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Tuberculosis and HIV

Evaluation of New Tests for Diagnostic Accuracy, Cost-Effectiveness and Effect on Treatment of Tuberculosis in Smear-Negative HIV-positive Patients in Uganda

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Dedication

For the ‘smla’ progeny: May yours and the generations beyond, be free from the twin curse of Tuberculosis and HIV.

ABSTRACT

Background: Despite tremendous progress in the control of the global tuberculosis (TB) epidemic during the past decade, it is still a major health problem even today. The burden of TB is particularly severe in sub-Sahara Africa where TB and HIV co-infection is common, especially in those who are smear-negative. Such patients are twice more likely to die from TB than their counterparts because of delayed diagnosis and treatment. This thesis focuses on the evaluation of currently available new tests which have been developed to facilitate rapid diagnosis and early treatment of TB.

Aims: The purpose of this thesis was to increase knowledge about the diagnostic accuracy, cost-effectiveness and effect on treatment of TB of the new tests in smear-negative HIV-positive patients in a context where resources are limited.

Methods: In **study I**, a systematic review was performed to summarize and compare the over-all accuracy for smear-negative TB of the existing traditional TB tests (WHO 2007 TB algorithm) and two new tests: Xpert MTB/Rif test (a molecular-based method) and Microscopic Observation Drug Susceptibility test (a culture-based method). In **study II**, a cross-sectional study was performed to collect primary laboratory data on the diagnostic accuracy for smear-negative TB of Xpert MTB/Rif (GeneXpert), Microscopic Observation Drug Susceptibility test (MODS) and Nitrate Reductase Assay (NRA) in HIV-positive patients. The results of the three tests were compared with traditional solid Löwenstein-Jensen (L-J) TB culture and a new conventional liquid (MGIT) TB culture method. In **study III**, we modelled the cost-effectiveness of using MODS or GeneXpert-based algorithm for diagnosis of pulmonary TB in HIV-positive patients. Finally, in **study IV**, we investigated how best to treat patients with presumptive pulmonary TB who were both Smear and GeneXpert negative.

Results: From **study I**, we found that the sensitivity of the tests for smear-negative TB was moderate (61-73%). The specificity was high for both GeneXpert (98%) and MODS (91%) but moderate for the WHO 2007 TB algorithm (69%). From **study II**, we found that GeneXpert, MODS and NRA had low sensitivity for smear-negative TB in HIV-positive patients (24-49%). However, the specificity of all three tests was high (92-98%). From **study III**, we found that utilizing a MODS-based algorithm for diagnosis of pulmonary TB in HIV-positive patients was more cost-effective than utilizing a GeneXpert-based algorithm (US$ 34 versus US$ 71 per TB patient diagnosed). From **study IV**, we found that a smear and GeneXpert-negative test result had a high negative predictive value for TB. Thus, despite the low-moderate sensitivity of GeneXpert for smear-negative TB, a majority of patients (88%) responded fully to antibiotic treatment and empiric TB treatment was initiated in only a few (8%) of them.

Conclusions: GeneXpert, MODS and NRA are useful for diagnosis of TB in smear-negative patients including those who are HIV-positive. The tests could be used to improve the existing WHO 2007 TB algorithm. But since they have low-moderate sensitivity, additional evaluation for TB is required in those who test negative using these new tests. The high specificity of GeneXpert, MODS and NRA implies they are highly reliable to initiate TB treatment in those with positive results. From an economic view point, utilizing a MODS-based algorithm for diagnosis of TB in HIV patients is more cost-effective than a GeneXpert-based algorithm. Therefore, where resources are limited, MODS could be used as an alternative to GeneXpert. Using an antibiotic treatment trial in HIV patients who are both smear and GeneXpert negative could be useful to reduce empiric TB treatment because of the high negative predicative value for TB of the two combined tests.

Key words: Accuracy, Algorithm, GeneXpert, HIV, MODS, NRA, Smear-negative, TB

LIST OF PUBLICATIONS

This thesis is based upon the following studies, which will be referred to by their Roman numerals throughout the thesis:

I. Simon Walusimbi, Freddie Bwanga, Ayesha de Costa, Melles Haile, Moses Joloba, and Sven Hoffner. Meta-analysis to compare the accuracy of GeneGeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infectious Diseases 2013, 13:507

II. Simon Walusimbi, Freddie Bwanga, Ayesha de Costa, Melles Haile, Sven Hoffner, Moses Joloba. Evaluation of the GeneXpert_ MTB/Rif test, microscopic observation drug susceptibility test and nitrate reductase assay, for rapid and accurate diagnosis of smear-negative tuberculosis in HIV patients.
International Journal of Mycobacteriology, 2013, vol 2, pages 148– 155

III. Simon Walusimbi, Brendan Kwesiga, Rashmi Rodrigues, Melles Haile, Ayesha de Costa, Lennart Bogg, Achilles Katamba. Cost effectiveness of Microscopic Observation Drug Susceptibility test compared to GeneXpert MTB/Rif test for diagnosis of pulmonary tuberculosis in HIV patients in Uganda. (Manuscript)

IV. Simon Walusimbi, Fred C. Semitala, Freddie Bwanga, Melles Haile, Ayesha de Costa, J. Lucian Davis, Moses L. Joloba, Sven Hoffner, Moses .R. Kamya. Outcomes of a clinical diagnostic algorithm for management of ambulatory smear and GeneXpert MTB/Rif negative HIV infected patients with presumptive Pulmonary TB in Uganda. (Manuscript).

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LIST OF ABBREVIATIONS

AFB	Acid-fast bacilli
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-Retroviral Treatment
CFU	Colony Forming Unit
COPD	Chronic obstructive Pulmonary Diseases
CXR	Chest X-Ray
DALY	Disability Adjusted Life Years
DST	Drug Susceptibility Testing
EPTB	Extra-pulmonary TB
EQA	External Quality Assurance
FM	Fluorescent Microscopy
HC	Health Centre
HIV	Human Immunodeficiency Virus
LED	Light Emitting Diode
L-J	Löwenstein-Jensen solid TB culture
MDG	Millennium Development Goals
MDR-TB	Multi Drug Resistant-TB
MGIT	Mycobacterium Growth Indicator Tube- liquid culture
MODS	Microscopic Observation Drug Susceptibility test
MTB	Mycobacterium tuberculosis
NRA	Nitrate Reductase Assay
NRH	National Referral Hospital
NTLP	National TB & Leprosy Control Programme
NTRL	National TB Referral Laboratory
PCP	Pneumocystis carinii (jirovecii) pneumonia
PEPFAR	President's Emergency Plan for AIDS Relief
PTB	Pulmonary Tuberculosis
Rif	Rifampicin
RRH	Regional Referral Hospital
SN-PTB	Smear-negative pulmonary tuberculosis
TB	Tuberculosis
VHT	Village Health Team
WHO	World Health Organization
ZN	Ziehl-Neelsen staining procedure

1 INTRODUCTION

1.1 MICROBIOLOGY OF MYCOBACTERIA

Mycobacteria are subdivided into three groups: The Mycobacterium tuberculosis complex (MTC), Mycobacterium leprae and Non-tuberculosis mycobacteria (NTM) [1]. In humans, disease from Tuberculosis (TB) is caused by Mycobacteria from the MTC group within which, *Mycobcaterium. tuberculosis* (MTB) is the principal agent. MTB can be distinguished from other agents within the MTC group by its ability to reduce nitrate to nitrite [2]**.**

Mycobacteria are non-motile and non-spore forming aerobic rods measuring 0.2-0.6 x 1 -10 µm in size. Their envelope structure consists of mycolic acids of 60 to 90 carbons, with a high content (61-71%) of guanine and cytosine in their DNA [3]. Species variations are characterized by variation in sugar substitutions in the cell wall of the mycobacteria. Further, their cell wall is rich in lipids, making the surface hydrophobic and resistant to many disinfectants, including common antibiotics and common laboratory stains [1, 4]. However, once stained, they cannot be decolorized with acid solutions, and are therefore acid-fast. Other important wall components include the glycolipid trehalose dimycolate [5], which is thought to induce cord-like growth of mycobacteria on artificial media and lipoarabinomannan (LAM) a glycolipid which may play a role in virulence [6]. Compared to other bacteria which typically reproduce within minutes, Mycobacteria grow extremely slowly with a generation time of 18-24 hours because of their complex cell wall [4]. In addition, Mycobacteria are fastidious in nature, requiring specially enriched media for growth and enhanced by the presence of 5-10% CO_2 in a limited temperature range of 35-37 ^{o}C [1, 4].

1.2 RE-EMERGENCE OF TB AND THE EARLY CONTROL STRATEGY

Tuberculosis (TB) disappeared from the global public health agenda in the 1960s and 1970s because of improved socioeconomic conditions and the discovery of TB medicines leading to its decline during this period [7]. However, with the onset of the HIV pandemic in the 1980s, coupled with increases in drug resistance, TB re-emerged in the early 1990s and was declared a ‘global health emergency’ by the World Health Organization (WHO) in 1993 [8].

For nearly 25 years since 1990-2015, an estimated 7-9 million new TB patients occurred annually. During this period, between 1.5-2 million individuals died from TB annually, reaching a peak in 2004 [9]. In sub-Saharan Africa, due to the HIV epidemic, the number of TB patients doubled or tripled in some countries. TB became the leading cause of mortality in the region with HIV/AIDS as the commonest underlying factor [10-12].

In response to the alarming TB situation, the WHO launched the DOTS strategy. The DOTS strategy aimed to use cure of the infectious cases as the best prevention for TB transmission [13, 14]. The target was to detect at-least 70% of those infectious cases and cure at-least 85% of them by the year 2000. The DOTS strategy relied on smear-microscopy for TB detection and a standardized short course regimen for TB treatment.

