Xpert MTB/RIF for diagnosis of tuberculosis:

performance variability and impact on patient-important outcomes

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Frederick Haraka

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

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Basel, February 18, 2020

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*I dedicate this PhD thesis to my beloved wife **Winnie** and our children **Patricia** and **Patrick***

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Acknowledgement

I would like to first thank God for the great love and opportunities he has bestowed upon me in my life thus far. I would like to express my sincere gratittude to my parents for being supportive throughout my studies for this PhD as they have always done on many other occasions. To my family to which I dedicate this work, my beloved wife Winnie , our children Patricia and Patrick for being patient, supportive and encouraging.

I`m real thankful to My PhD committee as follows:

To Dr. Klaus Reither, in a special way, for recruiting me in his team in IHI-Bagamoyo, mentor, supervise this PhD and trusted my knowledge and skills repeatedly over the past 10 years.

To Prof. Sebastien Gagneux a renowned TB researcher for his supervision, encouragement and guidance throughout the PhD period.

To Prof Jacob Zinsstag for accepting to be part of my PhD committee as a secondary supervisor

To Prof. Lutgarde Lynen for accepting to be part of my PhD committee as an external expert

My sincere appreciation extends to:

To Prof. Muhammad Kambi Bakari who is current the Chief Medical Officer of Tanzania who encouraged me when I made a decision and choice to persue research career over 12 years ago.

To Dr. Humphrey Shao who invited me to join the Ifakara Health Institute (IHI) and worked with me in the early days of my career.

To Prof. Marcel Tanner, former director of Swiss TPH for his extraordinary decision to support my masters training at the University of Nottingham in the UK.

To Dr. Samuel Schumacher, from FIND and Dr. Claudia Denkinger (former head of TB at FIND) for their exceptional collaborative attitude, support and allowing me to use data from FIND for part of this thesis.

To Dr. Amanda Ross, a senior medical stastician at Swiss TPH for her exceptional support, team spirit and encouragement during the development of the manuscripts

To my colleagues in the TB research programme team of IHI for their wonderful team spirit.

To the participants of all studies that generated the FIND database which I used to write two manuscripts, the research teams of these studies, the authors of the papers included in the Cochrane review, to all I say thank you.

To Ms. Christine Mensch and her team for the wonderful coordination throughout my PhD studies.

To Dr. Honorati Masanja, the director of IHI, for his passion in developing researchers,thus creating room for training.

To the Canton of Basel for the scholarship (Amt für Ausbildungsbeiträge Basel-Stadt) which facilitated this PhD.

To all of you, you have been part of my career and this PhD in one way or the other and I say THANK YOU

Summary

Accurate diagnosis and early treatment are key in achieving global targets to end tuberculosis (TB). Between 2000 and 2017, nearly 54 million deaths were averted due to timely diagnosis and treatment initiation. Xpert MTB/RIF ("Xpert"¸Cepheid, Sunnyvale, CA, US) is a molecular diagnostic test with integration of an automated sample processing system and hemi-nested real time polymerase chain reaction (PCR) in a single cartridge. It provides results within two hours and can be used at point-of-care. Xpert was endorsed by WHO in 2010 and currently is strongly recommended for adults and children suspected for MDR-TB or HIV and conditionally for all suspected with TB, should resources allow. By 2016, approximately 34 million Xpert cartridges were procured globally. Despite considerable development in evaluation and roll out of Xpert, important questions still remain to be addressed. Xpert has shown varibility in performance and it remains a question whether the observed variability is entirely explained by differences in populations and epidemiological burden or methodological differences in how Xpert is being evaluated such as effect of time to positivity (TTP) on a reference standard (culture) on sensitivity and how previous history of TB affects the specificity of Xpert. Accurate TTP can only be determined in automated systems such as MGIT culture system. Furthermore, studies that have assessed patient-important outcomes have shown inconsistent results. This doctoral thesis provides evidence on the overall population level pooled effect of Xpert on important patient outcomes based on the Cochrane review and evidence on effect of TTP and history of TB on sensitivity and specificity Xpert, respectively.

This thesis is based on three manuscripts:

Manuscript 1: Effect of time to culture positivity as a reference standard on Xpert MTB/RIF sensitivity for diagnosis of pulmonary tuberculosis

This manuscript shows the effect of TTP on the sensitivity of Xpert based on the analysis of data from a database of 16 different studies that evaluated Xpert with a total of 11,870 participants from ten different countries. The TTP was divided up in categories of five days up to 42 days. In all culture positive, sensitivity of Xpert in categories up to 15 days were: ≤ 5,

99.3% , (95% CI: 95-100, N=351) , >5≤10, 99.3 % (95%CI, 98.4-99.7, N=2231) and >10≤15, 96.8% (95%CI,94-98.3,N=1885). In smear negative-culture positive: ≤ 5, 99%, (95% CI: 62-100, N=37) , >5≤10, 98% (95%CI, 80-100, N=237) and >10≤15, 92% (95%CI,83-96 ,N=413). In HIV positive: ≤ 5, 96% , (95% CI: 63-100, N=51) , >5≤10, 98% (95%CI, 90-100, N=199) and >10≤15, 94% (95%CI,82-98 ,N=168) The sensitivity of Xpert in the first fifteen days was high in all three groups of analysis: all culture positive, smear positive-culture positive and smear negative-culture positive and irrespective of HIV status. A significant drop in sensitivity of Xpert when TTP of more than 15 days were assessd across all groups of analysis. Xpert sensitivity decreased with increasing TTP. This may explain some of the variation seen across different studies published on the diagnostic performance of Xpert. In settings with better TB control efforts, it is expected that patients are diagnosed earlier and TTP will be increased. Thus, reporting TTP of MGIT culture is important to ensure comparability of studies on Xpert as a diagnostic TB test.

Manuscript 2: Effect of a previous history of tuberculosis on the specificity of Xpert MTB/RIF

This manuscript shows the effect of history of TB based on the analysis of data from a database of 16 different studies that evaluated Xpert with a total of 11,870 participants. A total of 1,630 participants had a history of TB. The median time since previous TB treatment was 3 years (Interquartile range (IQR), 0.0-6). Among the 803 patients with a TB episode within two years of testing, the specificity of Xpert was 92.2% (95% CI 81-97). The specificity increased with time since previous TB. Between two and five years (373 participants), the specificity was 99.0% (95% CI; 86-100) and above five years (454 participants), 98.6% (95% CI; 85.4-99.8).This manuscript shows that a history of TB negatively affects Xpert specificity, and this effect is increased among those with TB in the last two years. This implies for a need of algorithms in patients with history of TB and positive Xpert to guide interpretation and management. The use of chest X-ray and clinical judgement remain relevant.

Manuscript 3: Impact of the diagnostic test Xpert MTB/RIF® on health outcomes for tuberculosis

This manuscript is based on a Cochrane review and summarizes the effect of Xpert on patient-important outcomes. The review included ten studies, seven of which were randomized controlled trials and three pre-post intervention studies. In the domains of the Cochrane risk of bias tool, most randomized studies had a low risk of bias. There was evidence of a positive effect of Xpert on tuberculosis confirmation in treated patients (RR 1.29 95%CI 1.11, 1.51 in the randomized trials) and reduction in pre-treatment loss to follow up (RR 0.59 95% CI 0.42-0.84). Overall there was a reduction on all-cause mortality of 12% (RR 0.88 (95% CI 0.73, 1.05) and 24% (RR 0.76 95% CI 0.58-1.00) among HIV positive participants. There was evidence that Xpert lead to an increase in the overall proportion of patients treated for TB (RR 1.10 95%CI 0.98, 1.23), the likelihood of being cured (OR 1.09 95% CI 1.02, 1.16), and that the proportion of those treated who were not microbiologically confirmed was reduced (RR 0.59 95%CI 0.41 0.85. This manuscript shows that compared with smear microscopy, Xpert reduces all-cause mortality by 12% although uncertainty around the effect estimate was high and the data was also compatible with reduction of up to 27% and an increase of up to 5%. The mechanisms by which Xpert could affect mortality is likely at least in part related to the reduction in pre-treatment loss to follow-up as well as the increase in the proportion of patients cured. Further studies should assess the role of empirical treatment on the impact of Xpert on patient outcomes.

Abbreviations

AIDS	Aquired Immunodeficiency Syndrome
BMGF	Bill and Melinda Gates Foundation
Ct	Cycle threshold in Xpert MTB/RIF
FIND	Foundation for Innovative New Diagnostics
HIV	Human Immunodeficiency Virus
IHI	Ifakara Health Institute
LJ	Löwenstein-Jensen medium
MGIT	Mycobacteria Growth Indicator Tube (“MGIT”; Becton Dickinson, Franklin Lakes, NJ, USA)
PEPFAR	The President`s Emergence Plan for AIDS Relief
Swiss TPH	Swiss Tropical and Public Health Institute
TB	Tuberculosis
TTP	Time to culture positivity
Ultra	Xpert MTB/RIF Ultra (“Ultra”¸ Cepheid, Sunnyvale, CA, USA)
USAID	The United States Agency for International Development
UNITAID	A global health initiative hosted by the World Health Organization
WHO	World Health Organization
Xpert	Xpert MTB/RIF (“Xpert”¸Cepheid, Sunnyvale, CA, USA)

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1 Chapter 1

1.1 Introduction

1.1.1 Biology, transmission and clinical manifestation

Tuberculosis (TB) in human is caused by intracellular bacteria known as *Mycobacterium tuberculosis sensu strictu* and *Mycobacterium africanuum* belonging to the *Mycobacterium tuberculosis* complex (MTBC) (1). The MTBC consists of highly related bacterial lineages with nucleotide similiarity of 99.9%, except *Mycobacterium canettii* which differs markedly from the rest of the MTBC members (1). Other animal-associated members of the MTBC are responsible for diseases in wild and domestic mammalian hosts.

Scientific evidence suggests that MTBC emerged about 10,000 years ago in Africa and migrated with modern human to the rest of the world during the neolithic period (2). However, the matching of MTBC and human mitochondrial phylogeny suggest much older interrelationship, that carriage of MTBC in human was present in the hunters and gathers who migrated from Africa nearly 70,000 years ago (2).

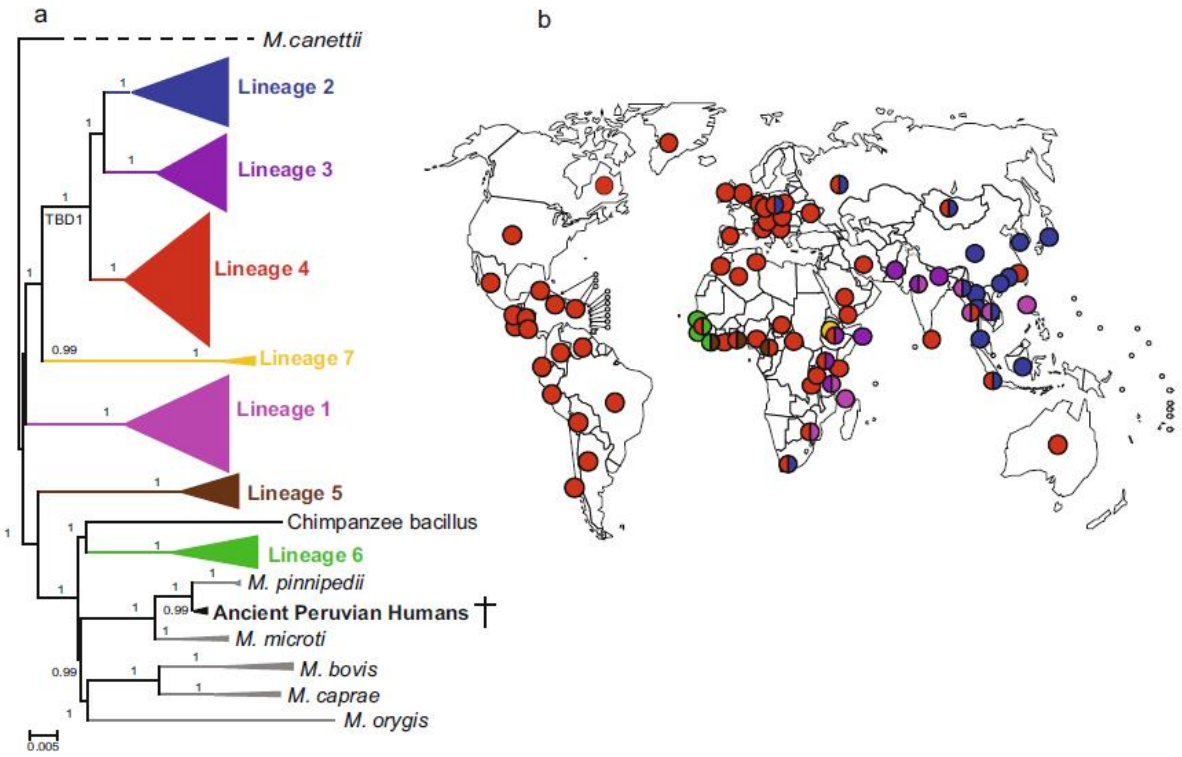
There are seven human adapted MTBC lineages. *Mycobacterium tuberculosis sensu strictu* comprises of lineages 1 to 4 and lineage 7. *Mycobacterium africanum* comprises lineage 5 and 6. MTBC lineages show geographic distribution variations, for example lineage 5 and 6 are restricted to West Africa and lineage 7 only occurs in Ethiopia, ***Figure 1.* 1***)*. Lineage 4 is the most widely distributed across the world (1). MTBC lineage variation has an impact on virulence and hence important for vaccine and drug development. The Xpert MTB/RIF test (Xpert), which is the central topic of this thesis, can detect the entire MTBC across all lineages.

TB transimission occur mainly through air from a TB patient to another person through released aerosals either by coughing, sneezing, speaking or spiting (3). It is estimated that a single sneeze can release up to 40,000 droplets and each of the droplets can potentially transmit TB (4). Untreated patients with active TB disease can potentially infect 10 to 15 individuals per year. The risk of TB transmission depends on many factors such as quantity of infectious

droplets released, immunity of uninfected person, virulences of the *Mycobacterium tuberculosis* strain, overcrowding, close contact and duration of exposure (5, 6).

Worldwide, about 1.7 billion people are latently infected with TB. These asymptomatically infected individuals have about 12% life time risk of progressing to active TB disease. If no active disease develops after five years, the risk drops to 2% and to 0.5% after 10 years (7). However, with HIV co-infection, the risk to progression increase to about 10% annually. These figures refer to a simplistic classification with latent, asymptomatic and not infectious TB on the one hand, and active, often infectious TB on the other hand. Recent evidence suggests, however, that TB infection should be considered as a spectrum, which ranges from eliminated disease to active, sub-clinical TB to active, clinical TB (7). Primary infection occurs when MTBC enters the lungs of an uninfected person. Depending on factors such as infectious dose and immunity of an individual, the infected person might control the infection and bacteria remain in the lungs without causing any symptoms. This is also known as latent infection, which can be stable or unstable. A stable latent infection is rapidly controlled and the organism may be sterilized, which minimises risk of reactivation (7). In the unstable latent infection the infection takes a waxing and waning course with high chances of reactivation or progression to active TB in the presence of precipitating factors e.g. malnutrition (7). Clinically, pulmonary TB presents usually with cardinal features of prolonged cough of more than two weeks or hemoptysis, fever, night sweats and loss of weight. Other symptoms may include fatigue and loss of appetite. TB manifestation outside the the lungs, also known as extra-pulmonary TB, may occur from haematogenous dissemination with varying symptoms depending on the organs affected by the pathogen.

Figure 1. 1 The Nature and Evolution of Genomic diversity of in the* Mycobacterium tuberculosis *Complex (1)



1.1.2 TB epidemiology and control

TB is one of the oldest dieases of humankind. In the past three centuries, TB was responsible for 25% of all death in Europe and America (8). TB is the leading cause of death from a single infectious disease (9). According to the recent World Health Organization (WHO) TB report of 2018, 1.2 million HIV-negative and 251,000 HIV-positive were estimated to have died of TB (9). In the same year, of the estimated 10 million new TB infections only 70% were reported (9). About 0.8 million cases were co-infected with HIV (9). Although TB cases were reported from all countries, two third of the total estimated TB were reported from eight countries; India, China, Indonesia, the Philippines, Pakistan, Nigeria Bangladesh and South Africa (9).

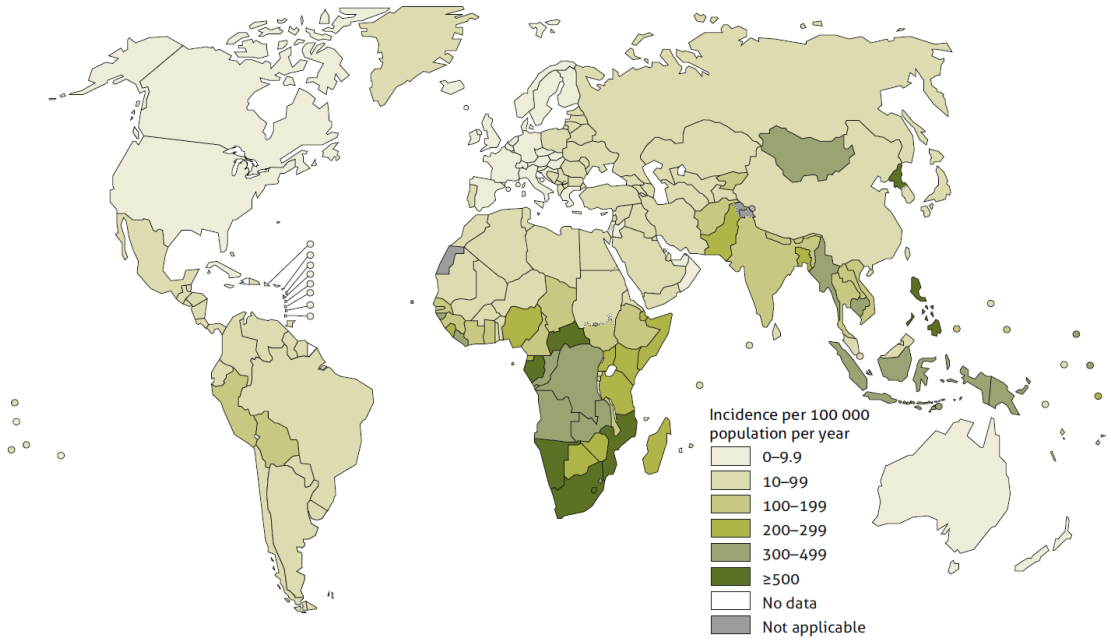
Drug resistant TB (DR-TB) was reported in 186,772 cases globally and about one in three was enrolled in treatment (9), accounting for only 25% of the total estimated global burden. China and India accounted for 43% of the DR-TB treatment gap (9). It is estimated that about 20% of all TB bacteria isolated globally is at least resistant to one major first or second line drug (10). Approximately 5% of patients worldwide are estimate to have TB, which is either resistant

to both isonizid and rifampicin known as MDR, or in addtion resistant to secondline floroquinolones or injectables of aminoglycosides (10).

In both drug sensitive and drug resistant TB, underreporting and/or underdiagnosis still exist. In 2018, India, Nigera, Indonesia and Phillipines acounted for more than half of the diagnostic gap (9). A number of challenges are related to the diagnostic gap and these include but not limited to: limited access to healthcare and lack of robust diagnostic tools at point of care.

In 2000, a partnership of 1,500 organizations, which include a wide range of stakeholders, was formed in efforts to elimiate TB. WHO endorsed the Stop TB Partneship to coordinate and spearhead efforts in TB control. The global end TB targets were made in 2015 and recognizes the need for a world free of TB. In order to achieve that, the Stop TB partneship has set targets to be achieved by 2035. Compared to 2015: (i) reduce the number of death by 95% (ii) reduce TB incidence rate by 90% and (iii) 0% TB-affected families facing catastrophic costs due to TB (11). In order to reliaze the end TB strategy targets, TB must have declined at a rate of 10% annually between 2015 and 2020, and should decline at a rate of 17% after 2020. As of 2018, the current decline of TB is only at 2% per annum (9). Furthermore, the end TB strategy calls for universal access to drug susceptibility testing among patients seeking healthcare. Indeed, optimization of current strategies and introduction of new tools particularly diagnostic tools cannot be overemphasized (11).

Figure 1. 2: Estimated TB incidence rates in 2018 (9)



1.1.3 TB diagnosis

1.1.3.1 Current standard of care for bacteriological confirmation of TB

Smear microscopy for TB was introduced approximately 100 years ago, and has been used to confirm TB ever since. The sensitivity of smear microscopy ranges between 20 and 60% (12). However, smear microscopy is known to be highly specific for TB, with specificity above 98% (12). Smear microscopy is relatively simple, inexpensive and identifies most infectious TB patients (12). Although the sensitivity for TB is limited, smear microscopy is still widely used in low and middle income countries where 95% of all cases and 98% of all TB related death occur (12). Furthermore, it has limited value in patients with low bacterial load particularly children and among HIV co-infected individuals. In drug resistance TB, smear microscopy has no value as it does not have capacity for resistance detection.

Culture is the the reference standard fo diagnosis of TB and was first described over 50 years ago. Ever since, culture has undergone several developments and to date, culture can be done

in both liquid and solid media. Mycobacteria growth indicator tubes (MGIT) liquid culture can show growth within 42 days after innoculation. Over time the MGIT culture evolved to current automated version known as BACTEC MGIT 960 system (MGIT Becton Dickson , Franklin Lakes, NJ, USA) (13, 14). MGIT 960 system consists of broth medium and a flurochrome compound, which leads to fluorescence as bacteria utilize oxygen while growing. (13, 14). TB is detected at a time to positivity when the estimated colony forming unit CFU per ml is 10^5 to 10^6 in the media (13). Negative culture is declared after 42 days of innoculation (13, 14).

