# Investigation pathways for tuberculosis among HIV-positive adults in South Africa

Yasmeen Hanifa

Thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy of the University of London

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Department of Clinical Research

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
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| **First Name(s)** | Yasmeen | | |
| **Surname/Family Name** | Hanifa | | |
| **Programme of Study** | Doctor of Philosophy | | |
| **LSHTM Email** (if this is no longer active, please provide an alternative) | yasmeen.hanifa@lshtm.ac.uk | | |

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# Abstract

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

## ABSTRACT OF THESES

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**Background and aims:** The World Health Organization (WHO) recommendation for regular tuberculosis (TB) screening of people living with HIV (PLHIV) using a symptom screen (WHO tool), with Xpert MTB/RIF (Xpert) as the initial diagnostic test has major resource implications. This thesis examined alternative investigation pathways, including Determine TB-LAM (LF-LAM) for TB screening, a clinical score to triage symptomatic individuals for Xpert, and repeating Xpert if the initial test was negative.

**Design and setting:** Prospective cohort of PLHIV, attending four HIV clinics in South Africa.

**Methods:** A systematic sample of adults attending for routine HIV care were enrolled in the XPHACTOR study, which tested a novel algorithm for prioritising investigation with Xpert. At enrolment sputum was collected from all and sent for immediate Xpert if any of: current cough, fever ≥3 weeks, body mass index (BMI) <18.5kg/$m^2$, CD4 <100 cells/$mm^3$ (or <200 if pre-ART) or weight loss ≥10%; otherwise, sputum was stored. Urine was stored if CD4 <200 cells/$mm^3$.
At attendance for immediate Xpert result, further investigations were facilitated per national guidelines. For those at highest risk of TB, who had negative initial Xpert result, a repeat sputum sample was stored. Participants were reviewed monthly to 3 months, when sputum and blood were taken for mycobacterial culture. At study completion stored sputa were tested with Xpert, and urine with LF-LAM.
We defined TB as "confirmed" if Xpert, line probe assay or culture for *M. tuberculosis* within six months of enrolment were positive, and "clinical" if TB treatment was started without microbiological confirmation.

**Results:** 3722 participants enrolled into XPHACTOR, and 167/3678 (4.5%) fulfilled case definitions for TB (124 confirmed, 43 clinical); 32.6% reported WHO tool symptoms. Amongst 424 participants with LF-LAM results, 56/424 (13%) had TB (40 confirmed, 16 clinical). Using grade 1 cut-off on pre-2014 reference card, LF-LAM sensitivity for confirmed TB (all clinical TB excluded) in CD4<100 vs. CD4 ≥100 was 16.7% (95% CI 4.7%, 37.4%) vs. 6.3% (95% CI 0.2%, 30.2%).
1048 participants who were WHO tool positive at enrolment provided data for development of a clinical prediction model for TB. The final model comprised ART status; BMI; CD4; number of WHO symptoms. When converted to a clinical score, a cut-off score of ≥3 identified those with TB with sensitivity and specificity of 91.8% and 34.3% respectively. If investigation was prioritised for individuals with score of ≥3, 68% (717/1048) symptomatic individuals would be tested, among whom the prevalence of TB

would be 14.1% (101/717); 32% (331/1048) of tests would be avoided, but 3% (9/331) with TB would be missed amongst those not tested.
Amongst 227 participants with an initial negative Xpert result, 28 (12%) had TB diagnosed during study follow-up (16 confirmed, 12 clinical); stored sputum tested positive on Xpert in 5/227 (2%).

**Conclusion:** Sensitivity of LF-LAM as a screening test is too low for use. Our clinical score, which requires external validation, may help prioritise TB investigation among symptomatic individuals. Amongst PLHIV with a negative Xpert result, further investigation using appropriate diagnostic modalities is more likely to lead to TB treatment than immediately repeating sputum for Xpert. More efficient TB case finding strategies are needed for PLHIV established in care, to minimise unnecessary investigation of large numbers who do not have TB.

## Acknowledgements

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I am immensely grateful to all those who so generously gave their time to take part in this research, so that others might benefit in the future; and for the opportunity I had to spend time with study participants at all sites. I am humbled by the difficulties that many have faced and continue to face in their lives, and I hope this experience has made me a better doctor.

I would like to dedicate this work to my father who died shortly after I submitted my thesis. His bravery and resilience never ceased to amaze me, his beautiful smile kept me going, and he showed me by example, that with hard work and dedication anything is possible. I miss him very much.

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## Acronyms

| | |
|---|---|
| AFB | Acid-fast bacilli |
| AIDS | Acquired immune deficiency syndrome |
| AOR | Adjusted odds ratio |
| ART | Antiretroviral therapy |
| AUROC/AUC | Area under the receiver-operating characteristic curve |
| BMI | Body mass index |
| CAO | Chronic airways obstruction |
| CART | Classification and regression tree |
| CD4 | CD4-lymphocyte count |
| CHC | Community health campaign |
| CI | Confidence interval |
| COPD | Chronic obstructive pulmonary disease |
| CPT | Cotrimoxazole preventive therapy |
| CRI | Credible Intervals |
| CRP | C-reactive protein |
| CXR | Chest radiograph |
| DHS | Demographic and health survey |
| DST | Drug-susceptibility test |
| EPTB | Extrapulmonary TB |
| EPV | Events per variable |
| FAST | Fast alcohol screening test |
| FIND | Foundation for Innovative New Diagnostics |
| HFIAS | Household food insecurity access score |
| HIV | Human immunodeficiency virus |
| HTC | HIV testing and counselling services |
| ICF | Intensified case finding |
| IGRA | Interferon gamma release assay |
| ILI | Influenza-like illness |
| IPC | Infection prevention and control |
| IQR | Interquartile range |
| IRIS | Immune reconstitution inflammatory syndrome |
| LAM | Lipoarabinomannan |
| LF-LAM | Lateral-flow LAM assay (Determine TB-LAM; Alere, USA) |
| L-J | Löwenstein–Jensen medium |
| LMIC | Low- and middle-income countries |

| | |
|---|---|
| LPA | Line probe assay |
| LRTI | Lower respiratory tract infection |
| LSHTM | London School of Hygiene & Tropical Medicine |
| MeSH | Medical subject headings |
| MGIT | Mycobacterium growth indicator tube |
| MDR | Multidrug-resistant tuberculosis |
| MSF | Médecins Sans Frontières |
| MTB | *Mycobacterium tuberculosis* |
| MUAC | Mid-upper arm circumference |
| NIMART | Nurse-initiated management of antiretroviral treatment |
| NPV | Negative Predictive Value |
| NTM | Non-tuberculous mycobacteria |
| OR | Odds ratio |
| PHC | Primary health clinic |
| PLHIV | People living with HIV |
| PCR | Polymerase chain reaction |
| PHQ9 | Patient Health Questionnaire-9 |
| POC | Point of care |
| PPV | Positive predictive value |
| PTB | Pulmonary TB |
| TB | Tuberculosis |
| ULTRA | Xpert Ultra (Cepheid, Sunnyvale, CA) |
| UN | United Nations |
| UNAIDS | Joint United Nations Programme on HIV/AIDS |
| URTI | Upper respiratory tract infection |
| USS | Ultrasound scan |
| VL | Viral load |
| WHO | World Health Organization |
| Xpert | Xpert MTB/RIF (Cepheid, Sunnyvale, CA) |
| XPHACTOR | Xpert for people attending HIV/AIDS care: test or review? |
| XTEND | Xpert for TB: evaluating a new diagnostic |

# 1) Introduction

This chapter provides a background to the human immunodeficiency virus (HIV) -related tuberculosis (TB) epidemic in sub-Saharan Africa, and the steps that have been taken to address it. It then describes the changing TB diagnostic landscape in 2011 and the implications thereof for resource-limited settings, which form the rationale for the research undertaken in South Africa for this thesis.

## 1.1. Background

### *1.1.1. Global burden of tuberculosis*

Tuberculosis (TB) has caused illness and death in human beings for thousands of years, and in 2016 was the tenth most common cause of death globally,[1] responsible for the deaths of an estimated 1.3 million HIV-negative and 300,000 HIV-positive individuals.[2] It disproportionately afflicts people living with HIV (PLHIV), who are at around twenty-fold greater risk of TB than HIV-negative individuals,[3] and much more likely to die during and after treatment.[4, 5] Untreated, the 10-year case fatality rate for pulmonary TB in HIV-negative individuals is estimated at 20% for smear-negative and 70% for smear-positive disease.[6] However, once infected with *M. tuberculosis* (MTB), most individuals with a competent immune system do not develop clinically manifest active TB,[7, 8] but are thought to have latent infection, "a state of persistent immune response to stimulation by MTB antigens."[9] Globally there is a large reservoir of latent TB infection (LTBI), around one-quarter of the world's population in 2014,[10] who have a 5-15% lifetime risk of developing disease;[7] the risk of reactivation is far greater in people living with HIV (PLHIV).[11]

Effective drug treatment for TB has been available since the 1940's, and prior to this TB incidence and mortality had been declining in Western Europe alongside improvements in living standards.[12] However, particularly in sub-Saharan Africa, the global HIV epidemic has resulted in a resurgence of TB.[11] In 1993 TB was declared a global health emergency by the World Health Organization (WHO)[13] when identified in the top six contributors to global disease by the 1990 Global Burden of Disease Study.[14] The burden of TB remains vast, mainly affecting South East Asia and Africa, and there were an estimated 10 million incident TB cases globally in 2017.[2] Around one-third of this estimated total were not notified to national TB programmes and deemed "missing"; presumably undiagnosed, unreported, or reflecting the uncertainty around the estimates.[2] Going forward the WHO

goal is to use national TB case notification data directly as a proxy for TB incidence; prerequisites for this are accurate reporting and diagnosis of TB, high quality TB surveillance systems and good coverage of quality healthcare.[15] For 2017, TB incidence estimates were derived for most countries from national case notification data adjusted to account for gaps in case-detection, and for the 23 countries accounting for 60% of global incident cases using data from TB prevalence surveys.[16]

### *1.1.2. HIV-associated tuberculosis*

HIV infection is the strongest risk factor for TB, increasing the risk of disease both after recent infection and due to reactivation of latent infection;[4] recent transmission plays a greater role amongst PLHIV in settings of high HIV and TB prevalence.[17] Immunosuppression caused by the destruction of $CD4^+$ T lymphocytes by HIV infection greatly increases the risk of developing active TB,[4] with risk increasing as CD4 cell counts progressively decline.[18] Sub-Saharan Africa, home to over half of all PLHIV,[19] has borne the brunt of the HIV epidemic, which has fuelled the dramatic resurgence of TB in this region. In 2017, 9% of global TB cases were attributable to HIV infection, with most (72%) residing in Africa.[2] TB remains the leading cause of death amongst PLHIV, responsible for about one-third of all HIV-related deaths,[20] of which again the majority occurred in Africa in 2017.[2]

Early diagnosis with prompt treatment is a key strategy to help reduce HIV-related TB morbidity and mortality, yet delay in treatment for pulmonary TB amongst adults in sub-Saharan Africa is well documented, due to delays in presentation for care and at health system level.[21-23] Tackling TB in PLHIV is made much harder by limitations in existing diagnostic tests, most of which lose accuracy as immunosuppression progresses, and the increased frequency of atypical presentations such as extrapulmonary and disseminated disease.[24] Classical chest radiograph features such as cavitation, known to be associated with increased bacillary load in sputum,[25] are less likely with advanced immunosuppression, [26] and PLHIV are more likely to have smear-negative pulmonary TB.[27-29]

### *1.1.3. Ending the tuberculosis epidemic*

The first global response to the resurgence of TB in the early 1990's was the WHO Directly Observed Treatment Short-course (DOTS) strategy.[30] DOTS standardised the anti-TB treatment regimen, and emphasised diagnosis using quality-ensured sputum microscopy to enable treatment of those most infectious, i.e. smear-positive TB, setting targets for detecting 70% of estimated TB cases and curing 85%. In addition, sustained political and financial commitment, good health-system infrastructure to enable an uninterrupted supply of anti-TB medication, and standardised data collection to enable monitoring of national TB programmes were prioritised. Subsequent global strategies to reduce the burden of TB broadened in scope to address HIV-associated and multidrug-resistant (MDR) TB and set targets firstly within the Millennium Development Goals (MDGs) and Stop TB strategy. MDG 6C to "halt and reverse TB incidence" was attained globally by 2015,[31] and the additional Stop TB partnership targets[32] to halve TB mortality and prevalence compared with 1990 levels by 2015 were almost reached globally at 47% and 42% respectively.[31]

Post-2015 targets lie within the broader Sustainable Development Goals (SDGs)[33] which succeeded the MDGs and the WHO End TB strategy.[34] SDG 3 focusses on health and includes aims to end the epidemics of HIV, TB, malaria and neglected tropical diseases by 2030, and achieve universal health coverage.[33] End TB aims for a 95% reduction in TB deaths and a 90% reduction in the TB incidence rate by 2035 compared with 2015, for zero TB-affected households to experience catastrophic costs due to TB by 2020, and universal drug susceptibility testing (DST).[34] Progress needs to be accelerated to reach the End TB targets. In order to reach the first End TB milestones by 2020 the decline in TB incidence which was 2% per year in 2015 has to reach 4-5%, and TB mortality has to decline from 16% of TB cases to 10%.[35] This slow decline in TB incidence is likely due to ongoing transmission because of delayed diagnosis in individuals with active TB, progression to active TB amongst individuals from the large reservoir of those with LTBI,[36] and a need to also address the social determinants of TB.[37] In September 2018, at a high-level United Nations (UN) meeting, political leaders reaffirmed their commitment to ending the global TB epidemic by 2030. Their pledges included screening all PLHIV regularly for TB, finding the missing people with TB, and by 2022 to have treated 40 million people for TB and 30 million for LTBI (including 6 million PLHIV).[38]

## 1.2. Addressing the burden of TB in PLHIV

In response to the huge burden of HIV-related TB, the WHO produced guidance in 2004[39] which was updated in 2012, recommending a 12 point package of collaborative TB/HIV activities.[40] These comprise activities to ensure delivery of integrated TB/HIV services; "the Three I's for HIV/TB" to reduce the burden of TB in PLHIV and initiate early ART; and to reduce the burden of HIV in patients with presumptive or diagnosed TB. The Three I's strategy comprises: intensified TB case-finding (ICF); providing isoniazid preventive therapy (IPT) for those eligible and early ART; and ensuring TB infection, prevention and control (IPC). ICF, treatment for LTBI and early ART are discussed in further detail below.

### *1.2.1. Intensified TB case-finding*

Intensified TB case-finding, provider-initiated regular screening to identify active TB, aims to diagnose TB earlier and therefore help to reduce suffering, mortality and TB transmission.[36] ICF also enables a healthcare worker (HCW) to rule out active TB in PLHIV prior to treatment for LTBI and ART initiation, thus avoiding inadvertent LTBI treatment for individuals who need TB treatment and minimising the risk of immune reconstitution inflammatory syndrome *(*IRIS).[41]

A **screening tool** for TB aims to distinguish those individuals *likely to have TB* from those who *probably do not have TB*. Individuals identified as likely to have TB require investigation with a **diagnostic test** to detect MTB.[36] In order to reliably rule out TB the tool used for screening requires high sensitivity, i.e. the proportion of individuals with disease correctly identified (*true positives*). The specificity of a screening tool refers to the proportion of individuals without disease who are correctly identified (*true negatives*). There is always a trade-off between sensitivity and specificity, hence a screening tool designed to maximise sensitivity will lose specificity, but combining it with a diagnostic test with high specificity can create an algorithm with high combined sensitivity and specificity.[36]

A TB screening tool with low specificity will result in a large proportion of those screened, many of whom will not have TB, requiring a diagnostic test for TB. This will place a strain on scarce resources in low- and middle-income countries (LMIC) and potentially hinder scale-up of LTBI treatment. For example, if 1000 HIV-positive individuals are screened for TB in a setting where TB prevalence is 5%, using a tool with sensitivity and specificity of

80% and 50% respectively (reflecting the performance of the recommended WHO TB screening tool in PLHIV), then 475 individuals would undergo unnecessary TB investigation (**Table 1-1**).

**Table 1-1 Relationship between result of screening tool with 50% specificity and identification of true negatives**

| Result of screening tool | TB disease | No TB disease | Total |
|---|---|---|---|
| Positive | 40 | 475 | 515 |
| Negative | 10 | 475 | 485 |
| Total | 50 | 950 | 1000 |

*Assumes prevalence of TB is 5%, and screening tool has sensitivity and specificity of 80% and 50% respectively

In the same setting, using a screening tool with sensitivity and specificity of 50% and 70% respectively (reflecting the performance of the recommended WHO TB screening tool in PLHIV on ART),[42] will result in fewer (285) individuals requiring unnecessary investigation for TB (**Table 1-2**).

**Table 1-2 Relationship between result of a more specific screening tool and identification of true negatives**

| Result of screening tool | TB disease | No TB disease | Total |
|---|---|---|---|
| Positive | 25 | 285 | 310 |
| Negative | 25 | 665 | 690 |
| Total | 50 | 950 | 1000 |

*Assumes prevalence of TB is 5%, and screening tool has sensitivity and specificity of 50% and 70% respectively

Health care workers (HCW) may not use a screening tool if they perceive it to be inaccurate and thus poor use of their time in already overstretched services. Cross-sectional surveys from South Africa of consecutive adults leaving primary health clinics, which enrolled those with symptoms suggestive of TB when screened at exit by research staff, found only 4-50% had been asked about TB symptoms and less than 25% to submit a sputum sample by clinic staff.[23, 43, 44] Even amongst those specifically attending for TB-related symptoms, less than 40% reported that clinic staff had requested a sputum sample.

Limitations of these studies include reliance largely on participant self-report of HCW asking about TB symptoms or for sputum, and a large number[44] or lack of data[23] regarding the number of individuals not screened for study eligibility which limits generalisability. The aforementioned studies were conducted between 2011 to 2015,[23, 43, 44] and the low rates of TB screening and investigation are concerning, particularly as the researchers identified bacteriologically confirmed TB in 5% of those who were not investigated by clinic staff.[23, 44] The WHO recommended tool for TB screening and further evaluation of those identified as more likely to have TB are discussed in more detail later, as this is central to the rationale for the research undertaken in this thesis.