1.3 GLOBAL INITIATIVES FOR TB CONTROL

Reports on the DOTS strategy revealed suboptimal progress towards the set targets [7, 10]. For example, a report of the DOTS strategy in 1998 showed that only a handful of countries had achieved the set detection and treatment success targets for TB [15]. In addition, there were persistent difficulties in TB diagnosis coupled with high TB mortality rates. It is during this period, that the momentum to set up new global initiatives for TB control picked up.

This momentum led to the creation of the STOP TB partnership and development of the first Global plan to Stop TB 2000-2005 [16]. The first 'Global plan to Stop TB 2000-2005' was intended to expand DOTS coverage, address HIV associated TB and drug resistant TB, and to pursue innovative research for new TB diagnostics, drugs and vaccines [17]. In parallel, the Global Fund was established in 2002 to increase resources to fight AIDS, Tuberculosis and Malaria [18]. The second 'Global plan to stop TB 2006-2015' set out the actions and funding needed to accelerate progress in TB control [19]. The targets in the second plan included reduction of the global TB prevalence and mortality by half relative to the 1990 levels by 2015. The key strategies in the second plan for achieving the set targets included scaling up existing interventions and the introduction of new technologies, notably new TB diagnostics.

1.4 CURRENT GLOBAL TB BURDEN

Globally, TB mortality and TB prevalence have fallen by 45% and 41% respectively since 1990 [20]. Despite the decline however, an estimated 9 million people continue to develop TB and 1.5 million continue to die from the disease annually (Figure 1). Furthermore, the current TB detection levels are suboptimal because nearly three million of the incident cases are not diagnosed, keeping TB a top cause of respiratory disease world-wide [21]

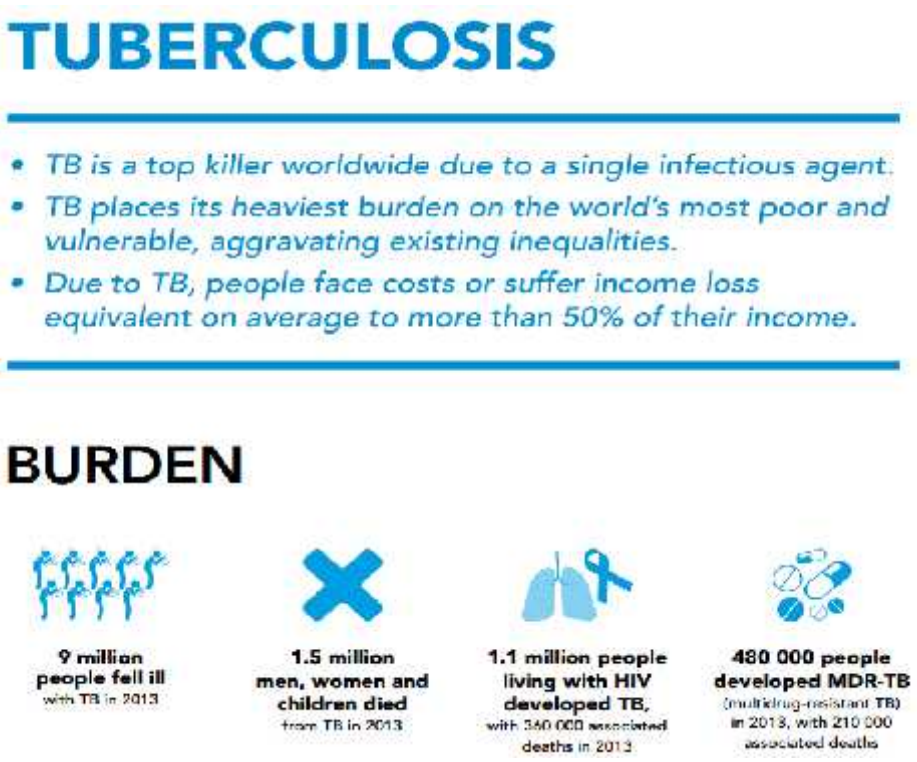

Figure 1: Graphic of the current global TB burden [22]

The factors that influence the continued high global TB burden are several. They classically include poverty, overcrowding and malnutrition. However, by far, HIV remains the strongest risk factor for TB. In an individual with HIV, the risk of developing TB is 20-30 times compared to an individual without HIV [23-25].

Of the estimated 9 million incident TB patients annually, 12% (1.1 of 9 million) are infected with HIV [20]. Furthermore, nearly half a million of these incident TB patients have multi-drug resistant TB (MDR-TB). Interestingly, despite the rapid deterioration and high mortality in HIV patients infected with MDR-TB, the association between MDR-TB and HIV infection is still unclear. One recent meta-analysis found that HIV increased the risk for MDR-TB [26], while another meta-analysis found no such association [27].

The highest burden of TB is in those aged 35-54 years which is the most economically productive age group. Although TB mainly occurs in men, nearly 40% of patients (3.3 out of 9 million) are women and 6% (550,000 of 9 million) are children aged less than 15 years [20].

Globally, only about 50% of new patients have a bacteriologically confirmed diagnosis of pulmonary TB. A substantial proportion (37%) are treated for pulmonary TB empirically while the rest (14%) are treated for extra-pulmonary TB. This pattern is evident in all regions of the world [20].

World-wide, the biggest burden of TB (80% of incident cases and 95% of deaths) occurs in twenty-two low or middle income countries [20]. In 2013, most of the incident TB patients were from Asia (56%) with India, China, Pakistan and Indonesia standing out, and from Africa (29%) with Nigeria and South Africa standing out (Figure 2).

Regionally, the burden of TB continues to be highest in sub-Saharan Africa [15, 20]. In 2013, the incidence of TB in sub-Saharan Africa was twice (281 compared to 126 per 100, 000 population) the incidence at global level. In addition, TB mortality in sub-Saharan Africa was nearly three times (42 compared to 16 per 100,000 population) the mortality at global level [20].

Within sub-Saharan Africa, the southern region is the most affected with some countries such as South Africa and Swaziland having TB incidence rates of 860 and 1382 per 100,000 population respectively (7-10 times the global rate). The region also has the highest levels of mortality from TB globally, particularly among those infected with HIV (≥ 40 compared to 16 per 100,000 population) [20]. The TB situation in East and West Africa is similar with TB incidence largely between 125-299 per 100,000 population and TB mortality between 10-20 per 100,000 population [20].

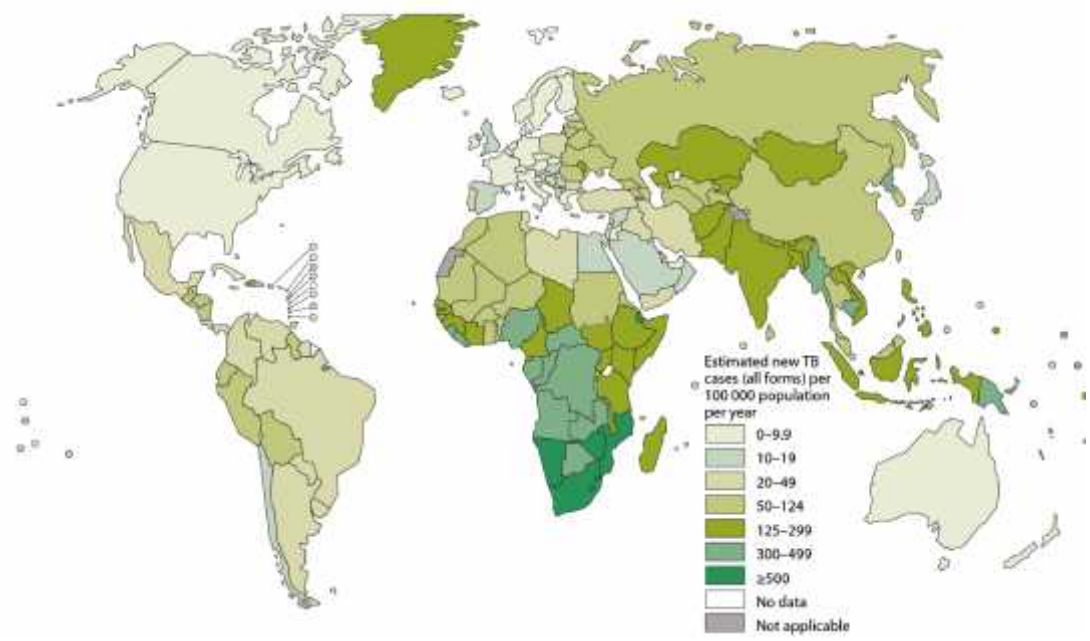

Figure 2: Graphic of the estimated global TB incidence in 2013 [20]

1.5 ECONOMIC BURDEN OF HIV-ASSOCIATED TB

Globally, over 130 million years of healthy life (DALYs) are lost due to illness and premature mortality from TB (43,650,000) and from HIV (91,907,000) annually [28]. Furthermore, the two diseases account for five percent of all the DALYs world-wide each year. But the DALYs due to TB and HIV are over four times in sub-Saharan Africa compared to the global average (8800 versus 1900 per 100,000) [29, 30]. This is because more young adults from the region die from TB or other HIV related causes than elsewhere.

For TB, these DALYS were estimated to result in economic losses equivalent to 3.4 trillion dollars (US $) between 2006 and 2015 globally if its control was not improved [31]. For sub-Saharan Africa, the economic losses during this ten year period were estimated to be 519 billion dollars (US $). Furthermore, eighty percent of these economic losses in sub-Sahara Africa were attributed to co-infection with HIV [31].