Löwenstein-Jensen (LJ) culture uses solid media e.g. egg based media. LJ has been used for many years and it is considered economical and easy to prepare. In general, LJ is associated with low rate of contamination. However, LJ culture takes longer time to grow, up to eight weeks after innoculation (14).

Establishment of both liquid and solid cultures require specified conditions of infection control. Biosafety level 3 facilities require expensive infrastructures and specialized trainings both of which limit the use of culture at point-of-care in low- and middle-income countries.

1.1.3.2 Development of new TB diagnostics

In the UNITAID Diagnostic Technology Landsscape report, the Foundation for Innovative New Diagnostics (FIND) classifies development of Nucleic Acid Amplification Tests (NAATs) diagnostics assay based on the degree of complexity of use (15). The majority of diagnostic technologies are in the development stages with few under WHO evaluation (15), *Appendix 1*. So far, the following have received WHO recommendations for TB or MDR detection: lateral flow-lipoarabinomannan (LAM) assay, loop mediated amplification (LAMP) assay, line probe assay (LPA), the current Xpert assay and Xpert MTB/RIF Ultra (Ultra) (15). The current diagnostic pipeline calls for developers to accelearate development of NAAT that meet targeted product profile and can be readily available at point-of-care. Xpert remains the most widely used at point of care of all NAATs endorsed by WHO.

1.1.3.3 Xpert MTB/RIF

1.1.3.3.1 Technical characteristics

Xpert MTB/RIF (Cepheid, Sunnyvale, CA, US) is a molecular diagnostic test with integration of an automated sample processing system and hemi-nested real time polymerase chain reaction (PCR) in a single cartridge (16). The Xpert plastic cartridge contains liquid sample processing, buffers and PCR reagents (17). Once a sample has been prepared and mixed in the cartridge, the cartidge is inserted in the GeneXpert instrument. The GeneXpert instrument controls intracartridge fluidics and performs real-time PCR (17). Xpert can simultaneously detect *M. tuberculosis* and susceptibility to rifampicin. Xpert amplifies sequences of the *rpoB* gene that are specific for MTBC and simultaneously probe for mutation in the Rifampicin Resistant Determinant Region (RRDR) of the *rpoB* gene (17). Xpert cartridge contain *Bacillus globigii* DNA as an internal control for sample processing and PCR (17).

Initial clinical validation and implementation studies showed Xpert can provide results within two hours and promising test performance in areas of intended use in both patients with pulmonary and extrapulmonary TB (18-20).

1.1.3.3.2 Perfomance

Xpert perfomance varies across different populations, type of TB, specimen used and when different case definitions for TB were used (21-24). In a recent Cochrane review including 70 studies and 37,237 participants, most studies done in high burden countries, the Xpert pooled sensitivity was 85% (82% to 88%) with a specificity of 98% (97% to 98%) among adults with pulmonary TB (25). For rifampicin resistance, including 48 studies and 8,020 participants, Xpert pooled sensitivity was 96% (94% to 97%) with a specificity of 98% (98% to 99%) (25). Among children, Xpert pooled sensitivity and specificity were 62% (51% to 73%) and 98% (97% to 98%), respectively, when expectorate or induced sputum were used (23). Xpert perfomance were comparable among studies that used gastric lavage samples (23). In a Cochrane review Xpert perfomance for extra-pulmonary TB was variable in different samples (cerebrospinal fluid (CSF), pleural fluid, urine, joint and bones) (22).

1.1.3.3.3 Policy and roll out

In 2010, the WHO endorsed Xpert in 2010 after an expert meeting which reviewed exisiting evidence on accuracy. By early 2011, the WHO recommeded the use of Xpert as an initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB, as a strong recommendation (26). Furthermore, conditional recommendations were made that Xpert should be used as a follow up test to smear microscopy in settings where MDR-TB and HIV are of lesser concern, particularly for further testing of smear negative patients (26). These recommendations were based mainly on studies done in adults. Following availability of new evidence, WHO convened another expert meeting in 2013. Additional two strong recommendations were made; (i) Xpert should be used rather than conventional microscopy, culture and DST as initial diagnostic test in children suspect of MDR-TB or HIV associated TB, (ii) Xpert should be used in preference of conventional microscopy and culture as the initial diagnostic for CSF specimen for patients suspected of TB meningitis. Further conditional recommendations included using Xpert as initial diagnostic test in non-respiratory specimens in extra-pulmonary TB and in all adults and children depending on resource availability (26).

FIND negotiated with Cepheid for concessional pricing for Xpert in the public sector and for non-govermental organizations (NGO) in 145 high burden developing countries for 17,000 USD per GeneXpert platform and 16.68 USD per cartridge (27). Following support from PEPFAR, USAID, UNITAID and BMGF the price was further reduced to 9.98 USD per cartridge (27). By 2016 approximately 23 million Xpert cartridge were procured in pubic sector in 130 countries and approximately 34.4 millon overall globally (28, 29). Out of 22 high burden countries, 10 had included Xpert in their national policy as the initial diagnostic for all presumed to have TB (29). The smear/ Xpert ratio in these countries decreased between 2014 and 2016 from a median of 32.2 smears for each Xpert test to 6 (29). South Africa had the highest decrease in ratio given that South Africa scaled up the use of Xpert to replace smear microscopy as initial TB test (29). Concessional pricing indeed contributed to rapid scale up of use of Xpert in developing countries. However, there are still concerns on costs among 10 out

of 22 high TB burden countries where TB is primarily diagnosed in the private sector (30). In these countries, the average price per cartridge in private healthcare was 68.73 USD, which often impedes patient`s access to Xpert .

The imperfect perfomance of Xpert, particularly in smear-negative and HIV- associated TB, led to the development a next generation assay call Xpert MTB/RIF ultra (Ultra), which has more abillity to detect TB even at very low bacterial load (24, 31). Ultra has 5% more sensitivity but 3.2% less specificity than Xpert (31). Higher sensitivity were seen more among HIV positive and smear negative-culture positive patients, while decrease in specificity was more among patients with previous history of TB. WHO called for an expert meeting which concluded that Ultra was non-inferior to Xpert for diagnosis of TB and rifampicin detection and that current WHO reccomendations for Xpert apply for Ultra as well (31).

1.1.4 Evidence gaps

Despite considerable development in evaluation and roll out of Xpert , important questions still remain to be addressed. Xpert has shown varibility in performance and it remains a question whether the observed variability is entirely explained by differences in populations and epidemiological burden or methodological differences in how Xpert is being evaluated. Furthmore, clinical impact in terms of patient-important outcomes remains an open question on the public health impact of Xpert in TB control. Clearly identified evidence gaps concern the effect of time to culture positivity on Xpert sensitivity, as well as the effect of a history of TB on the specificity performance of Xpert. Furthermore, the impact of Xpert on patient-important outcomes is still underresearched. These gaps are discribed in detail below.

1.1.4.1 Time to culture positivity

TTP has been shown to be a proxy of bacillary load of the sputum inoculum (32-35). Longer TTP have been shown to correlate with low bacillary load (32, 35, 36). Longer TTP have also been shown among smear negative patients whose sputum was induced. Xpert sensitivity was found to correlate with TTP (32). Longer TTP of 28 and 14 days were observed among patients with smear negative-culture positive and smear positive-culture positive TB, respectively (32).

Accuracy studies of Xpert have shown low sensitivity in smear negative-culture positive compared to smear positive-culture positive patients. It is hypthesized that variation in bacterial load may affect performance of Xpert and that differences in the perfomance of Xpert between studies may be partially be explained by different TTP. This remains to be proved in large study which takes into account patients from different population and epidemiological settings.

The variation in the type of culture (liquid or solid) and number of cultures may affect performance evaluation of Xpert. For example, Dorman *et al* in their additional analysis, showed higher sensitivity of Xpert when a single liquid culture was considered as a reference standard compared to multiple cultures. However, these analyses had small sample sizes, therefore lacking enough power for a definite conclusion.

1.1.4.2 A previous history of TB

Studies have reported incidences of false Xpert positive results among patients with a previous history of TB (37, 38). Arguably, in patients with a history of TB Xpert detects dead *M. tuberculosis* bacilli (37-39). False positives among previous TB patients was shown to be likely if the previous TB event was more recent, had a chest X-ray not compatible with TB, HIV co-infection and had high CT value consistent with low bacterial load (39, 40). A history of TB increases the pre-test probability for TB in these patients, and in settings of high HIV burden where rates of co-infections are high, a positive Xpert could either be false or true positive. A potential risk of overtreatment in this sub-population remains relevant. It is crucial that the time since previous TB event and the likelihood of a false positive is well characterized to better inform clinical decisions. Previous studies were either small or done in particular settings which affects generalizability. Thus, such evidences call for evaluation of such variability in large studies across different populations and epidemiological settings.

1.1.4.3 Patient-important outcomes

In terms of impact on patient-important outcomes, studies have shown inconsistent results (41-46). Evidence shows limited impact of Xpert on mortality (41, 46), whereas other studies have

shown impact of Xpert on reducing time to treatment initiation, particularly among HIV positive individuals (42, 44). To date, there have been seven trials that have assessed the impact of Xpert on patient relevant outcomes (41-47). A recent meta-analysis by Di Tanna GL *et al* 2019, on the impact of Xpert on clinical outcomes could not conclude with certainity any impact of Xpert on mortality among outpatients tested for TB (48). For the mortality analysis, this review included only three of the seven randomized controlled trial available so far (48). Little is known on the impact of Xpert on other intermediate patient-important outcomes such as pre-treatment loss to follow up. Furthermore, it remains crucial to quantify the effect of Xpert on bacteriological confirmation, treatment and treatment success. These outcomes were not reported by Di Tanna GL *et al* 2019. A gap still exists on the impact of Xpert on patient outcomes in drug resistant TB. While other studies have shown a positive effect of Xpert on reducing time to diagnosis and treatment initiation in drug resistance TB (49), others have shown negative effects on treatment outcomes among drug resistant TB (50). Patient-important outcomes are crucial in determining the health and quality of life of the population.

To summarize, further research using large sample sizes from TB patients in different epidemiological settings is needed to understand how time to culture positivity affects Xpert sensitivity and how much this effect contributes to the variation in Xpert performance variability. Furthermore, we need to better understand the effect of a previous history of TB and duration since previous TB on the Xpert specificity. The only review on impact of Xpert on mortality did not show any positive effect given the small number of studies included at the time. Reviewes with larger sample sizes are likely to detect effects on mortality of Xpert should these effects exist. Such reviews should include a wide range of patient-important outcomes, including pre-treatment loss to follow up, to allow comphrehensive assessment of the impact of Xpert on patient-important outcomes.

This doctoral thesis provides evidence on the overall population level pooled effect of Xpert on important patient outcomes based on the Cochrane review. Furthermore, it provides evidence on the effect of TTP and a previous history of TB on the sensitivity and specificity Xpert.

2 Chapter 2

2.1 Thesis aim

The main aim of this doctoral thesis was to understand factors influencing Xpert performance variability and its impact on patient-important outcomes. Two studies were conducted using database from samples collected from 10 countries by FIND (Belarus, Cambodia, Georgia, India, Italy, Moldovia, Peru, South Africa, Vietnam and Zimbabwe), representing different epidemiological settings. The third study was a Cochrane review.

Manuscript 1:The first study aimed to assess the effect of time to culture positivity as a reference standard on Xpert sensitivity for diagnosis of pulmonary TB. Additional analysis was done to assess Xpert performance depending on number of culture and type of culture used. Comparison was made in three groups: all culture positive, smear positive-culture positive and smear negative-culture positive.

Manuscript 2: The second study aimed to assess the effect of a previous history of TB on the specificity of Xpert. Categories of time since the previous history of TB were made and the specificity was estimated in each category. The risk of false positivity was calculated in different sub-groups including HIV positive and negative TB patients.

Manuscript 3: The third manuscript aimed to assess the impact of Xpert on health outcomes for TB. A Cochrane protocol **(Cochrane Systematic Review - Intervention - Protocol Version published: 27 February 2018,** doi.org/10.1002/14651858.CD012972**)** was published. The focus was on the following outcomes: mortality, pre-treatment loss to follow up and proportions of patients (treated, treated and confirmed bacteriologically and treated without confirmation). Comparison was made between Xpert and smear microscopy strategies.

3 Chapter 3

3.1 Effect of time to culture positivity as a reference standard on Xpert MTB/RIF sensitivity for diagnosis of pulmonary tuberculosis

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Manuscript to be submitted to the American Journal of Epidemiology

3.1.1 Abstract

Background

Xpert performance for tuberculosis (TB) detection is usually assessed against culture and varying sensitivity has been found across studies. This variation in Xpert performance may be due to substantially varying bacillary load and/or different choice of reference standards. We examined the sensitivity of Xpert against different reference standards (number and type of culture) and using time to culture positivity (TTP) from an automated Mycobacterium Growth Indicator Tube (MGIT) culture system as an indicator of the bacillary load.

Methods

A large database of sixteen studies performed for the purpose of supporting a biorepository was collated by the Foundation for Innovative New Diagnostics (FIND). Participants were recruited prospectively from sites in ten countries in South America, Africa, Asia and Europe. Participants gave two sputum samples each for liquid and solid cultures. TB was defined as at least one culture positive. Time to culture positivity (TTP) was estimated from automated Mycobacteria Growth Indicator Tube ("MGIT"; Becton Dickinson, Franklin Lakes, NJ, USA) as the difference in dates between inoculation and positivity. The TTP was divided up in categories of five days (≤ 5, >5≤10, >10≤15, >15≤20, >20≤25, >25≤30, >30≤35 and >35≤42 days). The sensitivity of Xpert was estimated as the proportion of culture positive patients who were Xpert positive using logistic regression, for different categories of TTP. The culture reference standard was defined in two cultures of each, MGIT and LJ. Predefined sub group analyses were done by smear and HIV status. The sensitivity of Xpert was calculated as the proportion positive of those positive by the reference standard. Clustering by study was taken into account using a random effect.

Findings

We included 9,894 participants from the 16 studies. Of the 5,850 participants with positive MGIT culture, 76.3% had a TTP of equal or less than 15 days. The overall median TTP was

11 days (Interquartile range (IQR) 8-15). The overall sensitivity of Xpert was 88.1% (95% CI 81.8 to 92.5). In all culture positive, sensitivity of Xpert in categories up to 15 days were: ≤ 5, 99.3% , (95% CI: 95-100, N=351) , >5≤10, 99.3 % (95%CI, 98.4-99.7, N=2,231) and >10≤15, 96.8% (95%CI,94-98.3,N=1,885). In smear negative-culture positive: ≤ 5, 99%, (95% CI: 62-100, N=37) , >5≤10, 98% (95%CI, 80-100, N=237) and >10≤15, 92% (95%CI,83-96 ,N=413). In HIV positive: ≤ 5, 96% , (95% CI: 63-100, N=51) , >5≤10, 98% (95%CI, 90-100, N=199) and >10≤15, 94% (95%CI,82-98 ,N=168) The sensitivity of Xpert in the first fifteen days was high in all three groups of analysis: all culture positive, smear positive-culture positive and smear negative-culture positive and irrespective of HIV status.

We observed a significant drop in sensitivity of Xpert when TTP of more than 15 days were considered across all groups of analysis. Using different TB case definitions, sensitivity of Xpert was highest against a single culture of either MGIT or LJ compared to multiple cultures in each. Sensitivity of Xpert was highest against a single MGIT: 93.4% (95% CI 87.3-96.6) in all culture positive patients and in smear negative culture positive patients 78% (95% CI 58.9-89.8). The lowest sensitivity was observed against multiple LJ (N=7260), 78.7% (95% CI 62.2-89.3) in all culture positive and in smear-negative culture-positive 64.9% (95% CI 49.7-77.5).

Conclusion

Xpert sensitivity decreases with increasing TTP. This may explain some of the variation seen across different studies. In settings with better control efforts, it is expected that patients are diagnosed earlier and TTP will be increased. Thus, reporting TTP is important to ensure comparability of studies. The comparison against different definitions of culture positivity demonstrate the importance of comparability of reference standards across studies.

3.1.2 Background

Accurate diagnosis and early treatment are key in achieving global targets to end tuberculosis (TB). Between 2000 and 2017, nearly 54 million deaths were averted due to timely diagnosis

and treatment initiation (9). Xpert has transformed TB diagnosis since its introduction and recommendation by the World Health Organization (WHO) in 2010 (51-53).

Compared to the standard method of smear microscopy, Xpert has the advantage that it is more sensitive and provides information on rifampicin resistance. A Cochrane review estimated a pooled sensitivity of Xpert to be 85% and a pooled specificity of 98% (54). However, the performance of Xpert has been shown to vary between studies, depending on population, settings and testing strategy (21-23). For example, the pooled sensitivity of Xpert in extra-pulmonary TB and in children varies depending on which specimen has been testing for Xpert sensitivity (22, 23). Further variation of Xpert sensitivity is dependent on bacillary load measured by smear microscopy and HIV co-infection across studies (25).

The reference standard used for the evaluation of Xpert is culture. In solid culture, the decontaminated sputum pellet is incubated usually for a maximum of 56 days (55). Liquid cultures using Mycobacteria Growth Indicator Tube ("MGIT"; Becton Dickinson, Franklin Lakes, NJ, USA) are observed for a minimum of 42 days (56, 57). However, across studies the type of cultures (solid and/or liquid culture) and number of cultures varies, and may affect performance estimates of the index test as well as the bacillary load, which may vary across populations. For example, differences in the performance of Xpert between studies may be at least partially explained by different reference standards (number and type of cultures). For example, an increasing number of samples examined with culture is likely to result in decreasing sensitivity of the index test. It is also likely that Xpert performance varies substantially with different TTP and this may lead to differences between accuracy studies.

Another reason for variation between these studies might relate to the patients spectrum. Depending on the control efforts in countries, patients might present earlier or later in their disease. This might affect the bacillary load. While smear status, is one criteria by which the patient spectrum can be defined, the time to positivity (TTP) of culture is better proxy of bacterial load of the sputum inoculum (33). Not surprisingly, smear and Xpert positivity is

associated with TTP, with patients with lower TTP are more likely to be smear or Xpert positive. (36, 58, 59).

The effects of TTP and the number of reference tests have not been systematically evaluated. We examined a large dataset from 16 studies to estimated the impact of time to culture positivity and different reference standard definitions on Xpert sensitivity.

3.1.3 Methods

We analysed data from sixteen previous studies on the accuracy of Xpert conducted as part of the biorepository efforts of FIND. While the studies were performed under different protocols, the protocols were aligned in respect to the aspects critical to this analysis and had used Xpert and a common reference standard testing with liquid and solid cultures that was standardized across studies. Further details on the individual studies are provided in the *Appendix 4.*

Participants

Participants were aged 18 years or older and were presumptive TB patients presenting in eleven countries (Belarus, Cambodia, Georgia, India, Italy, Moldovia, Peru, South Africa, Vietnam and Zimbabwe). Participants were excluded from the analysis if they were below 18 years of age or had contaminated or missing culture or indeterminate or missing Xpert results of their initial sputum sample. Patients were enrolled between 13 June 2011 and 6 March 2018.

Test Methods

Index test

The Xpert result of the initial sample only was considered in the analysis. Xpert results were reported as detected, not detected or indeterminate. Indeterminate Xpert results were excluded from the analysis.

Reference standard

The TB case definitions were based on all four available culture results from two different sputum samples. MGIT liquid culture, and solid culture on Löwenstein-Jensen medium testing were performed on each of the two sputum specimens. The presence of *M.tb* complex in solid or liquid culture was confirmed with MPT64 antigen detection and/or MTBDRplus, MTBC and CM/AS line probe assays (Hain Lifesciences, Nehren, Germany).

Definite TB was defined if at least one culture was positive with confirmed *M.tb*. We explored Xpert sensitivity using different case definitions of culture positivity. Multiple MGIT and LJ were defined if results were available from the two sputum specimens cultured and single was based on the assumption of only one being available. For sensitivity of Xpert, we calculated sensitivity of Xpert using MGIT only or LJ only when two cultures were available for each as reference standards. Second, we assumed if only one culture (MGIT or LJ) was available as a reference standard and defined it that as single MGIT or LJ. We explored Xpert sensitivity using different case definitions of culture positivity based on reference standards.

Time to culture positivity was estimated for MGIT only and was determined as the difference in days between the date of inoculation to MGIT vial and date of positivity or until 42 days. Cultures with negative results after 42 days were considered negative. Contaminated cultures were excluded from the analysis.