### *1.2.2. Antiretroviral therapy*

ART reduces the incidence of TB by about 65%, irrespective of baseline CD4 count, with the greatest reduction in those with advanced immunosuppression.[45] Since 2015 the WHO has recommended ART for all PLHIV ("treat-all"),[46] based on evidence of marked reduction in severe HIV-related (mainly TB and invasive bacterial disease) and non-HIV related illness and death,[47, 48] improved likelihood of restoration of immune function,[47] and reduction in HIV transmission.[49] This strategy increases ART uptake,[50, 51] and modelling using data from sub-Saharan Africa suggests that annual HIV testing with immediate ART could avert 40% of TB cases between 2015 and 2050.[52] Fast-Track targets established to rapidly scale up HIV treatment and prevention strategies for SDG 3, set the first milestone to reach "90-90-90" targets by 2020.[53] These comprise 90% of PLHIV knowing their status, 90% of those HIV-positive receiving ART, and 90% on ART to be virally suppressed; and thus for 73% of all PLHIV to have suppressed viral loads.

In South Africa, at a time when ART eligibility criteria comprised CD4 count ≤ 200 cells/mm$^3$ or advanced WHO clinical stage, almost 20% of ART-eligible individuals systematically screened for TB had previously undiagnosed bacteriologically-confirmed pulmonary TB.[54-56] A systematic review of studies published between 2000 and 2012 estimated a TB incidence rate of 4.17 per 100 person-years (P-Y) in individuals on ART in higher TB burden settings, greatest in those with lower CD4 cell counts.[57] TB is one of the most common causes of mortality within 12 months of starting ART in sub-Saharan Africa.[58] These data highlight the importance of screening for TB prior to and at regular intervals following ART initiation.

### *1.2.3. Preventive therapy for treatment of latent TB infection*

Treatment of LTBI in PLHIV reduces the risk of developing active TB by at least one-third,[59, 60] and is of greatest benefit in those with a positive tuberculin skin test (TST) in whom the risk of TB is reduced by about two-thirds,[59] when compared with placebo. Amongst adults attending HIV clinics in Botswana, extending IPT to 36 months was more effective, largely amongst those with positive TST, in preventing TB than the standard 6 month course; ART had an additive beneficial effect.[60] In South Africa, amongst individuals established or recently initiating ART, 12 months of IPT reduced TB incidence by 37%;[61] and a similar degree of benefit was shown in Ivory Coast when IPT was given for 6 months shortly after early ART initiation.[47] 2018 WHO LTBI guidelines recommend extended IPT (36 months rather than 6 months) in TB endemic settings for adults and adolescents with positive or unknown TST status.[9] TB disease must be excluded prior to preventive therapy, and scale up of LTBI treatment therefore requires a reliable TB screening tool. In South Africa TST is not required prior to IPT; for those on ART IPT duration is 12 months if TST-negative or TST–unknown, and at least 36 months if TST-positive.[62] Here HCW concerns about accurately ruling out TB have been identified as a barrier to IPT implementation.[63, 64]

### *1.2.4. Impact of the Three I's*

Advanced HIV disease related illness, in particular TB, remained the leading global cause of both admission and in-hospital mortality amongst PLHIV in a systematic review covering the decade commencing 2007.[65] Low CD4 cell counts were associated with admission for TB, and less than half of patients were on ART at admission.[65] A review of post-mortem studies of PLHIV in LMIC published between 1992 and 2012 reported a pooled estimate of autopsy prevalence of TB in adults of 40%.[66] A study which prospectively enrolled PLHIV from primary care clinics in South Africa reported a similar prevalence of TB on autopsy.[67] Almost half of TB cases in the review were only diagnosed at post-mortem, and in most cases TB was deemed the primary cause of death and disease was disseminated suggesting advanced immunosuppression.[66] The studies conducted in sub-Saharan Africa suggested an increase, by about 5% each decade between 1992 and 2012, in the autopsy prevalence of TB in adults.[66] However as autopsy studies are generally undertaken in a highly selected group of hospitalised patients, it is not possible to infer that this trend is truly representative of deaths amongst HIV-positive individuals during this period.

The aforementioned burden of TB in hospitalized PLHIV together with reports of poor adherence to TB screening[68] and diagnostic[23, 43, 64, 69] guidelines by healthcare workers in sub-Saharan Africa indicate that much more still needs to be done to tackle HIV-related TB. However recent descriptive analyses suggest a possible population level impact of ART on TB control in countries where HIV has driven the resurgence of TB.[2, 70] In six Southern African countries with HIV-related TB epidemics, between 2010 to 2017 (a period of rapid increase in HIV care and ART coverage) there has been a sustained decline in TB case notifications.[2] South Africa is one of these six countries and here TB incidence is estimated to have declined rapidly at an average annual rate of 7%.[2] An analysis of publicly available data from 2010 to 2015 identified a more rapid decline in estimated TB case notification rates amongst PLHIV than HIV-negative individuals in sub-Saharan Africa, with greater decline in countries with greater ART coverage.[70] It is not however possible to assign causality from these ecological studies, and factors other than ART rollout may also have played a role, e.g. strengthening of health care systems or expansion in IPT provision.[2, 70]

### *1.2.5. WHO 4-symptom TB screening algorithm (WHO tool)*

Screening for TB in order to rule out active disease is the entry point to LTBI treatment, and is recommended before initiation of ART, and at every clinical encounter in an effort to address HIV-related TB.[40] The performance characteristics and cost of the selected screening tool for ICF and the diagnostic test for TB, as well as the prevalence of TB in the population to be screened, are key considerations to ensure both appropriate care and efficient allocation of limited resources. Measures of diagnostic accuracy include predictive values which relate to sensitivity and specificity through disease prevalence. Positive predictive value (PPV) is the proportion of individuals with a positive test result who have disease, and negative predictive value (NPV) is the proportion of individuals who screen negative who are disease-free. The number needed to be screened (NNS) refers to the number of individuals who need to be screened in order to identify one case of TB, is dependent on the characteristics of the screening tool and varies with disease prevalence. NNS is 1/prevalence if the screening test is perfect (100% sensitivity and 100% specificity), and increases as disease prevalence decreases. PPV of a test increases as disease prevalence increases, with most of the gain occurring with increases from the lowest rates of prevalence,[71] and NPV reduces as disease prevalence increases. Another characteristic measured, which is not dependant on the prevalence of the disease, is the negative

likelihood ratio (LRN) which indicates the change in the odds of having a condition if a screening test is negative; the smaller the LRN, the greater the reduction in odds.

In 2008 absence of a simple, standardised, evidence-based tool to rule out TB amongst PLHIV in resource limited settings was identified as contributing to lack of scale up of IPT. Many TB screening studies have been published, from different settings and at different stages in an individual's pathway through HIV care, and each identified a different optimal combination of symptoms and/or clinical features.[55, 72-79] In order to produce a standardised tool WHO facilitated an individual participant data meta-analysis of observational screening studies published between 2002 and 2010,[80] using a gold standard of culture-confirmed TB from any specimen with most studies collecting only sputum samples. The screening tool developed comprises any one of four symptoms (current cough, fever, weight loss or night sweats). It was designed to rule out TB so maximises sensitivity (78.9%), and to minimise the LRN for TB, and for ease of use. However, a consequence of maximising sensitivity is lack of specificity (49.6%), so half of those without TB are incorrectly identified as requiring further evaluation (as illustrated in **Table 1-1**).

The WHO tool has a very high NPV, 97.7% at a TB prevalence of 5% in PLHIV, so absence of all symptoms effectively rules out TB; and in the meta-analysis a NNS of 12 (amongst all participants) was reported at 5% TB prevalence.[80] However its PPV is only 8% (at a TB prevalence of 5%), so the vast majority of those who screen positive will not have TB (475/515 [92%] as illustrated in **Table 1-1**), yet will require further evaluation for TB. This simple screening tool, primarily designed to rule out TB prior to preventive therapy for LTBI in PLHIV, is the recommended tool for ICF in PLHIV at every clinical encounter.[40, 81] Since 2010 WHO has recommended Xpert MTB/RIF (Cepheid, Sunnyvale, CA; henceforth Xpert) as the initial diagnostic test for TB in PLHIV.[82] The combination of a screening tool that lacks specificity with an expensive diagnostic test for TB poses a huge challenge in resource-constrained settings, as discussed further below.

The original WHO meta-analysis and a systematic review undertaken for the 2018 WHO LTBI guidelines to determine the accuracy of the WHO tool to rule out TB in individuals on ART are discussed in further detail in the literature review (**Chapter 2**).[9, 42, 80]

### *1.2.6. Diagnostic tests for active TB*

In 2017, only 64% (6.4 million), 51% (464,633), and 29% (160,684) of the global estimated number of people with an incident TB episode, HIV-related TB, and drug-resistant TB respectively were actually notified to national programmes and then reported to WHO.[2] 14% of notified incident TB diagnoses were extrapulmonary, and amongst pulmonary TB diagnoses only 56% were bacteriologically confirmed. Individuals with coincident pulmonary and extrapulmonary TB are notified as pulmonary TB, hence the actual proportion with extrapulmonary TB is likely to be greater.[83] Access to a rapid, accurate diagnostic test for TB is one key element to addressing the gaps between estimated and notified TB diagnoses, and the three main tests currently used (microscopy, culture and nucleic acid amplification assays), as well as testing for lipoarabinomannan (LAM) which is not recommended for general use are discussed below.

In 2014 WHO identified four high priority areas for new TB diagnostics.[84] These comprised three point-of-care (POC) tests, one non-sputum-based test to detect all forms of TB, one to triage patients for confirmatory testing, and one sputum-based test for pulmonary TB to replace smear microscopy. The fourth product identified was a rapid drug-susceptibility test (DST) for use in lower tiers of laboratory service closer to primary health care. As highlighted by this report, there is a great need in resource-constrained settings for a simple triage test for use at lower levels of care after screening for TB. A triage test would identify amongst symptomatic individuals those at greatest risk of TB who should have confirmatory testing, so that the volume of individuals requiring an expensive diagnostic test (Xpert) can be reduced. Minimum sensitivity (>90%) and specificity (>70%) requirements were agreed by consensus for this test.[84]

#### *Smear microscopy*

First developed in the 1880s, smear microscopy of sputum or other specimens using the Ziehl-Neelsen (ZN) bacteriological stain to identify acid-fast bacilli (AFB) is still widely used in resource-limited settings to identify mycobacteria. It is simple, inexpensive, rapid, and requires little infrastructure, thus making it suitable for use at peripheral laboratory level. However it is insensitive against a gold standard of culture-confirmed MTB, requiring an estimated 5,000 bacilli per milliliter of sputum for a positive result, and cannot distinguish MTB from non-tuberculous mycobacteria (NTM).[85, 86] The reported sensitivity of smear microscopy is highly variable, from 20% to 80%.[85] Sensitivity is further reduced

amongst PLHIV who are more likely to have paucibacillary and extrapulmonary disease.[54, 75, 87] Replacement of conventional microscopy by light-emitting diode (LED) fluorescence microscopy which is 10% more sensitive, inexpensive, and faster, is now recommended by WHO.[86]

### *Mycobacterial culture*

Mycobacterial culture is the current gold standard test for identifying MTB, requiring only 10 to 100 bacilli per millilitre of sputum. However, culture takes time, ranging from 7-14 days (longer if bacillary load is lower) for automated systems using liquid media which are more prone to contamination, to four weeks for a positive result using traditional solid media. Mycobacterial culture is also expensive, requiring infrastructure which may not be feasible at peripheral laboratories, and thus access is limited in resource-constrained settings.[85]

### *Cepheid® Xpert® MTB/RIF assay*

In 2010 WHO recommended Xpert as the initial diagnostic test for PLHIV, replacing sputum microscopy.[82] Xpert is an automated polymerase chain reaction (PCR) based test that provides rapid (turnaround time 2 hours) and simultaneous detection of both TB and rifampicin resistance, and requires only 131 bacilli per millilitre of sputum for detection. Sample processing and PCR take place within the GeneXpert cartridge, so that after the sputum sample is inserted all further processes are fully automated, enabling near patient testing with minimal operator requirements.

A 2014 Cochrane review reported pooled sensitivity and specificity of Xpert for culture-positive pulmonary TB in PLHIV of 79% (95% credible intervals [CrI] 70-86%) and 98% (95% CrI 96-99%) respectively, which is far superior to the performance of sputum smear microscopy.[88] Xpert performs less well for smear-negative, culture-positive pulmonary TB in PLHIV;[56, 89] with pooled sensitivity of 61% (95% CrI 40-81%) vs. 97% (95% CrI 90-99%) respectively for smear-negative vs. smear-positive, culture-positive TB.[88] There does appear to be an incremental yield from additional samples which is discussed further in the literature review (**Chapter 2**).

In order to further improve the diagnosis of HIV-related and paucibacillary TB, Cepheid have developed Xpert Ultra (henceforth Ultra), which is more sensitive than Xpert (90% vs. 77%) for sputum culture-positive TB in PLHIV. Overall, Ultra is also more sensitive than Xpert for sputum smear-negative and culture-positive TB (63% vs. 46%), however specificity is reduced (96% vs. 98%) and this is speculated to be due to detection of DNA from non-viable MTB, which may result in inappropriate TB treatment in individuals without TB.[90] Ultra uses the same GeneXpert platform as Xpert, and WHO has endorsed its use as an alternative to Xpert. A truly POC, battery-operated device, GeneXpert Omni, which is suitable for use in health care facilities is currently in development.[85] Cepheid have, in the interim, launched GeneXpert Edge, a portable, battery-powered, single module system compatible with both Xpert and Ultra cartridges.[91]

In 2013 the recommendations for Xpert were updated to include use in children, for extrapulmonary samples such as cerebrospinal fluid or lymph nodes, and if resource allowed for consideration as the initial diagnostic test in all adults.[92] Although designed to be used near-patient, rollout of Xpert to peripheral health centres has been limited by cost, requirements for an uninterrupted power supply, and a maximum recommended operating temperature of the GeneXpert platform of 30°C. The cost per Xpert cartridge, even with preferentially negotiated pricing which is available only to the public sector in LMIC, is US$9.98.[93] Laboratory cost analysis in South Africa indicates a cost per test conducted (including consumables, equipment, labour and overheads) of US$14.93 for Xpert vs. US$2.25 for ZN smear microscopy (US$3.40 for fluorescence smear microscopy). The cost for liquid culture (Mycobacterial Growth Indicator 960 automated liquid culture system [MGIT]) was US$12.16 in this analysis, so comparable to Xpert.[94] In spite of WHO recommendations, a 2017 Médecins Sans Frontières (MSF) survey of national TB policies and practices undertaken in 29 countries (all but three featuring in at least one WHO high burden category for TB), reported that only 15/28 (54%) had implemented on a wide scale Xpert as the initial diagnostic test for PLHIV and other high risk groups.[95]

In 2011, South Africa made a policy decision, to replace sputum microscopy with Xpert as the initial diagnostic test for TB. From 2010 to 2016, South Africa purchased both the most Xpert cartridges under concessional pricing (11 million) and the most GeneXpert Xpert MTB/RIF modules (four thousand), globally; and in 2016 alone purchased almost 2.5 million cartridges.[96] In spite of the cost implications of replacing microscopy with a much more expensive test, mathematical modelling predicted cost-effectiveness of this strategy in sub-Saharan Africa because of reduction in early mortality on ART[97] and increased TB case finding.[98] Subsequent modelling, using data collected during the rollout of Xpert in South

Africa, found that this strategy had little impact on either cost or cost-effectiveness of TB evaluation and treatment (within 6 months of the initial diagnostic test).[99] From 2017 South Africa commenced a phased rollout of Ultra to replace Xpert.

Two randomized trials in Southern Africa in "real-world" settings, which compared testing sputum with Xpert vs. microscopy in symptomatic patients attending primary health facilities, did not find differences in morbidity, mortality, or the overall proportion of adults starting TB treatment.[100, 101] TB-NEAT was a pragmatic multicenter trial, in which patients were randomly assigned to either on-site Xpert (performed by a nurse) or microscopy (performed by a technician at an attached or nearby laboratory) with provision of same-day test result.[101] Patients who could provide two spot sputum samples were enrolled. One sample underwent mycobacterial culture and the other either same day Xpert or microscopy; patients were asked to wait for the test results, during which time they underwent chest radiography. More patients in the Xpert vs. microscopy arm started same day TB treatment (17% vs. 9%), mainly based on positive Xpert. TB-NEAT reported a high level of empiric TB treatment, largely based on chest radiograph. This ease of access to chest radiography and point-of-care Xpert is not generalizable to most clinics in sub-Saharan Africa and does not reflect routine clinical care in these settings. Although about 15% of culture-confirmed cases were missed in the microscopy group, the proportions of culture-negative patients who received TB treatment did not vary between Xpert and microscopy arms.[101] XTEND was a pragmatic cluster-randomised trial, embedded within the national roll out of Xpert in South Africa, with clusters (laboratories) allocated to either Xpert or microscopy for adults investigated for TB by clinic staff. Investigators found between the two groups no difference in 6-month mortality, the proportion who had started TB treatment, or time to treatment in those with a positive test result. Although mycobacterial culture was not routinely done due to the pragmatic nature of XTEND, in accordance with TB-NEAT these findings also suggested that Xpert had replaced empirical treatment rather than improving TB case-finding.[100]

Analysis of programme data from Cape Town, over a period (2010 to 2014) spanning the national Xpert rollout (2011-2013), found amongst HIV-positive individuals a reduction in TB notification rates of 19% and halving of empiric TB treatment rates, with a slight increase of 3% in the rate of bacteriologically confirmed TB.[102] A similar pattern was seen in notification rates amongst HIV-negative individuals. At the end of the period, however, more than a quarter of HIV-positive TB patients were still treated empirically. The authors postulate that rates of empiric treatment and/or TB case notification rates may have declined during this period, but firm conclusions could not be drawn using this

observational data. The high levels of empiric TB treatment, also reported globally,[2] indicate a great need for accessible, low cost, non-sputum based diagnostic tests capable of detecting both pulmonary and extrapulmonary TB.[84]

### *Testing for urine lipoarabinomannan*

LAM, a cell wall lipopolysaccharide specific to mycobacteria that is detectable in urine, can be tested for using a POC lateral flow LAM assay (LF-LAM) (Determine TB-LAM; Alere, USA). LF-LAM has many attractive attributes, including relatively low cost (around US$ 3.50 per test), rapid results (within 25 minutes), low biosafety risk, and ease of sample collection; but it has poor sensitivity (45% in symptomatic PLHIV for bacteriologically-confirmed TB).[103] LF-LAM sensitivity, against a gold standard of bacteriologically-confirmed TB, is inadequate for use as a screening tool for TB either prior to ART initiation,[104, 105] or on receiving a new HIV-positive diagnosis;[106, 107] studies evaluating its utility as a screening test are discussed in the literature review.

