoarabinomannan is a structural carbohydrate antigen that is also part of the cell wall and helps the MTB by pass the host defense mechanism thus allowing it to multiply in the macrophages (Mrus et al., 2005).

The LAM has three major components; there is the glycolipid anchor that attaches it to the plasma membrane, a mannan core and a mannose anchor. The mannose anchor is what extends to the surface of the cell wall and the MTB uses it to attach on

mannose receptors on macrophages. The mannose anchor is the major virulent factor of MTB through it; it can inhibit phagosome maturation, apoptosis, and serves as a scavenger for toxic oxygen radicals among other factors. Hence, LAM is important for MTB survival in the host. (Lawn et al., 2012a).

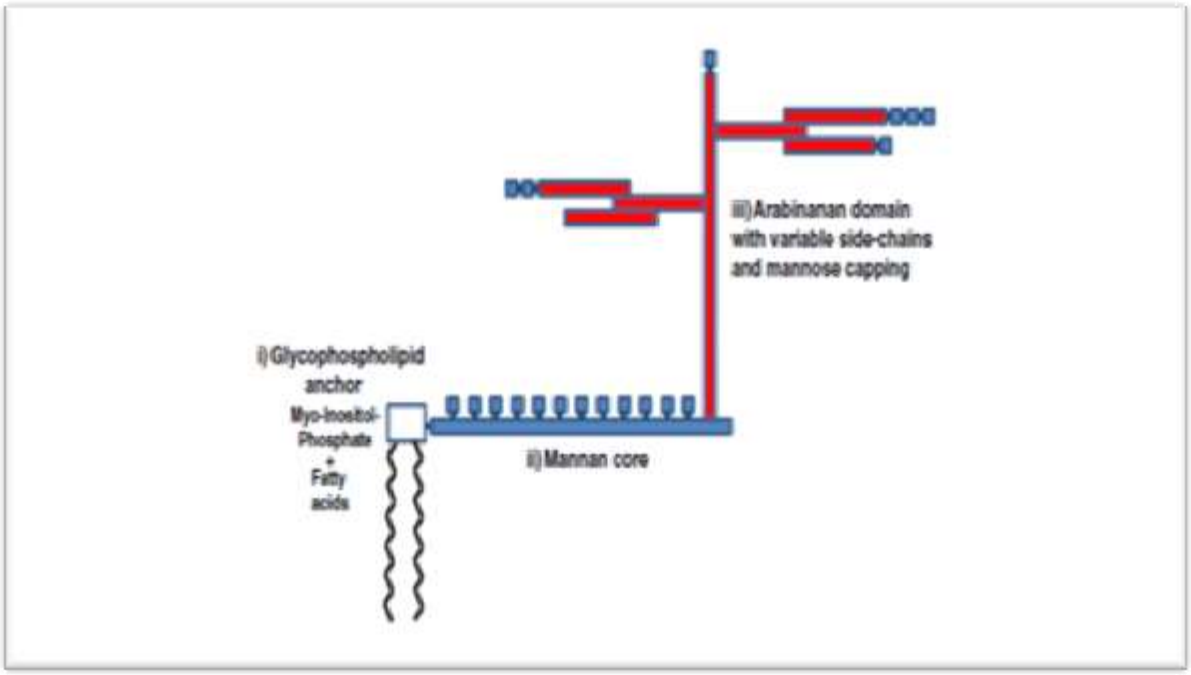


Figure 3: LAM antigen structure

2.2 Immune pathogenesis

Persons with active pulmonary TB are responsible for transmission of TB through aerosol droplets with 1droplet containing approximately three bacilli. The droplets can be spread by coughing, sneezing or talking with one cough containing approximately3000 droplets

Several factors determine whether the TB is transmitted or not. They can broadly be classified into host and environmental factors. Host factors influence the organism burden, whereby individuals with smear positive and cavitary disease is more contagious than those with smear negative disease are.

Environmental factors influence transmission and include ventilation; poorly ventilated and overcrowded places facilitate transmission due to prolonged exposure. Hence, persons in institutions, medical personnel and persons living in poverty are at higher risk of TB exposure.

Upon inhalation, the droplets settles in the sub-pleural airspaces of the middle and lower zones of the lung. The bacilli are engulfed by the alveolar macrophages that make up part of the innate immune system.

In the macrophage, if the bacilli are not phagocytosed they continue to multiply at a very slow rate. They multiply until they reach 100-10,000 copies that can elicit a cellular immune response, which manifests as a positive tuberculin skin test. The macrophage then bursts releasing the bacilli and in so doing attracts other cells of inflammation. This includes not only other macrophages but also T lymphocytes, specifically the CD 4 and CD 8 lymphocytes. The lymphocytes release interferon γ that activates the macrophage and stimulates phagocytosis of the bacilli.

The combination of the bacilli with the cells of inflammation form a tubercle in the lungs. Two factors determine how the disease progresses from here: host defense mechanisms and the virulence of the organism. Hence, it may progress to either active disease, or may become a latent infection, or the organism may be cleared.

In an intact cell, mediated immune response a granuloma is formed that walls in the bacteria thus prevents its spread. If the host immune defense mechanisms are deficient, this then progresses to primary disease, usually in the upper and middle lobe of the lung.

Reactivation disease occurs when there is a dormant bacillus that later becomes activated. This occurs in approximately 5-10% of healthy individuals with latent

infection over the course of a lifetime. Disseminated TB occurs when there is haematogenous spread of MTB to other sites. It is thus defined as isolation of MTB from blood, bone marrow, liver biopsy or two or more non-contiguous sites.

Factors that increase the risk of developing disseminated tuberculosis include age whereby the both extremities of age have an increased risk. Other associated risk factors include immunosuppression as occurs in PLHIV more so in those with advanced HIV. The most common manifestation of disseminated tuberculosis includes lymphadenopathy, pulmonary, bone and joint manifestation, genitourinary and gastrointestinal manifestation. Other less common manifestations are cutaneous and cardiovascular.

The clinical presentation of disseminated tuberculosis varies and it may have an acute, sub-acute or chronic presentation. It may present as a primary infection thus presenting as an acute fulminant disease with multiple organ failure, septic shock and acute respiratory distress (Ahuja et al., 1992).

It can also occur after a primary focus in the lung where the bacilli disseminate through the lymphatics and haematogenously spread to other organs like the liver, spleen, bone marrow and brain. These distant foci may be encapsulated and heal over weeks however if they fail to heal it progresses to sub-acute or chronic disseminated tuberculosis.

The chronic and sub-acute presentation of disseminated tuberculosis are more common with patients presenting with nonspecific symptoms like fever of unknown origin, failure to thrive and one or more organ dysfunction (Kim et al., 1990).

Due to the non-specific presentation the diagnosis of disseminated tuberculosis is often missed. A study conducted in Botswana found that out of the 128 post mortems

done in PLHIV 40% of them were diagnosed to have disseminated tuberculosis at the time of death (Ansari et al., 2002). Another study conducted in the United states found that approximately 20% of the disseminated tuberculosis cases were diagnosed at post mortem (Rieder et al., 1991).

This goes to show that the diagnosis of disseminated tuberculosis is a global dilemma with most of the cases being routinely caught at autopsy. LAM antigen assay has been shown to have a high sensitivity in PLHIV with low CD_4 counts or are seriously ill regardless of their CD_4 counts. Since it is a rapid test it can routinely be done at the bedside, use of TB LAM has the potential to reduce the time to initiation of treatment and thus reduced mortality.

Due to the extra pulmonary manifestation of disseminated tuberculosis, attaining a sample for diagnosis may be difficult hence making the diagnosis difficult. Thus, a high clinical index of suspicion is needed to make a diagnosis.

2.3 HIV and Tuberculosis

Not every individual who is infected with the MTB develops active disease. It estimated that approximately 5% of those who are infected will develop pulmonary disease within three years and another 5% will develop in the lifetime (Raviglione et al., 1995).

Several predisposing factors are implicated for one to develop active disease. HIV infection is one of the major risk factors. PLHIV have a much higher risk of developing tuberculosis than people not living with HIV. This is because there is increased risk of latent TB reactivation and has rapid TB progression due to the immunosuppression.

HIV targets CD_4 T helper cells that are central in anti-mycobacterial defense. Hence, there is an increased susceptibility to mycobacterial infections. CD_4 is a glycoprotein that is present on the surface of a variety of cells that include regulatory T cells, T helper cells, macrophages, dendritic cells and monocytes. These cells are involved in both innate and adaptive immune response. On the T cells, the CD_4 glycoprotein is part of the T cell co–receptor (TCR) where its role is to amplify the signal that is generated by the TCR. This then leads to interaction with the β_2 domain of the MHC class Π molecule that then activate the tyrosine kinase that is essential in the activation of the T cells (Havlir et al., 1999).

Tuberculosis also increases HIV viral replication by inducing macrophages to release tumor necrosis factor α, interleukin 1 and 6. Thus individuals who are HIV and TB co-infected have twice the risk of death compared with individuals who are only living with HIV (Havlir et al., 1999)

Tuberculosis can develop at any level of CD_4 count but the clinical and radiological presentation depends on the level of CD_4 count. Individuals with higher CD4 counts and low viral loads have a more typical presentation similar to the HIV negative individuals. While with decreasing levels of CD_4 and high viral loads the presentation is more atypical making definitive diagnosis more difficult (Huebner et al., 1995).

A study was conducted in Malawi by Harries et al to determine mortality rates of individuals after initiation of anti-TB medication. They found the highest mortality rate was in individuals with smear negative TB (60%) followed by those with EPTB (47%) in comparison to those with smear positive disease (30%). They attributed this higher mortality rates to the fact that smear negative TB and EPTB occur in more immune compromised individuals (Harries et al., 1998)

In this entity hence TB in the HIV co-infected has thus been identified as an AIDS defining disease in all its entities (Zumla et al., 2000).

2.4 Diagnosis of tuberculosis

Diagnosis of TB is typically based on clinical presentation, radiographic and laboratory findings. The typical clinical presentation is a triad of cough for any duration, fever for any duration, and drenching night sweats for 3 weeks or longer. However, to increase the sensitivity additional radiological findings are required. This mainly occurs in individuals not living with HIV (Cain et al., 2010).