Statistical analysis

Sensitivity was estimated as the proportion of patients testing positive by Xpert of those positive by the reference standard. Logistic regression was used to estimate sensitivity accounting for clustering by study using a random effect. We estimated the sensitivity of Xpert by TTP by grouping TTP into 5 day categories (≤ 5, >5≤10, >10≤15, >15≤20, >20≤25, >25≤30, >30≤35 and >35≤42 days). The sensitivity was plotted by category of time to positivity, with 95% confidence intervals. The sensitivity was estimated in three defined groups of culture

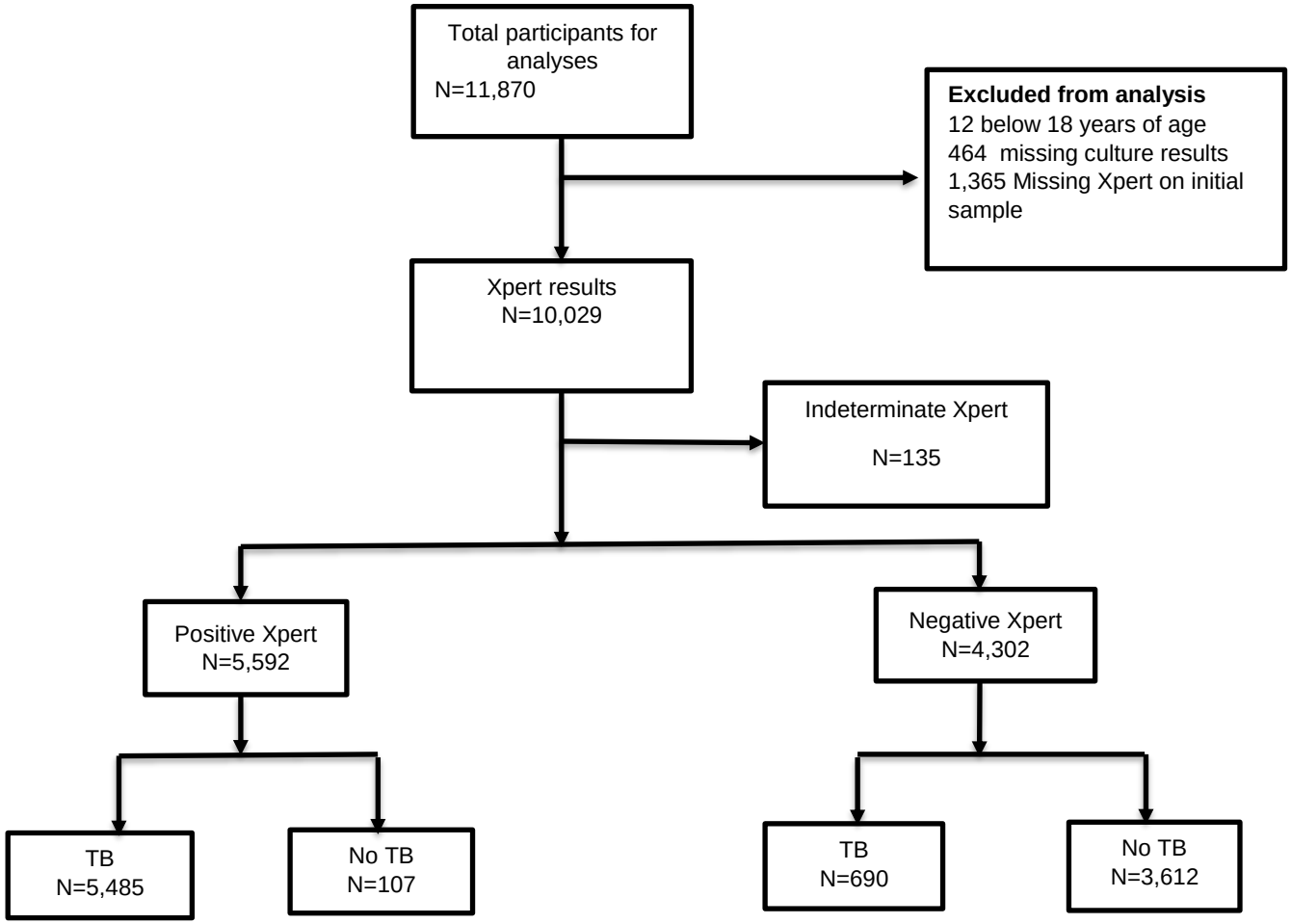
positive (all culture positive, smear positive-culture positive, smear negative-culture negative). Analyses were performed using Stata version 15 (stataCorp LLC, Texas, USA).

3.1.4 Results

There were 11,870 presumptive TB patients who were included in the 16 studies. We excluded 1,841 (15%) patients (1,389 did not have Xpert results on initial sputum sample, 464 were missing or had contaminated culture results and 12 who were below 18). A total of 9,894 had Xpert test results and were included in the final analysis. Of these 57% (5,592/9,894 had Xpert positive and TB was confirmed in 5,485 and 690 of Xpert positive and negative, respectively, ***Figure 3.*** **1** . Of the entire cohort, 62% (6,175/9,894) were confirmed to have TB with at least one positive culture result. The majority were newly TB diagnosed patients 83% (N=5,175). The largest number of participants came from studies in Peru, Vietnam and South Africa, **Table 3.** ***1***. Overall, 3,727 (37%) were female. The median age in the entire cohort was 35 years (IQR 26-49) with highest median age among patients in Vietnam 42 (30-54) and lowest in Peru 30 (23-45). HIV co-infection was common among patients in African countries (South Africa 60% (902/1,509) Zimbabwe 61% (51/102).

The overall sensitivity of Xpert was 88.1% (95% CI, 81.8 to 92.5) in all patients with at least one culture positive (6,175 participants). In smear-positive-culture positive participants, sensitivity was 98.6% (95% CI, 96.6 to 99.4) and in smear negative-culture-positive was 64.9% (95% CI, 50.0 to 77.5) **Table 3.** ***3***. Among patients who were co-infected with HIV, Xpert sensitivity was 86.7% (95% CI, 76.8 to 92.7) (686 participants). In HIV negative patients, the sensitivity was 89.7% (95% CI, 82.8 to 94.0) (4,798 participants).

Figure 3. 1: Flow diagram of included patients



Note:

TB-Tuberculosis
Xpert-Xpert MTB/RIF

Table 3. 1: Demographic clinical and diagnostic characteristics of the study participants

Demographic and clinical characteristics	Belarus N=92	Cambodia N=57	Georgia N=817	India N=431	Italy N=95	Moldovia N=718	Peru N=4083	South Africa N=1509	Vietnam N=1990	Zimbabwe N=102
Age	41 (29-56)	39 (28-65)	39 (28-54)	31 (23-47)	missing	38 (29-49)	30 (23-45)	35 (29-44)	42 (30-54)	38 (32-48)
HIV infection	2/92 (2.2%)	0[π]	6/817 (0.7%)	2/431 [α] (0.5%)	2/95[#] (2.1%)	32/718 (4.5%)	193/4083 (5%)	902/1509 (60%)	132/1990 (7%)	62/102 (61%)
Female sex	39/92 (42%)	33/57 (58%)	239/817 (29%)	179/431 (42%)	missing	183/718 (25.5%)	1756/4083 (43%)	697/1509 (46%)	550/1990 (28%)	51/102 (50%)
History of tuberculosis	18/92 (20%)	0	252/817 (31%)	125/431 (29%)	1/95 (1%)	231/718 (32%)	113/4083 (3%)	353/1509 (23%)	305/1990 (15%)	0
Diagnostic characteristics										
¥Prevalence	41/92 (45%)	36/57 (63%)	522/817 (64%)	297/431 (69%)	78/95 (82%)	650/718 (91%)	2378/4083 (58%)	694/1509 (46%)	1469/1990 (74%)	10/102 (9.8%)
S-C+	28/41 (68%)	10/36 (28%)	229/522 (44%)	93/303 (31%)	37/78 (47%)	251/650 (39%)	347/2378 (15%)	293/694 (42%)	299/1469 (20%)	7/10 (70%)
RIF resistance	13/41 (32%)	1/36 (3%)	423/522 (81%)	147/297 (49%)	9/78 (12%)	519/650 (81%)	373/2378 (16%)	301/694 (44%)	447/1469 (30%)	0

∞HIV test was not done in 98% in Brazil, α in India only 50% were tested for HIV, # in Italy 48% had unknown HIV status,π in Cambodia all tested negative

Two MGIT and LJ culutres were considered. ¥ At least one positive culture
MGIT-Mycobacterium Growith Indicator tuber for liquid culture
LJ-Lowestein Jensen for solid culture

Table 3. 2: Sensitivity of Xpert MTB/RIF by time to culture positivity

Categories for analysis	Pooled sensitivity of Xpert MTB/RIF using time to culture positivity as a reference standard (95% CI)							
	≤ 5 days (N=351)	> 5 ≤ 10 days (N=2231)	>10≤15 days (N=1885)	>15≤20days (N=808)	>20≤25days (N=296)	>25≤30days (N=119)	>30≤35days (N=75)	>35≤42days (N=45)
All culture positive	99.3% (95.0 to 100)	99.3% (98.4 to 99.7)	96.8% (94.0 to 98.3)	75.2% (60 to 86)	62% (47.8 to 74.5)	58.7% (34.5 to 79.2)	60% (42.8 to 75.1)	71.1% (56.3 to 82.4)
Smear positive-culture positive	99.6% (97.6 to 100)	99.6% (99.3 to 99.8)	99% (97 to 99.2)	95% (87 to 98)	91% (70 to 98)	75% (48 to 90)	87% (70 to 95)	92% (51 to 99)
Smear negative-culture positive	99% (62 to 100)	98% (80 to 100)	92% (83 to 96)	62% (43 to 77)	43% (31 to 56)	53% (20 to 83)	50% (35 to 65)	50% 30 to 70)

Sensitivity estimates and confidence interval (CI) were calculated allowing for random effect and clustering by site, only MGIT culture is considered.

Table 3. 3: Overall sensitivity performance of Xpert MTB/RIF test

Groups of analysis	Sensitivity	(95% CI; n/N)
All culture positive	88.1%	(81.8 to 92.5; 5485/6175)
Smear positive-culture positive	98.6%	(96.6 to 99.4; 4410/4563)
Smear negative-culture positive	64.9%	(50.0 to 77.5; 1062/1592)
HIV positive	86.7%	(76.8 to 92.7; 557/686)
HIV negative	89.7%	(82.8 to 94.0 ;4307/4798)

Sensitivity of Xpert MTB/RIF by time to culture positivity

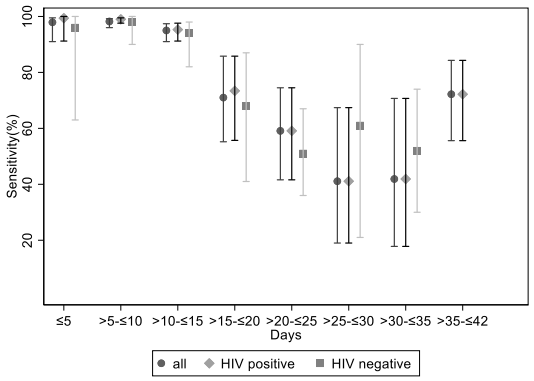
Generally, the sensitivity of Xpert decreased as time to culture positivity increased across categories of analysis of all culture, smear positive-culture positive and smear negative-culture positive ***Figure 3. 2***. The overall median time to culture positivity was 11 days (Interquartile range (IQR) 8-15). Of the 5,850 participants with positive MGIT culture 76% had TTP of equal or less than 15 days. In all culture positive patients, the sensitivity of Xpert in categories up to 15 days were: ≤ 5, 99.3%, (95% CI, 95-100, N=351), >5≤10, 99.3 % (95%CI, 98.4-99.7, N=2231) and >10≤15, 96.8% (95%CI,94-98.3, N=1,885). In smear negative-culture positive: ≤ 5, 99%, (95% CI, 62-100, N=37), >5≤10, 98% (95%CI, 80-100, N=237) and >10≤15, 92% (95%CI,83-96 ,N=413). In HIV positive: ≤ 5, 96% , (95% CI: 63-100, N=51) , >5≤10, 98% (95%CI, 90-100, N=199) and >10≤15, 94% (95%CI, 82-98 ,N=168). The sensitivity of Xpert in the first fifteen days was high in all three groups of analysis: all culture positive, smear positive-culture positive and smear negative-culture negative and irrespective of HIV status.

The sensitivity of Xpert in participants with a TTP of less than fifteen days was high in all three groups of analysis: all culture positive, smear positive-culture positive and smear negative-culture positive, **Table 3. *2***. This was also observed irrespective of HIV status ***Figure 3. 2***). There was a substantial drop in Xpert sensitivity for smear negative-culture positive patients

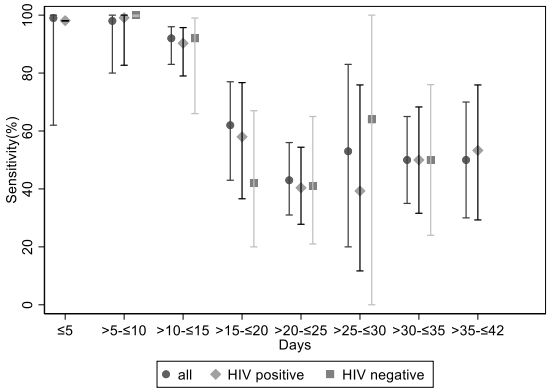
after 15 days of TTP. For smear positive patients, there was only a small drop in sensitivity by TTP **Table 3. *2***. A similar pattern was observed for both HIV positive and negative patients ***Figure 3. 2***. There was no substantial further reduction in the sensitivity of Xpert after 30 days to 42 days of TTP but numbers for analysis were low ,**Table 3. *2***.

Figure 3. 2: Variation in Xpert sensitivity performance

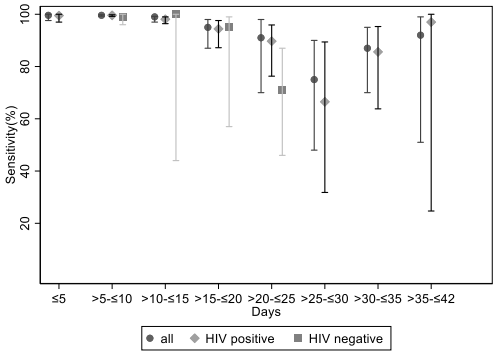
In all culture positive



In smear negative-culture positive



In all smear positive-culture positive



Sensitivity of Xpert by different case definitions

The overall sensitivity of Xpert was higher against a reference standard of either MGIT or LJ on a single sample compared to if multiple samples were used,**Table 3. *4***. The sensitivity of Xpert was highest against a single MGIT 93.4% (95% CI 87.3-96.6) in all culture positive patients. When only the smear negative culture positive participants when tested with a single MGIT were assessed, sensitivity was 78% (95% CI 58.9-89.8) .The sensitivity of Xpert was

91.4% (95% CI, 86.5-94.6) and 71.1% (95%CI,55.8-82.7) in all culture positive and smear negative -culture positive respectively against the use of multiple MGIT reference standards. The sensitivity of Xpert against single LJ reference standard were: 82.6% (95%CI, 64-92.7) in all culture positive and 68.4% (95%CI,55.1-79.3) in smear negative-culture positive. Against multiple LJ sensitivity of Xpert was 81.2%(95%CI,64.1-91-2) in all culture positive and 64.9% (95%CI,49.7-77.5) in smear negative-culture positive. Overall, the use of multiple LJ as the reference standard showed the lowest sensitivities.

Table 3. 4: Sensitivity performance of Xpert MTB/RIF using different tuberculosis case definitions

Reference standard	MGIT (N=9539)		LJ (N=7260)		All cultures (N=9894)
	Single	**Multiple**	**Single**	**Multiple**	
All culture positive	93.4% (87.3 to 96.6)	91.4% (86.5 to 94.6)	82.6% (64.0 to 92.7)	81.2% (64.1 to 91.2)	88.2% (81.8 to 92.5)
Smear positive-culture positive	98.4% (96.8 to 99.1)	98.5% (97.1 to 99.2)	98.7% (95.2 to 99.6)	98.4% (94.8 to 99.5)	98.6% (96.6 to 99.4)
Smear negative-culture positive	78% (58.9 to 89.8)	71.1% (55.8 to 82.7)	68.4% (55.1 to 79.3)	66.3% (54.2 to 76.6)	64.9% (49.7 to 77.5)

3.1.5 Discussion

We report the effect of the choice of TB definition and time to culture positivity on the performance of Xpert. Xpert sensitivity was consistently high in both smear positive and negative within 15 days of TTP, and decreased as TTP increased. Xpert sensitivity was also lower among smear negative-culture positive patients. We observed high sensitivity of Xpert when MGIT culture was used compared to LJ.

We observed varying performance of Xpert within different categories of TTP. It appears that the overall summarized sensitivity of Xpert corresponds with sensitivity of Xpert within 15 days of TTP and that; there is little benefit in terms of Xpert sensitivity after 20 days of TTP.

A longer TTP corresponds to low bacterial load and smear grading and studies have shown low sensitivity of Xpert in low bacterial load (58, 60) consistent with our findings. Low bacterial load is associated with smear negative culture positive results. In this secondary data analysis, we did not assess bacterial load based on cycle threshold (ct) values of Xpert to accurately estimate bacterial load at each category due to limitation of data availability. CT values are inversely proportional to the number of bacterial load. However, low bacterial load at this TTP remains a reasonable argument. We suspect that TTP beyond 35 days could be corresponding to the limit of detection of Xpert. However, this remains to be demonstrated in future studies.

Studies have reported low sensitivity of Xpert in smear negative-culture positive compared to smear positive-culture positive TB (54). Among smear negative- culture positive patients, Xpert sensitivity substantially decreased after 15 days among HIV-negative patients. This is consistent with previous studies on Xpert performance among smear negative-culture positive patients as shown in a Cochrane review (25). Imperfect smear microscopy performance among patients with low bacterial load could explain the drop in Xpert sensitivity in this group. The confidence intervals for sensitivity of Xpert in smear negative-culture positive among HIV negative were so wide, not to warrant any confident conclusion.

Xpert sensitivity dropped as TTP increased and its sensitivity beyond 20 days of TTP is comparable to that of smear microscopy and that at longer TTP Xpert sensitivity may be limited. This is relevant in sub-clinical TB.

The use of a single culture in either MGIT or LJ showed better performance of Xpert compared to use of multiple cultures. Generally, we observed MGIT sensitivity outperforming LJ, consistent with previous finding (57, 61, 62). The downside of MGIT higher sensitivity

compared to LJ is the higher cost of performing MGIT. In the base-case scenario, MGIT would require four more USD dollars per each culture done (63).

Our study benefits from a large dataset with patients from different epidemiological settings and where sample processing and testing were standards across all study sites. A limitation was the lack of precise bacterial load counts in the dataset. Such an analysis would have given a precise explanation to the drop of sensitivity of Xpert in longer TTP in relation to the limit of detection of Xpert.

We have demonstrated an effect of TTP on the accuracy of Xpert. This implies that studies assessing performance of diagnostic tests should also report and take into account TTP in order to understand differences in the performance of tests between study groups within one study or between different studies. In terms of clinical care, our findings underscore the need for clinicians to be vigilant in the diagnosis of patients with sub-clinical disease when using Xpert.

Ending TB will need diagnostics with good performance in those with clinical as well as those with sub-clinical disease burden. As efforts to control TB increase, a change in TB epidemiology will demand for diagnostics with good performance, in those with sub-clinical disease consistent with low bacterial load. WHO has recommended the newer version of Xpert known as Xpert MTB/RIF ultra (Ultra) with improved sensitivity compared to Xpert particularly in patients with low bacterial load (24, 54). However, this will need to be weighed against the loss in specificity with Ultra (24, 64).

In conclusion, Xpert sensitivity decreases with low bacterial load such as smear negative patients or those with longer time to culture positivity. Future evaluation studies on performance of diagnostic should take into account TTP and report these alongside test performance of diagnostics. A need to optimize existing tools such as Ultra for better accuracy and develop new diagnostics with better performance in those with low bacterial load remains relevant. Xpert sensitivity varied across different case definitions of reference standard and

that the use of single MGIT showed high sensitivity. More evidence is needed to establish whether the use of single MGIT for TB case definition is sufficient for accuracy assessment of diagnostics for TB.

Authors' contribution

FH CD KR AR and SS conceptualized the study and developed analysis plan

FH and AR analysed data and wrote the first draft

FH SGS AR AM SG KR and CD reviewed the final draft

Acknowledgement

We would like to acknowledge the support received from the data team at FIND and IHI (Ramadhani Abdul) during data extraction and data management

Conflict of interest

All authors had no conflict of interest to declare

4 Chapter 4

4.1 The effect of a previous history of tuberculosis on the specificity of Xpert MTB/RIF

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Manuscript accepted to the European Respiratory Journal

To the editor

Tuberculosis (TB) is the leading cause of mortality among infectious diseases. In 2018, 1.5 million people died due to TB (9). Early diagnosis of TB is key in achieving WHO End TB targets and molecular diagnostic tests have been developed. However, molecular tests cannot differentiate between viable and non-viable bacteria (33, 34), leading to challenges in the interpretation of positive test results in patients with recent TB.

Xpert MTB/RIF ('Xpert', Cepheid, Sunnyvale, USA), a widely used molecular diagnostic, amplifies sequences of the rpoB gene specific to *Mycobacterium tuberculosis* complex bacteria (16, 17). Xpert has a high specificity estimated as 98% (95%CI 97 to 99) in a meta-analysis (25). Growing evidence from case reports or small studies and one larger study by Theron *et al*. suggests a reduced Xpert specificity among patients with TB history (33, 39, 65, 66). The rate of Xpert false positives are higher the more recent the history of TB (67). This poses a clinical dilemma: while patients with recent TB have a high risk of reinfection or relapse, they may also have a high risk of a false-positive result (33, 66, 67).

To inform policy and clinical practice, we assessed the effect of TB history on the specificity of Xpert by time since the previous TB episode in settings with different levels of HIV-co-infection (68) in sixteen studies collated by FIND (Foundation of Innovative New Diagnostics, Geneva, Switzerland). While studies were carried out under different protocols, all used Xpert and a common reference standard with liquid and solid cultures that was standardized across studies.