LF-LAM sensitivity increased when evaluated in hospitalised HIV-positive patients with TB symptoms in Uganda and South Africa, particularly amongst those with advanced immunosuppression for whom very high specificity (99%)[108] suggests possible utility as a rule-in test for TB in this population.[109-111] Even in symptomatic, hospitalised PLHIV with advanced immunosuppression, LF-LAM sensitivity is suboptimal; therefore 2015 WHO guidance supports its use only to assist TB diagnosis in symptomatic PLHIV with CD4 counts ≤100 cells/mm$^3$, or those who are seriously ill irrespective of CD4 count.[112] This recommendation is supported by the recently published STAMP trial.[113] STAMP evaluated the impact on 56-day mortality of screening HIV-positive inpatients with urine LF-LAM, within 48 hours of admission.[113] Participants were randomly assigned to standard-of-care evaluation by the attending clinician, or additional urine LF-LAM test. At enrolment sputum was requested from all participants and tested with Xpert. The results of all TB screening tests were reported to the responsible clinical teams only as positive or negative so that the study groupings were not revealed. There was no difference in overall 2-month mortality between groups, but in three pre-specified high-risk groups (CD4 <100 cells/mm$^3$, severe anaemia, clinically suspected TB) mortality was lower in the group who underwent LF-LAM testing. A more sensitive LAM test, Fujifilm SILVAMP TB LAM, developed by the Foundation for Innovative New Diagnostics (FIND) is undergoing clinical evaluation.[114]

## 1.3. Context of the work conducted for this thesis

### *1.3.1. Country setting: South Africa*

South Africa is classified by the World Bank as an upper middle-income country, but has high levels of poverty disproportionately affecting black South Africans. It is one of the most unequal countries in the world, and levels of inequality have increased since the end of apartheid in 1994.[115] South Africa is home to the world's largest HIV epidemic, 19% of the world's HIV-positive individuals; and has the world's largest HIV treatment programme.[116] 2017 data estimate between seven[117] to eight[118] million PLHIV in a country with a population of just 56 million in 2016. Major strides have been made in the fight against HIV, and the country is in the process of ensuring universal health coverage through implementation of national health insurance.[119] In 2015 South Africa rolled out immediate lifelong ART for all PLHIV irrespective of CD4 cell count, and recommended viral load as the preferred option for monitoring treatment success.[120] The following year pre-exposure prophylaxis (PrEP) for key populations was approved.[121]

The Fifth South African national HIV prevalence survey, a cross-sectional household survey undertaken in 2017, reported adult (ages 15 to 49 years) HIV prevalence of 20.6% (26.3% vs. 14.8% in females vs. males), disproportionately affecting black Africans, adolescent girls, and young women; and with a marked geographical variation from 12.6% in Western Cape to 27% in KwaZulu-Natal.[118] In this survey 82% of households approached completed an interview, and amongst eligible adults (ages 15 to 64 years) 68% vs. 58% of women vs. men provided blood for HIV testing. Lower participation rates might explain the low reported HIV prevalence in males as it is not clear if the prevalence estimates reported were adjusted for non-response. The estimated annual HIV incidence in adults (ages 15-49 years) was 0.79%, a reduction compared with 1.79% in the previous 2012 survey.[122]

UNAIDS 2017 data estimate adult (ages 15-49) HIV prevalence of 18.8% and indicate South Africa has reached the first of the 90-90-90 targets, with 90% of all estimated PLHIV aware of their status, 61% of whom are accessing ART, and 47% of those on ART with suppressed viral load.[117] 2017 national survey data report attaining 85%-71%-86% with respect to the 90-90-90 targets.[118] Between 2010 and 2017, the annual number of new HIV infections reduced by 31%, and AIDS-related deaths reduced by 43%, to 270,000 and 110,000 respectively in all age groups.[117]

South Africa has one of the world's worst TB epidemics, driven by its HIV epidemic. In 2017 it ranked second in the world in terms of the percentage of TB patients who were HIV-positive (60%), TB mortality amongst PLHIV (99/100,000 population), and the overall per capita TB incidence (567 per 100,000 population); as well as accounting for 3% of the global total of incident TB cases.[2] 89% of TB cases notified (new and relapse) were reported to have pulmonary disease, and amongst those 65% were reported to be bacteriologically confirmed (largely by Xpert). South Africa appears in all three of the WHO high-burden country lists for TB, TB/HIV, and multidrug resistant (MDR) TB, and ranks ninth amongst the top ten countries with the largest gaps between notification of incident TB cases and best estimate of TB incidence.[2] WHO TB incidence estimates for South Africa are derived from case-notification data adjusted using expert opinion to estimate case-detection gaps. TB incidence estimates have recently been revised in light of the consistent downward trend in TB case notifications; a national TB prevalence survey is currently underway and will better inform incidence estimates. The overall prevalence of drug resistance was found to be high in the 2012-2014 South African TB drug resistance survey.[123] It was 2.8%, 4.6% and 9.3% respectively for MDR TB, any rifampicin and any isoniazid resistance respectively.[123] Compared with the previous 2001-2002 survey the overall MDR TB prevalence was similar, but the prevalence of rifampicin resistance in new cases was much greater; overall prevalence of both MDR and rifampicin-resistant TB was much greater in HIV-positive vs. HIV-negative individuals with TB (3.1% *vs.* 2.0% for MDR TB, and 4.9% *vs.* 3.2% for rifampicin resistant TB respectively).

South Africa performs well in terms of some, but not all, TB/HIV collaborative activity statistics. In 2017, 94% of TB cases had known HIV status, with 89% of those who were HIV-positive on ART, and 53% of PLHIV newly enrolled in care were on TB preventive therapy.[2] As in previous years South Africa accounted for the largest proportion of those newly enrolled in HIV care on IPT (39%) globally. 2015 National Department of Health (NDOH) ART[120] and 2014 TB[124] guidelines recommend regular symptom screening for TB in PLHIV based on the WHO tool, comprising any of: "current cough of any duration, persistent fever >2 weeks, unexplained weight loss of >1.5kg in a month, or drenching night sweats." Subsequent 2017 South African HIV clinicians society guidelines encourage ART initiation on the day of receiving an HIV diagnosis or CD4 count result, and recommend the WHO tool for TB screening prior to ART and IPT initiation.[62] These guidelines advise, prior to ART initiation, further investigation of those with any WHO tool symptom using both Xpert and mycobacterial culture on sputum, plus urine LAM if the CD4 count is <100 cells/$mm^3$. In addition they recommend, if feasible, sputum mycobacterial culture for all individuals with CD4 count <200 cells/$mm^3$ as part of TB screening prior to IPT initiation.

### *1.3.2. TB investigation pathways and HIV care in South Africa*

The research undertaken for this PhD was part of the XPHACTOR study ("Xpert MTB/RIF for people attending HIV care: an interventional cohort study to guide rational implementation"). XPHACTOR methodology and how this thesis links in are described in **Chapter three**.

XPHACTOR was conceptualized in 2011 at a time when the diagnostic landscape for TB was changing, and South Africa had decided to replace sputum microscopy with Xpert as the initial diagnostic test for TB across its entire laboratory service. Prior to this the only available diagnostic tests for TB were microscopy which is insensitive in PLHIV; and mycobacterial culture which is not available at lower tiers of laboratory, is slow and relatively expensive. The role of testing for LAM had yet to be defined for screening or diagnosis of TB in PLHIV, but it had become available as the POC LF-LAM.

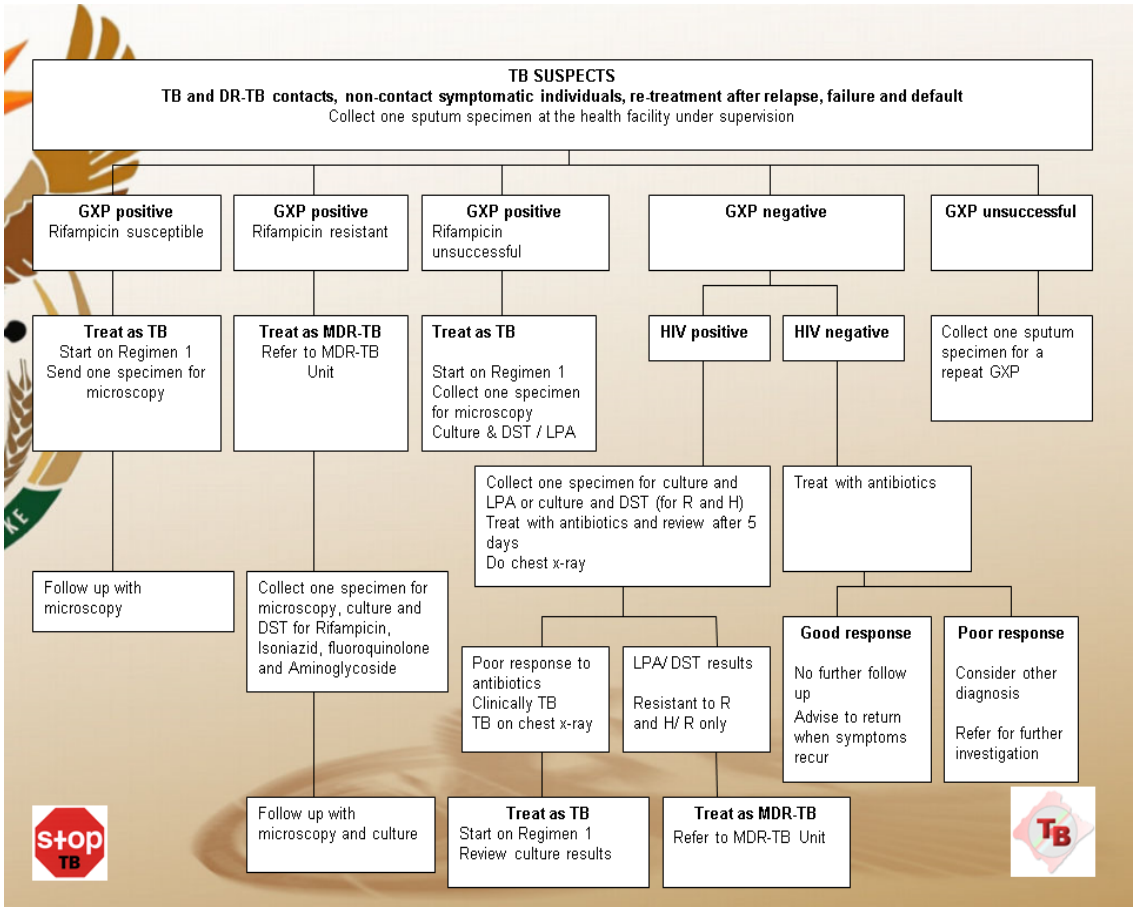
The replacement of microscopy with the far more expensive Xpert test, in the context of a highly symptomatic population of people attending for HIV care, was foreseen to have major resource implications. Resource constraints in LMIC were likely to limit use of this rapid diagnostic test, anticipated at the time to be "game-changing", in the very regions with HIV-related TB epidemics which stood to gain the most. Anecdotal experience from South Africa at the time was that in many clinics ICF guidelines for PLHIV were not followed, possibly because they were considered impractical, and therefore little screening or testing for TB was carried out.

XPHACTOR enrolment and follow-up were conducted between 2012 and 2014, during the national Xpert rollout in South Africa. At this time ART eligibility comprised CD4 ≤350 cells/$mm^3$ or WHO clinical stage ≥3. There was also a clear division between pre-ART care for PLHIV not yet eligible for ART, which was generally only provided in primary care clinics, and care for those on ART which was provided at all levels of care. Historically ART care had been provided at hospital-based clinics with doctor-led ART initiation, but since 2010 nurse-initiated management of antiretroviral treatment (NIMART) has been rolled out, enabling decentralisation of and expanded access to ART.

2014 NDOH guidance, which was also standard-of-care during the XPHACTOR study, recommends further evaluation of ambulatory PLHIV with a negative initial sputum Xpert result aligned with the 2007 WHO algorithm for smear-negative TB.[124] This comprises

clinical reassessment, chest radiograph if available, sputum for mycobacterial culture, and treatment with an antibiotic if clinically indicated (**Figure 1-1**).[124] Mathematical modelling using a decision model from South Africa[125] suggested that replacing sputum culture with a second Xpert (estimated to be cheaper than culture) would reduce loss to follow-up so 1% more patients would start TB treatment,[126] and save an estimated US$17.4 million per year.[126] This model assumed, based on limited data, the same sensitivity for the second Xpert test as for the first, guidelines would be correctly followed, and only 1% of those with TB symptoms would start TB treatment based on a clinical diagnosis.[125, 126] The strategy of sending a repeat Xpert for HIV-positive individuals whose initial Xpert result was negative had not been evaluated empirically at the time of the XPHACTOR study. However of note, current (2016) WHO ART guidelines, post-dating the XPHACTOR study, recommend that further evaluation in those with negative initial Xpert who are not seriously ill should include chest radiograph, clinical assessment and a repeat Xpert on a fresh sputum sample, with mycobacterial culture where feasible.[127]

**Figure 1-1. 2012 South African Department of Health algorithm for Xpert[124]**



## 1.4. Rationale

The rationale for the XPHACTOR study was that the combination of regular TB screening of PLHIV with the WHO tool, which would generate large numbers of patients requiring further investigation (of whom only a small proportion would have TB), and Xpert (the recommended initial diagnostic test), which is more expensive than smear microscopy, would pose a huge challenge in resource constrained settings. Targeting testing to those at greatest risk of TB in these settings was envisaged as a strategy to prioritise resources. XPHACTOR was a prospective cohort study that evaluated an algorithm (described in **Chapter 3**) which aimed to identify, among HIV-positive clinic attendees, those deemed "high priority" for immediate investigation with Xpert, and allowed watchful waiting for those assessed as lower priority.

XPHACTOR provided an opportunity to attempt to answer key questions arising from WHO ICF recommendations, as detailed in the thesis objectives below. Firstly, amongst those reporting WHO tool symptoms, could individuals at greatest risk of TB be identified to enable prioritisation of testing with Xpert? Secondly, amongst those for whom TB had been excluded but who continued to report symptoms, what were the causes for and "natural history" or evolution of these symptoms? Finally, LF-LAM had recently become available, affording an opportunity to evaluate its potential as a screening test for individuals attending for routine HIV care.

## 1.5. Aims and objectives of this thesis

The aim of this thesis is to explore, within the context of regular TB screening for individuals attending for routine HIV care, alternative screening and investigation pathways (to standard of care) at different stages of an individual's journey through evaluation for TB (**Figure 1-2**).

This thesis comprises four studies which address each of the following objectives.

**Objective 1 (Chapter 5 - Research Paper 1, "TB Screening with LAM in an HIV Clinic")**

Firstly, starting with TB screening itself, to evaluate the role of LF-LAM for screening individuals with advanced immunosuppression.

- To evaluate the diagnostic accuracy of LF-LAM among adults with advanced immunosuppression (CD4 cell count <200 cells/$mm^3$) established in HIV care, i.e. not those newly diagnosed HIV-positive.

**Objective 2 (Chapter 6 - Research Paper 2, "Clinical Score for TB in HIV-positive adults in South Africa")**

Secondly, to identify a simple "second step" algorithm (triage tool) to prioritise investigation amongst those identified by the WHO symptom tool as requiring further evaluation. This tool would help HCW decide whom to prioritise for immediate sputum test with Xpert. Again the focus was on individuals established in HIV care.

- To develop a clinical prediction model using data from the XPHACTOR study, comprising elements readily available in primary care, to predict the probability of TB in adults attending for routine HIV care screened for TB and found to be WHO tool positive.

**Objective 3 (Chapter 7 - Research Paper 3, "Investigating TB if initial Xpert is negative")**

Thirdly, amongst those who have been investigated for TB, but have a negative Xpert result, to evaluate the strategy of sending a second sputum sample for Xpert.

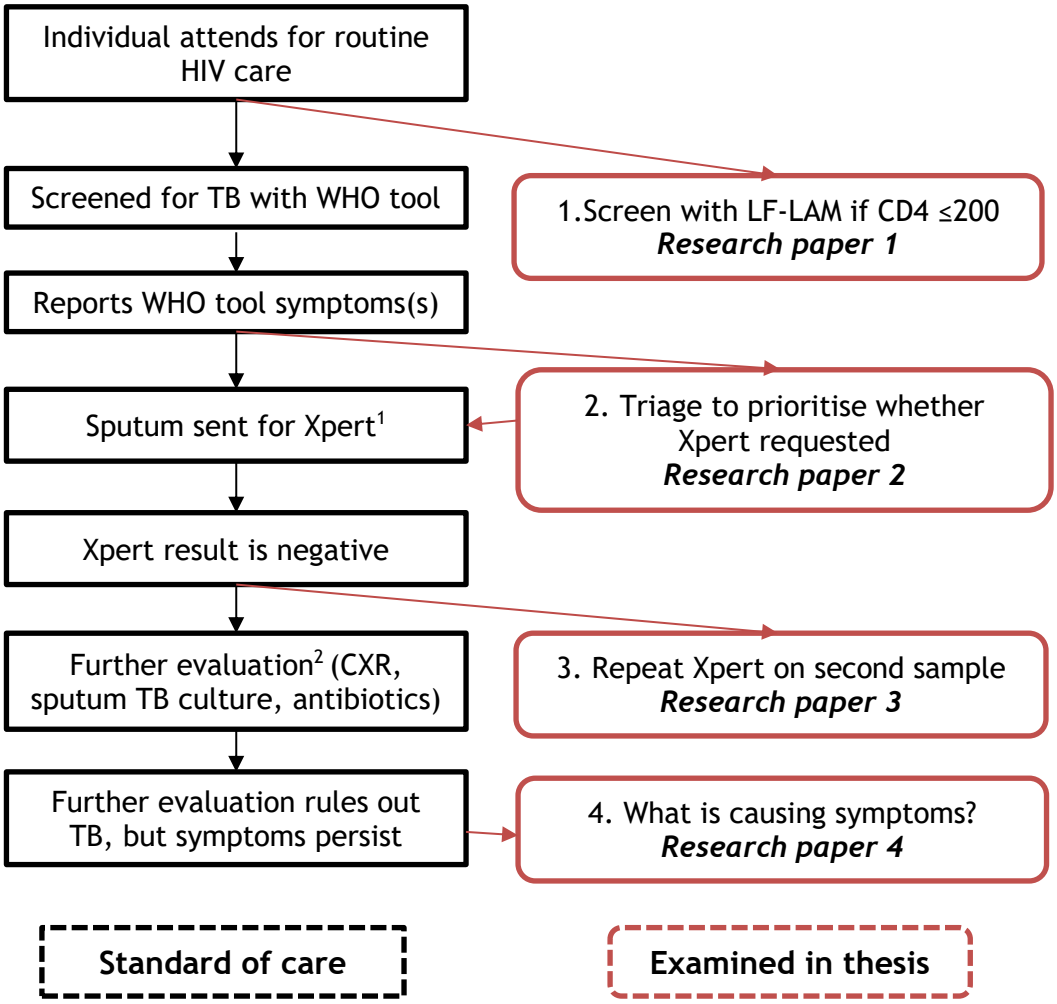
- To describe the diagnostic yield from two different strategies for investigating adults with HIV who are suspected of having TB, but whose first Xpert test is negative. These were immediate repeat sputum tested with Xpert, compared to sequential further investigation guided by NDOH recommendations (sputum for mycobacterial culture, chest radiograph, trial of antibiotics if clinically indicated).