Studies that have investigated the economic burden of TB, in patients from sub-Sahara Africa or from low-and middle-income countries found the costs of TB care corresponded to 35-58% of annual household income [32, 33]. Thus, for many households, TB care costs were catastrophic and in some situations, patients took loans or sold their household items to meet these costs. Costs of medicines, diagnostic tests and hospitalization were important drivers of the care costs and accounted for 20% or more of the total medical costs in many cases [32]. The largest costs however, were due to lost income (60%) which was incurred mainly before diagnosis and initiation of TB treatment [32]. In other situations, up-to 78% of the costs of TB care were due to direct non-medical costs such as transport and provision of special foods as part of therapy for the TB patients [34].

These huge economic losses at global and household level provided additional context for the preparation of the ‘Global plan to Stop TB, 2006-2015’. Moreover, the benefits of TB control were shown to exceed the costs of implementing the plan in all twenty-two high-burden TB countries. In sub-Sahara Africa, the benefits of implementing the plan exceeded the costs by roughly ten: one [19, 31].

1.6 EPIDEMIOLOGY OF HIV ASSOCIATED TB

Worldwide, an estimated 35 million people are infected with HIV [35]. As is the case for TB, HIV infection is most common in young adults aged 15-49 years. Since 2010 however, the number of new HIV infections has continued to decline by 13% [35]. In addition, since 2005, AIDS related deaths have fallen by 35%

after the global scale-up of HIV antiretroviral treatment (ART) through various global initiatives such as the '3 by 5 initiative' and PEPFAR [36, 37].

Just 15 countries account for 75% of the global HIV burden. Except for five of them (Brazil 2%; China 2%; India 6%; Russia 2%, United States 4%), the rest are from sub-Saharan Africa-making the region account for 70% (25 out of 35 million) of the global HIV burden [35]. Within sub-Sahara Africa, ten countries (Ethiopia, Kenya, Malawi, Mozambique, Nigeria, South Africa, Uganda, Tanzania, Zambia and Zimbabwe) account for 80% of all people living with HIV with slightly more women (58%) than men having the HIV infection [35].

Not surprising therefore, 80% of the 1.1 million TB patients co-infected with HIV globally, are from sub-Sahara Africa [20]. On average, 40% of TB patients in sub-Sahara Africa are co-infected with HIV, with the levels of TB-HIV co-infection highest in Southern Africa (68%) compared to Eastern (40%) and Western Africa (25%).

The epidemiology of HIV-associated TB has therefore remained un-changed since its earlier descriptions [38, 39]. Even in the era of ART, TB remains the main cause of mortality among HIV patients in sub-Saharan Africa [40-42]. ART however, reduces the risk of TB by 66% and the risk of death by 50% in people living with HIV [43, 44]. Thus, since 2013, the WHO recommends initiation of ART in HIV patients with CD4 count of 500 compared to CD4 count of 350 previously [45].

The risk of TB starts to increase soon after infection with HIV. The risk of TB doubles within one year and is sustained or increased during the following years [46]. In addition, HIV increases the risk of progression of latent TB to active TB while on the other hand, TB increases progress of HIV infection to AIDS [47, 48]. The rapid progression to AIDS explains why there are more deaths among TB patients co-infected with HIV during TB treatment compared to TB patients who are not infected HIV. At a global level, the proportion of TB patients who died in 2013 during TB treatment was more than three times higher among HIV-positive TB patients than in those who were HIV-negative (11% versus 3.4%) [20]. In sub-Saharan Africa, HIV-positive TB patients were twice more likely to die during TB treatment compared to HIV-negative TB patients (10% versus 5%) [20].

The poor treatment outcomes for TB in HIV-positive patients compared to those who are HIV negative is also related with the difficulty of diagnosis and the treatment delays that are associated with smear-negative TB [49-52]. Smear-negative TB is a common clinical problem in HIV patients and was described several years before. In one review, researchers described the dis-proportionate increase in smear-negative TB with the advent of the HIV epidemic and the higher mortality among this group compared to smear-positive patients [53]. A

subsequent review proposed the use of clinical algorithms for diagnosis of smear-negative TB since additional tests to TB microscopy were generally unavailable or inaccessible [54]. This was followed by a third review which proposed the urgent need to change the existing policies for the diagnosis of smear-negative TB in HIV patients from resource limited settings [55].

1.7 PATHOGENESIS OF HIV ASSOCIATED SMEAR-NEGATIVE PTB

TB is caused by the bacterium *Mycobacterium tuberculosis*-which is spread from person to person through the air. Pulmonary TB is therefore the most common form of the disease. It is also the most important for transmission control using the strategy of early diagnosis and treatment. Diagnosis of pulmonary TB is mainly achieved by examination of sputum examination as cough is the commonest sign/symptom [56]. TB microscopy is the most common method for sputum examination. However, HIV reduces the sensitivity of TB microscopy [53-55]. How this occurs is briefly described below:

In the normal host, on inhalation of the TB bacilli, they are engulfed and immediately killed by alveolar macrophages. This is achieved using different bactericidal mechanisms such as reactive nitrogen and oxygen intermediates [57]. However, the bacilli may survive in 25-50% of the infected individuals and continue to divide within macrophage cytoplasm [58]. The macrophages then present the Mycobacterial antigen to CD4+ lymphocytes, which become activated and initiate a cell-mediated response. The sensitized lymphocytes produce various cytokines such as TNF-α, IFN-γ, IL-6 and IL-12 which attract and activate more macrophages, enhancing their ability to kill the Mycobacteria [58]. Such activated macrophages become enlarged and differentiate into what are known as epitheliod macrophages to form a granuloma. The granuloma is a compact aggregate of epitheliod cells populated with many other cell types such as neutrophils, dendritic cells, natural killer cells and fibroblasts [59]. It is generally believed that the main purpose of the granuloma is to ‘wall off’ the bacteria in the host resulting in containment or cure in 90% of individuals [57, 58]. This view has recently been revisited however, and the granuloma is now thought to have a role in the dissemination of TB infection [59]

Considering that the granuloma is mainly a protective structure in the host, and that the CD4+ lymphocyte plays an important role in the formation of the granuloma, HIV has a detrimental effect on the formation of the granuloma by depletion of host CD4+ lymphocytes [60, 61]. This results in poor containment of the TB bacilli resulting in uncontrolled spread of the TB bacilli in the lung and elsewhere in the body. In addition, the poorly formed granuloma results into less cavitatory disease in the lungs. This results in less numbers of bacilli in the expectorated sputum than would normally be the case in the normal host. These numbers are often below the detection limit of microscopy (5000-10,000 bacilli

per ml) which results in smear-negative TB [62-64]. The situation is made worse by the weak respiratory effort in patients with advanced HIV disease resulting in sputum of poor quality with subsequent smear-negative results [65, 66].

2 OVERVIEW OF TRADITIONAL TB DIAGNOSTICS

2.1 MICROSCOPY

Microscopy is the main method for the laboratory diagnosis of pulmonary tuberculosis (PTB) for more than 100 years. This is achieved by direct microscopic examination for tubercle bacilli in sputum specimens stained using the Ziehl-Neelsen (ZN) procedure [67]. Sputum examination by microscopy is simple, inexpensive and efficient in detecting those cases of pulmonary tuberculosis that are most infectious [68]. Smear microscopy is also used to monitor treatment of patients and to establish cure. However, between 5,000-10,000 bacilli/ml of sputum are required for direct microscopy to be positive. But only a proportion of tuberculosis patients harbour such large enough numbers of organisms to be detected in this way [69]. Thus, the sensitivity of microscopy may be as low as 20% especially in HIV patients who have pauci-bacillary forms of TB [70]. In addition, it is virtually impossible to distinguish between the MTC and NTM forms of mycobacteria using the microscopy test which may result into incorrect treatment of NTM as MTC [53, 71]. Further, the yield of TB microscopy is highly dependent on skills of the operating technician and on the quality of reagents which may vary from one setting to another [69].

2.2 CHEST X-RAY

Like microscopy, chest X-ray has been used for over a century to diagnose pulmonary TB (PTB). In the pre-HIV era, PTB typically appeared on chest X-ray as cavitation with apical or bilateral distribution [54]. Interpretation of chest X-rays of individuals suspected to have PTB can be difficult. It is even more difficult among patients infected with HIV because they have atypical (pulmonary infiltrates with no cavities, lower-lobe involvement) appearance of TB on chest X-ray [72]. Moreover, chest X-ray can appear normal in nearly one-third of HIV-positive patients with TB as their immunity deteriorates even further [73, 74]. Studies that have evaluated the diagnostic value of chest X-ray in smear-negative patients found that it has low to moderate sensitivity and specificity of 28-78%, and 59-75% respectively [75, 76]. Nonetheless, chest X-ray is useful for diagnosis of extra-pulmonary TB such as pleural or pericardial TB and other non-tubercular chest diseases such as PCP or Kaposi’s sarcoma, which are common among people living with HIV [77].

A correct diagnosis of PTB on chest X-ray is dependent on the reader’s expertise because interpretation is currently not well standardized. Efforts are underway presently to introduce standardized scoring systems for interpretation of chest X-ray and thereby increase its sensitivity and specificity. These combine clinical and chest X-ray appearance. The available evidence indicates that the score systems could improve sensitivity remarkably (93-98%) although specificity could still remain low (35-50%) [78, 79]. A major limitation of chest X-ray is that it is not widely available

in the peripheral health facilities, and even where it is available, the quality of the X-ray is often poor.

2.3 SOLID CULTURE

Culture is the “gold standard” for TB diagnosis. Culture requires fewer bacilli (10-100/ml) for detection and therefore allows for detection of pauci-bacillary forms of PTB that are associated with HIV [4]. In addition, culture allows for species identification and drug susceptibility testing which have become important because of the increasing cases of drug-resistant TB [20]. However, implementing TB culture requires sophisticated infrastructure and highly skilled staff thus limiting the technology to mainly referral or research facilities.