We included all presumptive patients 18 years or older with Xpert and cultures performed. Patients were recruited in 10 countries (Belarus, Cambodia, Georgia, India, Italy, Moldovia, Peru, South Africa, Vietnam and Zimbabwe) between 9 June 2011 and 6 March 2018. We considered Xpert results for the first sample only. Patients with missing Xpert results were excluded from the analysis.

The reference standard was based on Lowenstein Jensen (LJ) and Mycobacterium Growth Indicator Tube (Bactec MGIT; BD Microbiology Systems, Cockeysville, MD, USA). Chest radiographs were considered compatible with TB, if the attending clinician rated the x-ray at least as likely for TB. We estimated the specificity of Xpert as the proportion of Xpert negative out of the culture negative patients. Time since previous TB treatment was calculated as the difference in dates between enrolment and the previous treatment completion. We estimated the specificity of Xpert using logistic regression with a random effect to account for clustering by study. All analyses were performed using Stata version 15 (stataCorp LLC, Texas, USA).
A total of 11,583 patients had Xpert and culture results. We excluded 1,627 (1,615 missing Xpert results, 12 below 18 years old), leaving 10,053 patients for the analysis. Of these, 59% (5,900/10,053) had a positive culture and 55% were positive on Xpert. Overall 16% (1,630/10,053) of patients had a history of TB. The median time since previous TB treatment was 3 years (Interquartile range (IQR), 0.0-6). Patients with TB history tended to be older (median age 39 [IQR 31-48] vs 35 [IQR 26-49], HIV positive and resistant to rifampicin.

False positive Xpert results were observed in 6.5% (95% CI 2.8 -13.7) of patients with TB history and 2% (95% CI 0.6-5.6) without a history of TB. In patients without previous TB, specificity was estimated as 98.0% (95% CI; 96.0-99.7; 3095/3164) while in those with it was 93.5% (95% CI; 80.4-98.1; 465/501). Among the 803 participants with a TB episode within two years of testing, the specificity of Xpert was 92.2% (95% CI 81-97). The specificity increased with time since previous TB, ***This study represents*** the largest dataset to date to assess the effect of TB history on the specificity of Xpert for TB. The specificity of Xpert was lower in those with TB history compared to those without it and increased as time since previous TB increased. In patients with TB history within two years, specificity was reduced. False positive was associated with negative chest X-rays even when adjusted for TB history and HIV, consistent with previous findings (69). This suggests that a negative chest X-ray could aid in deciding whether to start a patient with a positive Xpert result and recent TB history on therapy. This might be a viable strategy in patients without HIV. In HIV patients, the risk of not treating

a patient who may truly have TB might outweigh the risk of overtreatment. These results only provide an association and not causation. However, the decreased specificity in patients with recent TB history is coupled with increased sensitivity, as shown with Xpert MTB/RIF Ultra (67), which supports the causal link. A causal link is further supported by an association between false positive results and decreasing bacterial load in those with TB history. High Cycle threshold values (Ct) predict false Xpert positivity however, discriminatory power of Ct values in predicting false positive remain minimal (39). A study showed patients with false positive Xpert remained healthy and asymptomatic after 17 months of follow up (69). However, PET-CT showed positivity consistent with active disease at the end of therapy (33, 66), suggesting Xpert false positivity might not stem only from non-viable bacteria alone but could also be related to viable bacteria still present after a patient is clinically cured.

A limitation of our study was that Ct values for Xpert were not reported and that clinical outcome data were not available. Study strengths include the size of the dataset with a common reference standard. Data from different settings suggest likely generalizability. The overall specificity of Xpert in our study of 98% is identical with the pooled specificity reported in a recent Cochrane review including 95 studies (25).

Our results may have implications for the design of diagnostic algorithms in patients with TB history, particularly if the prior episode was within two years. Consideration of other conditions and careful clinical decision before treatment initiation in this group is important. The impact of false positive results on patient outcomes remain a relevant question for future studies and modelling studies, such as the one by Kendall *et al* for Xpert Ultra (70), should weigh the impact of likely overtreatment among patients with previous TB history in different epidemiological settings. Importantly, while our findings pertain directly to Xpert, similar results should be expected with other molecular TB assays. In conclusion, Xpert specificity is reduced among patients with previous TB history, and this reduction is greater among those with TB in the last two years.

Acknowledgement

We acknowledge all study teams, FIND data team and Ramadhani Abdul a statistician at Ifakara Health Institute for their support during data extraction and management

Figure 4. 1. Between two and five years (373 participants), the specificity was 99.0% (95% CI; 86-100) and above five years (454 participants), 98.6% (95% CI; 85.4-99.8). The rate of false positivity for TB detection was similar in both groups in patients with rifampicin resistance regardless of TB history. Of 247 patients with false positive results, 109 had chest X-ray results of which 75% were compatible with active TB. Patients with a non-compatible X-ray were more likely to have a false positive Xpert result with an odds ratio of 4.9 (95% CI; 2-11) adjusting for TB history, HIV, age and sex.

This study represents the largest dataset to date to assess the effect of TB history on the specificity of Xpert for TB. The specificity of Xpert was lower in those with TB history compared to those without it and increased as time since previous TB increased. In patients with TB history within two years, specificity was reduced. False positive was associated with negative chest X-rays even when adjusted for TB history and HIV, consistent with previous findings (69). This suggests that a negative chest X-ray could aid in deciding whether to start a patient with a positive Xpert result and recent TB history on therapy. This might be a viable strategy in patients without HIV. In HIV patients, the risk of not treating a patient who may truly have TB might outweigh the risk of overtreatment. These results only provide an association and not causation. However, the decreased specificity in patients with recent TB history is coupled with increased sensitivity, as shown with Xpert MTB/RIF Ultra (67), which supports the causal link. A causal link is further supported by an association between false positive results and decreasing bacterial load in those with TB history. High Cycle threshold values (Ct) predict false Xpert positivity however, discriminatory power of Ct values in predicting false positive remain minimal (39). A study showed patients with false positive Xpert remained healthy and asymptomatic after 17 months of follow up (69). However, PET-CT showed positivity

consistent with active disease at the end of therapy (33, 66), suggesting Xpert false positivity might not stem only from non-viable bacteria alone but could also be related to viable bacteria still present after a patient is clinically cured.

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Acknowledgement

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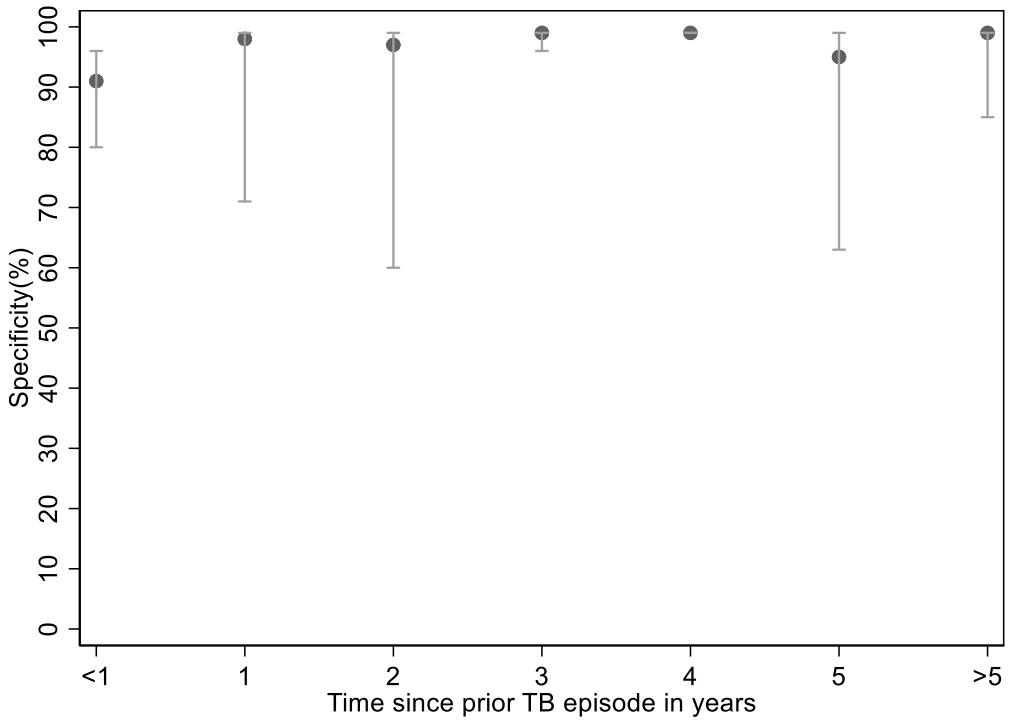
Figure 4. 1: Xpert MTB/RIF specificity by time since previous tuberculosis episode



Black circles: Specificity estimated using logistic regression with a random effect to account for clustering by study
Error bars: 95% confidence intervals
X-axis: Time period (in years)

5 Chapter 5

5.1 Impact of diagnostic test Xpert MTB/RIF® on health outcomes for tuberculosis

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Cochrane Database of Systematic Reviews 2018, Issue 2. Art. No.:CD012972. DOI: 10.1002/14651858.CD012972

Under review

5.2 Abstract

Background

Xpert is a diagnostic test for tuberculosis (TB) with a higher sensitivity than the standard test, smear microscopy. Xpert has been recommended by the World Health Organization and has been adopted by many countries in diagnostic algorithms. However, it is not known whether a test with greater accuracy would translate into improved outcomes for patients.

Objectives

To assess the impact of Xpert on important patient outcomes for people being investigated for tuberculosis. Our primary outcomes were; (i) all-cause mortality during trial follow-up from time of first contact with health care, (ii) proportion of tuberculosis cases reported, and number of drug-sensitive and drug-resistant tuberculosis cases (iii) proportion of patients treated (iv)

proportion of treated patients who were microbiologically confirmed (v) proportion of treated patients who were not microbiologically confirmed.

Search methods

We searched for publications without language restriction in the following databases: Cochrane Infectious Disease Group (CIDG) Specialized Register; Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE OVID; Embase OVID; CINAHL EBSCO; LILACS (Latin American and Caribbean Health Science Information database; BIREME); Science Citation Index Expanded (Web of Science), Social Sciences citation index (Web of Science), and Conference Proceedings Citation Index - Social Science & Humanities (Web of Science). We also searched the WHO International Clinical Trials Registry Platform, ClinicalTrials.gov, and the Pan African Clinical Trials Registry for ongoing trials.

Selection criteria

We included cluster-randomized trials, individually randomized controlled trials (RCTs), and quasi-experimental trials (pre-/post-implementation). We analyzed the randomized and non-randomized studies separately.

Data collection and analysis

For each study, two review authors independently extracted data using a piloted data extraction tool. We assessed the quality of studies using the Cochrane risk of bias and Effective Practice and Organization of Care (EPOC) tools and checked for evidence of publication bias. We used random effect meta-analysis to allow for heterogeneity between studies in setting and design.

Main results

We included ten studies, seven of which were randomized controlled trials and three pre-post intervention studies. In all domains of the Cochrane risk of bias tool, most randomized studies had a low risk of bias.

There was evidence of a positive effect of Xpert on tuberculosis confirmation in treated patients (RR 1.29 95%CI 1.11, 1.51 in the randomized trials) and reduction in pre-treatment loss to follow-up (RR 0.59 95% CI 0.42-0.84). Overall there was a reduction on all-cause mortality of 12% (RR 0.88 (95% CI 0.73, 1.05) and 24% (RR 0.76 95% CI 0.58-1.00) among HIV positive participants. There was evidence that Xpert lead to an increase in the overall proportion of patients treated for TB (RR 1.10 95%CI 0.98, 1.23), the likelihood of being cured (OR 1.09 95% CI 1.02, 1.16), and that the proportion of those treated who were not microbiologically confirmed was reduced (RR 0.59 95%CI 0.41 0.85).

Authors' conclusions

We found that, compared with smear microscopy, our estimate suggests that Xpert reduces all-cause mortality by 12% although uncertainty around the effect estimate was high and the data is also compatible with reduction of up to 27% and an increase up to 5%. The mechanisms by which Xpert could affect mortality is likely at least in part related to the reduction in pre-treatment loss to follow-up as well as increase in the proportion of patients cured. Further studies should assess the role of empirical treatment on the impact of Xpert on patient outcomes.

5.3 Plain language summary

Xpert for improving patient-important outcomes

Xpert is a test that has been recently introduced to improve the diagnosis of tuberculosis. It is more accurate than the standard test, smear microscopy. However, it is not known if the improved accuracy translates into improvements for patients in time to diagnosis, treatment initiation, cure and mortality. Our aim was to assess the benefits of using Xpert focusing on patient outcomes. We included studies, which reported patient outcomes in those diagnosed

using Xpert and smear microscopy.

What results say

We included ten studies that were conducted in low and middle-income countries and had low risks of bias.

Key findings

Compared to smear microscopy, Xpert had an estimated 10% increase in the risk of a patient being initiated on treatment, (95%CI; 2% less to 23% increase), a 29% (11%, 51%) increase in the chance of a treated patient being microbiologically confirmed, and an estimated reduction in the loss of patients before treatment initiation of 41% (16% to 58% reduction). Xpert increased chances of being cured by 9% (2%, 16%). There was an estimated 12% reduction (27% reduction to 5% increase) in the risk of death for Xpert compared to smear microscopy. Among HIV positive, the reduction in risk of death was 24% (42% reduction to no reduction. We could not confidently conclude whether or not Xpert has a small beneficial effect on mortality.

There is evidence that Xpert has a beneficial effect on some patient outcomes. Xpert reduces pre-treatment loss to follow up, increase in tuberculosis confirmation in those treated, and may lead to reduction in all-cause mortality. However, the range where the actual effect may be indicates that Xpert may make little increase in mortality.

5.4 Background

5.4.1 Description of the condition

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*, an obligate aerobe bacilli that belongs to the *Mycobacteria tuberculosis* complex (MTBC) (71). Transmission of tuberculosis most commonly occurs through the inhalation of droplets containing bacilli from a person with pulmonary TB who has coughed or sneezed. It is estimated that 1.7 billion people are infected by *M. tuberculosis* globally without having TB disease, and that about 5% to 15% will develop

TB disease (9, 72). The probability of developing TB is higher in immunocompromised individuals and among those infected with HIV (9). TB primarily affects the lungs (pulmonary tuberculosis); however, the disease can involve virtually any extra pulmonary sites in the human body.

In 2018, there were 10 million new TB cases globally and people living with HIV accounted for 8.6% of these (9). In the same year, TB was associated with 1.2 million deaths and a further 251,000 deaths from TB disease among people living with HIV (9). The proportion of TB cases living with HIV was highest in the World Health Organization (WHO) African Region, accounting for 87% of all TB-HIV co infected cases notified (9).

Both the emergence and under-reporting of drug resistance to antimicrobials used to treat TB remain major problems. In 2018, it was estimated that half a million people developed TB that was resistant to rifampicin (RR-TB) or multi drug resistant TB (MDR-TB), and only a third of these received appropriate treatment (9). MDR-TB diagnosis depends on culture infrastructures or line probe assays which are both expensive and not widely available at point of care (9, 73)

Commonly used diagnostic techniques for TB have limitations. Culture, which is the gold standard for diagnosis, is normally centrally located, requires a set of bio containment precautions and takes up to six or eight weeks for liquid and solid culture respectively before results can be obtained (74). For many years, sputum smear microscopy has been the method used to diagnose bacteriologically confirmed tuberculosis, particularly in low- and middle-income countries (LMICs) (74, 75), and remains the main diagnostic technique in primary healthcare facilities in LMICs. However, the sensitivity of smear microscopy is limited, ranging from 20% to 80% (75, 76), and sensitivity is further reduced in HIV-seropositive individuals (74). Other limitations of smear microscopy are that it is labour intensive, dependent on individual skills and experience, and unable to detect drug resistance (75). Consequently, there is a need for early, accurate and affordable diagnosis of tuberculosis, including universal drug susceptibility testing for all individuals being evaluated for tuberculosis (77).

5.4.2 Description of the intervention

New molecular diagnostic tools have been developed to improve TB detection and decentralize drug resistance testing. The Xpert assay is an automated nucleic-acid amplification test. It consists of a single-use multi chambered cartridge preloaded with the liquid buffers and lyophilized reagent beads required for sample processing, DNA extraction, and hemi-nested real-time polymerase chain reaction. The assay can be used with sputum samples and also, with varying sensitivity, with others specimens including cerebrospinal fluid, lymph node tissue or aspirates, pleural fluid, ascetic fluid, urine, dialysis fluid, and pus (78, 79). The assay can be performed at peripheral laboratories or health facilities without biosafety cabinets, and minimal training for laboratory staff is required (18, 19). Xpert can detect MTBC and rifampicin resistance within two hours (17).

A Cochrane Review update on the accuracy of Xpert for the detection of TB estimated the pooled sensitivity of the assay to be 85% and the specificity to be 98% (25). When compared with smear microscopy, Xpert sensitivity in smear-negative, culture-positive individuals was 67% (25). Sensitivity was estimated to be lower in people with HIV infection (81%) than in those without HIV infection (88%); additionally, the Xpert assay had a pooled sensitivity of 96% and specificity of 98% for the detection of rifampicin resistance (25).

In 2010, the WHO released a policy statement endorsing the Xpert assay and strongly recommending it as the initial diagnostic test for individuals with suspected MDR-TB and those with HIV. Conditional recommendations based on resource availability were made with regard to its use as a follow-on test for smear-negative individuals. Further recommendations for use in individuals with extra-pulmonary and paediatric TB were made in 2013 following new supporting evidence (80, 81). By the end of 2016, a total of 6,659 GeneXpert instruments and more than 23 million Xpert cartridges had been procured in the public sector among 130 LMICs under concessional pricing (52).

There have been practical problems with the introduction of Xpert: high rates of modular failure

linked to an interrupted power supply were observed among early Xpert adopters in LMICs (82). The full impact of Xpert depends partly on a stable power supply or alternative reliable sources of energy such as batteries or solar energy (27). A growing number of studies have investigated whether the increased sensitivity of Xpert translates into an impact on patient-important outcomes, such as time to treatment, morbidity, and mortality. These are the focus of this review.

Recently, new developments aimed at improving the sensitivity of Xpert have been made by introducing Xpert MTB/RIF ultra (Ultra). Ultra incorporates two new multi copy amplification targets, known as IS6110 and IS1081, and a larger amplification chamber. In a non-inferiority multicenter trial, the sensitivity of Ultra was 5% higher than that of Xpert (95% confidence interval (CI) +2.7%, +7.8%), but the specificity was 3.2% lower (95% CI 4.7% to 2.1%) (83). Ultimately, a Technical Expert Consultation Group convened by the Global TB Programme of WHO concluded that Ultra is non-inferior to Xpert in terms of the detection of both M. tuberculosis and rifampicin resistance (84). We will not focus on Ultra in this review. However, if we find that Xpert impacts patient-important outcomes, then it is likely that the Ultra will also have an impact.

5.4.3 How the intervention might work

If Xpert has a superior diagnostic accuracy compared with smear microscopy, the ability to detect resistance against rifampicin, near-patient utility and a fast turn-around time. It is expected that these features will have a positive impact on patient-important outcomes through beneficial diagnostic and therapeutic effects (85). The use of the assay may translate into a decrease in pre-diagnostic and pre-treatment loss to follow-up as well as reducing time to treatment. Pre-treatment loss to follow-up is associated with high mortality rates (86) and thus reduction in pre-treatment loss to follow-up may be expected to lead to reduction in mortality. Further, a larger proportion of true TB cases may receive effective therapy and fewer individuals may be falsely diagnosed with the disease on clinical grounds alone and incorrectly

treated empirically. Rapid detection of rifampicin resistance may lead to more rapid effective treatment initiation. These factors could improve important patient outcomes, such as treatment success, morbidity, mortality, and quality of life, and potentially have an impact on onward transmission.

5.4.4 Why it is important to do this review

Recent evidence from pragmatic trials in programmatic settings yielded largely inconclusive results for the impact of Xpert on different patient-important outcomes due to the limited size of individual studies and the large sample size required to detect (or rule out) effects of test-treatment interventions (87, 88). Some studies suggested limited benefits of on loss to follow-up and mortality (89, 90), whereas other studies have found that Xpert reduced time to treatment initiation, particularly among HIV-positive individuals (44, 91). A recent narrative review by (92) reported a limited impact on morbidity and mortality; however, the review included studies that demonstrated an impact of Xpert on increasing the diagnostic yield of bacteriologically confirmed TB and reducing the time to treatment initiation.

A recent individual-patient data meta-analysis including 8,567 patients, found that Xpert reduced the odds of death by 12% (odds ratio 0·88, 95% CI 0·68–1·14) (48). This analysis summarized the results of five of the seven RCTs conducted to date (93). A very large number of participants are needed to conclusively demonstrate the presence or absence of an effect of meaningful size on mortality (87, 88, 93), and to date no single published trial has been sufficiently powered to obtain a conclusive result. This review will help elucidate the impact of this diagnostic test (and other similar tests in future), and inform the further allocation of resources in LMICs.

5.5 Objectives

To assess the impact of Xpert on important patient outcomes for people with TB.

5.5.1 Types of studies

We include cluster- and individually- randomized controlled trials, and quasi-experimental trials (pre-/post implementation). We performed separate meta-analyses for randomized and non-randomized studies.

5.5.2 Types of participants

Individuals with suspected TB presenting with one or more symptoms of the disease and able to provide a sputum sample.