**Objective 4 (Chapter 8 - Research Paper 4, "Causes of TB symptoms in HIV-positive adults")**

Finally, amongst those who have been investigated for and found not to have TB, to attempt to identify the cause for persistent symptoms suggestive of TB.

- To determine causes for persistent or recurrent symptoms suggestive of TB amongst ambulatory adults attending for HIV care who have negative initial TB investigations.

**Figure 1-2. Aims of this thesis**




[1] TB treatment commenced if positive Xpert

[2] TB treatment commenced if positive mycobacteriology or indicated by CXR (chest radiograph) or clinical evaluation

CXR, chest radiograph

## 1.6. Structure of the thesis

This thesis is structured in research paper style format.

**Chapter 2** presents the literature review which addresses each thesis objective, focusing on existing evidence relevant to PLHIV in LMIC settings, and highlighting gaps in the knowledge. Firstly, evidence pertaining to the role of urine LAM as a screening test for TB in individuals attending for routine HIV care. Secondly, an overview of recommended strategies for developing clinical prediction rules to inform further investigation of patients, and then a review of current prediction rules for TB in PLHIV. Thirdly, the evidence pertaining to the utility of repeating Xpert on sputum in PLHIV who have an initial negative sputum Xpert result; and fourthly the causes identified for persistent symptoms suggestive of TB from studies amongst PLHIV in Sub-Saharan Africa.

**Chapters 3 to 4** provide an overview of the XPHACTOR study methods and present key results. Chapter 3 describes the study design, and how the objectives addressed in this thesis flow from XPHACTOR. The frequency of symptoms suggestive of TB in XPHACTOR study participants, both at enrolment and monthly follow up visits is reported in chapter 4. These data provide an indication of the potential volume of testing using Xpert required following screening with the WHO tool, and the extent to which repeat testing may be needed.

**Chapters 5 to 8** provide, in the form of papers which are either published or in press, the methods, results and discussions for each study. **Chapter 5** (**Paper 1**) provides the evaluation of LF-LAM to screen ambulatory PLHIV for TB, and has been published in PLoS One.[128] **Chapter 6** (**Paper 2**) presents the clinical score for TB as published in PLoS One.[129] **Chapter 7** (**Paper 3**) reports the diagnostic yield from repeat Xpert on sputum amongst HIV-positive individuals at high risk of TB, but with initial negative result. This paper has been published by Gates Open Research, but requires revision (which I am currently undertaking) before passing peer review. This chapter, therefore, presents the paper prior to revision. **Chapter 8** (**Paper 4**) presents the causes for persistent or recurrent symptoms suggestive of TB, as published in the International Journal of Tuberculosis and Lung Disease.[130]

**Chapter 9** summarizes the main findings of this research, the implications of these results for clinical practice and wider policy, limitations and generalizability, conclusions and recommendations for future research.

The **appendices** provide the ethical approval documents, consent form, XPHACTOR study enrolment questionnaire, standard operating procedures (SOPs) for Paper 4, and research posters describing the evaluation of the WHO tool for ruling out TB undertaken using XPHACTOR study data[131] and the frequency and seasonal variation of TB symptoms amongst participants on ART.[132]

## 1.7. Role of the candidate

I was the research manager for XPHACTOR, based in South Africa from 2012 to 2014, where I ran the study. The study was conceived and funding acquired by Prof Alison Grant and Dr. Katherine Fielding from the London School of Hygiene & Tropical Medicine (LSHTM), and colleagues at the Aurum Institute and the University of Cape Town in South Africa.

I contributed to protocol development, study design, and obtaining all required regulatory approvals and permissions. I consulted with stakeholders in South Africa at proposed study sites (community health clinics and hospitals), laboratories, and provincial and district level regulatory bodies. In collaboration with colleagues at the Aurum Institute and supervised by Prof Grant, I set up and managed the XPHACTOR study on a day-to-day basis. This included recruiting and training all research staff; monitoring study sites; developing all standard operating procedures (SOPs) and case report forms; assisting with database development, data entry and data cleaning.

I conceptualized and designed the standard set of investigations for participants with persistent symptoms suggestive of TB, assisted by Dr. Sandra Toro Silva from LSHTM, and we personally undertook clinical evaluation of participants together with two clinicians from the Aurum Institute. We also extracted relevant data from participants' medical records.

I conducted all the statistical analyses and interpretation reported in this thesis. I wrote the original drafts of all manuscripts for the research papers presented, incorporated feedback from co-authors, finalized and submitted them for publication.

## 1.8. Ethical clearance

Ethical approval for the XPHACTOR study was provided by ethics committees at the London School of Hygiene & Tropical Medicine (approval # 6165), University of the Witwatersrand in South Africa (approval # M120343), and University of Cape Town in South Africa (approval # 106/2012).

## 1.9. Funding

The research for this thesis was undertaken while I was a staff member at LSHTM. My salary support was provided through the funding awarded for the XPHACTOR study by the Bill and Melinda Gates Foundation (Grant number OPP1034523).

# 2) Literature review

## 2.1. Introduction

This thesis explored alternative pathways for finding TB in HIV-positive individuals established in care. The literature review, therefore, commences by examining the recommended tool for TB screening at each clinical encounter, the WHO symptom screen. The studies in the original systematic review which developed the WHO tool, in the update which investigated its performance in PLHIV on ART, and studies which report the frequency of WHO tool symptoms amongst those on ART are discussed. These studies provide comparison, with respect to the extent to which diagnostic testing for TB may be needed in the context of routine screening, for XPHACTOR which enrolled a population established in HIV care.

Subsequent sections of the review examine the published literature relating to alternative screening and diagnostic pathways for PLHIV in outpatient settings, starting with the utility of LF-LAM as an alternative to TB screening using the WHO tool. Published clinical prediction models are reviewed, both as alternative TB screening tools and also in a triage role, i.e. to prioritise diagnostic testing for individuals who have reported WHO tool symptoms during screening. The review then moves down the Xpert algorithm **(Figure 1-1)** to discuss the literature pertaining to the investigation of individuals with an initial negative Xpert result, specifically looking at the likely diagnostic yield from a repeat Xpert test and the factors that might improve this yield. Finally, in order to guide the investigation of patients who persistently report WHO tool symptoms on screening, the aetiology of these symptoms are summarised from studies investigating PLHIV in outpatient settings. The findings from the review put into context those from this thesis, and also helped to identify gaps in the literature which inform the objectives of this research.

## 2.2. WHO 4-symptom TB screening tool

### *2.2.1. Studies in the original meta-analysis*

The original WHO meta-analysis used data from twelve studies which, irrespective of the presence of symptoms suggestive of TB, undertook mycobacterial culture (mainly on sputum) for all participants.[80] Amongst almost 10,000 PLHIV, mostly from sub-Saharan

Africa, median CD4 count was 248 cells/$mm^3$ (available for 36% of participants), and overall TB prevalence was 5.8% (39% smear-positive pulmonary TB, 52% smear-negative pulmonary TB, 5% extrapulmonary TB only). The final tool comprised presence of any of current cough, fever, night sweats or unintentional weight loss. It had sensitivity and specificity for culture-confirmed TB of 78.9% (95% CI 58.3, 90.9) and 49.6% (95% CI 29.2, 70.1) respectively, and was more sensitive in a clinical setting.

The primary analysis was undertaken in just over 8,000 individuals with complete data from nine of these studies (**Table 2-1**), and investigated the performance of 23 combinations of five candidate symptoms, which were asked about in all studies and deemed easy to assess at all levels of healthcare (current cough, haemoptysis, fever, night sweats and weight loss). Participants in these nine studies were screened for TB in a wide range of settings including HIV testing and counselling (HTC) services;[73, 79] prior to ART initiation;[72, 133] and prior to the widespread availability of ART within the public sector, namely home-based pre-ART care,[76] community-based HIV-TB prevalence surveys,[74, 134, 135] and occupational health services for gold miners.[136] Most of the participants (60%) were contributed by three studies, two household prevalence surveys[74, 134] and one workplace survey.[135] These community-based studies unsurprisingly reported low prevalences of TB (0.4-1.9%), which might also relate to their more stringent criteria for defining culture-confirmed TB. In these studies, if the initial sputum was culture-positive for *MTB*, then further sputum samples were collected for microbiology and chest radiography was performed; and more than one sputum culture-positive for MTB was required to fulfil the case definition for confirmed TB.

The phrasing of the symptom screen naturally varied between studies, in particular with respect to the duration of symptoms, and the time frame for reporting presence of symptoms.[80] Cough > 21 days rather than current cough was stipulated by two studies.[73, 76] The timeframe for reported weight loss varied from presence of symptom within the last 1,[72, 134] 2[79] or 6[135, 136] months; and for night sweats and fever within the last 1,[72] or 2[79] months. These differences might have impacted on the overall diagnostic accuracy of the WHO tool which requires "current" presence of symptom(s). When utilised in a screening tool, stipulating a duration for a symptom will reduce sensitivity but improve specificity; increasing the period during which a symptom can be present will increase sensitivity at the expense of specificity.

Cain *et al*, whose data were included in the meta-analysis, evaluated more than eighty million combinations of between one to five variables (easily obtainable symptoms or

signs) to rule out culture-confirmed TB in clinic attendees prior to ART initiation.[72] Their best performing combinations of three or four predictors (sensitivity 93%, specificities 35-37%, NPV 97%), using data from 1748 participants of whom 15% had TB, all included cough, fever, and night sweats. The findings of their "exhaustive" search support the utility of these symptoms within the WHO tool, and suggest that even though the meta-analysis evaluated only five candidate symptoms, potentially little would have been gained if additional predictors had been included.

A strength of the meta-analysis was the use of individual participant data, but the studies included were largely conducted prior to the widespread availability of ART,[74, 76, 134-136] or if available in individuals who had not yet started ART,[72, 73, 79, 133] and only one study[72] collected non-sputum samples, limiting the ability to identify extrapulmonary TB. The WHO tool, therefore, cannot be assumed to perform similarly in a population on ART; and hence the rationale for undertaking a subsequent review, discussed later in this chapter, to determine its accuracy amongst individuals on ART.[42] Fifteen percent (1478/9629) of participants were excluded due to missing data, rather than using statistical methods such as imputation, which might have resulted in biased estimates of sensitivity and specificity.

The WHO tool has higher sensitivity (90.1%) in clinical settings and amongst individuals who have not been previously screened for TB (88%) (**Table 2-2**).[80] Sensitivity of a screening tool may decline if applied at regular intervals to the same population, and this may have implications when the WHO tool is used, as recommended, at every clinical encounter for PLHIV.[136] This decline in sensitivity can be explained in part by the successful detection of TB by the screening tool (followed by TB treatment) in prior rounds of screening.[137] This changes the characteristics of those remaining, who are less likely to have TB and are also likely to be less symptomatic. The proportion of individuals with active TB will be reduced and this will reduce the PPV of the WHO tool.[74, 136] The three studies which contributed to the assessment of the performance of the WHO tool in a previously screened populations were a household survey,[74] a workplace survey,[135] and one that enrolled gold miners at annual occupational health screening;[136] overall 52/3191 (1.7%) had culture-confirmed TB (**Table 2-1**). It seems unlikely that the sensitivity estimate for the WHO tool in previously screened populations of 40.5% (95% CI: 16.6, 69.9) can be generalised to active TB case finding in HIV clinics. The uncertainty around the sensitivity estimate is wide due to the small number of TB diagnoses. In these studies solid culture media, which is less sensitive than liquid media, was used and may have missed some TB diagnoses, impacting upon the estimation of the sensitvity of the WHO tool for TB in previously screened populations.

**Table 2-1 Characteristics of studies contributing data to the WHO meta-analysis[80]**

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number of PLHIV contributed to meta-analysis[80]<br>Median CD4 cells/mm$^3$ | C+ TB prevalence in PLHIV[80]<br>n/N (%) | Comments |
|---|---|---|---|---|---|
| Getahun[80]<br>2011<br>Metanalysis | • 12 studies (N=9626) including PLHIV<br>• Had to collect sputum & symptoms/sign from ALL, & ≥1 sample for MTB culture<br>• Identified 5 symptoms common to all studies (C,F,S,H,W) | C+ | Meta-analysis included 9 studies (N=8148) evaluable on C,F,S,H,W<br><br>CD4 248 (N=3489) | 557/9626 (5.8%)<br>• 288/557 (52%) SM-ve PTB<br>• 218/557 (39%) SM+ve PTB<br>• 28/557 (5%) EPTB only<br>• 23/557 (4%) site unknown | • 1478/9626 (15%) excluded as incomplete symptom data<br>• Sensitivity: 78.9% (58.3, 90.9)<br>• Specificity: 49.6 (29.2, 70.1) |
| Ayles[134]<br>2009<br>Zambia<br>X-sectional | Community TB/HIV prevalence survey; 2005<br>Randomly sampled households<br>Adults > 15y<br>ALL at enrolment: Symptom screen, 1 sputum (culture), HIV test on oral fluid<br>• Further evaluation if C+ MTB: 2 sputa (SM, culture) + CXR<br><br>>90% consented + had culture result<br>N=8044 in analysis<br>HIV prevalence 2297/8044 (28.6%) | • Confirmed: 2 C+; or 1 C+ & 1 SM+ve 3+<br><br>• Unconfirmed: 1 C+ & TB Rx (based on CXR / symptoms)<br><br>• Subclinical: 1 C+, & no symptoms & CXR normal, & no other positive microbiology | N=2145 | 41/2145 (1.9%) | ART data not collected, but low availability reported |
| Cain[72]<br>2010<br>Cambodia<br>Thailand<br>Vietnam<br>X-sectional | HIV clinic attendees; 2006-2008<br>Consecutive sample<br>Age > 6y (median age 31y)<br>ALL at enrolment: Symptom screen + exam; CXR; SM and culture on sputa (x3), urine, blood, stool, LNA if appropriate; FBC + CD4 | C+ on any sample<br><br>"Not TB": at least 1 sputum C-ve & 1 non-sputum C-ve | N=1721<br><br>CD4 242 | 267/1721 (15.5%) | Excluded those on ART |
| Chheng[73]<br>2008<br>Cambodia<br>X-sectional | HCT attendees<br>Consecutive sample, aged ≥ 19y<br>ALL at enrolment: Symptom screen, 3 sputa (SM, culture) | C+ or ≥2 SM+ve | N=123 | 20/123 (16.3%) | L-J media only |

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number of PLHIV contributed to meta-analysis[80]<br>Median CD4 cells/mm$^3$ | C+ TB prevalence in PLHIV[80]<br>n/N (%) | Comments |
|---|---|---|---|---|---|
| Corbett[135]<br>2007<br>Zimbabwe<br>Cohort | Employees; 2001-2002<br>ALL approached:<br>• HIV test at enrolment<br>• Access to OHS (TB Ix, HTC, HIV care [CPT, IPT, no regular TB screening])<br>• After 2 years - prevalence survey (>90% consented): symptom screen & sputum culture & HIV test.<br>o Further evaluation if TB symptoms or C+: CXR & sputum SM & culture<br><br>HIV prevalence 19%:<br>• Baseline 1233/6440<br>• After 2 years 874/4668<br>o 13/874 HIV-Pos fulfilled TB case definitions, of whom 5 C+ | Prevalent TB:<br>•Definite: 2 C+, or 1 C+ & CXR TB<br><br>•Probable: CXR TB & response to TB Rx within 2m | N=797 | 3/797 (0.4%) | Contributed to "previously screened" population in metanalysis<br>18/1233 (1.5%) on ART at enrolment<br>6% of PLHIV received IPT<br>If asymptomatic: pooled sputum cultured & SM read only if C+<br>L-J media only |
| Corbett[74]<br>2010<br>Zimbabwe<br>X-sectional | Community TB/HIV prevalence survey; 2005-2006<br>Randomly sampled households<br>Adults > 15y<br>ALL at enrolment: Symptom screen, 2 sputa (culture), HIV test<br>• Further evaluation if C+ or TB symptom: Sputum (SM, culture) + CXR<br>>90% consented and submitted sputum<br><br>HIV prevalence 1858/8979 (21%)<br>• 48/1858 fulfilled TB case definition | •Definite: 2 C+,<br>•Probable: 1 C+ & compatible symptoms / CXR, & response to TB Rx within 1m<br>•Probable C-ve MTB: C-ve MTB, & compatible symptoms / CXR, & response to TB Rx within 1m | N=1834 | 31/1834 (1.7%) | Contributed to "previously screened" population in metanalysis<br>If asymptomatic: pooled sputum cultured & SM read only if C+<br>L-J media only |
| Kimerling[76]<br>2002<br>Cambodia<br>X-sectional | Home based HIV care service; 2000<br>Adults ≥ 15y<br>ALL at enrolment: Symptom screen, 1 sputum (culture)<br>• 40/427 (9.4%) previously undiagnosed C+ MTB, & 14/441 already on TB Rx | C+ | N=393 | 36/393 (9.2%) | Unable to verify HIV status for all participants.<br>Only 441/787 (56%) registered with service were surveyed<br>L-J media only |