Until 2007, solid media (egg or agar-based) was the main type of media used for TB culture. The advantages of egg-based media include ease and ability to be prepared in-house, it allows for observation of colonies of mixed cultures and contaminants, and it may be stored for several weeks [4]. Löwenstein–Jensen (L-J) medium is the most commonly used egg-based solid medium for TB culture. L-J media containing glycerol is used to promote growth of M. tuberculosis growth, while L-J containing sodium pyruvate without glycerol is used to promote growth of M. bovis. Alternatively to reduce costs, L-J media without asparagin (Ogawa media) may be used [4]. A disadvantage of egg-based media is that when contamination does occur, it may involve the entire slant surface causing the entire culture to become lost. In addition, if specimens contain few bacilli it may take three to eight weeks before cultures become positive.

Agar-based media are less likely than egg-based media to become contaminated [62]. The most common are Middlebrook 7H10 and 7H11, which can be prepared in the laboratory using commercially available agar-powdered bases [62]. After preparation, the media is enriched with addition of Oleic acid, Albumin, Dextrose and Catalase (OADC). Because of the transparency of 7H10 and 7H11 plates, microcolonies of MTB with typical cord formation can be detected and counted using a microscope as early as one week after incubation [4]. Also, visibility of colonial morphology on agar plates is better than on egg-containing slants, aiding the identification of mycobacteria. A disadvantage of Middlebrook media is that the surface dries more rapidly than egg-based media.

2.4 EMPIRIC TB DIAGNOSIS

In the absence of adequate laboratory tools for TB diagnosis, the WHO recommended a clinical algorithm involving empiric TB diagnosis and treatment for HIV prevalent and resource constrained settings [80]. However, the way the algorithm was implemented was highly variable among clinicians. For example, empiric TB treatment was initiated in some situations based on clinical suspicion for TB following negative laboratory

investigations. In other situations, clinicians initiated empiric TB treatment exclusively based on clinician suspicion without any laboratory investigation particularly in seriously sick patients in peripheral health facilities. Thus, in some settings, nearly 50% of the patients could be treated for TB without smear results [81, 82].

Empiric TB treatment was of value however, in HIV-positive patients with advanced immunodeficiency because of the frequent occurrence of smear-negative TB in such patients [83]. Several autopsy studies provided support for this practice since half or more of the deaths in HIV patients were due to TB [29, 41, 84, 85] . Furthermore, a study that recently evaluated the impact of empiric TB treatment revealed that it averted 6-25% deaths and prevented 11-57% of incident TB cases in severely immune suppressed HIV patients (CD4 less than 100) [86].

3 OVERVIEW ON NEW TB DIAGNOSTICS

Since 2007, the pipeline for TB diagnostics expanded tremendously and new tests became available [87]. Between 2007 and 2015, the WHO developed many policies based on the new tests to improve TB diagnosis and treatment. These policies include; use of Xpert MTB/Rif test in HIV-positive patients [88], use of LED fluorescent microscopy as an alternative to Ziehl-Neelsen microscopy, [89], MODS and NRA to screen for MDR-TB [90], and use of liquid medium for TB culture and DST [91] among others [92]. An overview of these tests and implemented in this thesis is provided below:

3.1 GENEXPERT MTB/RIF TEST

Previously, molecular diagnostics for TB were limited to research laboratories. The introduction of the automated molecular test (Xpert MTB/Rif or GeneXpert) was considered a “game changer” in the use of molecular TB diagnostics because it provided a practical alternative to TB microscopy and culture [93]. While thousands of MTB must be present in each millilitre of sputum sample for PTB to be diagnosed using the microscope, GeneXpert can detect MTB at much lower concentrations of about 100-200 bacilli per millilitre of sputum and is able to distinguish between MTB and NTM with very high specificity [94, 95].

GeneXpert relies on real time polymerase chain reaction (PCR) to amplify a portion of the Mycobacteria gene where many of the mutations for resistance to the anti TB drug rifampicin, occur (81 base pair long rpoB hot spot region) [96]. If present, fluorescent dyed molecular probes of 16-20 base pairs long, are used for detection of the amplified Mycobacterial DNA in the clinical sample [95]. The steps involved to process the sample, amplification of nucleic acid and detection of the target sequences are automated. This is achieved by the use of single-use disposable GeneXpert cartridge that hold the PCR reagents and host the PCR process [95].

GeneXpert simultaneously detects *Mycobacterium. tuberculosis* and ‘drug resistance’ within two-three hours. Moreover, being an automated test, it does not require highly skilled staff to operate. Thus, the WHO currently recommends GeneXpert as the primary diagnostic test for HIV associated TB in adults or in children suspected of having TB or individuals suspected of having multi drug-resistant TB [97]. The test is currently implemented on a global scale. However, concerns have been raised about the cost-effectiveness of the GeneXpert because of its high cost but limited impact on patient morbidity and mortality [98-101]

3.2 LED MICROSCOPY

Efforts to improve TB microscopy were warranted since the method was the most widely used in several settings where TB was a high burden. With available evidence that Fluorescent microscopy (FM) was 10-20% more sensitive than ZN microscopy, diagnosis of TB could be improved with deployment of FM [102]. The most important advantage of fluorescent microscopy is that it uses a lower power objective lens (typically 25×) than traditional microscopy (typically 100×), enabling the microscopist to assess the same area of a slide in less time [103]. Other advantages include the simplicity of the fluorochrome staining method compared with Ziehl-Neelsen methods.

Fluorescent microscopy (FM), uses fluorochrome dyes such as auramine O or auramine-rhodamine with an intense light source. Previously, halogen or high-pressure mercury vapour lamps were used as the light sources. In comparison, traditional Ziehl-Neelsen microscopy could be performed using a conventional artificial light source or reflected sunlight [62]. Thus, wide spread implementation of FM was originally limited because of cost, the technical complexities of operating FM due to need for mercury vapour light sources, the need for regular maintenance and the requirement for a dark room.

The development of Light-emitting diodes (LED) as a source of the intense light required for fluorescent microscopy, offered the benefits of fluorescence microscopy without the associated previous costs. And following assessment of LED fluorescent microscopy with evidence that it was more sensitive than the traditional Ziehl-Neelsen microscopy and had qualitative and operational advantages, the WHO recommended that LED fluorescence microscopy be phased in as an alternative to Ziehl-Neelsen microscopy [89].

3.3 COMMERCIAL LIQUID CULTURE

Liquid culture is available on commercial or non-commercial basis. The use of liquid media for TB culture allows for increased detection of MTB of about 10% in 7-14 days compared to 21-42 days on solid media [62]. However, liquid culture is more prone to contamination compared to solid culture. And just like solid culture, liquid culture also requires high biosafety levels (biosafety level 3); highly skilled staff and costly maintenance of the infrastructure and equipment.

One of the most widely used commercial liquid culture tests is the BACTEC Mycobacteria Growth Indicator Tube System (MGIT 960). MGIT was developed as an alternative to the radiometric BACTEC 460 TB system [104]. MGIT is fully automated and continuously monitors growth of MTB in the culture. The system's culture tubes consist of modified Middlebrook 7H9 broth, growth supplement (OADC), and a mixture of antibiotics to prevent contamination of the media. MTB

growth is detected by the rate of oxygen consumption within the headspace of the cultures [105]

3.4 MODS AND NRA TB CULTURE

The Microscopic-observation drug-susceptibility test (MODS) [106], and the Nitrate Reductase Assay (NRA) [107], are common non-commercial liquid culture methods, used mainly to screen for multi drug-resistant TB [90]. The MODS relies on two well-known properties of *Mycobacterium tuberculosis* (MTB): First, the rate of growth of MTB in liquid medium is considerably higher than that on solid medium. Second, the morphology of MTB in liquid culture is characteristic and recognizable, consisting of so called "cord" like structures. Based on these two characteristics, MTB growth can be detected within 7-10 days using a microscope compared to conventional solid culture that takes 3-8 weeks [108, 109].

The NRA assay relies on the ability of MTB to reduce nitrate to nitrite. The presence of nitrite, indicating metabolically active mycobacteria, can easily be detected by addition of what is known as the 'Griess reagent' into the culture tube to produce a pink-purple colour change [4, 106]. By using liquid culture media for the NRA test, MTB growth can be detected within 10-14 days which is considerably quicker than that on solid medium [110].

Compared to the NRA, the MODS been developed further and used more extensively including different HIV care settings [111-113]. A 'MODS test kit™' has now been developed to address the difficulties faced by many laboratories in low- and middle-income countries to procure the consumables required for the test [114].

3.5 TB DIAGNOSTICS POST 2015

The landscape of TB diagnostics post 2015 will be different from the current one, with less reliance on TB microscopy and conventional TB culture in the near future [115]. Molecular based diagnostics will be expanded the most, with possible deployment of simple molecular tests at the point of care, and use of more complex molecular tests in specialised TB laboratories [116].

In the case of antigen and antibody tests for detection of TB, only one antigen test is currently on the pathway to WHO evaluation [116]. The test known as TB-LAM, is based on detection of a lipopolysaccharide called lipoarabinomannan, which is a major cell-wall component of *Mycobacterium tuberculosis* [117]. TB-LAM is a simple, cheap and rapid test with possibility for use as a point of care test. TB-LAM was evaluated in several studies and found useful in HIV patients with severe immune-suppression and with the highest risk of mortality if not treated [118-122]. For antibody tests, over fifty distinct antigens have been identified so far, with potential for inclusion in an antibody-based TB test [123]. Combinations of selected

antigens (mainly proteins) were found to have higher sensitivities than single antigens. Currently, there are several commercial antibody TB tests mainly based on detection of IgG or IgA antibodies. But they have inconsistent and imprecise estimates of sensitivity and specificity and the WHO does not therefore, currently recommend their use for TB diagnosis [124, 125].