The clinical presentation in PLHIV co-infected with TB t depends on the level of immunosuppression. CD_4 counts < 200 cells/mm^3, the presentation is atypical with a higher likelihood of extra pulmonary TB. Disseminated TB presenting as a chronic febrile illness with multiple organ involvements is more common (Mudenda et al., 2012).

2.4.1 Smear Microscopy

Smear microscopy relies on visual identification of acid-fast organisms with Ziel Neelsen stain. The acid-fast organisms include MTB and non-MTB organisms, which do not decolorize with acid alcohol hence retaining the primary stain.

Smear microscopy is the traditional primary method of diagnosing TB in low and middle income countries which have a high prevalence of TB (Hopewell et al., 2006). It is a simple, rapid and inexpensive way and can be used on various body specimens including sputum, cerebral spinal fluid, Broncho alveolar lavage, pleural, pericardial and peritoneal fluid, gastric washings and fine needle aspirates (Zumla et al., 2000).

Sputum smear microscopy is the first line in the diagnosis of pulmonary TB where two samples of sputum are collected one on the spot and another an early morning sample (Crampin et al., 2001). Presence of one positive smear is adequate to make a diagnosis of TB (Initiative & Organization, 2008).

Most low-income countries use conventional light microscopy that has been shown to have low sensitivity especially in PLHIV. Its sensitivity is between 50-60% hence it approximately misses 50% of the cases (Siddiqi et al., 2003). This figures are however lower in PLHIV with severe immune suppression. This is because its sensitivity is greatly reduced in low bacterial load for it requires at least 5000 bacilli/ ml (Initiative & Organization, 2008).

Smear microscopy is also coupled by the fact that it is not able to differentiate MTB from other variants of mycobacterium bacilli hence false positives. It also relies on the patient's ability to produce sputum; many patients with extra pulmonary and disseminated TB are not able to do so and thus cannot be routinely diagnosed using sputum microscopy.

2.4.2 The Xpert® MTB/RIF assay

Xpert® MTB/RIF assay is a fully automated nucleic acid amplification test (NAAT) for detection of TB and drug resistant TB.

A Cochrane review that was done in 2014 reported that in smear negative pulmonary TB, Xpert MTB/RIF had a sensitivity of 67% and a specificity of 99% regardless of the HIV status (Steingart et al., 2013).

The WHO has made strong recommendations for the use of Xpert® MTB/RIF assay as an initial diagnostic test for individuals suspected to have MDR TB or HIV

associated TB (Organization, 2013). In the setting of extra pulmonary disease WHO recommends further testing in the case of negative Xpert® MTB/RIF assay results (Organization, 2013).

Despite the fact that Xpert® MTB/RIF assay is able to generate results within 2 hours, it has several limitations. First, it requires stable and uninterrupted electrical supply to avoid interrupting the procedure (Organization, 2013). Secondly in the diagnosis of extra pulmonary TB, Xpert® MTB/RIF assay has been shown to have low sensitivity due to the paucibacillary nature hence WHO recommends further confirmatory tests to diagnose extra pulmonary TB such as pleural TB or TB pericarditis (Organization, 2013).

Xpert® MTB/RIF assay detects the presence of the whole MTB bacilli in the specimen, a study conducted at MTRH compared the sensitivity of Xpert® MTB/RIF assay on urine versus smear microscopy found that urine Xpert® MTB/RIF assay had increased sensitivity (30% versus 11%) and specificity (92% versus 89%) (IM, 2016).

However, currently WHO has not approved the use of Gene Xpert on blood, urine and stool (Organization, 2013).

2.4.3 Radiological diagnosis of tuberculosis

The classic radiological finding in PTB is an upper lobe cavitary lesion but this presentation is not as common in PLHIV who are more likely to develop EPTB or disseminated TB. CXR and chest contrast topography scan may be useful in diagnosing disseminated TB in patients with a classic miliary pattern. Hence, in this group of patients diagnosis based on radiological findings is more challenging.

2.4.4 Blood Cultures for isolation of mycobacterium tuberculosis

Blood cultures remains the gold standard of diagnosing disseminated TB. The advantage of blood culture is that it can detect low bacilli counts as low as 10 bacilli/ml of blood. However, the growth of the TB bacilli in traditional growth media, like Lowenstein-Jensen medium takes approximately 3 weeks. This thus delays the time to make a diagnosis and the initiation of treatment. Newer mediums that take a shorter period have been formulated but these are expensive hence not routinely available in resource limited settings (Zumla et al., 2000).

MTB is differentiated from other bacteria on culture by the production of niacin. However, culture media like Lowenstein-Jensen, which is an egg-based agar, are limited by the fact that they have a long incubation period (approximately median of 3 weeks). Hence, there is delay in initiation of treatment. Thus, an alternative diagnostic tool that hastens the diagnosis and allows an early initiation of treatment is paramount.

The sensitivity of blood cultures is further limited by the need to have bacilli present in the blood for it to grow. In persons living with HIV, this is a limitation because of the pauci-bacillary nature.

There are new automated cultures like MB/BacT, BACTEC 9000, Mycobacterial Growth Indicator Tube (MGIT) and the Microscopic Observation Drug Susceptibility assay (MODS) culture. These new culture media growths rely on detection of metabolism as the bacilli multiply which occurs earlier at approximately 9-16 days. There are even faster culture based tests that utilize bacteriophages and produce results in about 2 days. Though these cultures are faster, they are not routinely available especially in resource-limited settings.

2.4.5 Molecular technologies

These are newer molecular diagnostic tests that rely on nucleic acid amplification (NAAT). They use polymerase chain reaction (PCR) to detect the nucleic acid of the mycobacteria. Several available commercial kits detect various variation of the nucleic acid amplification that have different levels of accuracy. The two most commonly available tests are the amplified mycobacterium tuberculosis direct test and amplicor. These can be used on various body fluids including urine however they have been shown to have a higher accuracy level when used on respiratory samples as opposed to other specimens.

Their use however is technically challenging despite the fact that they have a high specificity. These is further limited by the fact that their sensitivity is highly variable meaning that though a positive test confirms disease a negative test doesn't necessary rule it out. They are also further confounded by the fact that these tests are not able to differentiation between alive and dead bacilli hence can have false positive results in patients who are already on anti-tuberculosis treatment.

2.5.6 The LAM Antigen Assay

Lipoarabinomannan is a lipopolysaccharide that forms part of the mycobacterial cell wall. It is produced by degrading and metabolically active MTB cells at a rate of 15mg/g of bacteria hence it is only present in active disease (Hunter et al., 1986).

Prior studies suggested that LAM antigen was released by the degraded bacterial cell wall in high concentration at the site of infection and then released into the bloodstream. Through simple filtration mechanisms it was then filtered in the kidneys from blood to urine (Boehme et al., 2005)

When it was discovered that LAM was a 17-kDa glycolipid that can easily transverse an intact basement membrane, there was the impression of the possibility of it being useful in the diagnosis of PTB and EPTB.

However, clinical studies have shown that the sensitivity of LAM antigen assay in the diagnosis of PTB to be very low at 20% and thus not routinely recommended for the diagnosis of PTB (Tonui, 2010). Thus the hypothesis that the presence LAM antigen in urine was released by degraded mycobacterial cell wall has thus been found to be invalid due to several postulated hypotheses.

First, LAM being an antigen in circulation it has to be bound to an antibody (anti LAM antibodies) or it would be incorporated to High Density Lipoproteins (HDL) (Sakamuri et al., 2013). This immune bound LAM antigen will not be able to pass through an intact glomerular basement membrane (Sakamuri et al, 2013). In this effect, no studies have been able to show any correlation between proteinuria and urine LAM positivity. Post mortem studies have also not shown any histological evidence of glomerular damage in patients with positive LAM antigen in urine (Cox et al., 2015).

Second LAM antigen has been postulated to be released from degraded bacterial cells yet upon initiation of anti-TB drugs, LAM antigen has not been shown to rise in parallel rise of anti-TBs concentration in blood (Wood et al., 2012).

Current hypothesis postulates that LAM antigenuria is due to renal involvement and haematogenous spread of MTB and not ultrafiltration mechanisms. Post mortem studies done on patients with LAM antigenuria, have shown histological evidence of renal TB further supporting the above hypothesis (Cox et al., 2015). This is further coupled by the fact LAM antigen assay sensitivity is high in patients with severe

immunosuppression specifically CD_4 counts < 50cells/mm^3, thus patients with greater risk of developing disseminated TB (Lawn et al., 2012b).

LAM antigenuria is thus a marker of disseminated TB and maybe used to aid diagnose disseminated TB(Lawn et al., 2012b).

Several studies have been conducted on LAM antigen assay to test its utility. One such study was conducted in western Kenya where investigators compared the diagnostic utility of LAM antigen assay in the hospitalized versus the ambulatory patients suspected to have TB. They found that LAM antigen assay had incremental diagnostic yield in patients who were hospitalized with very poor clinical condition. There was however no incremental diagnostic yield in the ambulatory patients (Huerga et al., 2017).

A study that was carried out in four Sub Saharan African countries demonstrated the utility of TB-LAM; PLHIV who were severely ill requiring hospital admission were randomly grouped into two groups. In one groups the decision to initiate TB treatment was based on LAM antigen assay positivity; while in the second group TB treatment initiation was based on routine TB tests that included chest radiographs, MTB cultures, Gene Xpert MTB/RIF or treatment based on clinical grounds. The group that was initiated TB treatment based on LAM antigen assay positivity, had a shorter time to treatment initiation within 0-3 days in comparison to the group that was initiated treatment based on routine tests that took approximately 8 days (p 0.024). Approximately 52% of the LAM antigen assay group was initiated TB treatment, in comparison to only 47% in the group that relied on routine tests (p 0.024). Hence, by relying on LAM antigen assay, more participants were started on TB treatment and this occurred earlier in comparison to participants who did not have a LAM antigen

assay done. So far, this is the first TB diagnostic test study that has been shown to have a mortality benefit. Studies of the impact of Gene Xpert MTB/RIF have demonstrated reduced time to treatment initiation but have not demonstrated a morbidity or mortality benefit (Peter et al., 2016).