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number of PLHIV contributed to meta-analysis[80]<br>Median CD4 cells/mm$^3$ | C+ TB prevalence in PLHIV[80]<br>n/N (%) | Comments |
|---|---|---|---|---|---|
| Lawn[133]<br>2009<br>SA<br>X-sectional | HIV clinic attendees prior to ART start<br>Adults ≥ 18y<br>ALL at enrolment: Symptom screen, CXR, 2 sputa (culture), urine stored (LAM)<br>58/235 (25%) fulfilled TB case definition | C+ | N=218<br><br>CD4 125 | 57/218 (26.1%) | |
| Lewis[136]<br>2009<br>SA<br>X-sectional | Gold miners at annual OHS screen (mini CXR + urine); 2000-2001<br>Alternate attendees sampled<br>ALL at enrolment: Symptom screen, mini CXR, 2 sputa (culture), urine HIV<br>• Further evaluation if C+, SM+, TB symptoms, CXR changes: sputa x3 (SM, culture) + CXR<br>HIV prevalence 567/1995 (29%)<br>• 20/567 (4%) fulfilled TB case definition | •Definite: 1 C+ & compatible symptoms / CXR; or 2 C+<br>•Probable: Compatible symptoms & response to TB Rx within 2 m & SM+/ new CXR abnormalities | N=560 | 18/560 (3.2%) | Contributed to "previously screened" population in metanalysis<br>ART not available<br>L-J media only |
| Shah[79]<br>2009<br>Ethiopia<br>X-sectional | New HIV-pos from HTC 2005-2006<br>Consecutive sample; Adults ≥ 18y<br>ALL at enrolment: Symptom screen + exam, CXR, sputa x3 (SM + culture); Blood (CD4)<br>32/438 (7%) fulfilled TB case definition (5 SM+ C-ve) | • SM+ TB: SM+ or C+<br>• SM- TB: 3 SM -ve & C+<br>• "Not TB": none of the above | N=427<br><br>CD4 181 | 27/427 (6.3%) | L-J media only |

ART, antiretroviral therapy; C,F,S,H,W = current cough, fever, night sweats, haemoptysis, weight loss, C+, culture-positive MTB; CI, confidence interval; CPT, cotrimoxazole preventive therapy; CXR, chest radiograph; FBC, full blood count, HIV-pos, HIV-positive; HTC, HIV testing & counselling services; IPT, isoniazid preventive therapy; LNA, lymph node aspirate; OHS, occupational health service; SA, South Africa; SM, TB microscopy; SM+, smear-positive; SM-, smear-negative; TB Rx, TB treatment; L-J, Löwenstein-Jensen media

**Table 2-2 Performance of the WHO tool in different populations[42, 80]**

| Population screened | TB prevalence % | WHO tool positive[1] % | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|
| *Original meta-analysis[80]* | | | | |
| Overall (N=8148) | 5.8% (557/9626) (range: 0.4-25.7) | 49.8% (3981/8148) | 78.9% (58.3,90.9) | 49.6% (29.2, 70.1) |
| Screened in clinical setting | | | 90.1% (76.3, 96.2) | |
| Not previously screened for TB | | | 88.0% (76.1, 94.4) | |
| Screened in community setting | | | 67.1% (41.7, 85.3) | |
| Previously screened for TB | | | 40.5% (16.6, 69.9) | |
| Any WHO tool symptom or abnormal CXR[2] (N=2805) | | | 90.6% (66.7, 97.9) | 38.9% (12.8, 73.3) |
| *Update from systematic review[42]* | | | | |
| On ART (N=4640) | 1.5%[3] (IQR: 0.6, 3.5%) | 29.7%[3] (IQR: 14.3, 45.7) | 51.0% (28.4, 73.2) | 70.7% (47.8, 86.4) |
| Not on ART (N=8664) | | 71.2%[3] (IQR: 46.7, 87.1) | 89.4% (83.0, 93.5) | 28.1% (18.6, 40.1) |
| Any WHO tool symptom or abnormal CXR[2] (N=646) | | | 84.6% (69.7, 92.9) | 29.8% (26.3, 33.6) |

ART, antiretroviral therapy; CI, confidence interval; CXR, chest radiograph; IQR, interquartile range
[1] Any of: cough, fever, night sweats or weight loss
[2] Any of: abnormal chest radiograph or any WHO tool symptom
[3] Median

### *2.2.2. Performance of WHO tool in individuals on ART*

A systematic review and meta-analysis were undertaken for the 2018 WHO LTBI guidelines, to determine the accuracy of the WHO tool to rule out TB in individuals on ART, and to further assess the impact of addition of abnormal chest radiograph to the WHO tool which had been reported to increase sensitivity in the original meta-analysis.[9, 42] **Table 2-2** summarises the findings from the original meta-analysis and the systematic review. The prevalence of TB amongst those on ART was much lower (1.5%) compared to the prevalence in the pre-ART population from the original meta-analysis (5.8%). The systematic review reported a lower pooled sensitivity of the WHO tool (51.0% vs. 89.4%) and greater pooled specificity (70.7% vs. 28.1%) for those on ART vs. those not on ART; estimates did not vary by CD4 count in meta-regression. The estimated NPV in those on ART vs. those not on ART was 99.3% vs. 99.6% at 1% TB prevalence, and 96.5% vs. 98.0% at 5% TB prevalence, enabling TB to be ruled out prior to preventive therapy. Addition of any abnormal chest radiograph findings to the WHO tool increased sensitivity to 84.6% for those on ART.

Seven studies provided data for individuals on ART for the systematic review and these are summarised in **Table 2-3**.[131, 138-143] These studies enrolled participants prospectively, and

evaluated all for TB irrespective of the presence of TB symptoms, against a reference standard of bacteriologically confirmed TB (smear, culture and/or Xpert). The study reporting the highest sensitivity for the WHO tool (49/50 [98%]) enrolled a convenience sample and findings will be biased. It is likely to have overestimated the sensitivity of the WHO tool.[140] Five studies excluded enrolled individuals for whom a final TB outcome could not be assigned, usually because sputum samples were not submitted at enrolment or results were contaminated or not available.[131, 138, 141-143] Three of these studies excluded almost 10% or more of enrolees from their analyses because of lack of sputum mycobacteriology and additional reasons including either data integrity concerns,[138] or failure to complete other study procedures (chest radiograph / TST), or missing data.[143] This will have introduced selection bias, perhaps improving sensitivity estimates, and impacts on the generalisability of findings of these studies. Individuals who cannot produce sputum are less likely to have reported current cough; which may also explain why cough is often the most commonly reported of the WHO tool symptoms in these studies (**Table 2-4**). Exclusion of individuals unable to produce sputum is likely to have impacted on the studies in the original meta-analysis, but it is not possible to assess this due to lack of clear reporting of whether these exclusions occurred.

Rangaka *et al* undertook a secondary analysis of data from their clinical trial which investigated the impact of IPT in individuals on ART, in order to evaluate the effect of ART on the performance of the WHO tool.[143] In the trial a consecutive sample of HIV clinic attendees underwent TB symptom screening and had sputum collected for mycobacterial culture. The WHO tool was retrospectively applied to data collected from these individuals, and all analyses in this study were performed on a dataset with complete data. 661/2090 (32%) of individuals without data for predetermined predictors, or unable to produce sputum, or without culture results were excluded from their analysis.[143] Patients were enrolled from a clinic where TB screening at every visit was already standard of care. Visit were undertaken at 2-4 weekly intervals for individuals preparing for ART, and 4-8 weekly for those on ART. Therefore, those enrolled to this trial had probably been repeatedly pre-screened for TB, and it is unlikely that symptomatic patients were considered for screening for the trial. These factors probably explain the very low proportion (6.6%) of participants who reported WHO tool symptoms, and thus the lack of sensitivity of the tool (23.8%), which as already discussed is less sensitive amongst individuals previously screened for TB.[80] In spite of this, the prevalence of culture-confirmed TB was high and was 5.4%, amongst those on ART. One might speculate that having experienced repeated rounds of screening, participants were more reluctant to

report symptoms or self-identify as symptomatic, or this might have arisen from the retrospective application of the WHO tool.

Two of the studies in the systematic review were conducted amongst pregnant and lactating women.[139, 141] Data for one study undertaken in Swaziland, which appeared to require duration of symptoms for fever or night sweats of two weeks to qualify as a positive symptom screen, were only available as a conference presentation.[139, 144] The WHO tool lacks sensitivity for culture-confirmed TB in pregnancy, which may reflect the impact of ART and previous TB screening. Furthermore, pregnancy itself may influence the presence of TB symptoms, in particular reported weight loss, and other measures of weight loss such as mid-upper arm circumference require evaluation. In this population, the addition of presence of TB symptoms in household members[141] or reported exposure to a TB case[145] is reported to improve the performance of the WHO tool.

Data from XPHACTOR, of which the research undertaken for this thesis forms part, are also included in the updated systematic review.[131] One hundred and forty-three individuals were excluded from the XPHACTOR analysis because of unclassifiable TB outcome (did not satisfy “TB” or “not TB” case definitions), but we did not exclude participants unable to produce sputum at enrolment, therefore our results are less likely to be biased. Strengths of the study include follow up of participants for 3 months, and collection of samples for mycobacteriology at both enrolment and 3-month visit, irrespective of presence of TB symptoms. All other studies presented in **Table 2-3** were cross-sectional with samples collected only at enrolment.

In summary, the findings of the updated systematic review indicate that the WHO tool is less sensitive but more specific amongst PLHIV on ART, compared with those pre-ART.[42] At a median reported TB prevalence amongst those on ART of 1.5% the NPV of the tool was high, enabling TB to be ruled out prior to the provision of IPT. Limitations of the studies in the updated review include exclusions of large numbers of participants who did not produce sputum samples and the inclusion of ANC attendees who might not be representative of PLHIV attending for routine care. A reference standard of culture-confirmed pulmonary TB was used, hence limiting applicability to diagnosing extrapulmonary TB.

**Table 2-3 Sensitivity and specificity of the WHO tool for TB in adults on ART**

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number in analysis<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | WHO-Positive n/N (%) | Sensitivity (95% CI) | Specificity (95% CI) | Comments |
|---|---|---|---|---|---|---|---|---|
| Ahmad Khan[138]<br>SA<br>2014<br>X-sectional | HIV clinic attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 3 sputa (2 smears & 1 culture), CXR | SM+ or C+ | 737 in analysis<br>• 522 on ART (70.8%)<br>CD4 = 365 | On ART: 31/522 (5.9%) | On ART: 233/522 (45%) | On ART: 51.6% (33.1,69.9) | On ART: 55.8% (51.3,60.3) | Excluded from analysis if: no sputum / submitted >14 days after enrolment (15), or data integrity concern (73) |
| Hanifa[131]<br>SA<br>2015<br>Cohort | HIV clinic attendees[1]<br>Systematic sample<br>ALL at enrolment: Symptom screen, 1 sputum (GXP)<br>All at 3 months: Blood & 1 sputum for culture | Confirmed = GXP+ / C+<br>Clinical = TB Rx without +ve MTB microbiology<br>Not TB: No +ve MTB microbiology (≥ 1 specimen) & alive ≥3m after enrolled. | 3229 in analysis<br>• 2439 on ART (75.5%)<br>CD4 = 439 | On ART:<br>Confirmed: 61/2421 (2.5%)<br>All TB: 79/2439 (3.2%) | On ART: 736/2439 (30%) | On ART: 60.7% (47.3,72.9) (confirmed TB) | On ART: 71.1% (69.2,72.9) | Excluded from analysis if: unclassifiable TB outcome (143) or clinical TB (18) |
| Kufa[140]<br>SA<br>2012<br>X-sectional | HIV clinic attendees<br>Convenience sample<br>ALL at enrolment: Symptom screen, 1 sputum & blood (culture), CD4, urine LAM | Confirmed = C+<br>Probable = SM+, compatible histology or CXR | 422 in analysis<br>• 196 on ART (68.1%)<br>CD4 = 264 | On ART:<br>Confirmed: 8/196 (4.1%)<br>All TB: 20/196 (10.2%) | On ART: 162/196 (82.7%) | On ART: 98.0%[2] (89.4,>99.9) (confirmed TB) | Not reported | Convenience sample |
| Calnan[139]<br>Swaziland<br>2016<br>X-sectional | Ante- & postnatal MCH clinic attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, sputum (GXP & culture), urine LAM, TST, IGRA, CXR, CD4 if HIV-pos | Confirmed = C+ | 990 in analysis<br>• 470 HIV-pos | 3.4% amongst HIV-pos | Not reported | 14.3% overall | 82.2% overall | Conference presentation<br>TB screening tool specifies duration of 2 w for fever & night sweats<br>Exclusions not reported<br>Sensitivity & specificity did not vary by HIV / pregnancy status<br>ART use not reported; but 90% PMTCT coverage nationally |

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number in analysis<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | WHO-Positive n/N (%) | Sensitivity (95% CI) | Specificity (95% CI) | Comments |
|---|---|---|---|---|---|---|---|---|
| LaCourse[141]<br>Kenya<br>2016<br>X-sectional | ANC attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 2 sputa (1GXP & 1 culture), Urine LAM, TST | C+ | 288 in analysis (CD4 = 437)<br>• 165 on ART (57.3%)<br>• 62 PMTCT | Overall: 7/288 (2.4%)[3] | Overall: 56/288 (19%) | Overall: 42.9% (9.9, 81.6)<br>On ART: 1/4 (25%) | Overall: 81.1% (76.1,85.5) | Excluded from analysis if: unable to produce sputum or contaminated culture (18) |
| Nguyen[142, 146]<br>Viet Nam<br>2011<br>X-sectional | HIV clinic enrollees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 2 sputa (smear & culture), TST, CXR | C+ | 397 in analysis[4] (CD4 = 336)<br>• 230 on ART (57.9%) | Overall: 28/397 (7.1%) | Overall: 147/397 (37.0%) | Overall: 50% | Overall: 64% | Excluded from analysis if: Did not complete all procedures or NTM / contaminated culture (39) |
| Rangaka[143]<br>SA<br>2012<br>X-sectional | HIV clinic attendees, on / about to start ART, undergoing screening for clinical trial<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 1 sputum (smear & culture) | C+ | 1429<br>• 775 on ART (54.2%)<br>CD4=289 | On ART: 42/775 (5.4%) | On ART[5]: 51/775 (6.6%) | On ART: 23.8% (12.1,39.5) | On ART: 94.4% (92.5,96) | Excluded from analysis if: unable to produce sputum within 1 m of enrolment or missing results; or missing symptoms or predetermined predictors (357 on ART; 304 pre-ART) |

ANC, antenatal clinic; ART, antiretroviral therapy; C+, culture-positive MTB; CI, confidence interval; CXR, chest radiograph; GXP+, Xpert-positive; HIV-pos, HIV-positive; IGRA, interferon gamma release assay; m, month; MCH, maternal child health clinic; NTM, nontuberculous mycobacteria; PMTCT, ART for prevention of mother-to-child transmission; SA, South Africa; SM+, smear-positive; TB Rx, TB treatment; TST, tuberculin skin test; w, week

[1] Analysis of data collected for the XPHACTOR study
[2] 49/50 of all participants who fulfilled case definition for either confirmed or probable TB were WHO tool positive
[3] One additional participant with positive Xpert result was classified as not TB
[4] Data stratified by ART status not reported
[5] WHO tool retrospectively applied

### *2.2.3. Frequency of WHO tool symptoms amongst PLHIV attending for routine care*

The frequency of WHO tool symptoms amongst HIV clinic attendees provides an indication of the volume of testing with Xpert that might be required when it is used for TB screening. **Table 2-4** summarises the frequency of WHO tool symptoms in seven published studies (four of which are already described in **Table 2-3**)[138, 141-143] which systematically enrolled and screened PLHIV established in care, as opposed to studies screening individuals at new HIV diagnosis or before initiation of ART. The proportion reporting any WHO tool symptom varies from 37%-45% in studies including those on ART from HIV clinics,[138, 142, 146, 147] 16-19% in ANC attendees,[141, 145] 33% in those postpartum,[148] and 71% in an exclusively pre-ART group.[138] The most common symptom reported was cough, except by those exclusively pre-ART who most often reported weight loss. The exclusion of individuals unable to produce sputum samples from four of these studies may have introduced selection bias and resulted in a greater proportion reporting cough than would otherwise be found in the context of routine HIV care.[138, 141-143] Adelman[147] *et al* investigated only those who were symptomatic, and Cranmer[148] *et al* did not report on TB diagnoses or investigations; hence it is not possible to estimate TB prevalence in these studies. However, these two studies which enrolled a representative sample of PLHIV, are likely to provide an accurate reflection of the proportion of individuals established in care who have WHO tool symptoms. The data presented in **Table 2-4** suggest that screening for TB using the WHO tool could identify one-third to almost half of individuals on ART, who are attending for routine HIV care, as requiring a diagnostic test for TB.

### *2.2.4. Summary*

The WHO tool was designed to rule out TB prior to IPT using individual level participant data from PLHIV who were pre-ART, and from a disparate range of studies, from community-based TB/HIV prevalence surveys (who supply most of the participants but few TB diagnoses), to HIV clinic attendees. The performance of the WHO tool amongst individuals previously screened for TB utilised data in the original meta-analysis from settings far removed from routine HIV care, i.e. community-based surveys and an occupational health service for gold miners. A subsequent systematic review suggests that it is less sensitive and more specific amongst those on ART.[42] The studies included in the aforementioned review are at risk of bias, having excluded participants unable to provide sputum at enrolment (possibly because they did not have cough) from analyses, limiting

generalisability and an accurate reflection of the impact of previous screening on the performance of the WHO tool. These studies indicate, however, that a minimum of one-third of those on ART report WHO tool symptoms (mainly cough and weight loss) and therefore require a diagnostic test.