5.5.3 Types of interventions Intervention

Diagnostic strategies that use Xpert.

Control

Diagnostic strategies that use smear microscopy.

Types of outcome measures

Primary outcomes

- All-cause mortality during trial follow-up by time from first contact with health care
- Proportion of TB cases reported, and number of drug-sensitive and drug-resistant tuberculosis cases
- Proportion of patients treated
- Proportion of treated patients who are microbiologically confirmed
- Proportion of treated patients who are not microbiologically confirmed
- Proportion cured (we defined in those cured and those completing treatment without evidence of failure)

Secondary outcomes

- Time from first contact to initiation of treatment
- Proportion of study participants with pre-treatment loss to follow up
- Proportion of study participants who were diagnosed with or treated for MDR/TB
- Number of visits prior to diagnosis

- Patient reported satisfaction

5.5.4 Search methods for identification of studies

We identified all potential trials regardless of language or publication status (published, unpublished, in press, and in progress).

Electronic searches

We searched the following databases using the search terms detailed in page 101 Appendix 3, from 2007 to 27 February 2018 and updated our search from 2017 to 31 July2019: Cochrane Infectious Disease Group (CIDG) Specialized Register; Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE OVID; Embase OVID; CINAHL EBSCO; LILACS (Latin American and Caribbean Health Science Information database; BIREME); Science Citation Index Expanded (Web of Science), Social Sciences citation index (Web of Science),and Conference Proceedings Citation Index - Social Science & Humanities (Web of Science). We also searched the WHO International Clinical Trials Registry Platform (www.who.int/ictrp/search/en/), ClinicalTrials.gov (clinicaltrials.gov/), and the Pan African Clinical Trials Registry (www.pactr.org/) to identify ongoing trials using (tuberculosis OR TB) AND (Xpert or GeneXpert or "sputum microbiology" or "sputum microscopy") as search terms.

Searching other resources

Conference proceedings

We searched the past two years' proceedings of the International Union against Tuberculosis and Lung disease (UNION) conference, the Conference on Retroviruses and Opportunistic Infections (CROI), and the International AIDS Conference (IAS). We reviewed reference lists of all included studies and relevant systematic reviews. We contacted leading researchers at the Foundation for Innovative New Diagnostics (FIND), the WHO, Centers for Disease Control and Prevention (CDC), and TB-REACH to identify unpublished data as appropriate.

Data collection and analysis

Selection of studies

Two review authors (FH and MK) independently screened studies for eligibility after the literature search, and coded studies as either 'potentially include' or 'exclude'. Based on the screening results, we assessed the full-text articles of studies in the 'potentially include' category for eligibility using an eligibility assessment form. Differences in opinion were resolved through discussion. Study authors were contacted whenever clarification was needed. Multiple publications of the same study were included only once of the same trial: we included the publication including the largest sample size for the outcomes assessed and the most detailed information. For the additional analysis on treatment success, which was done following a request by the WHO guideline development group (GDG), we included another publication (90) of the trial in Brazil by Trajman A *et al* (94). All excluded studies and reasons for exclusion are reported in the flow diagram.

Data extraction and management

Two review authors (FH and MK) independently extracted data using a piloted data extraction tool similar to a tool previously used by our group (85). We resolved disagreements through discussion or by consulting a third review author (AR). We extracted the following data: study details (first author, year of publication), participant details, intervention, control, outcome measured and how it was measured, covariates, length of follow-up, and measure of effect with 95% confidence intervals. For binary outcomes, we extracted the relative risk or odds ratio if available. For time-to-event outcomes, we extracted the log hazard ratio with standard error or confidence interval.

In addition, we recorded the number of participants and clusters randomized to each diagnostic arm, and the number of participants monitored for each outcome of interest and the number of events in each diagnostic arm. For cluster RCTs that were adjusted for clustering, we extracted the adjusted measures of effect for each outcome and method of adjustment. In studies that

are not adjusted for clustering, we extracted the number of clusters randomized or the mean cluster size and the intra class correlation coefficient (ICC), if available. We also extracted data relevant for the assessment of the risk of bias.

5.5.5 Assessment of risk of bias in included studies

Two review authors (FH and MK) independently assessed the risk of bias using the Cochrane 'Risk of bias' assessment tool for assessing risk of bias in randomized studies (95), and other tools for assessing risk of bias in non-randomized studies (EPOC resources for reviewers 2018: Schumacher SG 2016;). We contacted the corresponding study author for clarification or more information if data were missing or the procedures were unclear. We resolved all discrepancies through discussion or by consulting a third review author (AR). We assessed the risk of bias as low, high, or unclear.

We assessed the included studies for the method of allocation sequence generation and allocation concealment (adequate, inadequate, not done, or unclear (as defined by Juni P *et al* (96), blinding (describing who was blinded, noting that the outcome assessors, clinicians, and participants cannot be blinded); completeness of information about follow-up (proportion of those presenting for care who were treated, who completed treatment, and who were lost to follow-up), outcome reporting, and selective reporting bias. For cluster-randomized trials, we considered additional criteria, such as baseline imbalance and incorrect analysis.

Measures of treatment effect

For the outcomes that assess proportions, we present the impact of Xpert using risk ratios if available with their 95% confidence intervals. We aimed to present the impact of Xpert on time to treatment using hazard ratios, if available. We used the adjusted estimates presented by the authors unless the covariates used would affect comparability.

Unit of analysis issues

We carried out the intention-to-treat (ITT) analysis based on the intervention groups. For clustered studies that did not consider the cluster design in the analysis, we adjust the

estimated variance for clustering using the ICC before including estimates in the meta-analysis. If the ICC was not available, we aimed to use an ICC from another, similar study. If this was not possible, we include the study, but note that adjustment was not possible.

Dealing with missing data

We determined the reasons for missing data before extracting data from the studies by attempting to contact the respective corresponding study author.

Assessment of heterogeneity

We examined heterogeneity in the intervention effect between studies using a forest plot. We calculate the I^2 statistic (the proportion of variance in the meta-analysis that is attributable to study heterogeneity).

Assessment of reporting biases

We assessed reporting biases using funnel plots. We checked the funnel plots for symmetry or asymmetry. In case of asymmetry, we used the recommended test for funnel plot asymmetry (97).

Data synthesis

We conducted analyses using Review Manager 5 (RevMan 5) (98). We used random-effects model as the intervention effect was expected to vary between studies due to the participant mix, settings, and aspects of study design and health system factors.

Subgroup analysis and investigation of heterogeneity

We performed subgroup analyses in the HIV-infected patients. We conducted an additional subgroup analysis, which was not in the protocol by analyzing mortality restricting to studies, which reported mortality at six months. This analysis helped to estimate the effect of Xpert at the completion of TB treatment. We wished to performed sub-group analysis in children, adults and HIV uninfected patients but sufficient data was not available. Drug sensitive and resistant TB could not be analyzed since data was not available in both arms of the studies.

Sensitivity analysis

We did not perform any planned sensitivity analyses since the few unexpected circumstances we anticipated were not observed:

missing data that are likely to influence the outcome;

excluding studies with outliers that are suspected to influence the outcome;

Excluding studies with high risk of bias that are likely to affect the outcome.

Certainty of the evidence

We assessed the certainty of evidence using the GRADE approach (99, 100), and GRADEpro GDT software (101). We rated each important outcome as described by Balshem *et al* (102)

- High: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate: we are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect.
- Low: our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect.
- Very low: we have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect.

RCTs start as high quality evidence but could be downgraded if there are valid reasons within the following five categories: risk of bias, imprecision, inconsistency, indirectness, and publication bias. Studies could also be upgraded if there is a large effect, and if all plausible residual confounding would reduce a demonstrated effect or would suggest a spurious effect if no effect was observed (102).

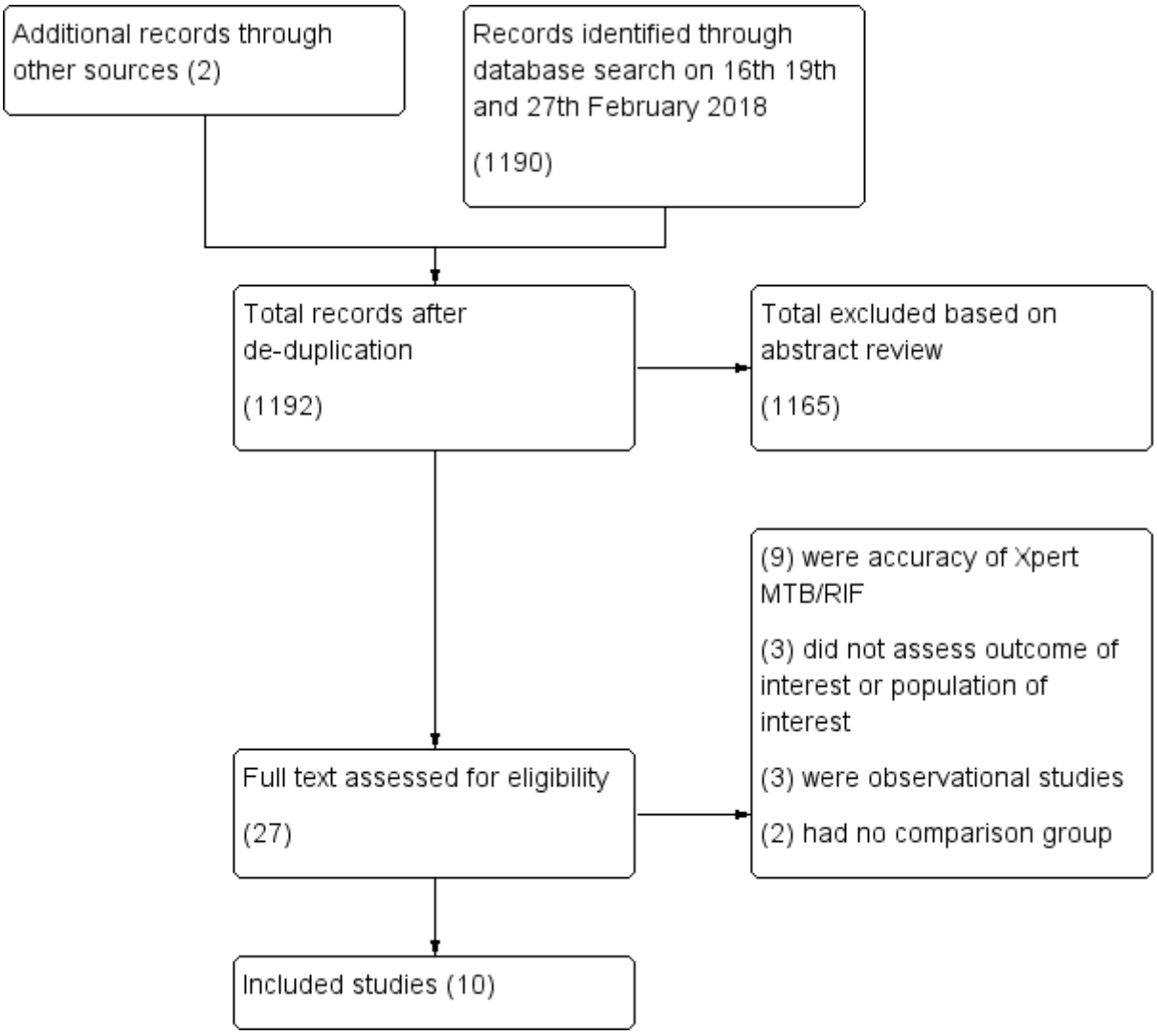
5.6 Results

5.6.1 Description of studies

Results of the search

Our search found 1,192 records, 1,190 from the databases search after duplicates were removed and two from additional sources. We excluded 1,165 records after screening the abstracts, the remaining 27 records were fully assessed for the eligibility criteria. Out of the 27 records, nine were studies on the accuracy of Xpert, three did not have outcome or population of interest, three were observational studies and two had no comparison group, with the remaining ten studies were eligible for inclusion, ***Figure 5. 1*** . Lessells RJ *et al* (47) assessed the impact of Xpert on patient-important outcomes, however this study was excluded because the comparison arm did not use smear microscopy.

Figure 5. 1: Flow diagram of included studies



5.6.1.1 Included studies

Designs

We included three individually randomized (44, 103, 104) and four cluster-randomized trials (45, 89, 91, 94), and three pre-and intervention period observational studies (49, 105, 106). Three cluster-randomized trials (45, 89, 91) used a parallel and one (94) a stepped-wedge design. The unit of the randomization for the cluster-randomized trials was primary care facilities served by a single laboratory (89, 94), HIV clinics (45) or calendar week in a single large primary care TB clinic (91). The length of follow up varied indifferent studies; two months (106) three months (103, 104), six months (44, 89, 91, 105) and 12 months (45).

Settings

Most of the studies were conducted in sub-Saharan Africa. There were four in South Africa (89, 91, 103, 105), one in Zimbabwe (104), one in Uganda (106), one in Malawi (45) and one multi-country study with sites in South Africa, Tanzania and Zimbabwe (44). The remaining two studies were conducted in Brazil (94) and Indonesia (49). The settings for the studies were the primary health facilities (44, 89, 91, 105), primary care laboratories (94), a specialized infectious disease hospital (104), an intensive care unit (103), a national referral hospital (106), public HIV clinics (45) and clinics for the management of MDR patients (49).

Participants

Seven studies included participants who were 18 years and above and two (49, 94) included all age groups. HIV co-infection was reported in all but one study (105), two studies conducted in an HIV clinic included only HIV-infected participants (45, 104). Patients were eligible to be included in the studies if they were evaluated for pulmonary tuberculosis (45, 89, 91, 94, 103, 105, 106), were at risk of MDR-TB (49)) or were infected with HIV and on ART (104) or newly infected with HIV and undergoing TB screening (45).

5.6.1.1.1 Interventions

All studies compared Xpert diagnostic strategies to smear microscopy strategies and conventional drug sensitive tests (DST), if used. Expectorate sputum samples were collected for Xpert and smear tests in all studies except Calligaro *et al* (103) where tracheal aspirate samples were used. All of the randomized studies collected two sputum samples for smear microscopy and one sputum for Xpert. In Theron *et al* (44) at least two expectorated sputum were collected, one sputum selected randomly was use for smear microscopy or Xpert and the other sputum underwent culture. In non- randomized trials, Yoon *et al* (106) collected and evaluated two sputum samples (early morning and spot) for smear microscopy and one extra spot sputum sample for Xpert, Schmidt *et al* (105) evaluated sputum as per National Health Laboratory Services (NHLS) South Africa guidelines and Van Kampen *et al* (49) evaluated one sputum sample for smear and culture pre-intervention and two samples during the intervention (one for Xpert, the second sample for culture and drug susceptibility testing (DST)).

5.6.1.1.2 Outcomes

5.6.1.1.2.1 Primary outcomes

All-cause mortality was assessed at different time-points across the studies: at 6 months by three studies (44, 89, 91), at 3 months by two studies (103, 104), 2 months by one study (106) and at 12 months by one study (45). Estimates for mortality were presented as relative risks (RR) with the exception of odds ratios (OR) by Theron *et al* (44). Since odds ratios and relative risks are similar when the proportion is less than 10% (107), we include the odds ratio in the meta-analysis, **Table 5. *1***.

The number of TB cases diagnosed by Xpert and smear microscopy were reported in all ten studies. Four studies reported the proportion of treated patients who were not microbiologically confirmed (45, 89, 91, 104). Treatment initiation was reported at different time points across the studies: within 48 hours by Calligaro *et al* (103) , two months by Theron *et al* (44), three months by Cox *et al* (91),six months by Churchyard *et al* (89) or within one year by Ngwira *et al* (45) of follow-up. The number of treated patients who were microbiologically confirmed was reported by five studies (45, 89, 91, 103, 104). Two studies (91, 94) contributed data for

analysis of cure outcome, **Table 5. *3***. Data from Durovni *et al* (94) was taken from another publication (90) of the same study.

Table 5. 1: Descriptive summary of studies included for all cause mortality assessment

Study, year	Country	Design	Settings	Sample size	Month mortality assessed	No. of patients tested in smear group	No. (%) deaths in smear group	No. of patients tested in Xpert group	No. (%) deaths in Xpert group	RR	P value
Churchyard 2015	South Africa	Cluster RCT	Outpatient-primary healthcare clinics	4656	6	2332	116 (5)	2324	91 (3.9)	1.1 (0.75-1.62)	0.61
Cox 2014	South Africa	Cluster RCT	Outpatient-primary healthcare clinics	1985	6	1003	38 (3.8)	982	33 (3.4)	0.89 (0.58-1.75)	0.51
Mupfumi 2014	Zimbabwe	RCT	Outpatient-specialized infectious disease clinic	424	3	214	17(9.9)	210	11 (6)	0.61 (0.29-1.27)	0.19
Ngwira 2017	Malawi	Cluster RCT	Outpatient-HIV primary healthcare clinics	1842	12	841	58 (8.6 per 100 person-years)	1001	55 (6.7 per 100 person-years)	0.78 (0.58-1.06)	0.1
Theron 2014	South Africa, Tanzania, Zambia, Zimbabwe	RCT	Outpatient-primary healthcare clinics	1502	6	758	63 (8)	744	58 (8)	*AoR 0.92 (0.61-1.39)	0.7

Table 5. 2: Descriptive summary of studies included for pre-treatment loss to follow up

Study, year	Country	Design	Settings	Total number tested positive for TB	Month pre-treatment loss to follow up assessed	Number of patients tested for TB in smear group	Number lost to follow up before treatment initiation in smear group N (%)	Number of patients tested for TB in Xpert group	Number lost to follow up before treatment initiation in Xpert group N (%)	RR	P value
Churchyard 2015	South Africa	Cluster RCT	Outpatient-primary healthcare clinics	374	1	174	26 (15)	200	34 (17)	0.96 (0.48-1.93)	0.91
Cox 2014	South Africa	Cluster RCT	Outpatient-primary healthcare clinics	424	3	167	41 (25)	257	32(13)	0.51 (0.33-0.77)	0.0052
Theron 2014	South Africa, Tanzania, Zambia, Zimbabwe	RCT	Outpatient primary healthcare clinics	367	6	182	28 (15)	185	15 (8)	-	0.03

Table 5. 3: Descriptive summary of studies included for cure

Study, year	Country	Design	Settings	Total number treated for TB	Number of patients treated for TB in smear group	Number cured in smear group N (%)	Number of patients treated for TB in Xpert group	Number lost to follow up before treatment initiation in Xpert N (%)	OR	P value
Cox 2014	South Africa	Cluster RCT	Outpatient-primary healthcare clinics	492	224	176 (78.6)	268	215 (80.2)	¶1.10 (0.75-1.71	0.75
Durovni 2014	Brazil	Step wedged cluster RCT	Outpatient primary healthcare clinics	4088	1856	1267 (68.3)	2232	1571 (70.4)	0.92 (0.79-1.06)	-

5.6.1.1.2.2 Secondary outcomes

Time to treatment initiation was defined as the time taken from diagnosis or confirmation of TB to starting treatment or notification date as reported by Durovni *et al* (94), this was reported in all studies except in Calligaro *et a*l (103). Pre-treatment loss to follow-up was reported by four studies (44, 89, 91, 104), **Table 5.** ***2***

None of the studies reported either the number of visits prior to diagnosis or patient reported satisfaction and so these outcomes were not assessed. Drug-resistant TB diagnosis was reported in five studies in the Xpert arm only (44, 89, 91, 94, 103) thus, we could not compare the health impact of Xpert on MDR-TB. Only one study (49) reported the impact of Xpert on patient outcomes in MDR-TB.

5.6.1.2 Excluded studies

We excluded 17 studies, ***Figure 5. 1*** . Nine studies assessed accuracy, three did not assess either the outcome of interest or the population of interest, three had no comparison group and two were observational studies.

5.7 Risk of bias in included studies

Allocation (selection bias)

Four randomized trials (44, 45, 89, 104) had sufficient information for generation of allocation sequence and had a low risk of bias. One trial, Cox *et al* (91), had a high risk of bias since the randomization list was generated by the investigator and insufficient details on concealment. All three (49, 105, 106) non-randomized pre and post trials had a high risk of bias.

Blinding (performance bias and detection bias)

All seven randomized trials (41, 43-46, 91, 103) had a low risk of detection bias, while the pre and post trials had unclear risks. For all of the trials blinding was not feasible but knowledge of the diagnostic test is part of the intervention and so there was a low risk of performance bias. Although Theron *et al* (44) reported that the central laboratory personnel were blinded to clinical based results, the authors did not clearly indicate if clinicians who performed morbidity

assessment score were blinded to the allocation arm. All pre and post trials had a high risk of performance bias, ***Figure 5. 2***.

Incomplete outcome data (attrition bias)

The five randomized trials were considered to have a low risk of attrition bias except one trial by Cox *et al* (42) with an unclear risk. Cox *et al* did not clearly state what proportion of the unfavorable outcome was linked to loss to follow-up, since the unfavorable outcome was reported as a combined outcome for defaulters, death and failure. The non-randomized trials had a low risk of attrition bias except Yoon et al (106) which had a high risk since loss to follow-up was significantly different between the pre- and post- intervention periods.