2.5 HIV and sepsis

Sepsis is associated with a high morbidity and mortality both in the non-HIV and HIV setting. However, HIV infected persons admitted with sepsis in the intensive care unit were found to have a poor prognosis in comparison to persons who were not HIV infected (Mrus et al., 2005).

The HIV setting, the virus mainly targets the CD_4 T cells that play an important role in the adaptive immune system. Thus, with increasing viral loads the CD_4 counts decreases hence weakening the immune system mainly the cell mediated immune system, thus there is increased susceptibility to infections ranging from viral, bacterial, fungal and protozoal.

Several factors have been found to determine the etiology of sepsis in the HIV settings and this include the age of the patient, the level of CD_4 counts, whether the infection is community or hospital acquired and the geographical area in that developed versus developing countries (Taramasso et al., 2016).

The isolation of MTB from blood culture defines disseminated TB though this may be rare in developed countries; it is common in developing countries that have a high incidence and prevalence of TB.

Studies conducted in areas with high prevalence of TB, found the prevalence of MTB as a cause of sepsis ranging between 17 and 54% (Jacob et al., 2013; Peters et al., 2004; Varma et al., 2010).

In addition to high prevalence of TB these countries, they are further coupled by the fact that they have also a high prevalence of HIV infection and poor access to medical care hence most patients present in advanced stages of HIV infection. Studies conducted in Uganda and Malawi found that HIV infected participants who had MTB bacteremia had lower CD_4 counts with median CD_4 counts ranging between 17 and 129cells/µl (Bedell et al., 2012; Jacob et al., 2013)

HIV infected persons with even lower CD_4 counts (<50cells/µl) are also at increased risk of bacteremia caused by atypical MTB (Control, Prevention, Adults, & Adolescents, 2017).

The gold standard of diagnosing and differentiating between MTB bacteremia and atypical MTB is blood cultures that have a median time of approximately 3 weeks. Thus there is delay of initiation of treatment hence a high mortality rate seen within one month of admission (Crump et al., 2012).

CHAPTER THREE: METHODOLOGY

3.1 Study site

The study was carried out in the adult medical wards at Moi teaching and referral hospital (MTRH) Eldoret, Kenya. The MTRH serves as the teaching hospital for Moi University Schools of Medicine (MUSOM), Public health, Dentistry, and Nursing.

The MTRH is the second largest National Teaching and Referral Hospital (level 6 Public Hospital) in the country serving a catchment population of approximately 20 million people, with a bed capacity of 991 patients, an average number of 1200 patients at any time and about 1500 out patients per day.

It mainly serves residents of Western Kenya Region (representing at least 22 Counties), parts of Eastern Uganda and Southern Sudan with a population of approximately 24 Million.

It is located along Nandi Road in Eldoret Town, Uasin Gishu County (310 Kilometers Northwest of Nairobi the Kenyan capital city).

It has a continued partnership with Academic Model Providing Access to Healthcare (AMPATH). AMPATH has 800 plus care setting serving approximately 160,000 of the 1.5 million Kenyans that live with HIV infection with MTRH serving as its main referral site.

Majority of the people in this region rely on public health service for hospital admission and care. The bulk of admissions at MTRH are mainly people who are HIV infected and TB co-infected.

3.2 Study population

The study population was adult PLHIV persons admitted with sepsis in the medical wards. The sampled population were all those who met the inclusion criteria and consented to participate in the study.

Sepsis was defined using the international sepsis two criteria that requires the presence of two or more of the following:

Suspected infection as per the admitting diagnosis

Plus two or more of the following:

Respiratory rate >20 beats per minute

Pulse rate >100beats per minute

Temperature >39^0C or <36^0C

WBC >12,000/mm or < 4,000/mm (Bone, 1992)

The international sepsis two criteria was used instead of the sepsis 3 criteria because it was found to have a higher sensitivity in the prediction of mortality in the non-ICU setting (Fernando et al., 2018).

3.3 Study design

This was a cross sectional descriptive hospital based study.

3.4 Sampling and Recruitment

3.4.1 Sampling Technique

Participants were recruited through a consecutive sampling technique until the desired sample size was achieved. Upon admission of individual participants, the principle investigator was informed within 4-12hours. The principle investigator then reviewed the participants and those who met the eligibility criteria and had their consent

obtained. The participants were informed that they were being screened for disseminated TB and urine and blood samples were taken. They were informed that the results would be provided to their primary clinicians and they were also provided with results.

3.4.2 Eligibility Criteria

Above 18 years

PLHIV.

Admitted with sepsis

3.4.3 Sample Size.

The sample size was derived using the Fisher et al 1992 formulae.

$$\pi = \frac{(\text{Z critical})^2 * P(1-P)}{d^2}$$

Where: $(\text{Z critical})^2$ at 95% confidence interval= 1.96

P = 0.5

Q= (1-P)

D level of precision at 5% = 0.05

Where;

π = sample size

Z = the value corresponding to 95% confidence = 1.96

α = significance level at 5% =0.05

p= estimated prevalence of disseminated TB in PLHIV admitted with sepsis

d= margin of error

Currently in Kenya there are no studies done on the prevalence of disseminated TB in PLHIV admitted with sepsis. However, a similar study was carried out in Uganda, they reported the prevalence of disseminated TB in PLHIV and had been admitted with sepsis at 23% (Jacob et al., 2013); since the setting in Uganda is almost similar to our setting:

$\pi = (1.96/0.05)^{2 *0.5*0.5} = 272$

With 10% attrition = 299 (to account for any invalid tests)

Rounded off to = 300

3.5 Study procedure

Once the participants met the inclusion criteria, age and gender, (social demographics characteristics) were recorded. The participants who were reported already known to have HIV infection, further history was taken about whether they were on HAART, their last viral load and CD_4 counts plus their facility of follow-up.

Thirty milliliter (30 ml) of urine was collected in a sterile container. For bed bound patients a Foleys catheter was inserted under sterile conditions and a spot urine sample was collected. Sixty microliter (60µl) of urine was then pipetted onto the LAM antigen assay-loading bay. The LAM antigen assay strip was then incubated for 25 minutes at room temperature and pressure.

Under standard indoor ambient lighting, the strips were then examined using the naked eye for bands within 25-35 minutes of urine loading. The bands were then compared and graded with the manufacture's reference card as per the January 2014 guidelines. Grade one being very low intensity while grade four being high intensity.

The results were described as follows as per the manufactures guidelines:

> Positive band was a band equal in intensity with any grade in the reference card
>
> Negative band was lack of a band in the patient's window
>
> Equivocal/indefinite was a band that was of lower intensity than any grade in the reference card or an incomplete band

The LAM antigen assay strips were stored indoors under lock and key. The unused strips as per the manufactures guidelines were stored at 4-25ºC and were only available to the researcher and the research assistants. Training on how to undertake the test had been done prior by a certified immunologist at the MTRH/AMPATH reference laboratory.

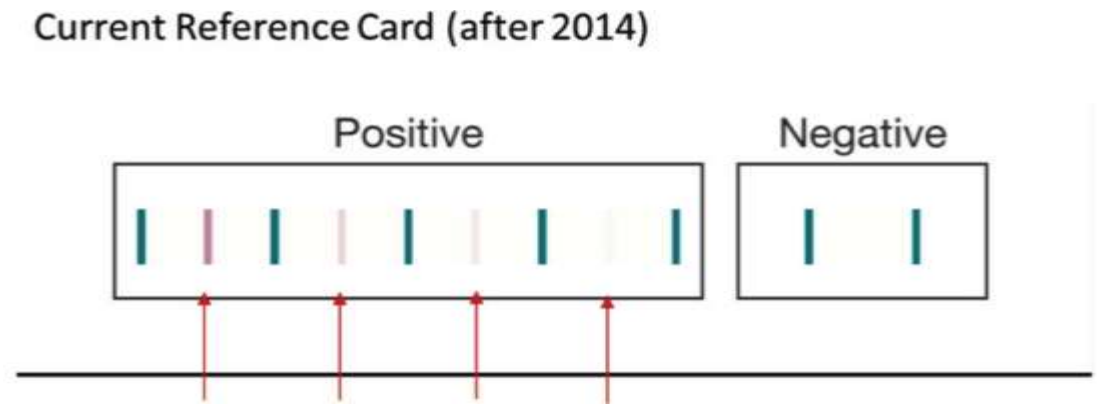
Results were then immediately provided to the primary clinicians; positive LAM antigen assay result was accompanied by a recommendation to start TB treatment and further test to confirm the diagnosis. A negative LAM antigen assay result was further accompanied with recommendation to consider further diagnostic workup for TB using other standard test if indicated.

Trained laboratory phlebotomist under aseptic conditions then took blood samples. Whereby 2ml of blood was put in an EDTA bottle for full blood count determination and another 2ml of blood for serum creatinine and urea determination. All these results were provided to the primary clinician caring for each participant and decision to start anti-TB medication was left at their discretion.

The participants were informed also of the results (both the urine and blood workups).

For participants who were already on follow up at our facility their records were retrieved for determination of their last viral load and CD_4 count.

Participants who were newly diagnosed or not follow up at our facility, the decision on CD_4 count and viral load measurement was left for the clinicians where they deemed it necessary.