**Table 2-4 Prevalence of WHO tool symptoms amongst HIV-positive adults attending for routine care**

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | Prevalence of TB symptoms % | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | WHO+ | C | W | F | N | |
| Ahmad Khan[138]<br>SA<br>2014<br>X-sectional | HIV clinic attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 3 sputa (2 smears & 1 culture), CXR | SM+ or C+ | 737 in analysis<br>• 522 on ART (70.8%) CD4 = 365<br>• 215 pre-ART (29%) CD4 = 200 | On ART: 31/522 (5.9%) | 45%[1] N=522 | 30% | 17% | 7% | 19% | Excluded if: no sputum / submitted >14 days after enrolment (15), or data integrity concern (73) |
| | | | | Pre-ART: 34/215 (15.8%) | 71% N=215 | 46% | 50% | 15% | 30% | |
| Nguyen[142, 146]<br>Viet Nam<br>2011<br>X-sectional | HIV clinic enrollees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 2 sputa (smear & culture), TST, CXR | C+ | 397 in analysis (CD4 = 336)<br>• 230 on ART (57.9%) | Overall: 28/397 (7.1%) | 37%[1] N=397 | 27% | 20% | 7% | 3% | Excluded from analysis if: did not complete all procedures or NTM / contaminated culture (39) |
| Rangaka[143]<br>SA<br>2012<br>X-sectional | HIV clinic attendees, on / about to start ART, undergoing screening for clinical trial<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 1 sputum (smear & culture) | C+ | 1429<br>• 775 on ART (54.2%) CD4=289 | On ART: 42/775 (5.4%) | On ART[1,2]: 51/775 (6.6%) | 5% | 2%[3] | NR | 1% | Excluded from analysis if: unable to produce sputum or missing results, or missing symptoms or predetermined predictors (661) |
| Adelman[147]<br>Ethiopia<br>2015<br>X-sectional | HIV clinic attendees<br>Consecutive sample<br>At enrolment: Symptom screen & **IF** symptomatic 3 sputa (smear, Xpert & culture) | GXP+ or C+ | 828 in analysis (CD4 = 420 [mean])<br>• 730 on ART (89%) | 13/217 (6.0%) who provided sputum had TB | 39% N=828 | 34% | 13% | 19% | 21% | First 5 symptomatic patients enrolled per day. Investigated only if symptomatic (n=321), of whom 35% did not provide sputum. |
| Hoffman[145]<br>SA<br>2013<br>X-sectional | ANC attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, sputa (smear & culture) | C+ | 1403 not on TB Rx at enrolment + 12 on Rx<br>• Amongst all 1415 (CD4 = 394)<br>• Median gest 24 wks<br>• 39% no ART agent | 35/1403 (2.5%) | 16% N=1403 | 8% | 7% | 4% | 3% | WHO tool sensitivity 28%, specificity 84% for previously undiagnosed TB |

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures<br>Exclusions from analysis | TB Case definition | Number<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | Prevalence of TB symptoms % | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | WHO+ | C | W | F | N | |
| LaCourse[141]<br>Kenya<br>2016<br>X-sectional | ANC attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 2 sputa (1GXP & 1 culture), Urine LAM, TST | C+ | 288 in analysis (CD4 = 437)<br>• Median gest 26 wks<br>• 26% no ART agent | 7/288 (2.4%) | 19%[1]<br>N=288 | 15% | 1% | 5% | 7% | Excluded from analysis if: unable to produce sputum or contaminated culture (18) |
| Cranmer[148]<br>Kenya<br>2017<br>X-sectional | Postpartum MCH attendees for 6-wk or 9-month infant immunisation<br>Systematic sample<br>ALL at enrolment: Symptom screen + if symptomatic further evaluation arranged by clinic. | N/A | N=498 (6 wks n=260, 9 months n=238)<br>• 313 on ART (63%)<br>• CD4 483 | Not known | 33%<br>N=498 | 19% | 11% | 15% | 9% | Analysis of data from HIV-positive mothers from national PMTCT-MCH survey.<br>No data re TB investigations / results.<br>15% reported IPT |

ANC, antenatal clinic; ART, antiretroviral therapy; C+, culture-positive MTB; CXR, chest radiograph; GXP+, Xpert-positive; MCH, maternal child health clinic; PMTCT, ART for prevention of mother-to-child transmission; SA, South Africa; SM+, smear-positive; TB Rx, TB treatment; TST, tuberculin skin test; WHO +, WHO tool positive

C W F N, cough, unintentional weight loss, fever, night sweats

[1] Sensitivity and specificity of WHO tool in this population presented in Table 2-2; [2] WHO tool retrospectively applied; [3] Self-report or documented

## 2.3. Urine lipoarabinomannan as an alternative TB screening tool for PLHIV

### *2.3.1. Introduction*

Testing for LAM is unique within the array of available TB diagnostics, as firstly it has greater sensitivity for TB in HIV-positive compared with HIV-negative individuals,[149, 150] and secondly amongst HIV-positive individuals sensitivity is greater in those with more advanced immunosuppression.[103] The test itself has gone through a number of iterations, with a commercially available laboratory-based urine LAM Enzyme-Linked Immunosorbent Assay (ELISA) (Clearview TB-ELISA; Alere, USA) preceding the current LF-LAM formulation; as has the reference card for LF-LAM. Prior to 2014, the manufacturer's reference card for LF-LAM comprised five grades of colour intensity. The least intense band was assigned grade 1, absence of a band graded negative, and absence of a control band deemed a failed test. In order to improve the specificity of the test, in January 2014 the reference card was revised to include only four bands, with band intensity grade 1 on the new card corresponding to grade 2 on the previous card. Therefore, evaluations of the diagnostic accuracy of LF-LAM have used varying cut-offs to define a positive LF-LAM test, which needs to be considered when comparing the performance of LF-LAM between studies.

A 2016 Cochrane review of LF-LAM for diagnosing TB in PLHIV reported its utility, against a reference standard of microbiologically-confirmed TB, firstly as a diagnostic test for individuals who were unwell (largely from inpatient studies) and secondly when used as a screening test in outpatient settings.[103] In the review a grade 2 cut-off on the pre-2014 reference card was deemed a positive LF-LAM result. For diagnosing bacteriologically-confirmed TB amongst symptomatic PLHIV the median pooled sensitivity and specificity were 45% (95% CrI 29%, 63%) and 92% (95% CrI 80%, 97%) respectively. Sensitivity was greater amongst those with CD4 ≤100 vs. >100 cells/mm$^3$, 56% (95% CrI 41%, 70%) vs. 26% (95% CrI 16%, 46%); although specificity was slightly less at 90% (95% CrI 81%, 95%) vs. 92% (95% CrI 78%, 97%) respectively.

Performance of LF-LAM for TB screening was reported from three studies in the Cochrane review, and sensitivity and specificity ranged from 0-44% and 94-95% respectively in these studies.[103] 2015 WHO guidance[112] and the Cochrane review[103] therefore advise against the use of LF-LAM as a screening test for TB because of its poor sensitivity in this context. However, because its sensitivity is better in symptomatic PLHIV, particularly amongst those with very low CD4 cell counts and its specificity is consistently high, it is suggested as an ancillary test to assist TB diagnosis in symptomatic PLHIV with CD4 counts ≤100 cells/mm$^3$, or those who are seriously ill (presence of any of respiratory rate > 30/minute,

temperature >39°C, heart rate > 120/minute, or unable to walk unaided) irrespective of CD4 count.[112] 2017 South African HIV clinicians society guidelines, in keeping with this guidance, recommend that all who have WHO tool symptoms when screened prior to ART initiation should be investigated with both Xpert and mycobacterial culture on sputum, and LF-LAM if CD4 count <100 cells/mm$^3$.[62]

At the time the research was commenced for this thesis, LF-LAM had recently become available, and the opportunity was taken to investigate how it performed as a TB screening test for individuals established in HIV care. Published studies reporting the diagnostic accuracy of LF-LAM as a screening test for PLHIV in outpatient settings, but not those undertaken in inpatient settings are discussed below.

### *2.3.2. Performance of LF-LAM as a screening test for TB in outpatient settings*

Eight published studies report LF-LAM performance for screening ambulatory PLHIV for TB. These can be divided into those screening PLHIV at first HIV-positive diagnosis (3),[106, 107, 151] prior to ART initiation (3),[104, 105, 152] and those established in care (2).[141, 153] Two of these studies are not discussed further, because LF-LAM performance data is not reported separately for inpatient vs. outpatients,[152] or because the data report the same population of participants from a subsequent study.[107] The remaining six studies are summarised in **Table 2-5,** four[104-106, 141] of which were included in the 2016 Cochrane review.

Two studies screened clinic attendees established in care, both using the current recommended cut-off to define positive LF-LAM result. Thit *et al* enrolled patients from a tertiary level hospital in Myanmar, a country where levels of HIV-TB coinfection are lower than in sub-Saharan Africa where most other LF-LAM studies have been performed.[153] Participants were followed for six months to confirm TB diagnoses, and 70% were on ART. Against a reference standard of bacteriologically-confirmed TB (Xpert or culture) they report an unusually high sensitivity of 63% and an unusually low specificity of 69%, using the current recommended cut-off for positive LF-LAM. The investigators do not report data regarding study exclusions or those who declined to take part, so selection bias is possible. All LF-LAM testing was undertaken by research doctors who were blinded to clinical details, and the investigators question whether the poor specificity arose from the particular batch of tests used, or the study doctors' reading of the test result. LaCourse *et al* screened ANC attendees, of whom around 80% were either established on ART or had commenced it as part of prevention of mother to child transmission (PMTCT).[141] In this

cross-sectional study, 20% of those screened declined to participate, and there were only seven culture-confirmed TB diagnoses, none of which were LF-LAM positive.

Drain *et al* screened individuals at new HIV-positive diagnosis, using LF-LAM performed by study nurses on fresh urine samples, and reported sensitivity of 30.9% and specificity of 92% for culture-confirmed TB, using the pre-2014 grade 1 cut-off to define a positive LAM result.[151] Using the same more sensitive cut-off, two other studies which screened participants prior to ART initiation, reported sensitivities and specificities for bacteriologically confirmed TB of 25.8-28.2% and 92.9-98.6% respectively.[104, 105] The aforementioned studies froze urine samples, for later laboratory-based testing with LF-LAM.[104, 105]

Only one study undertook prospective follow up to confirm TB diagnoses and collected extrapulmonary samples for TB culture (if clinically indicated), however submission of at least one sputum sample was one of the study inclusion criteria, introducing bias.[154] Lawn *et al* screened all participants at enrolment (unless pregnant) with chest radiograph.[105] Underdiagnosis, in particular of extrapulmonary TB is likely in these studies, because of limited investigation for extrapulmonary TB, and this might have resulted in underestimation of LF-LAM sensitivity. Very few participants enrolled to these studies are reported (where data are available) to have been unable to provide a urine sample, suggesting ease of sample collection. However it is likely that those unable to produce urine declined to participate, so urine sample collection might not be as straightforward as suggested.

**Table 2-5 Performance of LF-LAM for TB screening amongst PLHIV in outpatient settings**

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures | TB Case definition<br><br>LF-LAM cut-off | Number in analysis<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | LAM-Positive n/N (%) | Sensitivity n/N, % (95% CI) | Specificity n/N, % | Comments |
|---|---|---|---|---|---|---|---|---|
| ***Screened at new HIV-positive diagnosis*** | | | | | | | | |
| Drain[151]<br>2016<br>SA<br>X-sectional | Newly diagnosed HIV-pos (outpatients)<br>ALL at enrolment: Symptom screen, 1 sputum induced if necessary (solid & liquid culture), urine LF-LAM, CXR if indicated. | Definite TB: C+<br><br>Positive LF-LAM = Gd 1 pre-2014 card | 726/757 (95.9%) produced urine<br><br>675 in analysis<br>CD4 = 213 | Definite: 123/675 (18.2%) | 89/675 (13.2%) | Definite TB: 38/123, 30.9% | Definite TB: 508/552, 92.0% | Excluded from analysis if:<br>• No culture result (51)<br>• No urine (31)<br><br>LF-LAM by study nurse<br>Includes same study population as below[106] |
| Drain[106]<br>2015<br>SA<br>X-sectional | Newly diagnosed HIV-pos (outpatients)<br>ALL at enrolment: Symptom screen, 1 sputum induced if necessary (solid & liquid culture), urine LF-LAM, CXR if indicated. | Definite TB: C+<br>Clinical TB: Started TB Rx within 9 m without +ve MTB microbiology<br><br>Positive LF-LAM = Gd 1 pre-2014 card or Gd 2 | 351 enrolled<br><br>320 in analysis<br>CD4=248 (n=288) | Definite: 54/320 (16.9%)<br><br>Clinical: 14/320 (4.4%) | Either test<br>Gd 1: 43/320 (13.4%)<br>Gd 2: 32/320 (10.0%) | Definite TB:<br>Gd 1 either test: 22/54, 40.7%<br>• CD4<100: 55.6% (35-75)<br><br>Gd 2 either test: 15/54, 27.8%<br>• CD4<100: 37.0% (19-58) | Definite TB:<br>Gd 1 either test: 242/266, 91.0%<br><br>Gd 2 either test: 249/266, 93.6% | Excluded from analysis<br>• No LAM or TB culture result (31)<br><br>2 LF-LAM tests per sample by study nurse. Similar sensitivity / specificity to 1 test per sample |
| ***Screened prior to ART initiation*** | | | | | | | | |
| Balcha[104]<br>2014<br>Ethiopia<br>Prospective | HIV clinic attendees<br>ART-eligible<br>Consecutive sample<br>ALL at enrolment: Symptom screen, ≥1 sputum (liquid culture, GXP), blood (FBC, CD4), 50 ml urine<br>LNA (culture & GXP) if indicated<br>Outcomes reviewed after 6m | Definite TB: GXP+ / C+<br>Clinical TB: TB Rx without +ve MTB microbiology<br>Not TB: None of the above<br><br>Positive LF-LAM = Gd 1 pre-2014 card | 757/812 (93.2%) produced urine<br><br>757 in analysis:<br><br>CD4 = 211 | Definite: 128/757 (16.9%); 126 PTB<br><br>Clinical: 20/757 (2.6%); 15 PTB | 78/757 (10.3%) | Definite TB[1]: 33/128, 25.8%<br>• CD4<100: 52.8% (35.7–69.3) | Definite TB[1]: 566/609, 92.9% | Study exclusion criteria:<br>• Unable to produce sputum<br>Excluded from analysis if:<br>• No urine (55)<br><br>Urine frozen & tested when all enrolment completed<br>LF-LAM by lab technician blinded to clinical details |

| Author<br>Country<br>Year<br>Design | Study population<br>Procedures | TB Case definition<br><br>LF-LAM cut-off | Number in analysis<br>Median CD4 cells/mm$^3$ | TB prevalence n/N (%) | LAM-Positive n/N (%) | Sensitivity n/N, % (95% CI) | Specificity n/N, % | Comments |
|---|---|---|---|---|---|---|---|---|
| Lawn[105]<br>2012<br>SA<br>X-sectional | HIV clinic attendees prior to ART start<br>Consecutive sample<br>ALL at enrolment: Symptom screen, CXR, 2 sputa (liquid culture, GXP induced if necessary), urine for LF-LAM | Definite TB: C+<br><br>Positive LF-LAM = Gd 1 pre-2014 card | 595/602 (98.8%) produced urine<br><br>516 in analysis<br>CD4 = 170 | Definite: 85/516 (16.5%) | 30/516 (5.8%) | Definite TB: 24/85, 28.2%<br><br>CD4<100: 51.7% (95% CI, 32·5–70·6) | Definite TB: 425/431, 98.6% | Excluded from analysis if:<br>• Unable to produce sputum (60)<br>• Culture contaminated or no GXP result (19)<br>• No urine (7)<br><br>Urine frozen prior to testing in laboratory |
| ***Screening PLHIV established in care*** | | | | | | | | |
| Thit[153]<br>2017<br>Myanmar<br>Prospective | Hospital attendees (in & outpatients) – tertiary level<br>Consecutive sample<br>ALL at enrolment: Symptom screen + exam, 1 sputum induced if necessary (L-J culture, GXP), CXR, urine LF-LAM<br>Outcomes reviewed after 6m | Definite TB: GXP+ / C+<br>Clinical TB: TB Rx without +ve MTB microbiology<br>Not TB: None of the above<br><br>Positive LF-LAM = Gd 1 post-2014 card | 517 in analysis<br>• 463 outpatients<br>• 54 inpatients<br><br>CD4 = 270<br>On ART = 360/517 (70%) | Outpatients:<br>Definite: 46/463 (9.9%)<br><br>Clinical: 103/463 (22.2%) | Outpatients:<br>166/463 (35.9%) | Outpatients:<br>Definite TB[1]:<br>29/46, 63.0% | Outpatients:<br>Definite TB[1]:<br>217/314, 69.1% | Exclusions / number declining not reported, possible selection bias<br><br>LF-LAM by research doctor blinded to clinical details. Clinical team unaware of LF-LAM result. |
| LaCourse[141]<br>2016<br>Kenya<br>X-sectional | ANC attendees<br>Consecutive sample<br>ALL at enrolment: Symptom screen, 2 sputa (1GXP & 1 liquid culture), Urine LAM within 8 hours of collection, TST | Definite TB: C+<br><br>Positive LF-LAM = Gd 1 post-2014 card | 288 in analysis<br><br>CD4 = 437<br>On ART = 165/288 (57.3%)<br>PMTCT = 62/288 (20.3%) | Overall: 7/288 (2.4%)[3] | 13/266 (4.9%) | Definite TB: 0/7 | Definite TB: 95.1% | 76/388 (19.6%) screened, declined to participate<br>Excluded from analysis if:<br>• Unable to produce sputum (14)<br>Contaminated sputum (4) |

ANC, antenatal clinic; ART, antiretroviral therapy; C+, culture-positive MTB; CI, confidence interval; CXR, chest radiograph; GXP+, Xpert-positive; HIV-pos, HIV-positive;; PMTCT, ART for prevention of mother-to-child transmission; SA, South Africa; SM+, smear-positive; TB Rx, TB treatment

[1] Excluded clinical TB

### *2.3.3. Summary*

In spite of its relatively low cost and ease of sample collection, LF-LAM evaluations undertaken in outpatient settings demonstrate inadequate sensitivity (even if CD4 <100 cells/mm$^3$) to replace the WHO symptom screen for TB screening. Limitations of these studies include lack of prospective follow-up, and reliance on an imperfect reference standard of culture-positive pulmonary samples which will not identify TB in those who have solely extrapulmonary disease.