3.6 IMPACT OF NEW TESTS ON GLOBAL TB BURDEN

Implementation of the existing new diagnostics for TB will reduce the current burden of TB only moderately (36%). This is because the tests have difficulty in diagnosis of TB in children and extra-pulmonary TB, coupled with other factors such as ineffective TB treatment [126]. As a result, the current global decline in TB incidence is only about two percent annually which is too small to end the global TB epidemic (Figure 2).

The limited impact of the new TB diagnostics at population level has recently been described in some studies [127-129]. A combination of new tools, which are able to diagnose childhood TB and extra-pulmonary TB or prevent TB altogether is therefore highly desired [130].

In 2014, the WHO developed a new post-2015 TB strategy with a goal of ending the global TB epidemic by 2035 [131]. The strategy requires acceleration in the decline of the incidence of TB from two percent currently to ten percent during the next decade. To achieve this, there is need to optimally use the currently available tests and introduction of new TB medicines and a vaccine by 2025 (Figure 3).

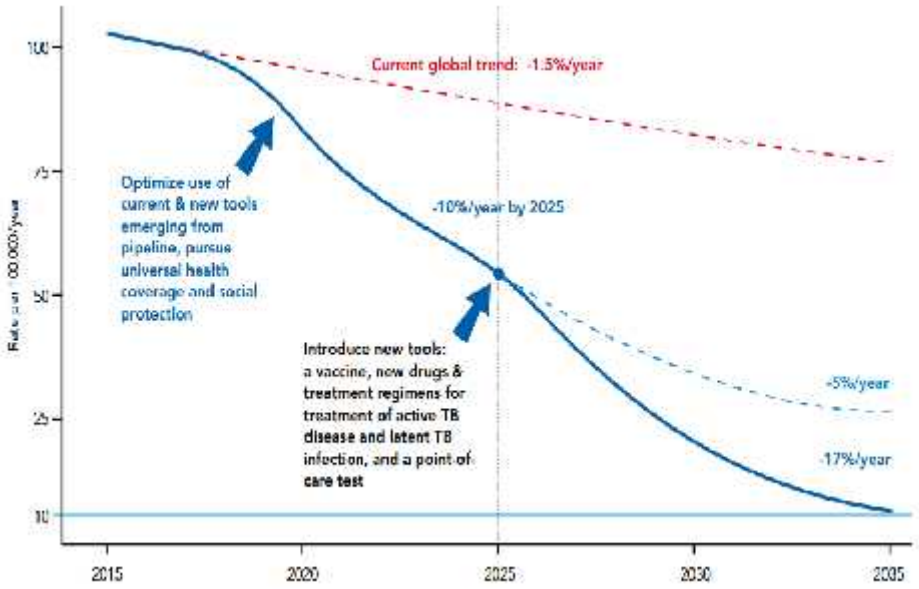

Figure 3: Graphic of the global targets for TB control by 2035 [131]

The status of these additional new technologies is as follows: According to the pipeline report for 2014, the TB drug pipeline currently features six compounds from four different classes [132]. Of them, only bedaquiline and pretomanid are likely to be used for a new TB regimen for treatment of drug- sensitive and drug-resistant TB by 2020. In the case of vaccines, the pipeline currently features 16 candidate TB vaccines which have undergone or are currently in various phases of clinical development [132]. These fall under three categories (i) Prime vaccines for replacement of the existing BCG vaccine using either live recombinant BCG (rBCG) or recombinant attenuated MTB, (ii) Prime-boost vaccines for administration as a booster in recently or remotely BCG primed individuals, and (iii) immunotherapeutic vaccines for use as an adjunct to chemotherapy to shorten TB treatment [133, 134]. Unfortunately, trials involving the new vaccines have so far been disappointing revealing there are still significant gaps in understanding the pathogenesis and immunology of tuberculosis [134, 135]

4 THE NATIONAL HEALTH SYSTEM OF UGANDA

The National Health System (NHS) of Uganda is made up of both the public and private health sector with each of them contributing about 50% of the reported outputs for health services [136]. The public health sector comprising Government of Uganda owned health facilities is structured into: National Referral Hospitals (NRHs); Regional Referral Hospitals (RRHs) and the District Health System (General hospitals, Health Centres 1-IV and Village Health Teams) [137]. The District Health System is further divided into Health Sub-Districts with either a Health Centre IV or General hospital functioning as the referral facility within the Health Sub-District (Figure 4). The private health sector consists of Private Not for Profit (PNFPs) providers, Private for Profit Health Practitioners (PFPs) and the Traditional and Complementary Medicine Practitioners (TCMPs). The PFP sector is concentrated in urban areas. The Government of Uganda (GoU) subsidizes the PNFP and a few private hospitals as part of a Public Private Partnership strategy for health services delivery. The subsidies cover the minimum package of health services that was developed by the GoU for all levels of health care for both the private and public sector. Currently, 72% of the households in Uganda live within five kilometres from a health facility [137].

A major challenge for delivery of health services in Uganda is the low staffing levels in health facilities. In 2011/12, the staffing level in the facilities was only 58% despite increased recruitment efforts by Government. In the rural setting, health services are largely provided by nursing staff because of shortage of doctors who prefer to work in urban settings [138]. In the case of the National TB and Leprosy Programme (NTLP), the staffing level stands at 43% which is lower than the national average. This severely constrains delivery of TB services particularly for laboratory services [139], despite being integrated within the general health services in the different tiers of the National Health System.

The TB laboratory network in the country comprises the National TB Reference Laboratory (NTRL) which is responsible for all the TB laboratories in the national, regional and district health structure. The NTRL mainly provides research and quality assurance services while the laboratories in the health facilities perform the direct diagnostic services for patient care. By the end of 2013, TB Culture and Drug Susceptibility Testing (DST) services were limited to only the NTRL and three other laboratories used for research. Accessibility to TB culture services was therefore limited for the general population. On the other hand, TB microscopy services based mainly on the Ziehl Neelsen procedure, were widely available up-to the Health Centre III level. Since 2010, LED Fluorescent microscopy (FM) has been scaled up to replace ZN in all National and Regional hospitals and some district hospitals. LED microscopy however, still represents just 5% of the TB microscopy network in the country.

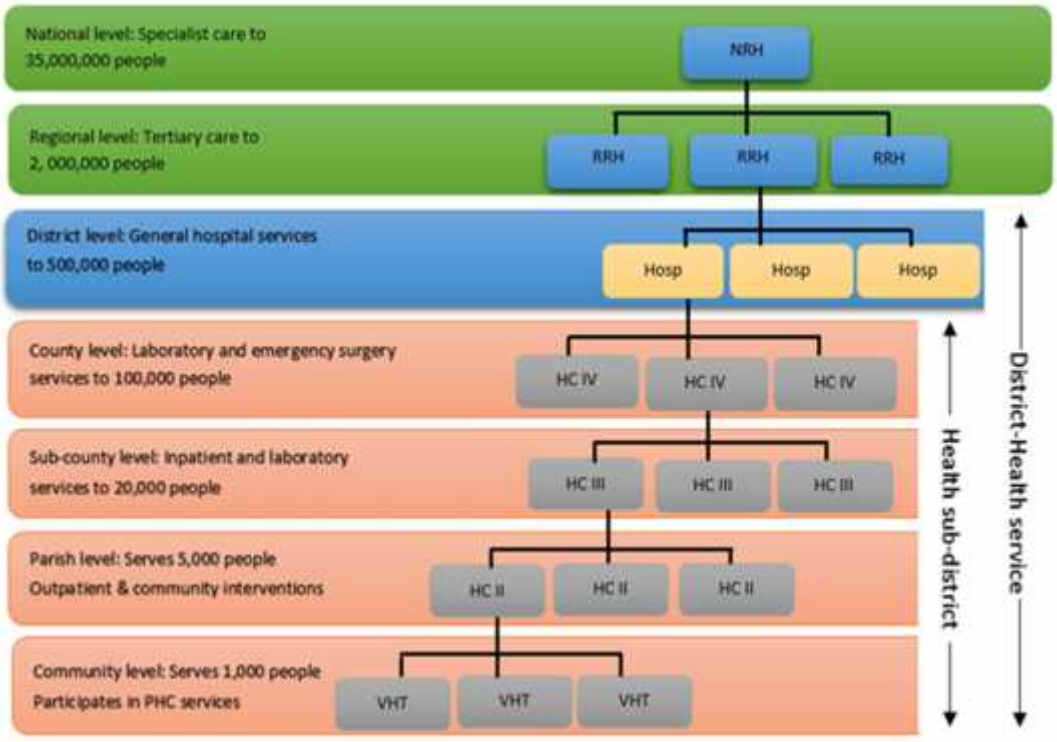

Figure 4: Graphic of the National Health System of Uganda. Adapted from ref [140]

5 STUDY RATIONALE

The purpose of this study was to evaluate new tests for early and increased diagnosis of pulmonary tuberculosis in smear-negative HIV-positive patients in Uganda.

Uganda has an estimated population of 35 million people [141]. Communicable diseases contribute over 50% of the disease burden in the country with malaria, HIV-associated illness and TB as the leading cause of ill health [142]. While the population HIV and TB prevalence have declined since the 1990s, the country remains a high HIV burden and high TB burden country. The current HIV prevalence for the general population is estimated at 7.3% while the TB prevalence is estimated at 154 per 100,000 population [143, 144].

From the economic viewpoint, TB and HIV account for 22% (6,935,000 of 31,400,000) of all the DALYs due to disease and injury in Uganda annually. This increases to 41% (16999000 of 31,400,000) considering DALYs due to infectious diseases and parasitic infections [30]. Thus, without improved TB control, it was estimated that Uganda would lose nearly 9 billion dollars (US $) between 2006 and 2015 [31].