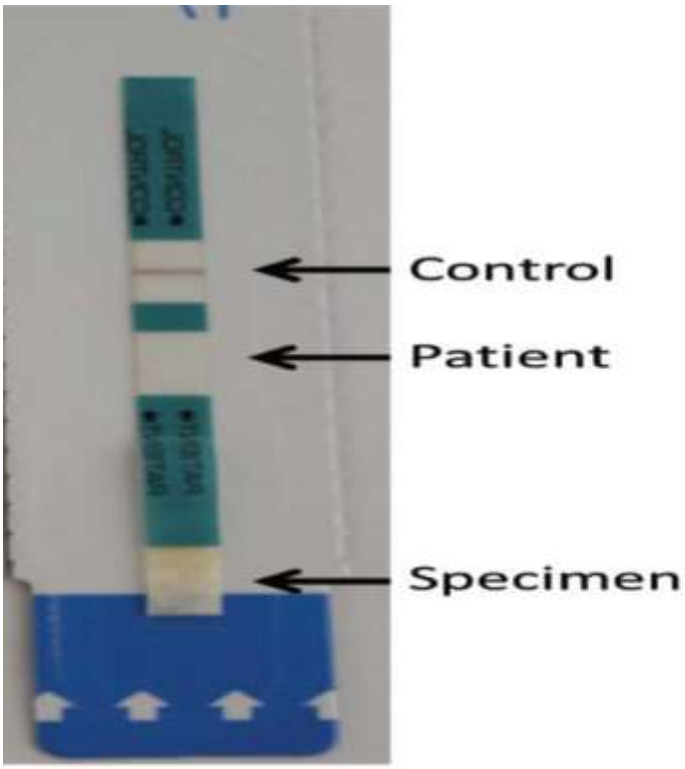


Figure 4: LAM antigen assay strip

Figure 5: The LAM antigen assay strip results

3.6 Data Collection and Management

3.6.1 Data collection

Data was collected between April and September 2017 using an interviewer administered structured questionnaire (Appendix 1). Medical forms were also reviewed and relevant clinical and laboratory data was obtained and entered in the interviewer administered structured questionnaire. The variables collected included demographic characteristics including age and gender; and HIV descriptors that included the knowledge of their HIV status prior to admission, and whether they were on HAART. Other variables that were collected included; laboratory parameters (CBC, serum urea and creatinine), and the admitting vital signs (axillary body temperature, pulse rate, respiratory rate and blood pressure). The results of the urinary LAM antigen assay were also recorded.

3.6.2 Data cleaning

All data variables were reviewed and inspected for distribution, missing data and inconsistencies.

3.6.3 Data entry

Data was entered into Epidata version 13

3.6.4 Data protection and safety

The completed questionnaires were stored under lock and key and were only accessible to the principal investigator. To ensure confidentiality, identifiable data collected was not shared. In the circumstance that the data collected was shared then anonymity of the participants was ensured through de-identification and data was also encrypted

Data that was stored in the computer was password protected. The computer had a password and Kaspersky antivirus. The questionnaires will be shredded after 3 years or upon publication of the study.

3.6.5 Data analysis

Data was analyzed using STATA version 13 special edition. Continuous variables were summarized as means and standard deviation while categorical data were summarized as frequencies and percentages with corresponding variable of proportion. Data that was not normally distributed was presented as medians and inter quartile ranges.

The statistical tests that were used were student T test or parametric equivalent (Wilcoxon rankson) and Chi square. Ninety-five percent (95%) confidence interval was calculated for the prevalence.

3.7 Ethical consideration

This study was conducted with ethical approval from the Institutional Research and Ethic Committee (IREC) of MTRH and Moi University (APPENDIX 4: MTRH AND IREC APPROVAL LETTERS). Permission to conduct this study was also obtained from the management of MTRH (APPENDIX 4: MTRH AND IREC APPROVAL LETTERS). A written informed consent was obtained from each participant that was recruited for the study and for the instances the participants could not give consent, their next of kin gave consent (appendix 2)

All participants including those that declined consent received the same level of care. There was minimal risk exposed to the participants except the physical pain and discomfort experienced during sample collection.

Confidentiality was maintained throughout the study period and recruitment was carried out in privacy. All participants including those that declined consent received the same level of care. There was minimal risk exposed to the participants except the physical pain and discomfort experienced during blood sample collection.

There was no conflict of interest in this study and no incentives were used to recruit the participants.

The results were availed to all the participants and their primary clinicians. The findings of this study shall also be availed to the scientific community and stakeholders, including AMPATH and ministry of health. Results of this; will also be published in a reputable journal, availed to the MUSOM library, and presented in professional conferences and seminars.

CHAPTER FOUR: RESULTS

The study was carried out between the months of April and September 2017. A total of 300 participants were recruited. Upon attaining consent, a spot urine sample was taken for LAM antigen assay testing. The results were provided to the clinician and recommendations to start treatment were made.

Data describing the demographics and clinical characteristics were collected.

The results are summarized below.

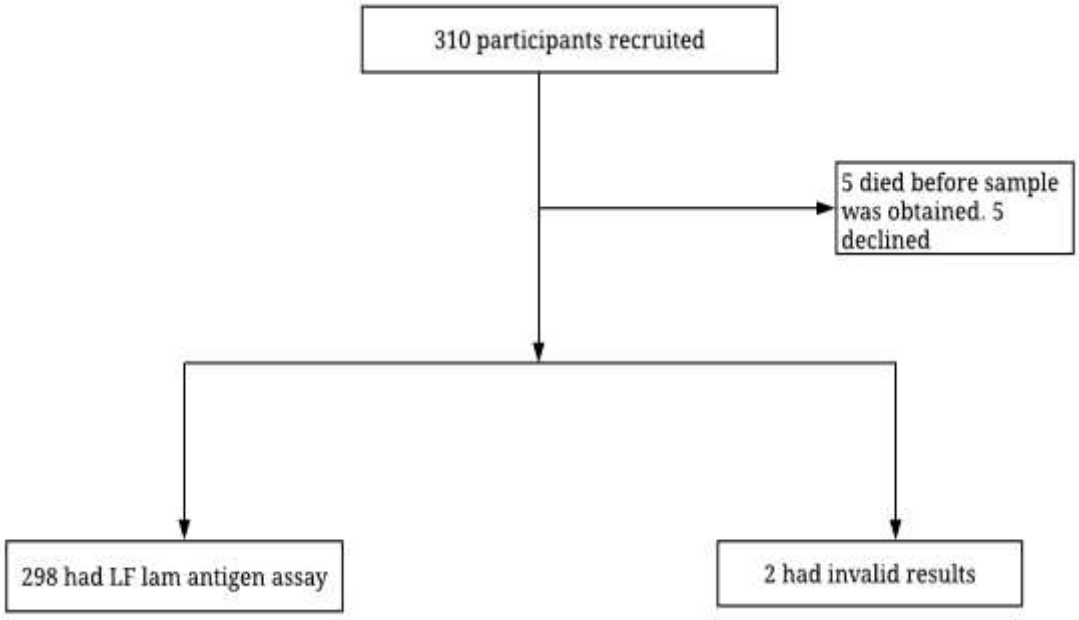


Figure 6: Recruitment schema

4.1 Demographic Characteristics

The median age was 41.68(SD11.72), with no difference in age between the LAM positive and LAM negative groups 41.62 (SD10.58) versus41.70 (SD12.13) respectively). There were more female participants than the male (173 (58%) versus 125 (42%) respectively). However, there was no significant association between sex and LAM positivity.

Table 1: Social demographic characteristics

Participant Characteristic	**Total** N= 298	**LAM** Positive n=77	**LAM negative** N=221	**P value**
Age Mean (SD)	41.68 (11.72)	41.62 (10.58)	41.70 (12.13)	0.96
Sex Male	125 (41.95%)	39 (31.20%)	86 (68.80%)	
Female	173 (58.05%)	38 (21.97%)	135 (78.03%)	0.072

The median age was 41.68 (SD11.72), with no difference in age between the LAM positive and LAM negative groups (41.62 (SD10.58) versus 41.70 (SD12.13) respectively).

There were more female participants than the male 173 (58%) versus 125 (42%). However this didn't affect LF LAM positivity with more male participants (51%) having a positive LF LAM antigen assay in comparison to females (49%)

4.2 Clinical Characteristics

Table 2: Admission vital signs

Participant Characteristic	Total N= 298	LAM positive n=77	LAM negative n=221	P value
Systolic BP(mmHg) Median (IQR)	100.89 (19.40)	97.70 (18.11)	101 (19.75)	0.09
Diastolic BP(mmHg) Median (IQR)	64 (22)	64 (23)	66 (23)	0.54
Temp (^{0}C) Mean (SD)	37.08 (1.40)	37.16 (1.61)	37.06 (1.32)	0.59
Respiratory rate(b/m) Median (IQR)	22 (4)	22 (4)	22 (2)	0.93
Pulse (b/m) Median (IQR)	110 (15)	110 (14)	114 (17)	0.02

The admitting vital signs that were taken into consideration included a blood pressure measurement, an axillary body temperature measurement, respiratory rate and pulse rate, which were all taken at the time of admission. The LAM positive group had a lower blood pressure with a median systolic of 98mmHg and diastolic of 64mmHg versus systolic of 101mmHg and diastolic of 64mmHg in the LAM negative group. All the participants were tachypnea with a median overall respiratory rate of 22 breaths/min with no difference in the two groups.

The radial pulse rate was the only admitting vital sign that was statistically significant (p0.02). All the participants were found to have tachycardia with an overall median pulse rate of 110beats/min. However, the LAM negative group had a relatively lower median pulse rate of 110beats/min in comparison to 114beats/min in the LAM negative group. The difference is not clinically significant.

Table 3: Laboratory markers

Participant Characteristic		Total N= 298	LAM Positive n=77	LAM negative n=221	P value
White blood cells(10^9/l)	Normal	127 (42.62%)	31 (24.41%)	96 (75.59%)	
	Leukopenia	105 (35.23%)	27 (25.71%)	78 (74.29%)	0.8
	Leucocytosis	66 (22.15%)	19 (28.71%)	47 (71.21%)	
Absolute lymphocyte count (/ml)	Normal (1001 – 3000)	114 (38.26%)	20 (17.54%)	94 (82.46%)	
	Lymphopenia (< 1000)	178 (59.73%)	55 (30.90%)	123 (69.10%)	0.04
	Lymphocytosis (> 3000)	6 (2.01%)	2 (33.33%)	4 (66.67%)	
Haemoglobin (g/dl)	Mean (SD)	10.28 (2.76)	9.71 (2.93)	10.47 (2.68)	0.04
eGFR(ml/min)	Median(IQR)	95.6(83.9)	86.8(97)	96.8(77.65)	0.53

Complete blood count and serum creatinine and urea levels were done. The only statistically significant findings in the complete blood count were the hemoglobin and absolute lymphocyte count levels.