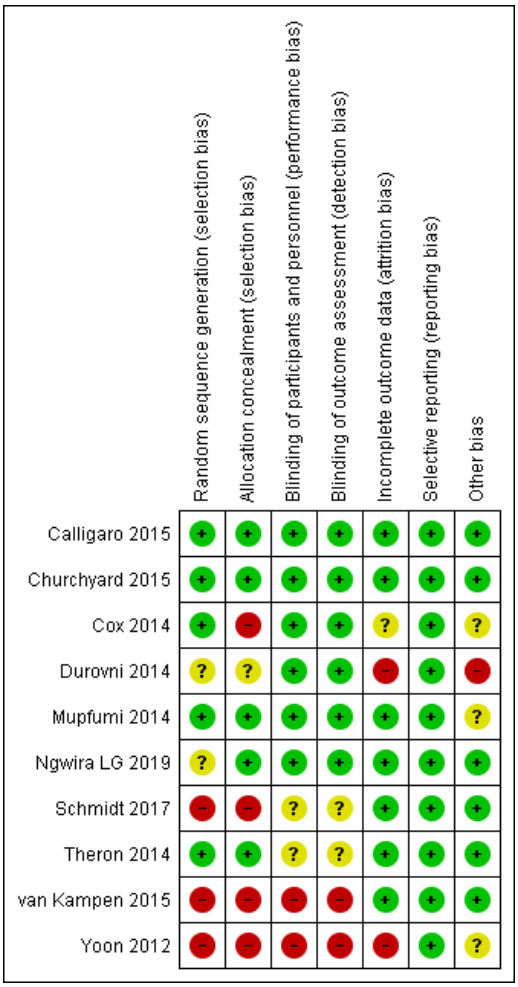
Selective reporting (reporting bias)

We did not detect any selective reporting either in the randomized or in the non-randomized trials.

Other potential sources of bias

We considered there to be an unclear risk of other biases in two randomized trials (42, 94) and one non-randomized trial (106) whereby the same team implemented both diagnostic strategies. We suspect that it is possible that healthcare workers might have introduced biases due to changes in behavior.

Figure 5. 2: Summary of risk of biases for all included studies



5.8 Effects of interventions

5.8.1 Primary outcomes

5.8.1.1 Mortality

Three randomized trials assessed the effect of Xpert on mortality at six months (42, 44, 89), at three months by Mupfumi *et al* (104) or 12 months by Ngwira *et al* (45). The mortality rate was 4.7% (248/5,265) in the Xpert compared to 5.7% (292/5,144) in the smear microscopy. The overall risk of mortality was estimated across these time points to be RR 0.88 (0.73, 1.05) for Xpert compared to smear microscopy (5 RCTs, 10409 participants), ***Figure 5. 3***. Restricting the analysis to the three studies which assessed mortality at six months only produced an estimate of RR 0.97 (95%CI: 0.77,1.23) (3 RCTs, 8143 participants*),* ***Figure 5. 5***. In studies that had included only HIV-positive participants, mortality risk was estimated to be 0.76

(95%CI: 0.58, 1.00) (2 RCTs, 2239 participants), ***Figure 5. 4***. In the non-randomized studies, mortality was reported in only one study (106), with a risk difference (RD) of 3% (-21%, 27%) between the baseline and Xpert implementation phases, favoring Xpert.

Figure 5. 3: All cause mortality

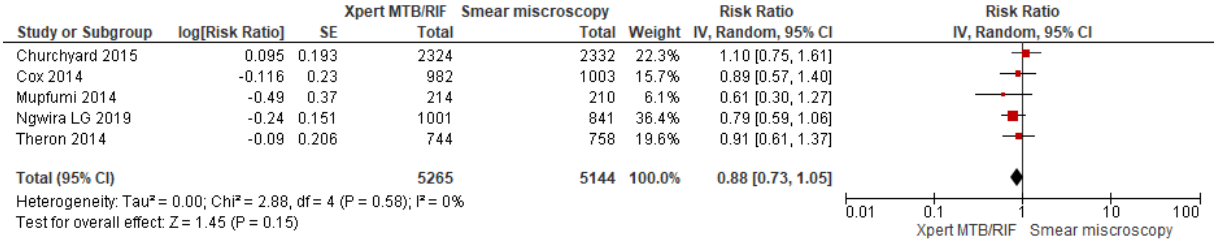


Figure 5. 4: Mortality among HIV positive population

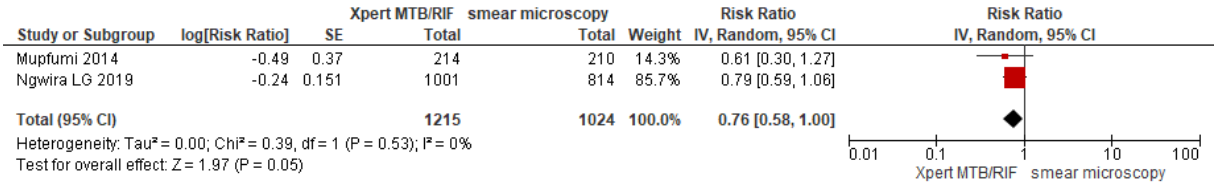
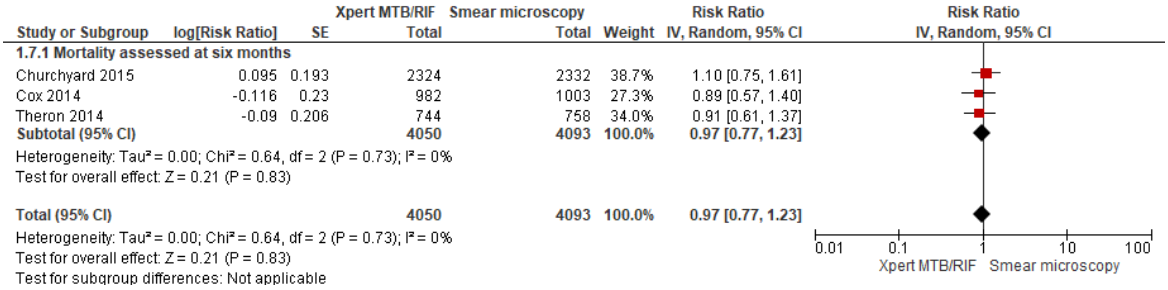


Figure 5. 5: Mortality assessed at six months



5.8.1.2 Number of TB cases reported

In six randomized trials (44, 89, 91, 94, 103, 104), the number of TB patients diagnosed was reported. Overall, there was a higher number of diagnosed TB patients with Xpert compared to smear microscopy (6,119 vs 5,750).

5.8.1.3 Proportion of patient treated

There was a tendency towards higher a proportion of patients being treated for TB in the Xpert compared to the smear microscopy arm, (RR 1.10 95%; 0.98, 1.23) (5RCTs, 8,793 participants), ***Figure 5. 6*** (42, 44, 89, 103, 104).

Those treated in the Xpert arm were more likely to be confirmed microbiologically than those treated in the smear microscopy arm were (RR 1.29 95% 1.11, 1.51) (3 RCTs, 780 participants), ***Figure 5. 7*** (45, 89, 91).

A lower proportion of treated patients in the Xpert arm were confirmed compared to the smear microscopy arm (RR 0.59 95% 0.41, 0.85) (4 RCTs, 367 participants), ***Figure 5. 8*** (45, 89, 91, 104).

Figure 5. 6: Proportion of patients treated

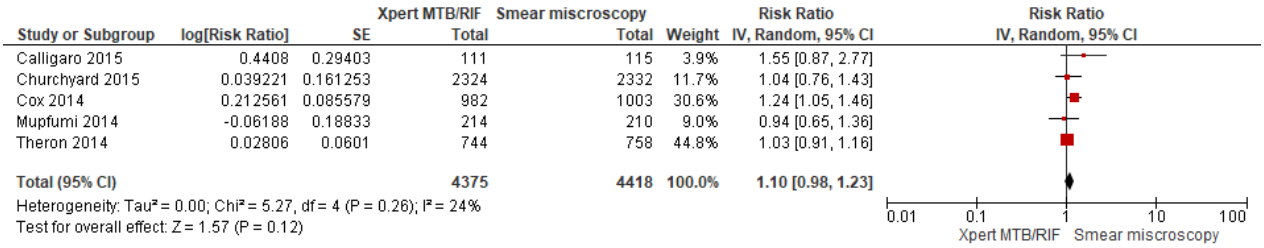


Figure 5. 7: Proportion of treated patients, microbiologically confirmed

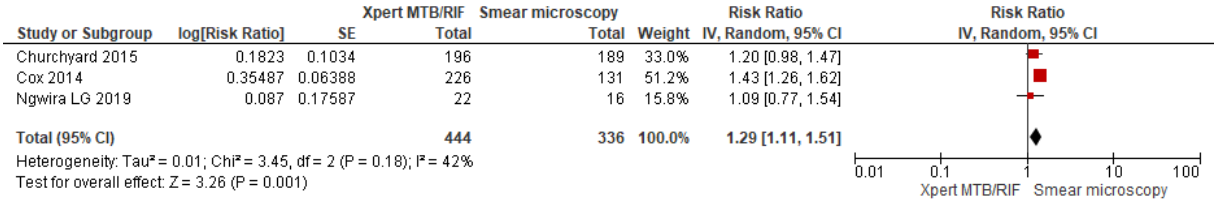
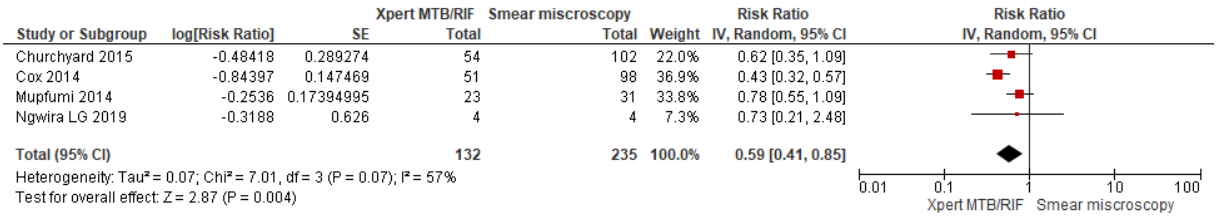


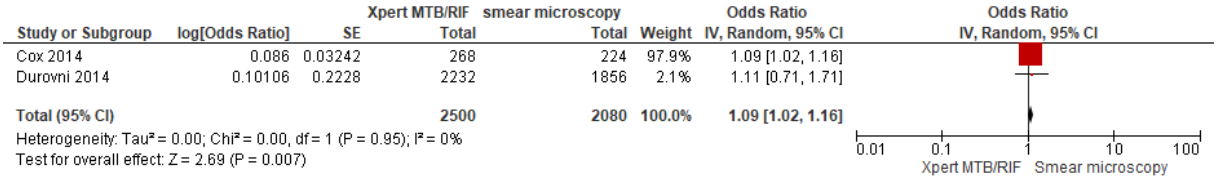
Figure 5. 8: Proportion treated, who are not microbiologically confirmed



5.8.1.4 Cure

Xpert significantly increased the odds of being cured compared to smear microscopy (OR 1.09, 95%CI: 1.02, 1.16) (2RCTs, 4,580 participants), ***Figure 5. 9*** (91, 94).

Figure 5. 9: Proportion of patients cured



5.8.2 Secondary outcomes

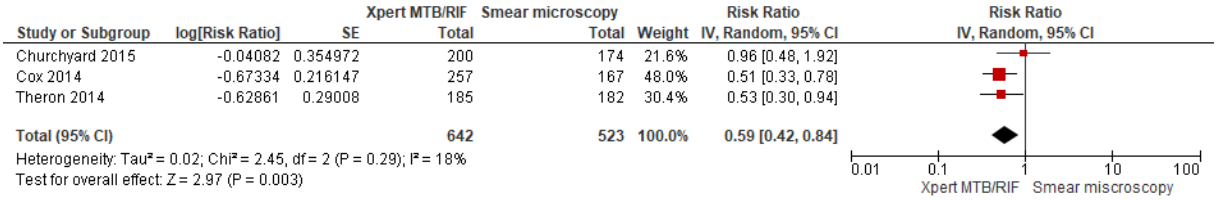
5.8.2.1 Time to treatment initiation

The hazard ratio for time to treatment initiation was reported for only one randomized study (91) (HR 0.76 (0.63, 0.92)) with medians of the skewed distributions for treatment initiation only reported in two further studies (89, 104). This outcome has been the subject of a recent individual patient meta-analysis by Di Tanna *et al* (48) which included four similar studies (44, 89, 91, 104) with an overall estimated hazard ratio of 1.00 (0.75, 1.32) adjusting for age and gender. We do not replicate it here.

5.8.2.2 Pre-treatment loss to follow-up

Across three randomized trials (42, 44, 89), Xpert was found to reduce the risk of pre-treatment loss to follow-up with an estimated risk ratio of 0.59 (95% 0.42, 0.84) (3 RCTs, 1165 participants), ***Figure 5. 10*** . (44, 89, 91).

Figure 5. 10: Proportion of pre-treatment loss to follow up



5.8.2.3 Drug-resistant TB

A comparison between Xpert and smear strategies for outcomes specific to drug-resistant TB could not be made because drug-resistant TB diagnosis was reported in the Xpert arm only. Only one non-randomized study (49) which had assessed patient outcomes among multi-drug resistant TB fulfilled the inclusion criteria. Time to MDR treatment initiation was reduced by 72 days, from median of 88 days pre-introduction to 16 days after Xpert was introduced.

5.8.2.4 Number of visits prior to diagnosis and patient reported satisfaction

Two pre-specified secondary outcomes (number of visits prior to diagnosis, patient reported satisfaction) could not be analyzed due to lack of data in all of the included studies.

Table 5. 4: Grade table: Xpert compared to smear microscopy in adults with signs and symptoms of pulmonary tuberculosis

Xpert compared to smear microscopy in adults with signs and symptoms of pulmonary tuberculosis						
Patient or population: adults with signs and symptoms of pulmonary tuberculosis **Setting:** **Intervention:** Xpert **Comparison:** smear microscopy						
Outcomes	**Anticipated absolute effects* (95% CI)**		**Relative effect (95% CI)**	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Comments**
	Risk with smear microscopy	**Risk with Xpert MTB/RIF**				
Mortality	Study population		RR 0.88 (0.73 to 1.05)	10409 (5 RCTs)	⨁⨁⨁⊖ MODERATE [1] [2] [3]	Compared to smear microscopy Xpert showed a tendency towards reduction on mortality. High rates of empirical treatment in settings in which studies were conducted and insufficient sample size, increase in proportion of cured and reduction in pre treatment loss to follow up potentially explain the lack of statistically significant effect of Xpert on mortality
	57 per 1,000	50 per 1,000 (41 to 60)				
Cure	Study population		OR 1.09 (1.02 to 1.16)	4580 (2 RCTs)	⨁⨁⨁⨁ HIGH [4]	Xpert increase the proportion of patients cured compared to smear microscopy.
	694 per 1,000	712 per 1,000 (698 to 724)				
Pre-treatment loss to follow up	Study population		RR 0.59 (0.42 to 0.84)	1165 (3 RCTs)	⨁⨁⨁⊖ MODERATE [5]	Xpert reduces pre-treatment loss to follow up.
	182 per 1,000	107 per 1,000 (76 to 153)				
	Study population					

Mortality in HIV-positive participants	71 per 1,000	54 per 1,000 (42 to 71)	RR 0.76 (0.59 to 1.00)	2266 (2 RCTs)	⨁⨁⨁⊖ MODERATE [6]	
*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI). __CI:__ Confidence interval; __RR:__ Risk ratio; __OR:__ Odds ratio;						
GRADE Working Group grades of evidence **High certainty:** We are very confident that the true effect lies close to that of the estimate of the effect **Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different **Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect **Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect						

Footnotes

[1] For all randomized trials, blinding of physicians to what test was done was impossible since knowing which test was done is part of the intervention itself. For example, the Xpert test has higher sensitivity than smear microscopy (and also produces RIF resistance results) and physicians must be allowed to take this into account when deciding about patient management. While outcomes between patients may therefore be different due to lack of blinding this was not judged to be a source of bias but rather the mechanism through which the intervention had an effect. Outcome measurement could theoretically have been influenced by the lack of blinding but this was deemed unlikely to cause bias of important magnitude. Overall, the lack of blinding was therefore judged not to put studies at increased risk of bias.Type a message

[2] No evidence of inconsistency, four studies in the direction of showing benefit.

[3] The 95% CI is wide likely suggesting imprecision. We caution about interpreting non-significance as no effect when the CI likely includes an effect that may be clinically important. We downgraded one level for Imprecision.

[4] Cure is the outcome of interest for patient important outcome. Studies have reported treatment success which includes those cured and those completing treatment without evidence for treatment failure . However, we did not downgrade for indirectness

[5] Variability in time for assessment of pre-treatment loss to follow up; Churchyard 2015 assessed within 28 days after enrolment, Cox 2014 assessed by three months after enrolment and Theron 2014 assessed by the end of the study (six months)

[6] Similarly, the 95% CI is wide likely suggesting imprecision. We caution about interpreting non-significance as no effect when the CI likely includes an effect that may be clinically important. We downgraded one level for Imprecision

5.9 Discussion

5.9.1 Summary of main results

We included ten studies that assessed the effect of Xpert on health outcomes. Compared to diagnostic strategies using smear microscopy, the use of Xpert showed a positive effect on TB confirmation, reduction in treatment without confirmation, reduction in pre-treatment loss to follow-up, and the suggestion of a moderate reduction in all-cause mortality. Similar effects on all-cause mortality reduction were evident in the HIV-positive population as well as when all-cause mortality was assessed by the end of treatment period. The use of Xpert increased the likelihood of cure and treatment completion in TB patients.

5.9.2 Overall completeness and applicability of evidence

This is the first Cochrane review on the impact of Xpert on patient-important outcomes. It includes studies carried out in low and middle income countries where 95% of cases and 98% of tuberculosis-related deaths occur (108). The various epidemiological settings in which the studies were conducted provide confidence in the generalizability of our findings in high burden settings. A limitation is that there have not been many studies conducted on impact of Xpert on patient outcomes. So far there are only seven randomized trials and none has assessed the impact of Xpert on patient-important outcomes in MDR-TB population (93). Another limitation is the variability in the duration of follow-up among studies, which may increase heterogeneity in the study effect estimates.

The studies included were carried out in sub-Saharan Africa, Asia and South America, areas with a high burden of TB. Five studies were set in South Africa, which was one of the earliest countries to replace smear microscopy with Xpert, and was the leading procurer of Xpert during the period of this review (29).

Our findings should be interpreted carefully in the context of healthcare systems. The quality of healthcare system in low and middle income countries is generally low (109). Patient-

important outcomes such as mortality depend on improvement of surrogate endpoints before the impact on patient outcomes can be realized (109). Studies have shown different experiences in terms of gaps in healthcare cascade, for example, South Africa performed generally better in terms of individuals accessing TB tests, but had poor treatment outcomes (110). Such observations underscore the need to interpret results in context of healthcare system and the effect of Xpert is likely to depend on linkage between diagnosis and treatment. Most studies included were from countries where TB is primarily managed in the public sector. Health effects of Xpert may be different in countries where TB is managed in the private sector with poor linkage to national programmmes. Comprehensive record keeping for test results and patient outcomes remain relevant in demonstrating health effect of Xpert.

We could not assess all objectives planned for this review due to limitations in data availability. Xpert could have an impact on outcomes such as patient satisfaction and visits to health facilities, which could not be assessed in this review. The outcome on cure was included following the request by the guideline development group for Xpert of the WHO.

5.9.3 Quality of the evidence

Overall, the quality of evidence on mortality and pre-treatment loss to follow up was judged as moderate whereas the quality of evidence on cure was judged as high. Mupfumi *et al*. did not specify the proportion of pre-treatment lost to follow-up but rather overall loss to follow-up at three months. We did not include this study in the analysis of pre-treatment loss to follow up nevertheless; a post hoc analysis with this study included did not change the direction of our findings. The lack of blinding in the pragmatic trials raises the possibility of performance and ascertainment biases, however included trials were pragmatically conducted hence exclude possibility for blinding. We judged low risk of biases in performance, ascertainment selection and attrition biases. An issue is the low number of trials carried out.

In endemic settings where most of the studies included were conducted, empirical treatment remains relevant. Empirical treatment is done when there is no bacteriological confirmation

and decision to treat is based on chest X-ray and/or clinical judgement. McCarthy et al reported that 15% of 561 patients on TB treatment in South Africa had been initiated on treatment empirically (111). Empirical treatment is more common when there is a high TB and TB/HIV burden, a high pre-test probability for TB or when a delay in initiating treatment could result in severe morbidity or mortality. Empirical treatment assigns patients to treatment without necessarily having test results leading into non-differential misclassification, which may mask benefits of a test towards mortality and has been discussed in a previous review (48).

5.9.4 Potential biases in the review process

A comprehensive search was carried out as far as possible without language restriction to ensure all studies meeting inclusion criteria were included. Experts in the field were contacted to identify any additional studies. We are confident that we managed to include all relevant studies. The studies included are similar to a recent review (48) which has some overlap in outcomes. We think it unlikely that there was any selection bias or that we had missed any potentially eligible studies.