LF-LAM's high specificity has been suggested as indicating a role as a rule in test for TB. However a retrospective record review of a small number of HIV-positive inpatients with disseminated nontuberculous mycobacterial (NTM) disease identified false-positive LF-LAM result in 19/21 who had negative TB microbiology.[155] These patients had advanced HIV disease, with median CD4 count of 5 cells/mm$^3$, and represented only a tiny fraction of the 1687 inpatients evaluated by the infectious disease consultation service in the tertiary level hospital in South Africa over a one-year period. However the authors highlight that caution is required to ensure that a positive LF-LAM result in a seriously unwell PLHIV, in whom TB treatment may well be clinically highly appropriate, does not preclude investigation and treatment for other likely diagnoses.

## 2.4. Clinical prediction models as alternative TB screening tools or as triage tools for symptomatic PLHIV

### *2.4.1. Introduction*

In order to reduce the volume of Xpert testing undertaken, the WHO has highlighted the need for a low-cost triage test, to identify amongst symptomatic individuals, those requiring confirmatory testing for TB.[84] A suitable triage test has not yet been identified and possible candidate tests are discussed in **Chapter 9.** A clinical prediction model, used as a "second step algorithm" in symptomatic individuals could fulfil this role. Clinical prediction models (also known as clinical prediction rules, prognostic models or risk scores) combine the characteristics of an individual and/or a particular disease (*predictors*), to predict a particular outcome. Diagnostic prediction models predict the likelihood that an outcome, e.g. TB disease is present, whereas prognostic models predict the probability that a particular event might occur in the future.[156, 157]

Clinical prediction models are increasingly abundant in the literature, with variable quality of construction as well as reporting, as highlighted by the TRIPOD statement which presents a recommended reporting framework,[157, 158] and the CHARMS checklist for systematic reviews of prediction modelling studies.[159] Developing a clinical prediction model is not a straightforward process, with consensus yet to be reached on key steps such as selection of candidate predictors and model building.

In this section I will present an overview of recommended strategies for developing a clinical prediction model,[159, 160] and subsequently using these assess published prediction models for active TB in PLHIV undertaken in outpatient settings in LMIC.

### *2.4.2. Recommended strategies for developing prediction models*

#### *Choice of model*

Regression models are the most widely used statistical models for clinical prediction, and multivariable logistic regression the most commonly used technique for developing diagnostic prediction models. Alternative statistical models include classification and regression tree modelling (CART) and artificial neural networks, both of which require large datasets.[156] The CART method is based on splitting patients into pairs of groups based on cut-off levels of predictors which maximally separate (discriminate between) the

two subgroups in terms of the outcome, but within the subgroups there is minimal variability.[156] The predictor which causes the largest separation is placed at the top of the tree, and splitting continues until a minimum size is reached or groups become homogenous. However, although these models provide a simple graphical display which is easy to understand, they must always categorise continuous variables thereby losing information, and have limited power as they quickly run out of "cases" within branches.

In neural networks the relationship between the outcome and the input variables is determined entirely by the data, with errors from initial predictions fed back into the network, so the network learns by example.[156] Candidate predictors based on clinical knowledge or literature review cannot be *a priori* included in the final model developed using a neural network. This type of model is less likely to be used in a clinical setting, where the structure of the model and the predictions need to be clinically credible to a healthcare worker, who holds responsibility for the consequences of all decision making.[161]

### *Candidate predictors*

Candidate predictors are all the variables that are considered for possible inclusion in the model, and not just those in the final selected multivariable model.[160] There is no consensus on the best method for selecting candidate variables, but suggested approaches include using literature review, clinical knowledge and studying the distribution of predictors in the study data.[156, 157, 160] Dichotomising continuous predictors is discouraged, as this results in both loss of information and statistical power.[162] Continuous predictors cannot be assumed to have a linear relationship with the outcome, and non-linearity should always be explored using appropriate statistical techniques.[160]

### *Sample size*

The sample size requirements for prediction studies are determined by the number of outcome events.[156, 159] In order to reduce the risk of overfitting, i.e. fitting a model that describes well the features of the data studied (including any quirks of the data), but does not predict reliably in new individuals the number of outcomes in the data relative to the number of predictive variables (events-per-variable [EPV]) is a key consideration.[156, 159] These predictive variables include not only all candidate predictors, but also the indicator variables for categorical predictors and transformations for continuous predictors. EPV of at least ten is recommended to ensure predictive accuracy.[163]

### *Handling of missing data*

The recommended strategy for handling missing data in prediction studies is to use the multiple imputation method, which replaces missing observations with values estimated from the available data. Omitting all data from participants who have missing values (complete-case analysis) risks developing a prediction model in a subset of individuals who are not representative of the original sample, [159] but might be considered if less than 5% of observations are missing.[160]

### *Model building*

A common method of selecting predictors for inclusion in the multivariable modelling (predictor pre-selection) is based on the strength of their univariable association with the outcome. This method risks predictor selection bias, i.e. predictors with large but spurious associations with the outcome are selected, and important predictors which may become associated with the outcome after adjustment for other variables are rejected.[159, 160]

There is no consensus on how best to select variables during multivariable modelling, but backward elimination and the full model approach are considered to reduce the risk of overfitting. In the full model approach, all candidate predictors are included in the model. In backward elimination the model starts with all candidate predictors, then a sequence of statistical tests is run to select predictors. The least significant candidate predictor at each step is sequentially eliminated according to a pre-specified criterion, e.g. Wald *p*-value >0.05 if logistic regression is used. Backward elimination risks predictor selection bias, but the full model approach is not straightforward as it may not be possible to define the full model, or practical to include all candidate predictors.[157, 159, 160]

### *Assessing the performance of a prediction model*

The performance of a clinical prediction model is commonly described using two statistical measures, discrimination and calibration. Discrimination refers to the ability of a model to differentiate individuals with from those without disease. Calibration refers to the accuracy of the model in predicting the outcome, i.e. the agreement between model-predicted outcomes and the actual observed outcomes. The concordance (C) statistic or

index is often used to quantify discrimination, and in logistic regression models corresponds to the area under the receiver operating characteristics (ROC) curve or AUROC. An AUROC of 0.5 indicates that the model cannot discriminate between those with and without disease. AUROC values of 0.7-0.79, 0.8-0.89, and ≥0.9, are respectively considered acceptable, excellent and outstanding discrimination.[164] Calibration is assessed visually using calibration plots which plot the predicted outcome probabilities against the observed outcome frequencies within quantiles of predicted risk. Calibration can also be assessed statistically using the Hosmer-Lemeshow test, and a *p*-value of <0.05 indicates lack of model fit (poor calibration), but this test has limited statistical power to detect poor calibration unless the sample size is large and the outcome frequent.[160]

### *Evaluation of a prediction model*

A prediction model is designed to optimally fit the data from which it was developed, so there is a potential that a model will be overfitted, and therefore the assessment of its predictive performance (C index) is likely to be "optimistic"; this is particularly so if the number of outcomes is small and the EPV is small.[165] Internal validation, using the data in which the model was developed, is recommended to estimate overfitting and optimism in model performance. Strategies for internal validation include the commonly used method of splitting the sample (randomly, non-randomly, or temporally), with model development in one portion (development sample), followed by assessment of predictive performance in the second portion (validation sample).

The split sample method is considered statistically inefficient as not all available data is used to develop the prediction model, and the development and validation samples tend to be similar. The preferred method for internal validation is to use a resampling procedure called the bootstrap.[159] Bootstrapping draws with replacement (to introduce a random element) a study sample of the same size as the original dataset from the entire dataset; thereby mimicking the process of sampling from the underlying population. Firstly, a prediction model is constructed using the entire dataset, and its performance assessed. Then, several hundred bootstrap samples are drawn, and each step of model development is repeated in every sample. Different models may be yielded in each bootstrap sample, and the performance of each of these models is evaluated in the original dataset. This enables estimation of the optimism in performance of the model developed in the original sample, and adjustment for this to the C index and the

estimated regressions coefficients in the final model.[156, 165] This adjusted performance therefore corrects for optimism and helps to "fine-tune" the model to the data.

In order to be clinically useful a prediction model needs to accurately predict the outcome in individuals outside of the development data. External validation, the process of evaluating the performance of the model in new data, is strongly recommended for all prediction models. This enables the updating or adjustment of the model if it performs poorly in the new data, thereby improving its generalisability.[166]

### *2.4.3. Prediction models for prevalent active TB amongst PLHIV*

The aim of this section is to describe and assess the quality of clinical prediction models for previously undiagnosed prevalent active TB in HIV-positive adults. The review focusses on models designed to identify prevalent active TB when screening PLHIV in LMIC outpatient settings.

#### *Search strategy*

The Medline database was searched for publications in the English language up to 1st May 2019. The Cochrane Library, and abstracts of world conferences of the International Union Against Tuberculosis and Lung Disease from 2013 to 2018 were also searched. In addition a systematic review of prediction models for pulmonary TB in adults published in 2017 was checked for further references.[167] This review identified six studies in total, only two of which reported models developed for screening PLHIV in outpatient settings.[143, 146] and which are discussed below. The authors concluded that the level of reporting on model development and evaluation of the studies in the review was poor, and that those reported were not useful for TB screening.

Recommended search terms for diagnostic prediction studies were used in Medline, including the Ingui search filter[168] updated with an additional search string as recommended by Geersing *et al*.[169] The search strategy is detailed in **Table 2-6**.

**Table 2-6 Search terms used in MEDLINE to identify clinical prediction studies**

| | | |
|---|---|---|
| Ingui Filter[168] | #1 | validat* OR predict*[Title] OR rule* |
| | #2 | predict* AND (outcome* OR risk* OR model*) |
| | #3 | (history OR variable* OR criteria OR scor* OR characteristic* OR finding* OR factor*) AND (predict* OR model* OR decision* OR identif* OR prognos*) |
| | #4 | decision* AND (model* OR clinical* OR logistic models[MeSH Terms]) |
| | #5 | prognostic AND (history OR variable* OR criteria OR scor* OR characteristic* OR finding* OR factor* OR model*) |
| Geersing updated search string[169] | #6 | Stratification OR ROC Curve [MeSH Terms] OR discrimination OR discriminate OR c-statistic OR c statistic OR area under the curve OR AUC OR calibration OR indices OR algorithm OR multivariable |
| Additional string for clinical score | #7 | clinical scor* |
| Tuberculosis search string | #8 | "Tuberculosis" [Mesh Terms] OR tuberculosis OR TB |
| HIV search string | #9 | "HIV"[MeSH Terms] OR acquired immune deficiency syndrome[MeSH Terms] OR HIV OR human immunodef* OR AIDS OR acquired immune def* OR acquired immunodef* |
| **Final search** | | **(#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7) AND (#8 AND #9)** |
| **Filters applied** | | **Humans; English; Adult: 19+ years; Adolescent: 13-18 years** |

MeSH: Medical subject headings

### *Inclusion and exclusion criteria*

Studies were included if they developed or validated a clinical prediction model for prevalent active tuberculosis in an outpatient setting and included HIV-positive individuals. Those which excluded or did not report enrolment of PLHIV, were conducted in high income countries or inpatient settings, or used tests which were unlikely either to be routinely available or used for tuberculosis investigation (e.g. biomarkers undergoing evaluation or computed tomography [CT] imaging) were excluded.

### *Results*

3809 publications were identified, and studies were excluded based on title or abstract. Four studies which developed prediction models for smear-negative TB in PLHIV,[170-173] and one which developed a model for incident TB in PLHIV were excluded,[174] as the focus of this thesis was screening individuals for prevalent TB as part of routine HIV care. Only

three studies were identified that fulfilled the eligibility criteria for this review, and these are described in **Table 2-7**.[146, 175] Two of these studies developed prediction models to identify prevalent active TB in PLHIV, the majority of whom were on ART, who were screened for TB in outpatient settings.[146, 175] The other study developed a model to triage individuals for further investigation, amongst those who had WHO tool symptoms when screened prior to ART initiation.[176] The final models and their performance are detailed in **Table 2-8**, and the studies themselves are discussed below.

**Table 2-7 Studies developing clinical prediction models for prevalent TB in the context of TB screening for PLHIV**

| Author<br>Country<br>Year<br>Design | Aim of model<br>Setting / population<br>Enrolment procedures<br>Exclusions | Type | Participants<br>TB case definitions | Outcome<br>In analysis | Candidate predictor selection<br>N | Events per variable | Model development | Validation | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Balcha[176]<br>Ethiopia<br>2014<br>Cohort | To triage WHO+ve PLHIV identified by screening for TB investigation<br>ART-eligible clinic attendees (N=812), CD4 212<br><br>Consecutive sample<br>Not eligible: if had ART, TB Rx within 2w<br><br>ALL screened at enrolment: symptom, 2 sputa (C, GXP), FBC, CD4.<br>If indicated: LNA (C & GXP)<br>Outcomes reviewed after 6m | D | N=625 WHO+ve<br><br>• Definite: C+ / GXP+<br>• Clinical: TB Rx without +ve bacteriology<br>• Not TB: Negative bacteriology & no TB Rx within 3m | Definite TB N=116<br><br>Total in analysis = 569 | Preselected by univariate analysis<br><br>N=15 | <10 | Multivariable logistic regression + backwards elimination<br><br>Complete case analysis<br><br>Continuous variables categorized | Not done | Enrolled only if able to produce paired sputa – not generalisable<br><br>•Excluded from analysis: Missing values 56/625 (9%)<br>•Clinical TB 21<br><br>Predictor selection bias<br><br>Transformation to score: 1 point assigned to each item |
| Nanta[175]<br>Thailand<br>2011<br>Cohort | To predict TB in PLHIV screened for TB<br><br>PLHIV attending OPD, ART & TB clinics, and IP<br>Not eligible: if had IPT or TB Rx within 1y<br><br>ALL screened at enrolment: symptom, CD4, FBC<br>• TB investigations arranged as part of routine care<br>Followed for 2m | D | N=257<br><br>• TB = any of: SM+, C+, compatible histology, clinical or radiological response to TB Rx. | TB N=66<br><br>Total in analysis = 257<br>171 (66.5%) on ART | Preselected by univariate analysis<br><br>N not reported | <10 | Multivariable logistic regression + backwards elimination<br><br>Complete case analysis<br><br>Continuous variables categorized | Not done | Sampling unclear, exclusions and missing data not reported. Likely selection bias + enrolment from TB clinics<br><br>Verification bias. Reference standard relied on routine investigation<br><br>Proportion IP not reported<br><br>Transformation to score: Weighted by coefficient from logistic regression model |

| Author<br>Country<br>Year<br>Design | Aim of model<br>Setting / population<br>Enrolment procedures<br>Exclusions | Type | Participants<br>TB case definitions | Outcome<br>In analysis | Candidate predictor selection<br>N | Events per variable | Model development | Validation | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Nguyen[146]<br>Viet Nam<br>2011<br>X-sect | To predict C+ PTB in PLHIV screened for TB<br><br>HIV clinic attendees (N=436), CD4 336<br><br>Consecutive sample<br>Not eligible: if screened for TB within 3m, TB Rx within 1y<br><br>ALL at enrolment: Symptom screen, 2 sputa (smear & culture), TST, CXR | D | N=436<br>• TB: C+ | TB N= 28 (6 AFB+)<br><br>Total in analysis = 397<br>230 (57.9%) on ART | Clinically important predictors & additional preselected by univariate analysis<br><br>N not reported | <10 | Multivariable logistic regression<br><br>Complete case analysis | Not done | Excluded from analysis:<br>• Did not complete all procedures (36)<br>• NTM / contaminated culture (3)<br><br>Symptom screen positive if present ≥2w in previous 4w<br><br>Final model includes diagnostic tests (CXR, sputum smear), not feasible for screening |

C, mycobacterial culture; C+, Culture positive; CXR, chest radiograph; D, development; FBC, full blood count; GXP, Xpert; HIV-P, HIV-positive; IP, inpatient; OP, outpatient; SA, South Africa; SM, smear microscopy; SM-Neg, smear-negative; TB Rx, TB treatment; TST, tuberculin skin test; WHO+ve, WHO-tool positive; X-sect, Cross-sectional

**Table 2-8 Performance of clinical prediction models for prevalent TB in the context of TB screening for PLHIV**

| Author<br>Country<br>Year | Outcome predicted<br>Population<br>n/N (% with outcome) | Final model / score | Performance of score |
|---|---|---|---|
| Balcha[176]<br>Ethiopia<br>2014<br>Cohort | C+ or GXP+ve TB<br><br>WHO+ve ART-eligible individuals, screened in primary care<br><br>137/791 (17.3%) | 1 point each:<br>• Cough<br>• Karnofsky ≤80<br>• MUAC <20 cm<br>• Lymphadenopathy<br>• HB <10 g/dL | Amongst WHO+ve (N=569)<br>≤1: PPV 20/255 (7.8%, 95% CI 4.9, 11.9)<br>2-3: PPV 77/280 (27.5%, 95% CI 22.4, 33.1)<br>≥4: PPV 19/34 (55.9%, 95% CI 37.9, 72.8)<br><br>Amongst N=791: WHO tool followed by investigation if score ≥4 AUC 0.75 vs. 0.70 using WHO tool alone |
| Nguyen[146]<br>Viet Nam<br>2011<br>X-sect | C+ TB<br><br>HIV clinic attendees, screened for TB<br><br>28/397 (7.1%) | Any of:<br>• CD4 <200<br>• Sputum AFB+ve<br>• CXR compatible with TB | Amongst WHO+ve (N=147)<br>• Sensitivity 100%, Specificity 59%<br>• PPV 20%, NPV 100%<br><br>AUC 0.83 (N=397) |
| Nanta<br>Thailand<br>2011<br>Cohort | TB (confirmed or clinical)<br><br>PLHIV attending hospital OP, ART or TB clinics, or inpatients; who were screened for TB<br><br>66/257 (25.7%) | Points assigned<br>• BMI <19 = 2<br>• Cough > 2w = 3<br>• Shaking chills ≥1w = 3<br>• On ART = 3<br>• CD4 ≤200 = 2<br>• Previous TB = 3 | ≤2: PPV 1/126 (0.8%, 95% CI 0.02, 4.3)<br>3-7: PPV 21/57 (36.8%, 95% CI 24.4, 50.7)<br>>7: PPV 16/22 (72.7%, 95% CI 49.8, 89.2)<br><br>AUC 0.92 (N=257) |

AUC, area under ROC curve; C+, Culture positive; CXR, chest radiograph; GXP, Xpert; HB, haemoglobin; HIV-P, HIV-positive; IP, inpatient; OP, outpatient; WHO+ve, WHO-tool positive; X-sect, Cross-sectional

### Prediction models for prevalent active TB in PLHIV undergoing TB screening

Nanta *et al* enrolled PLHIV from two hospitals in Thailand who were attending outpatient clinics or had been admitted, between 2009 and 2010.[175] All participants underwent symptom screen and had blood collected for CD4 and full blood counts, but investigation for TB was only undertaken as part of routine care; all were followed for two months. A very high proportion, 66/257 (26%) of those enrolled, fulfilled study case definitions for TB (bacteriologically confirmed or clinical), and over half were on ART. The final prediction model for TB, developed using multivariable logistic regression, comprised six items (BMI, cough, shaking chills, ART status, CD4 count, previous TB) which are easily obtainable at primary care level in LMIC. After transformation to a clinical score, the authors report AUC of 92%. ROC AUC is not the best measure for assessing utility of screening tests, as it assigns equal importance to sensitivity and specificity; whereas better screening tests

need to increase sensitivity at high specificity in order to reduce the number of false-positives requiring further evaluation.[177] The aforementioned study has many limitations, in particular the sampling strategy is unclear, participants were enrolled from TB clinics, the proportion enrolled from inpatient departments and study exclusions were not reported, and no information was provided regarding handling of missing data. The reference standard was poor, relying solely on investigation undertaken as part of routine care, although the authors did follow participants for two months in order not to miss TB diagnoses. The proportion diagnosed with TB was very high due to selection bias as participants were enrolled from TB clinics and inpatient departments, but the number of candidate predictors was too large (EPV < 10) and the model is likely to have been overfitted. The study is likely to be extremely biased in terms of participant selection and assignment of reference standard, the findings may not be generalisable to individuals attending clinics for routine HIV care, and validation was not undertaken. However, the final model is simple and the predictors are clinically logical, although "shaking chills" may not translate well in all settings.