Sixty percent (60%) of all the participants had lymphopenia that was defined as absolute lymphocyte count less than 1000 cells/ml. Of those with lymphopenia, 31% had a positive LAM antigen assay result and 69% had a negative LAM antigen assay result (p0.04).

Overall, all the participants had anemia with a median hemoglobin level of 10.28g/dl with the LAM positive group having a lower median hemoglobin level of 9.71g/dl versus 10.47g/dl in the LAM negative group (p 0.04). The median estimated glomerular filtration rate (eGFR) that was calculated using the CKD EPI formula was 95.6ml/min overall. The LAM positive group had a lower median eGFR at 86.8ml/min versus 96.8ml/min in the LAM negative group though this was not statistically significant (p 0.53).

The eGFR was calculated using the CKD epi formula that has been shown to take to account racial variation into consideration. The median eGFR was 95.6ml/min with participants who had urine LAM antigen assay positivity found to have a lower median e GFR at 86.8ml/min in comparison to 96.8ml/min in the participants who did not have urine LAM antigen assay positive.

4.3 Prevalence

Using LAM antigen assay the prevalence of disseminated TB in PLHIV admitted with sepsis was 26% (95% CI 20.96% to 31.20%). Out the 298 participants that had LAM antigen assay done, 77 of them had a positive result while 221 had a negative result.

4.4 The HIV descriptors

Table 4: LAM and HIV diagnosis

Participants characteristics		Total N=298(%)	LAM positive n=77(%)	LAM negative n=221(%)	P value
HIV Status	Newly diagnosed	88 (29.53%)	26 (29.55%)	62 (70.45%)	
	Known	210 (70.47%)	51 (24.29%)	159 (75.71%)	0.344

Seventy percent (70%) of all the participants were aware of their HIV status prior to admission with 24% of them having LAM antigen assay positive. There was no association between knowledge of HIV status and TB LAM results.

Table 5: LAM and HAARTs use

Participants characteristics		Total N=298(%)	LAM Positive n=77(%)	LAM negative n=221(%)	P value
HAART use	Not on HAART	193 (64.77%)	58 (30.05%)	135 (69.95%)	
	On HAART	105 (35.23%)	19 (18.10%)	86 (81.90%)	0.02

One hundred and ninety-three (64.8%) participants were not on antiretroviral therapy at the time of admission despite 70% of participants being aware of their status prior to admission. Not being on HAART was significantly associated with having a positive LAM assay result (p0.02).

Table 6: LAM positivity and viral loads/CD_4 counts

Participant Characteristic		Overall N= 298	LAM Positive	LAM Negative	P Value
Viral load	Undetectable	36 (35.29%)	6 (7.79%)	30 (13.57%)	
	Detectable	66 (64.71%)	17 (22.08%	49 (22.17%)	0.39
	Missing	196 (65.77%)	54 (70.13%)	142 (64.25%)	
CD4 mm3	Median (IQR)	105 (301)	121 (319)	94 (105)	0.42

Not all participants had viral loads and CD_4 count as part of their work up by the time of this study. This is due to the new guidelines by WHO in HIV care that recommended only a CD_4 count upon enrolment and a viral load after 6 months, then every 6 months after that for patients who are already on care (Organization, 2016).

The LAM negative group had better viral suppression with only 81% with detectable viral load vs 91% in the lam positive group.

Overall, median CD_4 count was 105 cells/mm^3 (IQR 301) with the participants with LAM antigen assay positive having a high CD_4 count at 121cells/mm^3 in comparison to 91cells/mm^3 in the LAM negative group.

However, because not all participants had CD_4 counts and viral loads could explain why these findings were not statistically significant. It was thus difficult to analyze the two groups in terms of CD_4 and viral load measurement.

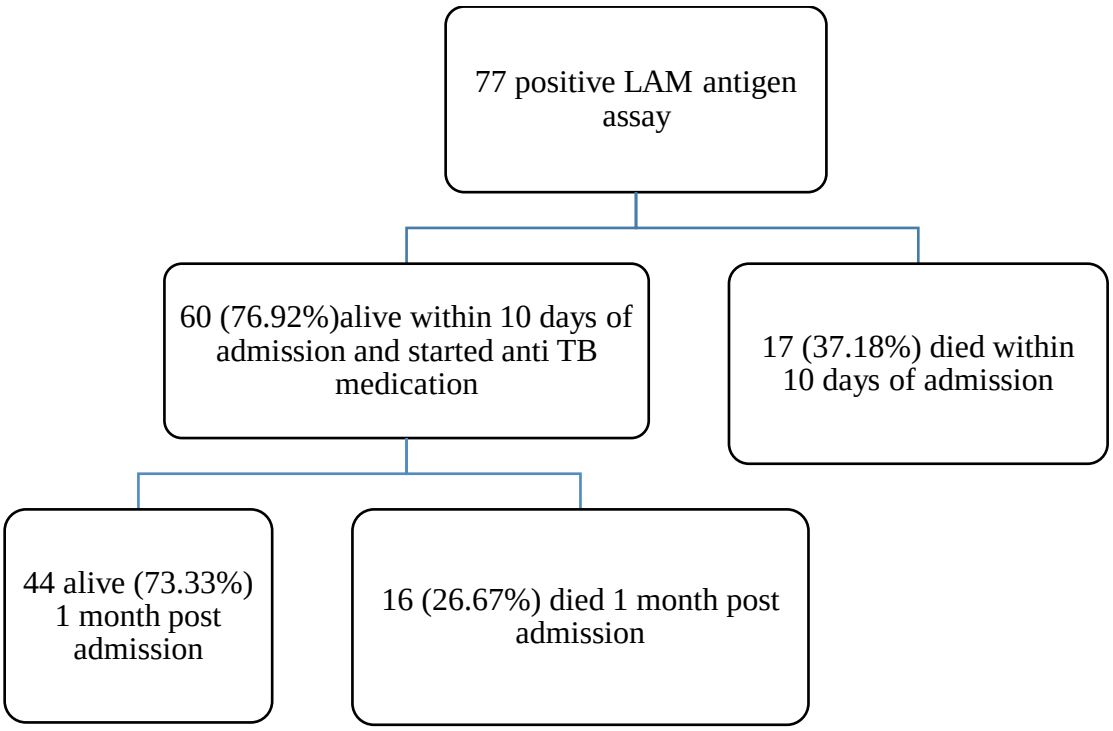


Figure 7: LAM positivity and mortality

There was statistical higher mortality seen in the lam positive group with 37% dying within 10 days of admission an additional 27% within 1 month of diagnosis($p < 0.001$).

CHAPTER FIVE: DISCUSSION

5.1 Prevalence

The prevalence of disseminated TB in PLHIV admitted with sepsis at MTRH, in this study was 25.84 % (95% CI 20.96% to 31.20%). This is a high prevalence.

Similar findings were reported in a study carried out in Uganda. Investigators recruited HIV infected persons who had been admitted with sepsis at two Ugandan hospitals and found a disseminated TB prevalence of 23% (Jacob, 2013). The slight difference in prevalence between these two studies could be explained by the fact that the Ugandan study relied on the gold standard to diagnose disseminated TB (MTB blood cultures) while this study utilized urinary LAM antigen assay.

A study conducted in South Africa found a prevalence of 10% among PLHIV attending outpatient clinic using urinary TB LAM (Kerkhoff, 2017). A lower prevalence is expected in outpatients compared with inpatients presenting with signs of sepsis. (Kerkhoff, 2017). Currently the WHO clearly recommends that the LAM antigen assay should not be used as a screening tool in asymptomatic individuals (WHO, 2015).

Both the WHO and Kenyan government currently recommend the LAM antigen assay as a point of care diagnostic tool in those who have advanced HIV determined either by low CD_4 counts or WHO stage 3/4. They also recommend that it can be used regardless of the WHO staging or CD_4 counts in the setting of severe illness requiring admission.

5.2 Demographics

The mean age in this study was 41.62, however the population used in Uganda was a much younger population with mean age of 34 and 37 in the multicenter analysis study(Jacob et al, 2013:Peter et al, 2016).

In this study, there was no difference in age between the LAM antigen assay positive group and the negative group with a median age of 41.68. Similarly, in the Uganda study there was no difference in age between the MTB bacteremia and the group without MTB bacteremia. (Jacob, 2013). The age in the Ugandan study was more widely distributed with a median age of 34 years unlike this study that which was more evenly distributed.

A South African study also found no difference in age between those with and without MTB bacteremia. The age like the Ugandan study was more widely distributed with a median age of 36years (Kerkhoff et al., 2017).

However, in all the three studies there were more female participants than males. This study had 173 females versus 125 males, the Uganda study had 194 females versus 174 males, and the South African study had 249 females versus 161 males (Jacob et al., 2013; Kerkhoff et al., 2017).

This finding may be explained by a study carried out in Nairobi slums by Muriithi et al that revealed that females have a more health seeking behavior than their male counterparts (Muriithi, 2013).

Despite more female participants, there were more male participants (51%) with LAM antigen assay positivity in comparison to 49% females. Though this was not statistically significant (p 0.072), it is clinically significant. This was reported in the

Ugandan study by Shaven T et al, where male gender was identified to be an independent risk factor for MTB bacteremia (Jacob et al., 2013).

A DHS survey conducted in 29 sub-Saharan countries that aimed to determine the uptake of HIV testing in those countries, reported that in 22 of those countries (Kenya was included) women were more likely to have been tested at least twice for HIV in the past year. The increased uptake in women was explained by the fact that there was increased testing in antenatal care (Staveteig et al., 2013).

The fact that more women were likely to have been tested prior to admission and started on HAART could explain why fewer women were reported with LAM antigen assay positive. However, this study was not powered to test for any associations.