Nearly 50% of the notified TB patients are co-infected with HIV, with the mortality rate among the TB-HIV co-infected patients twice that in TB patients without HIV infection [144]. Until 2010, only about 50% of the estimated incident TB patients (166 per 100,000 population) were detected each year. This increased to nearly 70% in 2013 because of implementation of the WHO intensified TB case finding policy [145]. However, a substantial proportion (30%) of the notified TB patients each year are smear-negative and are therefore treated for TB on empirical grounds [144].

Microscopy remains the main method for TB diagnosis in Uganda since it is the most accessible. Unfortunately TB microscopy has a sensitivity of only 40–60% under field conditions, falling as low as 20% in the presence of HIV co-infection [70]. To improve the diagnosis and treatment of smear-negative TB, Uganda adopted the WHO 2007 TB algorithm for management of smear-negative PTB in HIV prevalent settings [80]. The algorithm begins with TB microscopy and, if negative, followed by chest X-ray or culture. However, implementation of the algorithm was problematic: (1) There were inadequate medical workers to implement the algorithm fully, (2) the algorithm outlined a lengthy diagnostic pathway that required the patient to visit the clinic four times resulting in high dropout rates of patients during the diagnostic process, (3) access to quality chest X-ray was limited in many of the health facilities in the periphery, (4) TB culture was only undertaken at central level laboratories and results were seldom available in a clinically relevant timeframe. TB diagnosis among smear-negative HIV patients was therefore often delayed, which resulted in high mortality rates.

With the advent of new TB diagnostics from 2007, such as liquid culture and molecular tests, there were proposals to incorporate the new tests into the existing WHO 2007 algorithm to improve TB diagnosis and treatment, particularly for HIV-positive patients who were smear negative. However, the diagnostic accuracy, cost-effectiveness and impact on patient care of these tests in Uganda was not yet evaluated.

6 GENERAL OBJECTIVE

The over-all aim of this thesis was to increase knowledge of using new tests on the diagnostic accuracy, cost-effectiveness and effect on treatment of TB in smear-negative HIV-positive patients in Uganda..

6.1 RESEARCH OBJECTIVES

- To determine and compare the over-all accuracy of the existing (WHO 2007 TB algorithm) and new (GeneXpert, MODS, NRA) tests for diagnosis of smear-negative TB.
- To determine and compare the accuracy of GeneXpert, MODS and NRA for diagnosis of smear-negative TB in HIV patients.
- To determine the cost-effectiveness of using MODS versus GeneXpert for diagnosis of TB in HIV patients.
- To describe the effect of using GeneXpert on treatment of smear-negative HIV patients.

7 METHODS

7.1 STUDY AREA AND POPULATION

Study I involved secondary data analysis obtained from publications on the accuracy of the existing and new TB tests for diagnosis of smear-negative TB. Study II, III and IV were conducted at Mulago National Referral Hospital and Complex in Kampala, Uganda. Participants were enrolled from in-patients admitted to the medical department of the hospital, Figure 5, and out-patients attending the Mulago-Immune Suppression Syndrome (Mulago-ISS) clinic, Figure 6.

The Mulago-ISS clinic is a doctor led out-patient HIV care facility supported by the Makerere University Joint AIDS Program (MJAP). MJAP was funded by the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) since 2004 to provide free comprehensive HIV care services including TB treatment to adults and children. MJAP pioneered TB/HIV integrated care in Uganda, at Mulago in 2006. The TB/HIV integrated care services include routine screening for TB symptoms among HIV patients, implementation of routine TB infection control interventions and early initiation of anti-retroviral-therapy (ART) for all HIV infected who are patients co-infected with TB. As of March 2012, there were 14,000 patients receiving HIV care at the Mulago-ISS clinic.

The laboratory tests in study II, III and IV were conducted at the Mycobacteriology laboratory of the Department of Medical Microbiology, in the College of Health Sciences, Makerere University, located within Mulago Hospital Complex. The department has an established training program for graduate students pursuing research in TB. The laboratory previously supported the training of two doctorate students in basic TB research.

Figure 5: Mulago National Referral Hospital, in-patients department

Figure 6: Mulago-MJAP TB-HIV out-patients clinic

7.2 MAIN RESEARCH METHODS

Several study designs were used to approach the research objectives and are represented in the figure 7 below. We used structured questionnaires to collect the data for all the studies.

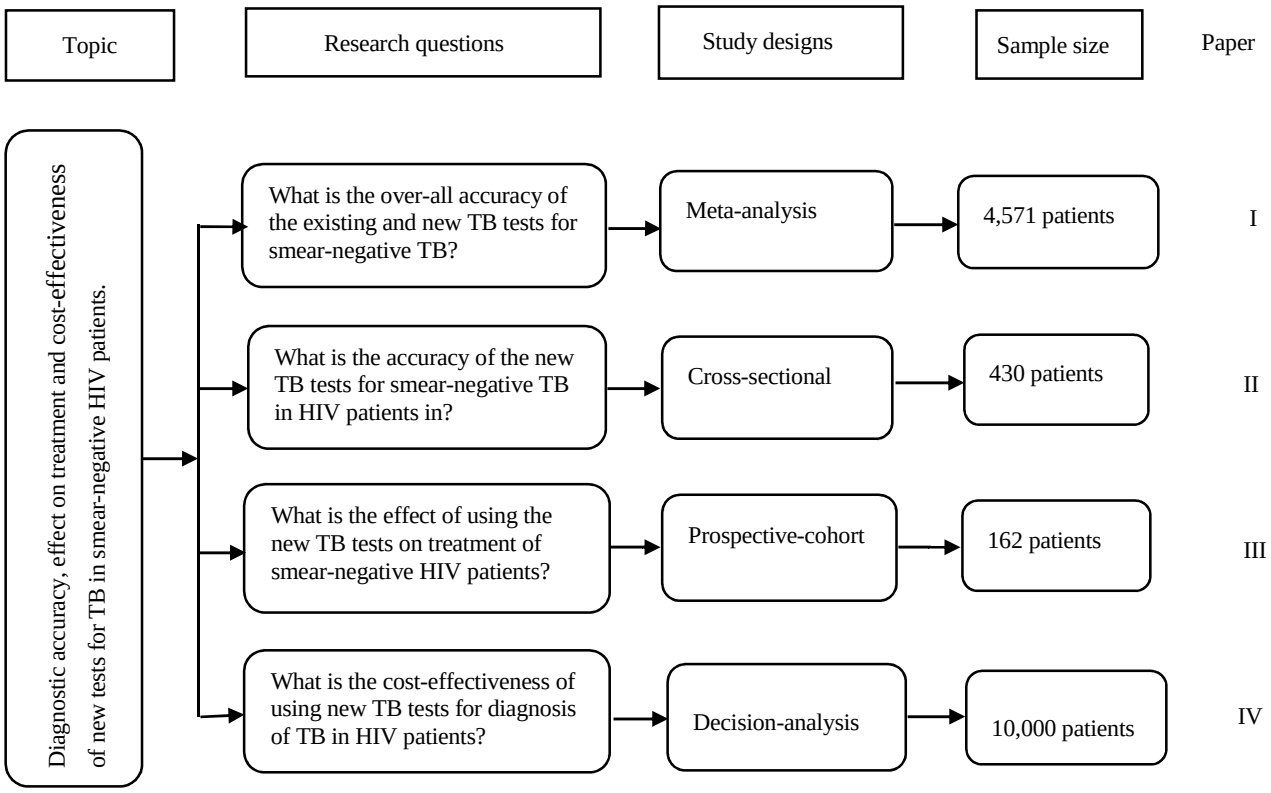

Figure 7: Overview of the study designs used in the thesis

DATA COLLECTION METHODS

7.3.1 Study I

A systematic review followed by meta-analysis that involved 4,571 patients was performed to summarize the over-all accuracy of two new methods (Xpert MTB/Rif and MODS) and the existing method (WHO 2007 TB algorithm), for diagnosis of smear-negative pulmonary tuberculosis: We searched for publications in English without year restriction in Pubmed using the following search terms: 'GeneXpert', 'Microscopic observation drug susceptibility', and 'WHO TB algorithm'. We reviewed the obtained abstracts and retrieved publications for full review based on whether the research was a diagnostic accuracy study or not. After reading the selected publications, we reviewed their references for additional publications. We then searched Google scholar to assess if relevant publications were missed. Studies were eligible if they used TB culture as the reference method and reported data to allow for first hand computation of sensitivity and specificity for detection of smear-negative PTB. In papers where this was not reported, we contacted the corresponding authors to provide the required data. For each study, data was independently extracted by one author using a standardized form and verified by a second author. The quality of studies was assessed using a standard tool-QUADAS [146].

7.3.2 Study II

We enrolled 430 consecutively presenting HIV patients into a cross-sectional study, to determine the diagnostic accuracy of Xpert MTB/Rif, MODS and NRA for smear-negative TB. We used a combination of solid and liquid TB culture as the reference test. Patients were eligible if they had a cough for two weeks or more, with or without fever, night sweats, loss of weight, or blood-stained sputum. Consenting patients provided a spot and morning sputum sample, which were examined using LED fluorescent microscopy (FM). Samples that were FM negative were then examined further using the Xpert MTB/Rif, MODS, NRA and combined solid/liquid TB culture

7.3.3 Study III

We used a decision analysis model to compare the cost-effectiveness of an algorithm using MODS or Xpert for diagnosis of PTB in HIV patients from the provider's perspective in Uganda. The model involved a hypothetical cohort of 10,000 HIV patients with a PTB prevalence of 20%. Cost data included equipment, overhead, staff, space, reagents, training and quality control. The estimates for diagnostic accuracy of the two tests were obtained from systematic reviews.