5.9.5 Agreements and disagreements with other studies or reviews

The estimated effect of Xpert on mortality was a 12% reduction (-27%, +5%). A similar conclusion was reached in a recent meta-analysis of five randomized trials using patient level data where an impact on mortality could neither be ruled in or out (48). In the review by (48), three studies (44, 89, 91) contributed data for the analysis of all-cause mortality. Our review included five studies, increasing the sample size, for the analysis of all-cause mortality. Insufficient power to detect mortality in randomized trials of diagnostic impact has been discussed previously (48, 93) and that even larger sample size are needed to detect impact of Xpert on mortality. The lack of statistical significance on impact of Xpert on mortality should be carefully interpreted not as the lack of public health impact of Xpert on mortality rather as evidence for lack of evidence on the difference (112, 113). Reduction of mortality of 12% to as high as 27% reduction by Xpert could result in large impact in terms of tuberculosis control

compared to smear microscopy. However, the evidence for existence of this difference remains to be demonstrated in studies with larger sample size. Mortality is an outcome that depends on a series of health system processes and intermediate outcomes, such as time to treatment initiation, before an impact of mortality can be realized. The impact of Xpert on mortality is likely to depend on the general improvement of healthcare systems in these settings (109). Among HIV-positive patient mortality, we detected a larger effect in Xpert arm compared to smear microscopy. A similar observation was made by Di Tanna *et al* (48).

In a narrative literature review of eight trials, which assessed impact of Xpert on patient outcomes, none of the individual trials had reported a significant impact of Xpert on mortality (92). All eight studies are also included in our review. There are high rates of empirical treatment in settings where most trials of impact of Xpert have been carried out and this may influence the estimates (48). In these settings, the lack of availability or access to confirmation of TB particularly for smear negative patients results in the probability of disease in the patient lying above treatment threshold, hence empirical treatment (114). Furthermore, the standard of care in randomized controlled trials, such as chest X-ray availability, is generally above that which a TB patient would receive in programmatic settings (115). This has two possible implications; (i) an increase in both pre and post-test probability particularly in those previously treated and hence increase chances for empirical treatment (ii) artificially reduce pre-treatment loss to follow-up and increased chances for empirical treatment (93). It remains debatable whether high standards of care in randomized controlled trials introduces biases or limits generalizability. Arguably, high interaction between research teams and participants in terms study procedures and follow up in the included studies limits the intended pragmatism (115). Of the included studies, only Durovni *et al* minimally interrupted the usual standard of care. The use of additional resources in randomized trial above what is available in usual care and strengthening of routine systems reduces chances to detect effect on mortality. While high internal validity in randomized trials remains important for strong evidence, use of routine collected data would likely replicate usual care and demonstrate effect of Xpert in pragmatic

settings(115).

Patients in the Xpert arms were more likely to be confirmed bacteriologically than those in the smear microscopy arms. Similar observation were reported in a recent meta-analysis where Xpert was shown to be superior for tuberculosis bacteriological confirmation (116). This finding is not surprising given the greater accuracy of Xpert compared to smear microscopy. In South Africa, the introduction of Xpert in 101 primary healthcare facilities was shown to increase the rate of bacteriological confirmation and reduce the rate of empirical treatment over a period of four years in a large population based programmatic cohort(117). Similar observations were made in Nepal (82). Empirical treatment has long been recommended by WHO particularly in resource-limited settings (118). Generally, an increase in bacteriological confirmation and reduction in empirical treatment has been linked to an overall reduction in tuberculosis notification (82, 117). It is likely that TB incidence might have decreased or that the patient had other disease conditions, however the impact of Xpert on TB confirmation is likely to reduce over diagnosis of TB.

Xpert significantly reduced the risk of pre-treatment loss to follow-up by 41% (CI 52% to 16%) compared to smear microscopy and has a turnaround time for results of two hours(18) which enables same day results. A recent review of 23 studies from 14 countries reported pretreatment loss to follow-up of smear or culture positive patients ranging from 4-38% (86). In this review, pre-treatment loss to follow-up was commonly reported in studies from Africa and Asia and mortality among lost to follow up was high. In Africa where the HIV/TB co-infection rate is high, pre-treatment loss to follow-up was associated with mortality. In Asia, high rates of private practice are linked to pre-treatment loss to follow-up. Long waiting times, repeated visits and delays in receiving results have also been associated with pre-treatment loss to follow-up (86).

5.9.6 Authors' conclusions Implications for practice

We found that, compared with smear microscopy, Xpert has a beneficial impact on some

patient outcomes supporting its use in practice. Our estimate suggests that Xpert reduces all-cause mortality by 12% although uncertainty around the effect estimate was high and the data is compatible with a reduction of up to 27% and an increase of up to 5%. We found that the use of Xpert decreased pre-treatment loss to follow up and increased the proportion of TB patients cured. Xpert was shown to increase TB confirmation and reduce empirical treatment. The mechanism by which Xpert may affect mortality is likely at least in parts related to the reduction in pre-treatment loss to follow-up as well as increases in the proportion of TB patients cured. The impact of Xpert on pre-treatment loss to follow-up will depend on other health system factors such as linkage to healthcare, proper recording and how fast a patient is initiated on treatment following positive results.

5.9.7 Implications for research

Future studies on newly developed molecular point of care tests such as the newly GeneXpert Omni (Omni) platform and Xpert ultra should incorporate the assessment of patient outcomes. Such studies are particularly valuable when carried out in settings close to patients such as primary healthcare facilities. Future trials should also report the impact of Xpert on patient outcomes in patients with drug-resistant TB. Gaps in knowledge has been identified and the number of visits to health facilities and patient satisfaction could also be included in future studies. The effect of empirical treatment should also be investigated.

5.9.8 Acknowledgements

We thank Paul Garner, Anne-Marie Stephani, Deirdre Walshe, Philomena Hinds, and Vittoria Lutje for their support and comments. The editorial base of the Cochrane Infectious Diseases Group is funded by UK aid from the UK Government for the benefit of developing countries (Grant: 5242).We extend our gratitude to European Developing Countries Clinical Trial Partnership (EDCTP) for the fellowship grant to Mwaka Kakolwa. This grant facilitated Mwaka Kakolwa during the review.

5.9.9 Contributions of authors

FH, MK, and AR wrote the first draft of the review. KR, AR, SGS, CMD, SG, and RRN reviewed the review. FH, KR, and AR wrote the final review.

5.9.10 Declarations of interest

FH has no known conflict of interest. RRN has no known conflict of interest. SGS has no known conflict of interest. MK has no known conflict of interest. CMD has no known conflict of interest. SG has no known conflict of interest. KR has no known conflict of interest. AR has no known conflict of interest.

6 Overall discussion

6.1 Summary of findings

Overall, Xpert perfomance varied according to different methodological definitions of reference standard in sensitivity and population of interest in specificity assessment.

Xpert sensitivity varied within different categories of TTP. Xpert sensitivity was consistently high in both smear-positive culture-positive and smear-negative culture-positive within 15 days of TTP, and decrease as TTP increased until 30 days of TTP. A substantial drop in Xpert sensitivity after 30 days of TTP among smear-negative culture-positive patients was observed. Sensitivity reached a plateau for 30 and 42 days of TTP. In additon, Xpert sensitivity was higher when MGIT compared to LJ culture was used as a reference standard for case definition. The sensitivity of Xpert was highest in single MGIT compared to multiple cultures.

Xpert specificity varied in patients with a history of TB. Specificity was reduced among patients with a history of TB. A substantial reduction in specificity was observed if the history of TB was within two years and chest X-ray not compatible with TB was associated with a false-positive Xpert result.

Regarding the impact of Xpert on patient relevant outcomes, we found that the use of Xpert showed a positive effect on TB confirmation, reduction in treatment without confirmation, reduction in pre-treatment loss to follow-up, and a suggestion of a moderate reduction in all-cause mortality compared to the use of smear microscopy. Similar effects on all-cause mortality reduction were evident in the HIV-positive population as well as when all-cause mortality was assessed by the end of treatment period. The use of Xpert increased the likelihood of cure and treatment completion in TB patients.

6.2 Interpretation of results

Xpert peformance has been widely assessed against a microbiological reference standard of culture. Different studies have assessed Xpert perfomance against single or multiple MGIT and/or LJ cultures, whereby sensitivity of Xpert is assessed as the proportion of culture positive

that test positive by Xpert in all patients throughout the evaluation period (54). This provides the best average estimate of Xpert performance. However, little evidence exist on how Xpert perfoms, particularly how sensitivity varies as a function of the time to culture positivity. Such evidence provides better understanding of Xpert sensitivity in the context of changing epidemiological trends as efforts for TB control are being escalated. Xpert sensitivity varies in different patient groups. For example, Xpert sensitivity has consistently been shown to be lower in smear-negative culture-positive patients compared to smear–positive culture-positive patients. In this thesis, similar sensitivity across the three groups of analysis within 15 days of TTP i.e. all culture-positive, smear-positiveculture-positive and smear-negative culture-positive were observed. Shorter time to positivity is generally associated with high bacterial load (58). It appears that at shorter TTP, the negative smear results observed in smear-negative culture-positive could likely be a result of imperfect smear microscopy perfomance.

It appears also that the overall sensitivity of Xpert corresponds to the sensitivity of Xpert within 15 days of TTP and that there is little benefit in terms of Xpert sensitivity after 20 days of TTP. The lower sensitivity of Xpert at longer TTP calls for better diagnostics with improved sensitivity such as Ultra.

The use of MGIT reference standard against Xpert showed better sensitivity compared to LJ, consistent with previous findings (57, 61, 62). Single culture showed better sensitivity of Xpert compared to when using multiple cultures. The downside of MGIT higher sensitivity compared to LJ is the higher cost of performing MGIT and high rates of contamination from previous studies (61-63). Evidence from Chihota VN *et al* suggests that in the best-case scenario, MGIT would require four more USD dollars per culture compared to LJ (63). Furthermore, MGIT culture infrastructures are expensive to establish and maintain. In the studies analyzed in this thesis a low rate of contamination of less than one percent was observed, which is most likely explained by the use of reference laboratories and standardize protocols. The use of MGIT alone for *M. tuberculosis* isolation has been showed to miss around six percent *M. tuberculosis*

which would grow on LJ beyond six weeks of negative MGIT (61). Overall, in settings with better control efforts, it is expected that patients are diagnosed earlier and TTP will be increased. Thus, reporting TTP is important to ensure comparability of studies to allow for better interpretation of results. Furthermore, the comparison against different definitions of culture positivity demonstrate the importance of comparability of reference standards across studies.

In terms of Xpert specificity, the observed drop in specificity among patients with a history of TB was associated a negative chest X-ray result, even when adjusted for TB history and HIV. This suggests that a negative chest X-ray could aid in the decision-making process whether to start therapy in a patient with a positive Xpert result and recent TB history. This might be a viable strategy in patients without HIV. However, in patients with HIV, the risk of not treating might outweigh the risk of overtreatment. This thesis has demonstrated variability in Xpert performance in both sensitivity and specificity. WHO recognises the need for clinical diagnosis in patients in which bacteriological tests tend to have low sensitivity and in patients in which delaying treatment may lead to catastrophic outcomes (119). In such circumstances, WHO recommends the use of chest X-ray to aid rapid diagnosis even when the risk of false positive clinical diagnosis is high or the positive predictive value is low (119). A combination of clinical judgement and the use of chest X-ray will remain relevant for the diagnosis of TB at longer TTP and in those with a shorter history of TB. The decrease in specificity of Xpert is similar to the one reported for Ultra among patients with a shorter history of TB (24). Further investigations are needed to detemine if repeating Xpert on a second sample in this sub-population may improve specificity as recommended for Ultra (31).

Xpert showed an impact on some patient-important outcomes. The estimated effect of Xpert on mortality was a 12% reduction (-27%, +5%). In a narrative literature review of eight trials, which assessed impact of Xpert on patient outcomes, none of the individual trials had reported a significant impact of Xpert on mortality (92). All eight studies were also included in

the Cochrane review included in this thesis. In the review by Di Tanna *et al* (48), which had similar findings on mortality, three studies (44, 89, 91) contributed data for the analysis of all-cause mortality. The Cochrane review in this thesis included five studies, which led to an increased sample size for the analysis of all-cause mortality. Yet, the statistical power remained overall insufficient to reliably detect effects on mortality, previously discussed for those randomized trials on health impact (48, 93)

The lack of statistical significance on impact of Xpert on mortality should be interpreted carefully not as the lack of public health impact of Xpert on mortality, but rather as a lack of evidence on the difference (112, 113). The observed reduction of mortality of 12% by Xpert could result in a large impact in terms of TB control compared to smear microscopy. Mortality is an outcome that depends on a series of health system processes and intermediate outcomes, such as time to treatment initiation, before an impact on mortality can be realized. The impact of Xpert on mortality is likely to depend on the general improvement of health care systems in settings, in which Xpert is rolled out (109).

High rates of empirical treatment in settings where most trials of impact of Xpert have been carried out may influence the estimates (48). In these settings, the lack of availability or access to confirmation of TB particularly for smear-negative patients results in the probability of disease in the patient lying above treatment threshold, hence empirical treatment (114). Furthermore, the standard of care in randomized controlled trials, such as chest X-ray availability, is generally above that which a TB patient would receive in programmatic settings (115), leading to (i) an increase in both pre- and post-test probability, particularly in those previously treated, and hence increase chances for empirical treatment, (ii) artificially reduce pre-treatment loss to follow-up,. It remains debatable whether high standards of care in randomized controlled trials introduces biases or limits generalizability.

Arguably, high interaction between research teams and participants in terms study procedures and follow up in the included studies limits the intended pragmatism (115). Of the included studies, only Durovni *et al* minimally interrupted the usual standard of care through the use of

routine databases. The use of additional resources in randomized trial above what is available in usual care and strengthening of routine systems reduces chances to detect effect on mortality. While high internal validity in randomized trials remains important for strong evidence, use of routine collected data would likely replicate usual care and demonstrate effect of Xpert in pragmatic settings (115).

Patients in the Xpert arms were more likely to be bacteriologically confirmed than those in the smear microscopy arms. Similar observation were reported in a recent meta-analysis where Xpert was shown to be superior for TB bacteriological confirmation (116). This finding is not surprising given the greater accuracy of Xpert compared to smear microscopy. Although empirical treatment has long been recommended by WHO, particularly in resource-limited settings (118), increase in TB confirmation will likely reduce empirical treatment as demonstrated in studies in South Africa (117) and Nepal (82). Generally, an increase in bacteriological confirmation and reduction in empirical treatment has been linked to an overall reduction in TB notification (82, 117). It is likely that TB incidence might have decreased or that the patient had other disease conditions, however the impact of Xpert on TB confirmation is likely to reduce over diagnosis of TB.

Xpert significantly reduced the risk of pre-treatment loss to follow-up by 41% compared to smear microscopy and has a turn-around time for results of two hours (18) which enables same day results. A recent review of 23 studies from 14 countries reported pretreatment loss to follow-up of smear- or culture-positive patients ranging from 4-38% (86). In this review, pre-treatment loss to follow-up was commonly reported in studies from Africa and Asia, and mortality among loss to follow-up was high, ranging from 0 to 82%. In Africa, where the HIV/TB co-infection rate is high, pre-treatment loss to follow-up was associated with mortality. In Asia, high rates of private practice are linked to pre-treatment loss to follow-up. Long waiting times, repeated visits and delays in receiving results have also been associated with pre-treatment loss to follow-up (86).

6.3 Strength and weaknesses

This thesis has the following stengths: the findings of variability in Xpert perfomance are reported from the analysis of a large dataset with patients from different epidemiological settings of HIV co-infection, suggesting generalizability. Furthermore, laboratory procedures were standardized across different protocols in all sites with a common reference standard, which minimizes any misclassifications. This thesis report includes the first Cochrane Review that provides direct evidence on the impact of Xpert on patient outcomes and informed a WHO guideline development group. Included studies in the review were carried out in sub-Saharan Africa, Asia and South America, areas with a high burden of TB, and should thus be generalizable in settings of intended use.

On the other hand, few limitations were observed and can be summarized as follows; (i) inability to assess the corresponding bacterial load counts in different categories of TTP for sensitivity variability analysis because the data on Ct values for Xpert were not reported in the database, hence not available. Such an analysis would have given a more precise explanation for the drop of sensitivity of Xpert in longer TTP in relation to the limit of detection of Xpert as well as assessing the corresponding bacterial load in reduced specificity; (ii) in the Cochrane Review, a limitation was that there have not been many studies conducted on impact of Xpert on patient outcomes. So far, there are only seven randomized trials and none has assessed the impact of Xpert on patient relevant outcomes in MDR-TB population; iii) not all planned objectives for this review were assessed. This is due to limitations in data availability. Xpert could have an impact on outcomes such as patient satisfaction and visits to health facilities, which could not be assessed in the review but were initially planned.

6.4 Implications

Xpert is a widely used molecular diagnostic tool and is currently recommended by WHO as initial diagnostic tool compared to smear microscopy, should resources allow (26). This conditional recommendation, together with other strong recommendations for MDR-TB and

paeditric TB, have contributed to Xpert being widely used in resouce limited settings, not to mention the concessional pricing (26, 29). To date, there are over 34 million Xpert cartridge that have been procured by the public sector (29). Being the most widely used molecular diagnostic tool, Xpert is expected to impact TB trends and to contribute to the control of TB. This thesis has demostrated that Xpert indeed impact patient-important outcomes and thus does contribute to TB control. The evidence generated from this thesis is direct. The findings of the Cochrane review has been presented to the WHO Guideline Development Group (Geneva, 3-6 December 2019) and contributed to the policy decisions for the use of Xpert. WHO end TB strategy aims at reducing TB incidence by 90% in 2035 (11). The reduction of TB incidence will indeed change the epidemiology of TB across different populations. This will require a better understanding of the perfomance of Xpert, particularly in sub-clinical TB. Such understanding will guide proper policy decisions on use of Xpert in the future. This thesis has shown marked reduced Xpert sensitivity at longer TTP which very likely corresponds to sub-clinical TB. Clinical judgement and the use of chest X-ray will remain relevant in TB diagnosis, particularly in those with sub-clinical TB. As efforts to control TB increase, a change in TB epidemiology will demand for diagnostics with good performance, particulary in those cases with low bacterial load. WHO has recommended the newer version of Xpert known as Xpert MTB/RIF Ultra (Ultra) with improved sensitivity compared to Xpert, particularly in patients with a low bacterial load (24, 54). However, Ultra gains in sensitivity come with the expense of a loss in specificity (24, 64). Furthermore, the observed reduction in specificity in patients with a previous history of TB, particularly in those whose history of TB is within two years, calls for guidance in interpreting Xpert results in this sub- population. TB programmes will need to develop guidelines and algorithms on positive results in these group, and decision for indicaations to treat or not treat. Furthermore, consideration of other conditions and careful clinical decision before treatment initiation in this group of patients is warranted. Key questions for future research remain on the impact of false-positive results on patient outcomes as well as modelling studies, such as the one by Kendall *et al* for Xpert Ultra (70), and should weigh

the impact of likely overtreatment among patients with previous TB history in different epidemiological settings.

6.5 Conclusion

TB remains the major killer from a single infectious agent, and diagnosing TB is still a challenge. The introduction of Xpert, a molecular diagnostic tool at point-of-care provides significant contribution to TB control and has been shown to impact patient-important outcomes. In order to reliaze full impact of Xpert, the strength of the health system, in which Xpert is rolled out, remains relevant. Methodological aspects of assesment of Xpert perfomance have shown variablity in Xpert sensitivity. Future studies reporting Xpert sensivitivity should consider reporting TTP as well to better understand performance characteristics. Finally, the need for algorithms guiding Xpert positive results in patient with a recent history of TB cannot be overemphasized.