5.3 Clinical Characteristics of participants

5.3.1 Admission Vital Signs

The admitting vital signs that were taken into consideration for the purpose of this study were; blood pressure, axillary body temperature, radial pulse rate and respiratory rate. Majority of the participants with LAM antigen assay positive had higher fevers (44.6% vs 39.8%). Similarly the Uganda study (46% vs 34%, p= 0.02) (Jacob et al., 2013). There was however no difference in terms of SBP in both groups

The only statistically significant finding was that participants who were LAM antigen assay negative had a higher pulse rate. At 114 beats per min in comparison to 110 beats per min in the LAM antigen assay positive group (p 0.02).

Tachycardia is a common presentation in sepsis and is attributable to sympathetic overdrive. This finding explains why all participants in this study had tachycardia which for the purpose of this study was defined as a pulse rate >100 beats per min.

A study carried out by Peter et al in Malawi that aimed to describe the characteristics of patients with sepsis and severe sepsis. Found that patients with severe sepsis tended to have higher pulse rate at 120 beats per minute in comparison to 104 beats per minute in patients with just sepsis (Waitt et al., 2015). This Malawian study was carried out before the sepsis 3 definition of 2016 that abolished the term severe sepsis and grouped sepsis into sepsis and septic shock (Singer et al., 2016).

According to the initial definitions of 1992, severe sepsis was defined as ≥2 Systemic Inflammatory Response Syndrome (SIRS) plus organ dysfunction, while sepsis was ≥2 of SIRS with no organ dysfunction (R. C. Bone et al., 1992).

SIRS (Systemic Inflammatory Response Syndrome) was defined as two or more of:

Temperature >38°C or <36°C

Heart rate >90beats per minute

Respiratory rate >20 breaths per minute or $PaCO_2$ <32 mm Hg (4.3 kPa)

White blood cell count >12 000/mm^3 or <4000/mm^3 or >10% immature bands

(R. C. Bone et al., 1992)

The Uganda study however reported slightly different findings where the participants who had MTB bacteremia were actually found to have a higher pulse rate than the group without MTB bacteremia. They went further to define heart rate, among other factors, as one of the non-HIV associated predictors of MTB bacteremia (Jacob et al., 2013).

However this study had a small sample size with only 86 participants with MTB bacteremia thus it would be difficult to generalize these findings (Jacob et al., 2013).

Hence, the finding that LAM antigen assay positive group had a lower pulse rate than the LAM negative group does not correspond with other studies done in this region.

Further studies with larger study populations may be required to assess for this association.

5.3.2 Laboratory Markers of participants

5.3.2.1 Hemoglobin Level

According to Lawn, et al, in addition to HIV status and CD_4 count the sensitivity of LAM antigen assay increases in patients who are severely ill, hospitalized and severely anemic.(Lawn et al., 2013)

Anemia is defined as hemoglobin levels <13g/dl for males and <12g/dl for females.

As per the WHO classification anemia is further classified into three categories:

Mild anemia is defined as hemoglobin concentration between 11.0-12.9g/dl in males and 11.0-11.9g/dl in non-pregnant females.

Moderate anemia is defined as hemoglobin concentration between 8.0-10.9g/dl for both males and non-pregnant females.

Severe anemia is defined as <8.0g/dl for both males and non-pregnant females (Organization, 2012).

Anemia is the most common hematological abnormality in HIV setting. A study conducted in south east Nigeria reported the prevalence of anemia in HAART naïve HIV positive individuals to be at 67% at diagnosis (Anyabolu et al., 2016).

The etiology of anemia in HIV infection is multifactorial but the most common mechanism is through anemia of chronic disease, whereby there is redistribution of iron mediated by hepcidin. Hepcidin is an acute phase reactant that inhibits the absorption of iron in the duodenum and the release of iron from hepatocytes and macrophages (Ganz et al., 2015).

A South African study to determine the characteristics of participants with a positive LAM antigen assay reported that the participants with a positive LAM antigen assay had lower hemoglobin concentrations in comparison to those with a negative LAM antigen assay.(Lawn et al., 2012). Kerkhoff et al also did another study in South Africa and they reported that 95% of the participants who had disseminated TB, had either moderate or severe anemia with median hemoglobin level at 7.7g/dl (Kerkhoff et al., 2017)

Shevin et al in Uganda reported similar findings, where they found participants with MTB bacteremia had lower hemoglobin levels than those with no MTB bacteremia. (7.4g/dl versus 9.3g/dl). In their risk stratification tool, they identified low hemoglobin levels as one of the non-HIV attributable risk factors that increased the predictive accuracy of correctly identifying severely ill HIV patients with increased risk of MTB bacteremia (Jacob et al., 2013).

The current study also had similar findings, with the LAM antigen assay positive group having lower hemoglobin concentrations at 9.71g/dl versus the LAM antigen assay negative group at 10.47g/dl (p 0.04).

Similarly a study carried out in Iran reported the prevalence of anemia in PLHIV to be at 71% (Meidani et al., 2012).

Like all other pathogens MTB requires iron for growth, hence high hepcidin concentrations ensures loading of iron in the macrophages thus promoting growth of MTB. Thus anemia occurring prior to TB diagnosis has been identified as an independent predictor of early incident TB in HIV setting, due to the fact that it allows a conducive environment for the growth of TB bacilli in the macrophages (McDermid et al., 2013)

Kerkoff et al found the prevalence of TB in HAART naïve PLHIV with anemia, was strongly and directly correlated with the severity of anemia, whereby those with severe anemia had a higher prevalence of TB(Kerkhoff, Wood, Vogt, & Lawn, 2014).

TB also causes anemia of chronic disease also by increasing the synthesis of hepcidin from hepatocytes. Hepcidin has already been alluded to traps iron in macrophages and inhibits intestinal absorption of iron. These two facts explain why despite an overall low hemoglobin level in all participants, the participants that had HIV/TB co-infection had even lower hemoglobin levels.

Anemia in the setting of HIV/TB co-infection has also been associated with increased mortality and progression to AIDS (Mocroft et al., 1999). Similar findings were reported in the South African study, where those with severe anemia were found to have lower CD_4 counts, higher viral loads and more likely to be in WHO stage 3/4, thus more likely to be immunosuppressed. These same participants with severe anemia and TB/HIV had also higher 90 day mortality and poorer prognosis (Kerkhoff et al., 2014).

5.3.2.2 Absolute Lymphocyte Count

Lymphocytes are broadly classified into B and T lymphocytes. The T lymphocytes are further classified into the helper CD_4 T lymphocytes and cytotoxic CD_8 T lymphocytes.

HIV virus targets the CD_4 T lymphocytes in the gut associated lymphoid tissue leading to overall reduction of the CD_4 T lymphocytes in the circulation. Initiation of HAART plus the cytotoxicity of the CD_8 T lymphocytes helps to control the viremia and in so doing increases the CD_4:CD_8 ratio above one. T lymphocytes are central in

controlling the replication of MTB, thus the low T lymphocytes count in PLHIV increases their susceptibility to MTB infection.

A CD_4 count and percentage measurement helps determine the level of immune suppression, and the risk of developing opportunistic infections, with increased risk with CD_4 counts<200cells/µl. CD_4 cell count measurement not only is it an expensive and labor-intensive test but also in most resource limited setting the results are not readily available requiring a second visit.

The normal reference range for absolute lymphocyte count is between $1.0–3.0\times10^9$/l while that for a total lymphocyte count ranges between $4.0\text{-}10.0\times10^9$/l. In the currentstudy,60% of the 298 participants had lymphopenia (absolute lymphocyte count$<1\times10^9$/l), with 31% of them having disseminated TB in contrast to 70% who had lymphopenia and LAM antigen assay negative (p 0.04).

With this in mind, hence Jacobson et al carried out a study in San Francisco, where they compared absolute and total lymphocyte count with CD_4 counts threshold. They correlated the three parameters and found that an absolute lymphocyte count <750 cell/µl and a total lymphocyte count <900 cell/µl had a 98% positive predictive value of a CD_4 <200 cell/µl. They thus concluded that an absolute and total lymphocyte count had equal utility in correlating with CD_4 threshold and recommended a higher cut off point for total lymphocyte so as to achieve the same sensitivity and specificity as the absolute lymphocyte count(Jacobson et al., 2003)

A study conducted in New Delhi that correlated absolute lymphocyte count and CD_4 count found using lower thresholds <1200cells/µl had a low sensitivity at 64%. They found that out of the 49 participants who had absolute lymphocyte counts <1200cells/µl only 77.6% of them had CD_4 counts <200 cells/µl. They thus then

compared the cut off <1200cells/µl with that of <1500cells/µl. They found that a cut off ≤ 1500/µl had a higher sensitivity at 78% for a CD_4 count <200 cells/µl. They thus recommended a higher cut off point for absolute lymphocyte count to reduce the number of the false negative that may be missed by using a lower cutoff of 1200cels/µl (Kakar et al., 2011)

This was similar to the San Francisco study that found that an absolute lymphocyte count <1500 cell/µl had a 79% sensitivity and 75% specificity for a CD_4< 200 cell/µl (Jacobson et al., 2003). However a study that was conducted in an emergency department in a hospital in Arizona America, found that an absolute lymphocyte count < 950cells/µl had a sensitivity of 76%. The participants in this study were seen in an emergency department requiring admission; this could explain why this study found a lower threshold in comparison to the New Delhi and San Francisco study where the participants were stable seen at an outpatient facility (Napoli et al., 2011).

In this study 59.73% of the 298 participants had lymphopenia, which for the purpose of this study was defined as a lymphocyte count$<1\times10^9$/l, with 71.43% of them having disseminated TB as per the LAM antigen assay (p value 0.04)

Similar findings were reported in the Uganda study whereby the participants with MTB bacteremia had a lower lymphocyte count (0.8 versus 1.1) compared with the non-MTB bacteremia group. (p0.007). On further multivariate analysis, they identified a lower CD_4 count as one of the risk factor for 18-day mortality in participants with MTB bacteremia (Crump et al., 2012).

However, for the purpose of this current study it was not possible to correlate the absolute lymphocyte count and CD_4 count. Further research can test for the association with LAM antigen assay with low absolute lymphocyte count.