7.3.4 Study IV

We performed a prospective cohort study with six months follow-up involving 162 smear and Xpert negative HIV patients with presumptive PTB. Patients were evaluated for extra-PTB or other respiratory diseases using available diagnostic tools in the hospital. Where no alternative diagnosis was made, patients were treated with broad spectrum antibiotics or got empiric TB treatment and then followed up for six months to assess response to treatment. The outcomes of interest at the end of the follow-up period were cure or lost-to-follow-up or dead.

7.4 DATA ANALYSIS

7.4.1 Study I

True positive, true negative, false positive and false negative values for each study were compiled in Microsoft Office Excel 2007 initially. The data were then entered and analysed using Meta-disc software version 4 [147], to obtain pooled sensitivity and specificity values of the GeneXpert, MODS and the WHO 2007 algorithm. The pooled estimates for sensitivity and specificity of the three test methods were presented graphically as Forrest plots. We also generated summary Receiver Operating Characteristic (sROC) curves for each test and computed the Spearman correlation coefficient between sensitivity and specificity to explore for heterogeneity in the studies.

7.4.2 Study II

The laboratory results were compiled in Microsoft Access 2003. SPSS version 17 was used to analyse the compiled data to obtain the sensitivity, specificity, positive and negative predictive values and the 95% confidence limits for each test. A combination of solid (L-J) and liquid (MGIT) TB culture was used as the reference test.

7.4.3 Study III

Cost data was compiled and analysed in Microsoft Office Excel 2007. Capital equipment was annualized at a discount rate of 3% using standard tables. Cost-effectiveness analysis was performed by in-putting the diagnostic accuracy probabilities and the calculated unit costs into TreeAge software version 3.5. A base-case analysis was performed using the pooled estimates for diagnostic accuracy, unit cost and TB prevalence. Sensitivity analysis was performed using the upper and lower limits of the three parameters in the model.

7.4.4 Study IV

The obtained data was compiled in Microsoft Office Access 2007. Analysis was performed using Stata version 11. Numerical data was summarized using the mean or median. Categorical data was summarized using frequencies or proportions. Normally distributed numerical data was compared using the T-test and non-normally distributed numerical data was compared using the Mann–Whitney rank-sum test. The Chi-square or Fisher's exact tests were used to compare categorical data. The odds of response to antibiotics were compared to the odds of no-response to the antibiotics to obtain crude odds ratios for each patient characteristic. Multivariate logistic regression using the crude odds ratios was performed to obtain adjusted odds ratios. We selected patient characteristics with p-values of less than 0.2 or those with a biological plausibility to influence response to antibiotic treatment.

7.5 ETHICAL CONSIDERATIONS

We obtained ethical permission for the research studies from the Uganda National Council for Science and Technology (permit no: HS 1214). The main ethical concerns involved study II and IV because they required direct interaction with individual patients and access to their private medical information. We therefore enrolled participants into these two studies after they provided informed consent. Another ethical issue in these two studies was the psychological harm to the participants from anxiety or sadness that could arise following a diagnosis of duo HIV and TB infection. Participants diagnosed with TB/HIV co-infection therefore got additional supportive counselling throughout the course of their treatment. Since studies I and III involved secondary data analysis, they did not generate major ethical concerns.

8 RESULTS AND FINDINGS

8.1 Study I

Overall, we obtained fifteen studies on GeneXpert involving 2046 patients, five studies on MODS involving 1462 patients and four studies on the WHO 2007 TB algorithm involving 1063 patients. The new tests (GeneXpert and MODS) had moderate sensitivity and high specificity for smear-negative PTB. The existing WHO 2007 TB algorithm equally had moderate sensitivity but moderate specificity (Table 1). There was substantial variability in the studies, as measured by the inconsistence index (I-square). For sensitivity, the I-square values were 71% for GeneXpert, 90% for MODS, and 81% for the WHO 2007 algorithm. For specificity, the I-square values were 70% for GeneXpert, 93% for MODS, and 89% for the WHO 2007 algorithm. Overall, GeneXpert had the highest discriminating ability of true positivity from false positivity (accuracy) as measured by the area under the receiver operating characteristic curves (AUC) shown below in Table 1.

Table 1: Over-all accuracy measures of GeneXpert, MODS and WHO 2007 algorithm

		Pooled estimate (range)		
Test	No. of studies	sensitivity	specificity	AUC
GeneXpert	15	67 (62-71)	98 (97-99)	0.94
MODS	5	73 (66-79)	91 (89-93)	0.88
WHO 2007 algorithm	5	61 (55-67)	69 (66-72)	0.69

8.2 Study II

The sensitivity of GeneXpert, MODS and NRA for smear-negative PTB in the study population was low. The sensitivity was lowest for MODS (24%), compared with NRA (42%) or GeneXpert (49%). But all three tests had high specificity values of 90% or above. Further, despite the low sensitivity of the tests, they all had high negative predictive values: 96% for GeneXpert, 93% for NRA and 91% for MODS. This was because of the lower PTB prevalence of about 11% in the study patients compared to 20-30% we expected prior conducting the study, Table 2.

Table 2: PTB prevalence extrapolated from sensitivity and specificity values of the tests

Test	No. culture positive	No culture negative	Total	Prevalence %
GeneXpert	43	326	369	12
NRA	41	325	366	11
MODS	41	323	364	11

8.3 Study III

The use of MODS for diagnosis of PTB in HIV patients, was more cost-effective compared to GeneXpert (US$ 34 versus US$ 71 per TB patient diagnosed). This was mainly because of the cheap cost of the MODS test (US$ 6.53 versus US$ 12.41). Furthermore, although equipment and overheads were proportionally higher for MODS than GeneXpert as expected, they accounted for just over 30% of the total cost of the MODS test (Table 3). The threshold value for cost, where using GeneXpert would be optimal over using MODS was US$ 5.92. This implied that in order to make the GeneXpert economically competitive with MODS, the current concessional price of the GeneXpert of about US $ 10, needs to be reduced further by nearly half.

Table 3: Details of the costs of MODS and GeneXpert by type of input (2014$)

	Method, (% of total)	
Type of cost	MODS	GeneXpert
-Consumables	3.84, (59)	10.37, (84)
-Equipment	1.76, (27)	1.37, (11)
-Staff (salary and training)	0.46, (07)	0.15, (01)
-Overheads (utilities and space)	0.29, (04)	0.29, (02)
-Quality control	0.18, (03)	0.23, (02)
Total (US$)	6.53	12.41

8.4 Study IV

The majority 88% (141 of 160) of the patients with smear and GeneXpert negative results fully responded to treatment with broad-spectrum antibiotics and empiric TB treatment was initiated in only 8% (12 of 160) of them, Table 4. The odds of improvement on antibiotics were not significant with any of the socio-demographic (age, gender, education level, employment status, marital status) or clinical (CD4 cell count, ART status, duration on ART, previous TB treatment) characteristics except for stage of HIV. The odds were lower (0.05) and statistically significant in patients with advanced HIV disease than in patients with early HIV disease (OR 0.05; 95% CI 0.0-0.42). This significance was still retained after multivariate adjustment (AOR; 0.038 (0.005-0.307). Of the twelve patients who got empiric TB treatment, only one was culture positive. The rest were culture negative (eight) or culture status was undetermined (three) because of contamination of the culture or missing result.

Table 4: High response to antibiotics treatment and low empiric TB treatment.

Response to antibiotic treatment	Counts, (N=160)	Percentage (%)
Full response to antibiotics	141	88
Initiated on TB treatment	12	8
Other treatment*	7	4

*(Kaposi's sarcoma-1, Lung carcinoma-1 and COPD-5)

9 DISCUSSION

9.1 MAIN FINDINGS

The purpose of this thesis was to increase knowledge on the diagnostic accuracy of GeneXpert, MODS and NRA for smear-negative TB with focus on HIV-positive patients, as well as their cost-effectiveness and effect on patient treatment.

We found that the tests had moderate sensitivity (67-73%) for smear-negative PTB which reduced (24-49%) in HIV-positive patients. The finding of the moderate sensitivity of GeneXpert from our meta-analysis, was similar to that by the Cochrane group in a study conducted around the same period [148], and updated a year later [149]. In addition, based on a sub-analysis of studies from high HIV prevalence settings, we found that the sensitivity of GeneXpert for smear-negative PTB reduced, contrary to what was generally believed at the time. This reduced sensitivity was supported by our laboratory study and another study from the same study area [150]. The two studies showed that in HIV-positive patients, GeneXpert missed half of the TB patients that were smear-negative.

There are new efforts now, to improve the sensitivity of GeneXpert for smear-negative PTB. Three strategies have been used to achieve this: (1) detection of two additional targets of Mycobacteria (IS6110 and IS1081), (2) using fully nested PCR to improve the quality of the DNA products, (3) testing increased volumes of the PCR products. Preliminary results of the new GeneXpert test indicate may have sensitivity equal to culture [151].

Our meta-analysis also provided a global summary of the accuracy of the MODS test for smear-negative PTB. A subsequent study one year later, got comparable results to our study (73% versus 88%) [111]. Both studies indicated the relatively superior sensitivity of MODS (a culture-based method) to GeneXpert (molecular-based method) in the diagnosis of PTB among HIV infected patients. We also highlighted the variable implementation of the WHO 2007 TB algorithm in our meta-analysis. Our findings could be used as a baseline for evaluating future TB diagnostic algorithms after the new tests are incorporated into the existing ones.

We were surprised by the low sensitivity of the GeneXpert, MODS and NRA tests in our laboratory study. Moreover, the MODS had the lowest sensitivity contrary to what we expected from our meta-analysis study. In the case of GeneXpert, there are two views which have been provided to explain our results. The first view suggests that the study participants had early TB disease and therefore had very low bacilli load in their sputum. This view explains why GeneXpert-negative patients are less likely to die during follow-up despite delay to initiate TB treatment [152]. The second view suggests that the study patients had advanced HIV disease which resulted in low bacilli load in their sputum not detectable by the GeneXpert [153, 154].