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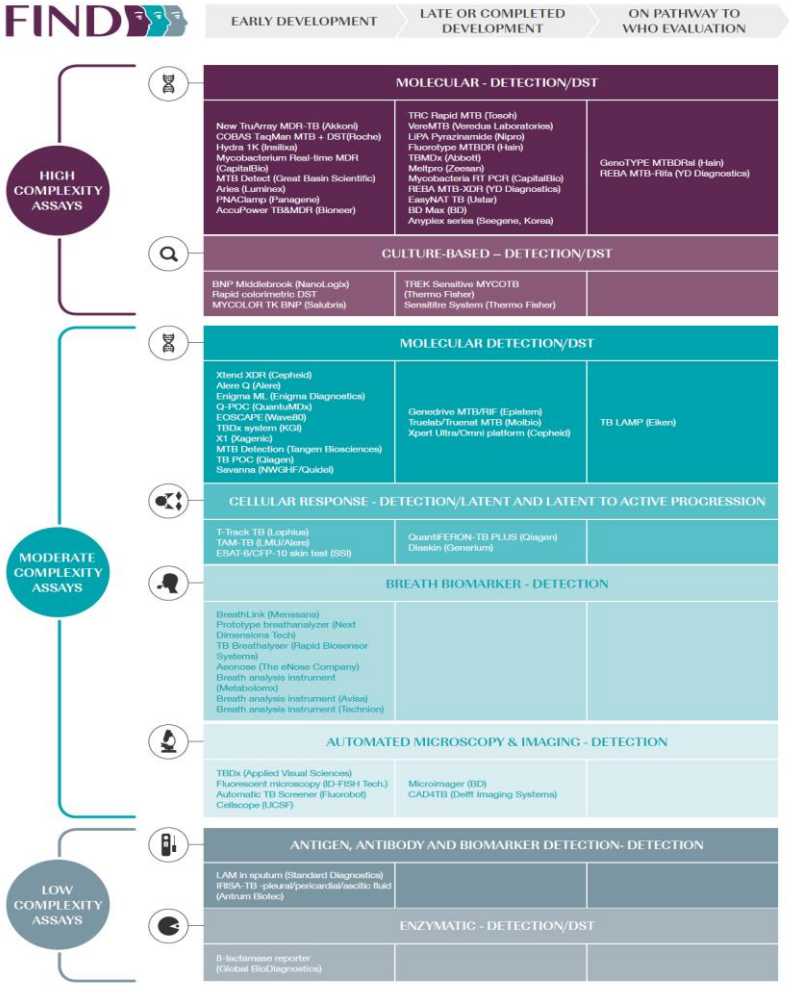
8 Appendix 1:

8.1 Current diagnostics pipelines listing development phases and types of technologies reported by FIND (15)



9 Appendix 2:

9.1 Characteristics of included studies in the Cochrane review

Study ID	Methods	Participants	Intervention	Outcomes	Notes
Calligaro 2015	Individual randomised controlled trial in ICUs in four hospitals	Participants: mechanically ventilated patients suspected for tuberculosis 18 years old and above, admitted between Aug 1 2010 and 31 July 2013. with no TB treatment in the previous 60 days Female: 40% in the Xpert arm, 41% in the smear microscopy arm HIV infection: 27% Xpert, 32 % smear Settings: intensive care units (ICUs) in four tertiary and secondary hospitals in Cape Town Country: South Africa Sample size: 317 patients in total	Smear and culture (control), or Xpert MTB/RIF and culture (intervention) of tracheal aspirate samples.	**Primary outcome**:proportion of culture-positive patients started on anti-tuberculous treatment in each trial group 48hrs after enrolment **Secondary outcomes** • time to bacteriological diagnosis, • time to treatment initiation, the proportion of culture-positive patients started on antituberculous treatment by the end of the study, • proportion of patients given empirical anti-tuberculous treatment, • mortality	

Churchyard 2015	Two-arm, parallel, cluster-randomised trial. A cluster was defined as a laboratory and two primary care clinics served by but not co-located with that laboratory	Participants had suspected tuberculosis: a systematic sample of adults giving sputum for TB investigation 18 years old and above Female: 62% overall HIV: 62% overall of whom 33% had ever been on antiretroviral therapy Setting: primary healthcare clinics and laboratories in medium burden districts in four provinces Country: South Africa Sample size: 4658 patients in total, 10 clusters in each arm	In the Xpert group, participants had one spot sputum specimen collected for Xpert MTB/RIF testing at the associated laboratory. In the microscopy group, participants had two sputum specimens collected for fluorescence microscopy.	**Primary outcome**: mortality, measured 6 months after enrolment **Secondary outcomes**: proportion with a positive index test result; in participants with a positive result, initial loss to follow-up, defined as the proportion not started on tuberculosis treatment within 28 days of enrolment; proportion of the overall cohort starting tuberculosis treatment by 6 months from enrolment	
Cox 2014	Pragmatic prospective cluster randomised trial. The study took place in one large primary care facility with randomisation by week to the intervention or routine care	Participants were presumptive pulmonary TB presenting at Ubuntu clinic in Khayelitisha Cape town 18 years old and above Female 44.7% in the Xpert group and 46% in smear microscopy group	Randomization was done on weekly basis. Each week during the study period was randomised to either Xpert MTB/RIF or smear microscopy Intervention: Xpert MTB/RIF Routine care: (smear, culture and DST for high risk of drug resistance)	**Primary outcome:**proportion of bacteriologically confirmed TB cases that had not initiated appropriate treatment by 3 months after enrolment **secondary outcomes**: • time to diagnosis, • time to TB treatment, • all-cause mortality, • the number of clinic visits prior to	Target condition: Tuberculosis case definition: Bacteriological confirmation of TB (smear, Xpert or culture)

		HIV infection: 59% Xpert group 59.7% in smear microscopy group Setting: primary healthcare clinic Country: South Africa Sample size:982 Xpert MTB/RIF arm and 1003 smear microscopy arm		appropriate TB treatment	
Durovni 2014	Stepped wedged cluster randomised trial. All 14 laboratories started with smear microscopy. Two laboratories then switched overnight to the Xpert arm every month, so that by the eighth and final month of the trial, all clusters were in the Xpert arm. The unit of comparisons were laboratories and the clinics which use their services.	Patients who had sputum samples sent to the study laboratories for the diagnosis of pulmonary TB between February and October 2012 All age groups Female: 35.6% Xpert, 35.9% smear microscopy HIV infection: 7.4% Xpert, 9.8% smear Settings: primary healthcare facilities which used the laboratories in the study Country: Brazil, the study was conducted in the cities of Manaus (three	The diagnostic test for pulmonary TB was Intervention: Xpert Comparison arm: sputum smears.	**Primary outcome** • notification rate of laboratory confirmed pulmonary TB by clinics relying on study laboratories' services measured by the differences and ration of rates in the intervention versus the baseline period • time to treatment initiation, estimated by the notification date minus the laboratory result date **Secondary outcomes** notification rates: for	

		laboratories) and Rio de Janeiro (eleven laboratories) Sample size: 2610 Xpert, 2050 Smear microscopy arm , 14 clusters		• pulmonary TB despite a negative test result, • pulmonary TB without any laboratory result reported, • overall pulmonary TB irrespective of laboratory test result. • the rate of Xpert tests positive for rifampicin resistance • proportion of patients with a rifampicin-resistant Xpert result confirmed by conventional DST (PPV)	
Mupfumi 2014	Single centre pragmatic individually randomised controlled trial	Participants included were consecutive symptomatic and asymptomatic HIV-infected patients initiating anti-retroviral . 18 years old and above Female: 55% HIV infection 100% (HIV clinic) setting:Specialized infectious disease hospital Country: Zimbabwe	Patients provided 2 spot sputum specimens at least 1 hour apart. If patients were unable to expectorate sputum, attempts were made to induce sputum using nebulized 6% hypertonic saline. Samples in the microscopy group had a direct smear performed on each sample followed by staining with auramine O (Leica, Germany). Xpert MTB/RIF assays were performed on direct sputum	Primary outcome:proportion of patients who were diagnosed with ART-associated TB or who died within 3 months of randomisation	Target condition: Tuberculosis Case definition: Patients with at least 1 positive Xpert or microscopy (for “scanty” samples, both smears needed to be recorded as “scanty”)

		Sample size:424			
Ngwira LG 2019	Cluster randomised trial in 12 primary healthcare centres in rural Thyolo district Malawi	Participants included were newly diagnosed with HIV 18 years old and above Female: 55% HIV infection 100% (HIV clinic) setting: primary healthcare facilities Country: Malawi Sample size:1842	Primary healthcare clinics were randomised to either screen TB in newly HIV patients by Xpert MTB/RIF or light emitting diode fluorescence microscopy (LED FM). Symptom screening and sputum evaluation were performed on-site by trained study personnel, and results were provided to participants on the same day. Participants testing positive for active TB were referred for treatment.Participants with TB symptoms but negative Xpert or LED FM results were asked to return in one month and provided IPT at that time if asymptomatic.All patients with positive Xpert or LED FM results had sputum taken for confirmatory culture performed at a central laboratory.All participants were asked to return to study clinics for assessment every three months (with one extra visit when on IPT).	Primary outcome: • all-cause mortality within 12 months following HIV diagnosis Secondary outcomes: • TB treatment outcomes, • TB incidence, • and mortality in subgroups of age (≤35 versus >35 years old), sex, clinical stage (stage I/II versus III/IV), and ART eligibility/CD4 count	Target condition: Tuberculosis in newly diagnosed HIV patients Case definition: Positive Xpert MTB/RIF, LED FM or positive culture
Schmidt 2017	Before and after evaluation cohort to evaluate impact of roll out of Xpert MTB/RIF on detection and treatment of new adults with pulmonary tuberculosis	18 years old and above Adults suspected of pulmonary tuberculosis were included	Data were collected from the electronic NHLS database that records all microbiological tests for TB in the region, including the type of test (sputum smear microscopy, Xpert MTB/RIF or liquid culture) and the result of each test. Unique individuals tested for pulmonary TB were identified by unique laboratory	Primary outcome:Tuberculosis detection Secondary outcome: • Median time to diagnosis	

		Female:45.9% pre, 44.1% post HIV infection was not reported Setting:Primary healthcare in the Cape Winelands East in Weastern Cape. The Cape Winelands is a semi-rural area with a very high estimated total TB case notification rate of 1 400 per 100 000 population Country. South Africa Sample size:15629 pre/10741 post	identifiers. Data from the two periods were compared for the proportion of patients investigated for TB who tested positive by sputum smear microscopy, liquid culture or Xpert MTB/RIF, and the proportion of sputum smear microscopy, liquid culture or Xpert MRB/RIF tests that were positive	• Median time to treatment	
Theron 2014	Pragmatic randomised parallel group multi-centre trial, Eligible patients were randomly assigned to undergo either Xpert MTB/RIF or smear microscopy	Eligible patients had one or more symptoms of TB according to WHO criteria, able to spontaneously expectorate two sputum samples, had not received anti-TB treatment within the previous 60 days, gave informed consent, 18 years old and above Female :43% HIV infection 60%	Intervention group received Xpert MTB/RIFsputum testing and control group smear microscopy sputum testing	**Primary outcome**:Tuberculosis related morbidity (graded using TB score and Karnofsky performance score) **Secondary outcomes:** Feasibility of point-of-care Xpert MTB/RIF testing (accuracy, failure rates, operator protocol adherence, and user appraisals);	Target condition: Tuberculosis case definition: culture positive patient

		Setting: five peri-urban primary healthcare tuberculosis clinics with attached or close-by treatment facilities and microscopy laboratories Countries: South Africa, Tanzania, Zambia, Zimbabwe Sample size: 758 patients randomised to microscopy, 744 to Xpert		Time to diagnosis (overall and at days 1, 2, 3, 14, 28, and 56); Time to anti-tuberculosis treatment initiation (overall and at days 1, 2, 3, 14, 28, and 56); Proportion of culture-positive patients not started on anti-tuberculosis treatment (dropout) or lost to follow up (culture-positive patients started on treatment who were not retained in the study	
Van Kampen 2015	Pre and post study	Criteria: Individuals at high risk of MDR TB according to guidelines, March 2011-March 2013 All age-groups Female: pre 40%%, post 38% HIV: Pre 0.8%, post 2.9% Setting: Three clinics offering programmatic management of drug resistant TB in East, Central and West Java in Indonesia Country: Indonesia	The diagnostic approach in the pre period was to collect one sputum sample from each individual and conduct smear microscopy and culture on solid or liquid media. If the culture was positive for TB, an isolate was re-cultured for first-line DST. During the intervention, one sputum sample was collected for Xpert testing and a second sample was used for diagnostic workup with culture and first-line DST	Proportion of individuals positive for TB Second-line treatment initiation in rifampicin resistant TB patients Time from client registration to diagnosis Time from diagnosis to treatment start	

		Number of eligible patients: pre 871, post 966			
Yoon 2012	A Multicentre implementation study of Xpert MTB/RIF with two phases: baseline and implementation phase using a cohort of patients at Mulago national referral hospital	18 years old and above History of cough more than two weeks but less than six months 48% female HIV infection ,76% settings: Mulago national referral hospital Country:Uganda Sample size:477	In the baseline phase (August 2009–March 2010), Xpert MTB/RIF results were not reported to clinicians or used for patient management. This phase allowed for the collection of baseline data on study outcomes and was necessary for local validation of Xpert MTB/RIF performance compared with conventional laboratory methods. In the subsequent implementation phase (March 2010–August 2010), Xpert MTB/RIF results were provided to clinicians and were used to inform TB treatment decisions. Each sample underwent smear microscopy, Xpert MTB/RIF and Culture	Primary outcome. Two month mortality secondary outcome: time to TB detection and treatment	

10 Appendix 3:

10.1 Search strategy

MEDLINE OVID

1 Xpert*.mp. 2 geneXpert*.mp. 3 Cepheid.mp. 4 near* patient.mp. 5 1 or 2 or 3 or 4
1 (smear adj3 microscop*).mp
2 (sputum adj3 microscopy).mp
3 Sputum/ch, cy, mi [Chemistry, Cytology, Microbiology]
4 6 or 7 or 8
10 exp Tuberculosis/
11 tubercul*.ab. or tubercul*.ti.
12 TB.ab. or TB.ti.
13 Mycobacterium tuberculosis/
14 10 or 11 or 12 or 13
15 5 or 9
16 14 and 15
17 limit 16 to yr="2007 -Current"

This search strategy was adopted for all other electronic databases.

11 Appendix 4

11.1 Eligibility criteria

Patients were eligible to be included in the analysis if they had: (i) two culture available for both first and second samples in both Mycobacterium Growth Indicator Tube (Bactec MGIT; BD Microbiology Systems, Cockeysville, MD, USA) and Lowenstein Jensen (LJ), (ii) had Xpert results available,(iii) were 18 years old and above (iv) were presumptive TB patients enrolled in studies in which clinics were linked to reference laboratories and (v) had a final status in the database that could be defined based on culture results and/or clinical decision. Patients were excluded if they had received treatment within 60 days prior to presentation at the clinic.

12 Curriculam vitae

Personal Information

Name	Dr. Frederick Haraka. MD, MScApEpi
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Brief Personal Statement

I am a clinical epidemiologist working in tuberculosis clinical research programme at Ifakara Health institute. My research focuses on clinical studies and randomized control trials of anti-tuberculosis drugs, diagnostics, test-treatment trials and evidence synthesis. Over the past nine years, my role in clinical research has evolved from a study doctor to currently a principal investigator of several clinical studies. I am experienced in research leadership, supervision of site teams, and hands on in clinical care, management of study participants, research grant application and collaborations. I am currently the site principal investigator of multi-centre, multi-country trial phase 2c of anti-tuberculosis regimen of bedaquilline pretomanid and pyrazinamide combination. I am also a principal investigator of a diagnostic trial, which evaluates the use of C reactive proteins (CRP) test as a triage test for tuberculosis. I have participated and led several projects under TB research programme. Based on the Cochrane review I led on the impact of Xpert MTB/RIF on patient-important outcomes, in December 2019, I was invited by the Global tuberculosis programme of the World Health Organization (WHO) to attend and present the evidence to the guideline development group in the meeting for updating WHO policy.

Education

2016 to 2020	PhD Epidemiology, University of Basel, Switzerland Thesis topic: “Xpert MTB/RIF for diagnosis of tuberculosis: performance variability and impact on patient-important outcomes”
2013	MSc in applied epidemiology, University of Nottingham, United Kingdom Dissertation: Influence of Parental Smoking on Longitudinal Changes in Smoking Behavior from Adolescence to Adulthood: A Historical Cohort Study Using the British Cohort Study-1970**.** (**MSc with merit**)
2009	Doctor of medicine (MD), Muhimbili University of Health and Allied sciences, Dar es Salaam Tanzania Elective: Factors affecting HIV counselling and testing among adults in Muheza District, Tanzania (Publication link: https://www.ajol.info/index.php/thrb/article/view/55817)

Employment history including current position(s)

2010 to date	Research scientist Ifakara Health Institute, Bagamoyo,Tanzania

2013 to date Principal investigator/Project leader, TB research programme, Bagamoyo Tanzania

2011 to 2013 Co-principal Investigator in TB research programme, Bagamoyo, Tanzania

2009 to 2010 Intern doctor, Amana referral Hospital, Dar es Salaam, Tanzania

Teaching positions

2017 to date Lecturer for the Msc in public health research offered by the Ifakara Health Institute under the umbrella of the Nelson Mandela African Institute of Science and Technology- Tanzania*(Sessions: Advance epidemiology, scientific report writing,Tuberculosis)*

2016 to 2017 Part time lecturer for the Msc in public health at Hubert Kairuki Memorial University-Tanzania *(Sessions: Study designs, Randomized controlled trials)*

Major scientific achievement (s)

Invited by the Global TB programme (GTB) of the WHO **to attend and present** evidence to the Guideline Development Group for WHO policy update on molecular assays intended for initial diagnostic of tuberculosis, 3-6 December 2019 Geneva, Switzerland

First authored publications

2018 **Haraka F**, Nathavitharana RR, Schumacher SG, Kakolwa M, Denkinger CM, Gagneux S, Reither K, Ross A. Impact of diagnostic test XpertMTB/RIF® on health outcomes for tuberculosis. *Cochrane Database of Systematic Reviews* 2018, Issue 2. Art. No.:CD012972. DOI: 10.1002/14651858.CD012972

2015 **Haraka F**, Glass TR, Sikalengo G, Gamell A, Ntamatungiro A, Hatz C, et al. (2015) A Bundle of Services Increased Ascertainment of Tuberculosis among HIV-Infected Individuals Enrolled in a HIV Cohort in Rural Sub-Saharan Africa. *PLoS ONE 10(4): e0123275. doi:10.1371/journal.pone.0123275*

2012 **Haraka F**, Rutaihwa L, Battegay M, Reither K, *Mycobacterium intracellulare* infection in a non-HIV infected patient in a region with high burden of tuberculosis*. BMJ Case Reports 2012; doi:10.1136/bcr.01.2012.5713*

2012 **Haraka F**, Mohamed A, Kilonzo G and Shao H, Factors affecting HIV counselling and testing among adults in Muheza District, Tanzania *Journal of Health Research Volume 14, Number 1, January 2012*

2008 **Haraka F**, Bakari M, Assessment of Awareness attitude and perception on HIV vaccine trials among Students at University of Dar es Salaam. *Tanzania medical journal vol 23 No 2 September 2008*

Co-authored publications

2019 Tweed CD, Dawson R, Burger DA, Conradie A, Crook AM, Mendel CM, Conradie F, Diacon AH, Ntinginya NE, Everitt DE, **Haraka F**, Li M, van Niekerk CH, Okwera A, Rassool MS, Reither K, Sebe MA, Staples S, Variava E, Spigelman M. Bedaquiline, moxifloxacin, pretomanid, and pyrazinamide during the first 8 weeks of treatment of patients with drug-susceptible or drug-resistant pulmonary tuberculosis: a multicentre, open-label, partially randomised, phase 2b trial. *Lancet Respir Med. 2019 Dec; 7(12):1048-1058.*

2018 Sabi I, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, Kamwela L, **Haraka F**, Hoelscher M, Paris DH, Saathoff E, Reither K, Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study ***J Infect.*** *2018 Jul 20. pii: S0163-4453(18)30214-7. doi: 10.1016/j.jinf.2018.07.002.*

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2017 R. E. Aarnoutse , G. S. Kibiki, K Reither , H. H. Semvua, **Haraka F**, C. M. Mtabho, S. G. Mpagama, J van den Boogaard , I. M. Sumari-de Boer , C Magis-Escurra, M Wattenberg, J. G. M. Logger,L. H. M. te Brake , M Hoelscher , S. H. Gillespie, A Colbers ,P. P. J. Phillips, G Plemper van Balen , M. J. Boeree , for the PanACEA consortium, Pharmacokinetics, tolerability and bacteriological response of 600, 900 and 1200 mg rifampicin daily in patients with pulmonary TB. *Antimicrob Agents Chemother. 2017 Oct 24; 61(11). pii: e01054-17. doi: 10.1128/AAC.01054-17*

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2013 Pohl C, Jugheli L, **Haraka F**, Mfinanga E, Said K, Reither K Pulmonary Aspergilloma: A Treatment Challenge in sub-Saharan Africa *PLoS Negl Trop Dis 7(10): e2352. doi:10.1371/journal.pntd.0002352*

Grants

2019 European and Developing Countries Clinical Trial Partnership (EDCTP) 700,000 Euro (Co-applicant (SIMPLICI TB consortium) (**Co-applicant**)

2019 European and Developing Countries Clinical Trial Partnership (EDCTP) 100,000 Euro (CAP-TB consortium) **(Co-applicant)**

2016 Foundation for innovative new Diagnostics award 200,000USD for the CRP trial (**Main applicant/Principal investigator)**

2016 Stop TB partnership| TB-REACH initiative award 298,000USD Omni/Ultra trial (**Main applicant/Principal investigator)**

Awards

2012 University of Nottingham developing solutions masters scholarship

2013 European AIDS clinical society exchange programme fellowship

2016 Canton of Basel PhD scholarship

Currently approved research projects

Simplified Short Treatment for Tuberculosis: An **Open-Label, Partially Randomized Trial** to Evaluate the Efficacy, Safety and Tolerability of a 4-month Treatment of Bedaquiline plus Pretomanid plus Moxifloxacin plus Pyrazinamide (BPaMZ) Compared to a 6-month Treatment of HRZE/HR (Control) in Adult Participants with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis (DS-TB) and a 6-month Treatment of BPaMZ in Adult Participants with Drug Resistant, Smear-Positive Pulmonary Tuberculosis (DR-TB)

Funds: TB Alliance/EDCTP; total budget 12Mio Euro; IHI: 700k Euro

Duration: 5/2019 - 4/2022

Role: Site Principal Investigator.

CAP-TB: Close the Gap, Increase Access, Provide Adequate Therapy.

Funds: EDCTP; total budget 5.6 Mio Euro; IHI: 100k Euro

Duration: 9/2019 - 8/2022.

Role: Investigator; PhD studentship; trial currently on-hold because device in new development cycle.

CRP TRIAL: Impact of a point of care C-reactive protein assay used as a triage test on diagnostic yield in tuberculosis screening among outpatients in Tanzania; a pragmatic cluster randomized trial
Duration: 9/2018-June 2020, total budget 200k USD; IHI: 150K USD

Role: Principal investigator; trial currently under ethical review

Memberships

Data and Safety Monitoring Board (DSMB)

Trial Safety Board: TB-IRON TRIAL, ETH, Zurich

Review (Journals):

Tanzania journal of health research

Journal of diabetes

Infectious diseases

Active memberships in scientific societies, academies

Union against Tuberculosis and Lung Disease

Tanganyika medical council

Medical association of Tanzania

References

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18 February 2020