However considering that the LAM antigen assay group had an opportunistic infection, disseminated TB, they were thus more immunosuppressed than the LAM antigen assay negative group. Disseminated TB is an opportunistic infection that tends to occur in PLHIV with CD_4 counts<200cells/µl.

5.3.3 The HIV Descriptors

The HIV descriptors that were determined in this study included whether the participants were on ART at the time of recruitment and if they were aware of their HIV status prior to admission.

Participants who had LAM antigen assay positive, 66% of them were actually aware of their status prior to admission and only 34% diagnosed at admission. Though this finding was not statistical significant (p 0.34), similar findings were also found in the Uganda study with only 32% were unaware of their HIV status prior to the admission (Jacob et al., 2013).

One hundred and ninety-three (193) participants were not on HAART at the time of admission. Despite this 66% of the participants with LAM antigen assay positivity actually aware of their HIV status prior to admission. Seventy five percent (75%) of them were not on HAART at the time of admission (p value 0.02). It could be they were either lost to follow up, considering that the Consolidated HIV guidelines of 2016 recommends to test and treat regardless of WHO staging and CD_4 count (WHO, 2016). However, it also could be that there was poor linkage between diagnosis and treatment. In addition, some of these participants could be had been diagnosed before the test and treat guidelines of 2016 and were actually HAART naïve. However, it was difficult to ascertain when the diagnosis was made and if they were defaulters or

HAART naive. The only character of interest that thus taken into consideration was whether they were taking HAART at the time of admission.

Out of the 193 participants not on HAART, 30% of them had LAM antigen assay positive versus 18% who were on HAART and had with LAM antigen assay positive(p 0.02). The Ugandan study, only 8% were actually on HAART at the time of admission in comparison to 92% in those with no MTB bacteremia ($p<0.001$). They thus identified the absence of HAART in PLHIV as one of the independent predictors for MTB bacteremia/disseminated TB (Jacob et al., 2013).

The study in Uganda reported almost similar findings whereby 67% of the participants were actually aware of their status prior to admission with 77% of them not being on HAART (Jacob et al., 2013). This goes to show that there is a good uptake of HIV testing however there could be loss of linkage between those who are tested and those who are started on HAART.

The South African study, 81% of the participants were aware of their HIV status at the time of recruitment with 58% of them not on HAART. On further characterization of the participants who were not on HAART, only 12% of them had disseminated TB. This finding in contrast to the current study and the Ugandan study, was not statistically significant (p0.11) (Kerkhoff et al., 2017).

Currently the WHO recommends testing and treating for HIV; however, in all three countries there was discrepancy between those who were aware of their HIV status and those on HAART. Further research is thus needed to identify why there is still discrepancy between the number tested and those actually on HAART despite the test and treat guidelines and ways to combat this discrepancy.

A DHS survey carried out in 29 sub Saharan countries between 2003 and 2011 found that the uptake of HIV testing has actually increased by 10fold in all participating countries, with Kenya being one. Hence the discrepancy is not in the uptake of testing but rather the linkage of treatment (Staveteig et al.,, 2013).

A systematic review done by Sydney Rosen et al that looked at retention in HIV care between testing and treatment in Sub Saharan countries, found that there was loss to follow up at each stage of care. This systematic review was done before the current recommendation of test and treat, they thus categorized retention to care in 3 stages from testing to initiation of HAART, and in each stage there was significant loss to follow up (Rosen et al., 2011).

This current study, participants who were not HAART had a higher chance of having LAM antigen assay positive in comparison to those who were on HAART (30% versus 18% (p0.02). This is despite the fact that a quite majority of them were aware of their status prior to admission, thus further research is required to identify why there is a discrepancy between the number tested and those actually on HAART.

As per the WHO consolidated HIV guidelines of 2016 they recommend starting HAART to every individual upon testing positive. This is regardless of their CD_4 counts and WHO staging as had been stated in the prior 2013 guidelines. They further recommend viral load measurement 6 months after diagnosis then 12 months after, then every 12 months in stable patients. These new guidelines they recommend in centers that can do routine viral loads to stop doing serial CD_4 count measurement (Organization, 2016).

Hence as per these guidelines, it was not possible to have all our participants with viral loads and CD_4 measurements. Out of the 298 participants, only 102 had viral

load, 36 were virally suppressed with viral loads count <1000cells/ml. Six out of the 36 who were virally suppressed, had LAM antigen assay positive with the remainder being LAM antigen assay negative. On further characterization of the participants in terms of a LAM antigen assay results, the lam antigen assay positive group had a higher proportion (91%) who had detectable viral loads in comparison to 81% in the LAM negative group. However, this was not statistically significant with p value of 0.39 it corresponds with the South African study.

Kerkoff et al study in South Africa defined viral suppression as viral copies <400 cells/ml while in this study it was defined as <1000 copies/ml viral copies (Kerkhoff et al., 2017). A cut off a 1000 cells/ml was used based on the fact that the risk of HIV transmission and disease progression to advanced HIV is low at levels <1000 copies/ml (Organization, 2016).

The South African study reported that of the forty-one participants who had MTB positive blood cultures only four were virally suppressed (p value 0.003) (Kerkhoff et al., 2017).

This is supported by the fact that disseminated TB and positive LAM antigen assay has been associated with individuals with advanced HIV disease. As per the WHO they defined advanced HIV disease as CD_4 counts <200cells/mm^3 or individuals who are HIV positive and are severely ill.

Similarly, the Uganda study, participants who had MTB bacteremia were found to have lower CD_4 counts in comparison to those who did not have MTB bacteremia (median CD_4 counts 17 versus 64 cells/µl respectively). This finding similar to the South African study and in contrast to this current study was statistically significant

($p<0.001$) They thus identified low CD_4 counts as independent factors associated with increased risk of MTB bacteremia in the HIV setting (Jacob, 2013).

In this study the overall median CD_4 count was 105 cells/mm^3 (IQR 301) with the participants with LAM antigen assay positive having a high CD_4 count at 121cells/mm^3 in comparison to 91cells/mm^3 in the LAM negative group, this finding however was not statistically significant (p value 0.42).

The Uganda study the MTB bacteremia group had a lower median CD_4 count at 17cells/mm^3 in comparison to 64 cells/mm^3. This finding in contrast to this study was statistically significant $p < 0.001$ they identified a low CD_4 count as an independent risk factor for the development of MTB (Jacob, 2013).

The south Africa study found findings that were similar to the Uganda study, participants with disseminated TB had a lower median CD_4 count at 42 cells/mm^3 in comparison to 102 cells/mm^3 in the participants who didn't have MTB bacteremia and this finding was statistically significant ($p<0.001$) (Kerkhoff et al., 2017).

5.3.4 LAM antigen assay positivity and mortality

Out of the seventy-seven participants with LAM antigen assay positive, sixty (76.92%) of them were alive 10 days from admission and they were started on anti TB medications. Seventeen (37.18%) of them died within 10 days of admission (p value <0.001).

One month from admission, out of the sixty that had LAM antigen assay positive forty-four (73.33%) were alive and sixteen (26.67%) of them died (p value <0.001). For the participants that died within one month from admission it was difficult to ascertain the cause of death.

The Ugandan study found almost similar findings whereby a high mortality were reported. The eighty-one participants who had MTB bacteremia forty-three (53.1%) of them died within 30 days in comparison to eighty-three (31.9%) out of two hundred and sixty who died and did not have MTB bacteremia. This finding was also statistically significant (p 0.001).

However, a lower mortality was seen in a multicenter study that was done in 4 sub Saharan countries. They had two arms in the study, one arm they started anti TB medication based on LAM antigen assay positivity while the other arm they based on convention tests. They found that in the arm that was started anti TB medication based on LAM antigen assay positivity had a lower mortality (21%) in comparison to conventional tests (25%). This study showed that starting anti TBs based on LAM antigen assay had a mortality benefit (Peter et al., 2016)

5.3.5 Renal Involvement and LAM antigen assay positivity

Previously studies had postulated that LAM antigenuria is due to simple ultrafiltration mechanisms, however recent studies have shown that this hypothesis is invalid.

Cited hypothesis include; first LAM being an antigen in circulation it has to be bound to either anti-LAM antibodies or high-density lipoproteins. Hence, this immune-complex of bound LAM cannot pass an intact glomerular basement membrane due to the tight fenestrations junctions (Haraldsson, 2008).

Secondly, it was thought that the LAM antigenuria was due to high disease burden. However, this too has been proven invalid due to the findings that early after initiation of TB treatment, serum LAM increases however, urine LAM has not been shown to increase. This is further supported by the fact that LAM antigen assay has been found

to have a low sensitivity in the diagnosis of pulmonary TB even in high burden disease stages (Wood, 2012)

Third, participants with LAM antigenuria could be having an underlying glomerular pathology. This too has been shown invalid since no studies have shown any association between urine LAM antigen positivity and proteinuria considering that proteinuria is a marker of glomerular damage. In addition, postmortem studies that have assessed renal biopsies of participants who had LAM antigen positive have not reported any glomerular damage. (Peter, 2012The presence of the LAM antigen in urine has been proposed due to actual detection of the whole MTB bacilli in urine and not just the antigen. A study by Lawn et al found that urine that was LAM antigen assay positive, when Gene Xpert MTB/RIF assay done on the same urine sample, it was able to detect more that 50% of the cases. The Gene Xpert MTB/RIF assay detects the whole MTB bacilli by amplification of the DNA and not just the MTB antigens (Lawn, 2011)

This has been further shown in a study conducted in Uganda, where they assessed the kidney biopsy of participants who had urine LAM antigen assay positive. There was histological evidence consistent with renal TB involvement in 62% of the biopsies. It is thus postulated, urine LAM antigen assay positivity, is due to the haematogenous spread of the MTB bacilli and the consequent associated renal involvement. This is further supported by the fact that urine LAM antigen assay has been shown to have increased sensitivity in those who have advanced HIV and have a higher risk of developing renal TB hence disseminated TB (Lawn, 2016)

This study aimed to determine if there were any renal derangements in participants who had urine LAM antigen assay positive. We calculated the estimated glomerular

filtration rate (eGFR) using the CKD EPI formula, the median overall eGFR was 95.6ml/min with participants with LAM antigen positive having a lower median eGFR at 86.8m/min versus 96.8ml/min in those who had LAM antigen assay negative, though this was not statistically significant (p 0.53).