As both kinds of patients would be seen during routine clinical care, the most plausible explanation is that the minimum number of bacilli required for detection by GeneXpert is far above that required for detection by culture (100-200 versus 10-100 bacilli per ml) [4, 95]. Furthermore, in our study, it is possible that the start bacilli load in the patients' samples was diluted even more by addition of sample reagent in the ratio of 3:1. Although this was the recommendation by the manufacturer, a recent study indicates that if the sample reagent is added in a ratio of 2:1, it improves the sensitivity of GeneXpert [155].

The low sensitivity of MODS and NRA was likely because the two tests were performed on samples stored in a fridge (4–8^{o}C) typically for one week. Some loss of viability could therefore have occurred during the storage period. Moreover, the tests were declared negative after fourteen days initially which was short for any viable Mycobacteria to grow to a level where they could be detected, especially when using a microscope as is the case for the MODS test.

We found that using a MODS-based algorithm was more cost-effective compared to using a GeneXpert-based algorithm for diagnosis of HIV-associated PTB. Given, the emerging evidence of the limited effect of GeneXpert on the clinical outcomes of patients [98, 100, 101], the cost effectiveness of using GeneXpert in resource limited settings is currently questioned because of affordability and sustainability concerns. On the other hand, the MODS test was specifically developed for resource limited settings and has superior diagnostic accuracy to GeneXpert [111, 149]. As scale-up of conventional culture methods have been limited by the high set-up and maintenance costs [156], the MODS could be a suitable alternative because it is cheap. MODS offers the opportunity to decentralize TB culture services. This could increase TB detection and access to drug-susceptibility testing which is important for early diagnosis and treatment of drug-resistant TB. A recent study from Peru suggests this is possible and our cost-effectiveness study supports this move [157].

Nevertheless, cost-effectiveness analysis is only one of the considerations for decision making to adopt a new technology or not. Other factors to take into account include human resource and training requirements, impact of the new technology on the health system as a whole, the infrastructure required to support the new technology, quality assurance, procurement and maintenance issues among others [158]. One way to improve the cost-efficient use of the GeneXpert is to deploy the test in populations where the prevalence of TB is very high for example in prisons, or referral hospitals instead of deployment in district level hospitals or Health Centres.

While sensitivity and specificity of a test describe how well it performs against the gold standard (independent of prevalence of disease), the positive and negative predictive values of a test are population specific and will depend on the prevalence of disease within the population being tested [159, 160]. Generally, a higher

prevalence will increase the positive predictive value of a test and decrease the negative predictive value and vice versa.

Thus, despite the low sensitivity of GeneXpert, MODS and NRA, they had high negative predictive values of 90% or above because of the lower than expected PTB prevalence in the study participants. This low PTB prevalence was possibly due to the high antiretroviral treatment (ART) coverage of 80%, in the study population. It is established now that ART can prevent TB in HIV patients [43, 44]. Nevertheless, TB remains the leading cause of mortality even in HIV patients on ART. Empiric TB treatment could therefore, still be useful given the low-moderate sensitivity of the tests among patients who are smear-negative. Moreover, there is more benefit of empiric TB treatment if initiated early because TB incidence rates are three times higher during the first three months of ART than three months post ART [161, 162].

Empiric TB treatment however, is poorly defined and is not standardized as shown by our meta-analysis study. The WHO definition of empiric TB treatment refers to initiation of treatment in peripheral health facilities exclusively based on clinical suspicion (before TB investigations) for seriously sick patients [80, 163]. In practice, empiric TB treatment is also initiated at referral health facilities based on clinical suspicion of TB when there is no bacteriological confirmation (after TB investigations). This common practice of empiric TB treatment is now feared to undermine the potential effect of the new TB tests such as GeneXpert [164]. An important question therefore, is what impact the new TB tests will have on patient treatment, specifically on the practice of empiric TB treatment. This is important because over-treatment for TB results in misuse of the few resources available to the TB programmes, overlooking other treatable diagnoses and exposure of patients to potentially toxic medicines which they do not require in the first place.

In our fourth study, we investigated how best to treat smear and GeneXpert negative patients with the aim of lowering empiric TB treatment. We found that the combination of a negative smear and GeneXpert test had a high negative predictive value for PTB in our study population. This could be explained by the lower than expected PTB prevalence (11%) in the study population. Although this prevalence was reported elsewhere [165], it is lower than the commonly observed PTB prevalence (20-30%) in HIV-prevalent settings [75, 76, 101].

In light of the low PTB prevalence in the study population, and the high negative predictive value of a smear and GeneXpert negative result, we found that provision of broad-spectrum antibiotics resulted in cure for the majority of patients. Less than 10% of the patients got empiric TB treatment way below the current national levels of 30% [144]. Trials are currently underway to address the question of empirical TB treatment with focus on its benefit for prevention of mortality in HIV patients with advanced disease and the clinical predictors HIV patients for empiric TB treatment [166]. Overall

however, there are still few studies that have investigated the impact of the new TB diagnostics on patient-important outcomes or health systems-level [158, 167].

9.2 METHODOLOGICAL ISSUES

9.2.1 Study I

The key strengths of the meta-analysis study included the use of a standard protocol for conducting the review [168], and assessment of the quality of the studies [146]. We also used various overlapping search strategies which enabled the retrieval of several relevant publications. In addition, we explored for heterogeneity using several methods such as sub-group analysis and generation of sROC curves [168, 169]. The study however, was limited by the wide differences in the publications. This defeated the logic of pooling results, although it was addressed by generation of the sROC curves. In addition, we found few publications involving the MODS test and the WHO 2007 TB algorithm. The small number of publicationstudies on MODS could explain why the sensitivity for the test was 73% in our study compared to 88% in the later systematic review [111]. As there has been no recent systematic review involving the WHO 2007 algorithm, an update of our study is necessary to include the recent publications on the diagnostic accuracy of the algorithm [75, 76].

9.2.2 Study II

The main strength of our laboratory study was the enrollment of consecutively presenting patients into the research. This reduced bias in patient selection [160]. In addition, we used a combination of solid and liquid culture which is the recommended gold standard for TB diagnostics [104]. However, the MODS and NRA methods tests were performed on stored samples. This possibly decreased the viability of the Mycobacteria resulting in low sensitivity of the tests in comparison to the gold standard. Future studies involving freshly processed samples could be considered.

9.2.3 Study III

We modelled the cost-effectiveness of using a MODS-based algorithm versus a GeneXpert-based algorithm for diagnosis of PTB. While models are useful alternatives to costly trials, they are limited by the assumptions used to implement them [167]. Therefore, modelling studies still need to be supported by real-world data. Moreover, such studies would provide additional information which is of more practical relevance than models can provide [163]. These data include time from diagnosis to initiation of treatment or the drop-out rate of patients from the diagnostic pathway.

9.2.4 Study IV

The major strength in this study was the prospective six-month follow-up of the smear- and GeneXpert-negative patients following broad spectrum antibiotics or empiric TB treatment. However, the study did not have an appropriate control group. As an alternative to a randomized control study, a better design could have been to compare the results from the prospective follow-up and results from retrospective program data, possibly of one year. Thus, although we found only a few patients got empiric TB treatment, it is not surprising that our results are contrary to those from the recent trials involving Xpert [100, 101]. In these trials, empiric TB treatment was about 20% and therefore, the overall benefit of our strategy for reducing empiric TB treatment was possibly over-estimated.

10 CONCLUSIONS

GeneXpert, MODS and NRA increase detection of PTB in patients with a smear-negative outcome after examination by microscopy. However, additional investigations are required in those who test negative because the three tests have low-moderate sensitivity for PTB in patients with this outcome. The tests are reliable for initiation of TB treatment because of their high specificity.

The diagnostic accuracy of GeneXpert for smear-negative PTB is superior to that for MODS and the current WHO algorithm for TB diagnosis. Incorporation of GeneXpert could therefore improve the diagnostic accuracy of existing algorithm.

From an economic view point, using MODS for diagnosis of PTB in HIV-positive patients is more cost-effective than using GeneXpert. Therefore, where resources are limited, the MODS could be used as an alternative to GeneXpert.

A Smear and GeneXpert negative test result has a high negative predictive value for PTB in HIV patients with high ART coverage. In such patients, initial treatment with broad spectrum antibiotics could be useful to reduce empiric TB treatment. A trial of antibiotic treatment could therefore, be incorporated in new GeneXpert-based algorithms.

11 AREAS FOR FUTURE RESEARCH

The findings from the studies in this thesis are useful to researchers involved in test development, policy makers, and medical practitioners involved in TB care.

The currently available new tests have only low-moderate sensitivity for Smear-negative TB. Future research is therefore required to develop tests with sensitivity equivalent to culture. For some of the current new tests such as MODS and NRA, there is need to research ways to reduce the training requirements for operating them and to standardize the test procedures.

Policy makers, need to research the over-all benefit of using the new TB tests to the health care system. In addition, policy makers need to research the best ways to scale-up implementation of the new tests. For example, at what tier in the health system should the new tests be deployed to achieve maximum effect for the invested resources.

More research is also required to estimate the epidemiological impact of the new tests to support decisions for allocating resources to adopt the new tests. Furthermore, real-world data is required to support the economic view that using MODS for diagnosis of TB in HIV-positive patients is more cost-effective than using GeneXpert.

Clinicians will need to research which combination of the traditional and new tests results in increased diagnosis of smear-negative TB. There is still need to research how best to reduce the practice of empiric treatment for smear-negative TB using the new tests.

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