The South African study also assessed the eGFR and the median overall GFR was 125ml/min (IQR 18-227) with participants with disseminated TB having a lower mediane GFR of 126ml/min (IQR 80-167) versus 129ml/min (IQR 95-166) (p 0.96) (Kerkhoff, 2017)

The Ugandan study however assessed only the creatinine levels and did not calculate the eGFR, the median overall creatinine level was at 97.24 (IQR 0.8-1.7) with no much difference reported in those with MTB bacteremia (Jacob, 2013).

However, in all three studies the sample size of those with disseminated TB was small, this study had a sample size of 77, Kerkoff et al in South Africa had a sample size of 41 and Shevin et al in Uganda had a sample size of 86. None of these studies were powered to assess for any correlation between urine LAM antigen positivity with eGFR. Further studies with a larger sample size are thus required to further assess for this association (Jacob, 2013: Kerkhoff, 2017).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion.

There was a high prevalence of disseminated TB (26%) in PLHIV admitted with sepsis in the medical wards at Moi Teaching and Referral Hospital. The associated risk factors in our setting included: not being on HAART, anemia, tachycardia and lymphopenia.

There was higher mortality seen in the LAM positive group within 1 month of admission.

6.2 Recommendations

1. The LAM antigen assay should be a routine diagnostic test in PLHIV who are admitted with sepsis at MTRH
2. There is need for a larger study to determine other risk factors associated with LAM antigen assay positivity.
3. Further studies to ascertain the agreement of LAM antigen assay and blood cultures in our setup.
4. A lam antigen assay should be done promptly and anti TB medication started immediately this is due to the high mortality seen in this group

6.3 Strengths and Limitations

Strengths

This study relied on the use of LAM antigen assay the only point of care test that has been shown to have a mortality benefit in the diagnosis of TB.

Limitations.

This study could have under-estimated the prevalence of disseminated TB by only concentrating participants who were HIV infected and had been admitted with sepsis.

This was a cross sectional study where exposure and outcome were accessed simultaneously hence difficult to establish casual influences.

Also not all of the participants that were recruited for the study attended the outpatient clinic at MTRH hence it was difficult to attain their medical records. Thus, not all participants had viral loads and CD_4 count measurements.

Finally, the diagnosis of disseminated TB were not confirmed with the gold standard that is blood cultures.

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APPENDICES

Appendix 1: Consent Form

English Version

You are being invited to participate in a research study on the prevalence of disseminated tuberculosis in HIV patients in WHO stage 3 and 4 admitted Moi Teaching and Referral Hospital with sepsis. Dr. Caroline Gituku who is a resident at Moi University School of Medicine Department of internal medicine is conducting this study.

The study shall involve about 10 minutes where your urine sample will be taken and a free LAM antigen assay done at the bed site. In addition, a blood sample shall be taken for a full blood count and serum urea and creatinine measurement.

The benefit of participating in this study to you as an individual is to facilitate a quick diagnosis of disseminated tuberculosis. All results shall be communicated to your primary physician to facilitate further management.

This research has minimal risk to you and will cause no harm. There are no costs to you for participating in the study.

The results of the study will be stored in a database that is password protected and only accessible by those conducting the study. No one will be able to identify you or your results, should the data be published, no individual information will be disclosed. Your participation in this study is voluntary. If you decide to participate, you can change your mind later and quit the study before the end of the study. If you decide not to participate, or if you quit the study, it will not affect the health care services you receive at the clinic. By signing this document, you are voluntarily agreeing to participate. You are free to decline to answer any particular question you do not wish to answer for any reason.

If you have any questions about the study, please contact Dr. Caroline Gituku on cell phone number 0723209276. The Institutional Review and Ethics Committee of Moi University and Moi teaching & Referral Hospital has granted us permission to conduct this study.

The chairman IREC

Moi Teaching and Referral Hospital,

PO Box 3,

Eldoret.

My cell phone number 0723209276

YOUR CONSENT:

ADULTS ABOVE 18 YEARS OF AGE

I have been adequately informed that I am recruited in a study to find out if I have disseminated TB. The investigator has also informed me that my participation in this study is voluntary and will not exclude me from routine care even if I opt out. She has also informed that I will not be required to pay for the tests for the purposes of this study.

Sign ……

Name …….

Date……

Kiswahili

Unakaribishwa kushiriki katika utafiti juu ya kuenea kwa kifua kikuu (disseminated Tb), kwa wagonjwa wenye virusi vya ukimwi ambao wamelazwa katika hospitali kuu ya Moi Teaching na Hospitali. Utafiti huu unafanywa na daktari Caroline Gituku ambaye ni mwanafunzi was shahada la juu (masters) katika Chuo Kikuu la Moi, shule ya udaktari (internal medicine)

Utafiti huu utachukua dakika 10 ambapo sampuli ya mkojo itachukuliwa na upimaji wa LAM antigen utafanywa hapo kitandani. Pia sampuli ya damu itachukuliwa kwa hesabu kamili ya damu na serum urea na kipimo cha creatinine.

Faida ya kushiriki katika utafiti huu kwa mtu binafsi, ni kuwezesha uchunguzi wa haraka wa kifua kikuu (disseminated TB). Matokeo yote yatatumwa kwa daktari wako wa msingi ili kuwezesha usimamizi zaidi.

Utafiti huu una hatari ndogo kwako na hautafanya madhara yoyote. Hakuna gharama kwako kwa kushiriki katika utafiti.

Matokeo ya utafiti utahifadhiwa kwenye daraka ambalo neno la siri linalindwa na linapatikana na wale wanaofanya utafiti. Hakuna mtu atakayekufahamu wewe au matokeo yako. Amabapo pale matokea itapatiwa wenginehakuna maelezo ya mtu binafsi yatafunuliwa. Ushiriki wako katika utafiti huu ni kwa hiari. Ikiwa unaamua kushiriki, unaweza kubadilisha mawazo yako baadaye na kuacha kabla ya mwisho wa utafiti. Ikiwa ukiamua kuacha kushiriki, haitaathiri huduma za huduma za afya unazopokea kwenye hospiatli. Kwa kusaini hati hii, unakubali kushiriki kwa hiari. Wewe ni huru kupungua kujibu swali lolote ambalo hutaki kujibu kwa sababu yoyote.

Ikiwa una swali lolote kuhusu utafiti, tafadhali wasiliana na Dk. Caroline Gituku kwenye nambari ya simu ya mkononi 0723209276. Kamati ya Ukaguzi na Maadili ya Taasisi ya Chuo Kikuu cha Moi na Mafundisho ya Moi & Hospitali ya Rufaa imetupatia ruhusa ya kufanya utafiti huu.

Mwenyekiti IREC

Hospitali ya Mafunzo na Hospitali ya Moi,

PO Box 3,

Eldoret.

Namba yangu ya simu ya mkononi 0723209276

HIDHANI YAKO:

Walio na miaka 18 na zaidi

Nimeelezwa ipasavyo ya kwamba ninashiriki katika uchunguzi wa usomi utakao chunguza iwapo ninaugua kifua kikuu (disseminated TB). Ugonjwa ambao mimi kwa ajili ya kuwa na virusi via ukimwi naweza kuwa nao. Mchunguzi pia amenieleza kuwa sitakosa matibabu yangu ya kawaida iwapo nisishiriki katika uchunguzi huu. Pia nimeelezwa kuwa sitahitajika kulipia chochote kinachohusiana na uchunguzi huu.

Ishara.....

Jina.....

Tarehe……

Appendix 2: Interviewer administered structured questionnaire

Demographics

IP

NO……Age……Gender……

Admission vital signs

Pulse rate……

Temperature……

Blood pressure……

Respiratory rate……

HIV descriptors

Was participant aware of their HIV status prior to admission?

Yes ☐ No ☐

Was participant on HAART Yes ☐ No? ☐

Laboratory markers

White blood cell count……

Absolute lymphocyte counts……

Hemoglobin level……

Serum creatinine……

Serum urea……

Lam antigen assay results Positive ☐ Negative ☐

Appendix 3: Procedure for vital signs measurement

Blood pressure will be taken using an Omron M2 compact upper arm blood pressure monitor.

The participant will be seated in a relaxed position at a quiet place. The participant will have no tight clothing on the upper arm.

The participant shall sit upright with the back straight and placed on the table so that the cuff is on the same level as the heart. The cuff shall be wrapped around the right arm such that the bottom of the cuff is at least above the elbow. It shall then be fastened snuggly. The start button on the machine shall then be pressed and the cuff shall automatically begin to inflate and the machine shall take a reading.

The blood pressure as well as the heart rate reading shall then be displayed on the screen.

Should an error occur the cuff shall be deflated and the process repeated. High blood pressures shall be confirmed with a manual blood pressure measurement using mercury sphygmanometer.

For very sick participants who will not be able to sit upright. A blood pressure reading following the above procedure shall be taken with the participant lying supine

The blood pressure machine shall be calibrated every week.

The respiratory rate shall be calculated manually as the participant remains seated in a relaxed position. The total at the end of a full minute shall be taken as the respiratory rate.

The temperature shall be taken using a clinical thermometer placed at the participant’s armpit.

APPENDIX 4: MTRH AND IREC APPROVAL LETTERS

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471020

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

Reference: IREC/2016/160
Approval Number: 0001758

27th September, 2016

Dr. Caroline Gituku,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 SEP 2016
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Dr. Gituku,

RE: FORMAL APPROVAL

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

"Prevalence of Disseminated Tuberculosis in PLHIV Who Stage 3 or 4 Admitted with Sepsis at MTRH using LAM Antigen Assay".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1758*** on 27th September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th September, 2017. 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,

For

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

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

30th September, 2016

Dr. Caroline Gituku,
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:-

"Prevalence of Disseminated Tuberculosis in PLHIV Who Stage 3 or 4 Admitted with Sepsis at MTRH using LAM Antigen Assay".

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

30/09/2016

DR. WILSON ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

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