Nguyen *et al* developed a clinical prediction model for use as a TB screening tool in PLHIV attending for routine HIV care in Viet Nam.[146] The investigators enrolled consecutive HIV clinic attendees and at enrolment, all underwent symptom screen, TST, chest radiography and had two sputa collected for mycobacterial culture. Over half of participants were on ART and 28/397 (7%) fulfilled their reference standard of culture-confirmed TB. However, around 10% of those enrolled were excluded from model development because of missing data. This will have introduced bias, if for example those who did not return for TST reading were unable to do so because they were too unwell because they had TB, and alternative statistical methods for dealing with missing data should have been considered. The final selected model comprised three items (CD4 count, sputum microscopy result and chest radiograph compatible with TB), hence including indicator variables the EPV was less than 10. Two of the three variables in Nguyen's final model were the results of diagnostic tests, sputum microscopy (which would have contributed to the reference standard) and chest radiograph; so the model's reported sensitivity of 100% amongst individuals with WHO tool symptoms is unsurprising. A model that requires chest radiograph or sputum investigation is not suitable for use as a screening tool for PLHIV at every clinical encounter for TB. Individuals who had been screened for TB in the preceding three months were excluded from this study, limiting applicability to routine HIV care settings at the time the study was conducted, when PLHIV are likely to have attended on a monthly basis for ART pick up. No form of validation was undertaken by the authors.

### Triage tool for prevalent active TB for use after WHO symptom screen

Balcha *et al* performed a secondary analysis of data collected for a study undertaken in Ethiopa, which compared the diagnostic yield of Xpert on sputum with microscopy and culture, amongst adults screened for TB prior to ART initiation.[176] The authors developed a prediction model for triaging those who reported WHO tool symptoms for TB investigation. 116/569 (20%) of those who reported WHO tool symptoms fulfilled their case definition for bacteriologically-confirmed TB. The authors selected candidate predictors using univariate analysis and excluded 9% of those eligible for the analysis because of missing data. EPV was less than ten, suggesting an overfitted model, and validation was not undertaken. A major limitation of this analysis, is that the original study appears to have excluded individuals who could not produce a paired sputa sample, introducing bias. This also limits generalisability of the study to routine clinic settings, where not everyone is able to produce even one sputum sample.

The authors' final model comprised five predictors (cough, Karnofsky score, mid-upper arm circumference [MUAC], peripheral lymphadenopathy and haemoglobin), but did not perform much better than using the WHO tool alone when assessed using the receiver-operating characteristic curve (AUROC). In busy routine clinic settings, where HIV care is often delivered by nursing staff, and adherence to TB screening algorithms is poor,[43] it is unlikely that a second step triage tool which incorporates Karnofsky score, recent haemoglobin level and examination for peripheral lymphadenopathy will be utilised. ROC AUC is not the best measure for assessing utility of screening tests as it is an average across all possible cut-offs for a test, including those that might not be clinically relevant; and is not an intuitive concept to understand. Furthermore it assigns equal weighting to sensitivity and specificity, but a better screening test is one that increases sensitivity at high specificity, so that the number of false-positives requiring further evaluation is reduced.[177]

### Other screening tools developed using statistical modelling

An additional two studies, which are relevant to TB screening in PLHIV, but do not strictly fulfil the eligibility criteria for this review are discussed below.[72, 143] These comprise Cain *et al*'s algorithm,[72] which was included in the original WHO meta-analysis (**Table 2-1**), because it uses CART modelling to guide investigation; and Rangaka *et al* who evaluated

whether additional predictors might improve the discriminatory ability of the WHO tool.[143] The latter study has already been described in detail as it was included in the systematic review to determine the accuracy of the WHO tool to rule out TB in individuals on ART (**Table 2-3**).

Cain *et al* developed a TB screening algorithm for PLHIV prior to ART initiation which has been implemented and evaluated in a number of HIV clinics in Cambodia, Viet Nam and Thailand.[72] The improving diagnosis of TB among people living with HIV (ID-TB/HIV) algorithm, comprises cough of any duration, fever of any duration, or night sweats lasting ≥3 weeks in the preceding 4 weeks. The presence of any of the aforementioned symptoms triggers investigation for TB. Derivation of this tool has already been discussed, and it has higher sensitivity (93%) but lower specificity (36%) than the WHO tool. The investigators used CART analysis to develop a simple prediction model to guide further investigation of those who had a positive symptom screen, and in particular to prioritise mycobacterial culture. The diagnostic algorithm provides an alternative to the smear-negative pathway and started with collection of two sputum samples for microscopy, followed by chest radiography if both sputa were negative. If the radiograph was abnormal then empiric TB treatment could be commenced whilst awaiting confirmatory mycobacterial culture result, as 33% in this group had confirmed TB. If the radiograph was normal, but TB was still suspected, then the risk of TB was further stratified by CD4 cell count. If CD4 count was <350 cells/mm$^3$ empirical TB treatment (as 10% had TB in this group) with confirmatory mycobacterial culture could be considered. In those with CD4 count ≥ 350 cells/mm$^3$, as only 5% in this group had culture-confirmed TB, then a strategy of monitoring instead of mycobacterial culture could be considered. The symptom screen has been evaluated in Kenya in individuals aged > 7 years who were newly diagnosed HIV-positive and performs similarly in terms of sensitivity and specificity to the WHO tool.[178] The yield from the entire screening and diagnostic algorithm has been investigated in clinics in Thailand, Viet Nam and Cambodia, amongst ART-naive adults attending for routine HIV care; but culture was not undertaken systematically for all participants, limiting ability to compare sensitivity and specificity of this algorithm with the WHO tool.[179]

Rangaka *et al* undertook a secondary analysis of data collected for an IPT trial, and limitations of this analysis, in particular selection bias, have already been discussed **(Table 2-3)**. The authors investigated whether additional predictors might improve on the performance of the WHO tool amongst individuals on ART. Six predetermined predictors (all categorised) were added in multivariable logistic regression to a model which comprised only the WHO tool. The authors undertook backwards elimination, starting with

the full model, retaining variables deemed statistically significant, and compared the final model with the WHO tool using AUC. In multivariable analysis, BMI, CD4 count, and ART duration of less than three months were independent predictors of TB amongst those on ART. Addition of these variables to the WHO tool improved the discrimination of the tool, as measured by the AUC, from 59% (95% CI, 53%–66%) to an acceptable 70% (95% CI, 60%–79%) amongst those on ART. ROC AUC is not the best measure for assessing utility of screening tests, as already discussed above. The authors' findings are however pertinent when choosing predictors for prevalent TB in clinical prediction model development, and this is relevant to Research Paper 2.

### *2.4.4. Summary*

There is a paucity of published clinical prediction models developed for use either as a screening tool for TB in PLHIV attending for routine care, or as a second step triage tool to prioritise investigation for those who report symptoms when screened using the WHO tool. Published models suffer from methodological flaws, in particular inadequate sample size, selection bias, failure to deal adequately with large volumes of missing data, and lack of validation.

## 2.5. Investigation pathways for PLHIV following a negative Xpert result

### *2.5.1. Introduction*

In 2012, at the time the research for this thesis commenced, the algorithm devised for investigating PLHIV with a negative Xpert result[124] (**Figure 1-1**) was identical to the 2007 WHO algorithm for diagnosing smear-negative TB in ambulatory adults which commenced with sputum microscopy for all with chronic cough.[180] The aim of the 2007 algorithm was to expedite treatment of smear-negative TB in PLHIV, within a maximum of four visits, and thereby reduce the high mortality previously arising from protracted evaluations for TB in this population. Additionally, the use of mycobacterial culture and chest radiograph early in the pathway aimed to improve the accuracy of diagnosis. The case definition for smear-negative pulmonary TB was revised for HIV-prevalent settings, requiring either one positive mycobacterial culture and compatible symptoms; or two sputa negative on TB microscopy, with compatible chest radiograph and a decision to commence TB treatment. If the initial sputum microscopy was negative, then at the second visit which was envisaged to occur the following day, all of chest radiography, a second sputum sample (for microscopy and mycobacterial culture), and a clinical assessment regarding empiric TB treatment were required. The third visit was for review of the chest radiograph and second microscopy result; and if TB was deemed unlikely then to provide antibiotics to treat, as appropriate, either bacterial or *Pneumocystis jirovecii* pneumonia. The purpose of the fourth visit was to assess the response to the antibiotic trial and to re-evaluate for TB those patients whose symptoms had not resolved. The antibiotic trial was not intended as a diagnostic aid, but rather to treat any commonly co-existing bacterial infection in PLHIV, with advice to reattend if symptoms that responded to treatment recurred.

The Xpert-negative algorithm for PLHIV, compared with the smear-negative pathway, reduced the number of attendances required for evaluation from four to three, by providing the antibiotic trial at the second visit (**Figure 1-1**). This pathway is still onerous, and still requires good access to chest radiography and a lengthy wait for the result of mycobacterial culture. Innovative ways of getting around this include upfront collection of two sputum samples from all who are symptomatic, with testing of the second sample determined by the result of the initial Xpert (e.g. mycobacterial culture if the initial Xpert result is negative), as is policy in Cape Town, South Africa.[181, 182] If resources permit, then access to on-site chest radiography or laboratory would be the ideal solution, but this is rarely feasible outside of a clinical research setting in most LMICs.[101, 181]

Schnippel *et al* modeled the costs and impact, for symptomatic PLHIV investigated for TB who had a negative initial Xpert, of replacing the entire pathway with a second Xpert test.[126] The authors concluded that this strategy could save an estimated US$17.4 million per year at national programme level in South Africa. The assumptions made in their model included sensitivity estimates for Xpert based on Boehme *et al*'s implementation study,[183] i.e. 100% and 79% for smear-positive and smear-negative culture-confirmed TB, respectively; 1% of symptomatic individuals started empiric TB treatment based on antibiotic trial and/or chest radiograph;[125] loss to follow-up of 13% and 26% following the first and second visits respectively; and it did not consider extrapulmonary TB. It is unclear if the model assumed that everyone with a negative Xpert result was able to produce a further sputum sample, but this would have equally impacted either pathway, as both require a second sputum sample. Repeating the Xpert test is very attractive compared to the Xpert-negative algorithm, as it is far simpler and could markedly reduce diagnostic delay. However, in the aforementioned model all further evaluation stopped if the repeat Xpert result was negative, risking missing TB diagnoses, which the authors estimated at 2% fewer diagnoses made compared with the culture-based pathway. Interestingly, 2016 WHO ART guidelines do recommend a repeat Xpert test on a fresh sample, but this is in addition to chest radiograph and clinical assessment, and if feasible submission of sputum for mycobacterial culture.[127]

The rationale for a repeat Xpert in the 2016 WHO ART guidelines appears likely to be the evidence of an improved diagnostic yield from repeating Xpert, which is described in diagnostic accuracy or TB screening studies that have tested multiple sputum samples collected at study enrolment using Xpert. These studies are discussed below, together with factors likely to improve yield. At the time the research for this thesis was undertaken, the strategy of repeating the Xpert test had not been empirically evaluated in the context of investigating PLHIV established in HIV care, who had been identified as needing confirmatory testing by TB screening. It is likely that the sensitivity of any diagnostic pathway will be lower in PLHIV identified through TB screening, a scenario where the prevalence of TB will be lower and individuals will be less symptomatic, compared with patients attending because of TB symptoms, or those preparing for ART who are at greater risk of TB and likely to have higher sputum bacillary load.

### *2.5.2. Studies performing Xpert on multiple samples obtained at enrolment*

Data regarding the yield from a second sputum sample tested with Xpert are derived from two main sources, firstly the original multicenter evaluation of Xpert amongst HIV-positive and -negative individuals attending for care because they were unwell, and secondly studies screening PLHIV for TB, which investigated all using Xpert irrespective of the presence of symptoms. In general, in both scenarios, multiple sputum samples were collected either at a single visit or for the initial diagnostic process, which does not reflect how the Xpert-negative pathway is followed in real life. Studies were generally cross-sectional in design, with no prospective follow-up.

Boehme *et al* undertook a large, multi-country evaluation in individuals with symptoms suggestive of TB, collecting three sputum samples (two spot and one morning sample) which appear to have been spontaneously produced.[184] Two samples were first decontaminated, followed by centrifugation, then sputum deposits underwent microscopy after resuspension in phosphate buffer; following this, each sample underwent both Xpert test (after further processing) and mycobacterial culture (on both liquid and solid media, therefore four cultures in total). The third sample underwent direct microscopy and Xpert testing. Overall, 50.6% (741/1462) of participants had culture-confirmed TB, and 40% of participants were HIV-positive. The sensitivity of testing one (untreated sample) vs. two vs. all three samples using Xpert was 92.2% (675/732) vs. 96.0% (1423/1482) vs. 97.6% (732/741) for all culture-confirmed TB; and 72.5% (124/171) vs. 85.1% (296/348) vs. 90.2% (157/174) for smear-negative, culture-positive TB. The denominator for testing on two samples included two observations per participant, the first observation combined the first and third samples, and the second observation combined the second and third samples. The sensitivity data for repeat testing was not reported stratified by HIV status. However, the investigators did report that amongst HIV-positive participants, there was no difference between the sensitivity of Xpert on decontaminated vs. untreated sputum, which is of relevance for research studies which store decontaminated pellets for later testing with Xpert.

6% of those fulfilling study eligibility criteria were not enrolled to the aforementioned study, mainly because they could not produce three sputum samples, so the authors are likely to have overestimated the sensitivity of Xpert.[184] Almost half of all participants had previously been treated for TB. False-positive Xpert results can arise from detection of dead bacilli, thus potentially reducing test specificity, but specificity was high in this evaluation (99.2% vs. 98.6% vs. 98.1% for one vs. two vs. three samples) .[185, 186] The study

was undertaken only in reference facilities, limiting generalizability to other settings, and in routine care settings patients are unlikely to be able to produce this many sputum samples.

Lawn *et al* investigated the diagnostic accuracy of Xpert in a different context in South Africa, namely for screening PLHIV for TB prior to ART initiation.[56] Median CD4 count in participants was 171 cells/mm$^3$. Two sputum samples were collected from all participants; the first was a spot sample (induced if necessary), and the second was induced for all participants. The findings from studies which induce sputum samples are not generalizable to routine HIV care settings. Both sputum samples were decontaminated and processed as detailed above,[184] prior to undergoing microscopy, testing with Xpert, and mycobacterial culture using liquid media. About 15% of those enrolled did not contribute to the analysis as they could not produce two sputum samples, and about one-quarter of those enrolled had previously been treated for TB. This exclusion potentially results in an overestimation of the sensitivity of Xpert and underestimation of its specificity. In this study the prevalence of culture-confirmed TB was 17.3% (81/468; 5.3% smear-positive, and 12% smear-negative), and the reported sensitivities of Xpert for one vs. two samples were 58.3% (42/72) vs. 72.2% (52/72) for all culture-positive TB, and 43.4% (23/53) vs. 62.3% (33/53) for smear-negative, culture-positive TB.

Cavanaugh *et al*, screened consecutive individuals at enrolment to HIV care in 24 Kenyan facilities, specifically excluding those who had received TB treatment within the preceding one year to reduce the risk of false-positive Xpert results.[187] Three sputum samples (one morning, and two spot specimens) were requested, lymph node aspiration (LNA) undertaken if appropriate, and stool samples were collected at three facilities. Xpert was undertaken on one spot sputum (unprocessed), and on the morning sample (processed as detailed above to enable mycobacterial culture)[184] only if the sample was of sufficient volume. Mycobacterial culture using liquid media was undertaken on two sputum samples (one morning and one spot), LNA, and stool. Median CD4 count in participants was 343 cells/mm$^3$, and the case definition for TB of positive mycobacteriology on culture or Xpert was fulfilled by 11.3% (88/778) of participants, of whom 8% (7/88) were diagnosed by Xpert alone. Amongst 74 TB diagnoses made in participants with two sputa tested with Xpert, 57% (42/74) vs. 66% (49/74) respectively were identified by the spot vs. morning samples. The morning sample identified 20% (14) TB diagnoses undetected by the spot sample, suggesting that a morning sample might improve yield compared with a spot specimen; and therefore, sensitivity of Xpert for one vs. two samples of 42/74 (57%) vs. 56/74 (76%). Amongst 69 participants with TB diagnosed and CD4 cell count results, two

Xpert tests were reported to identify more TB diagnoses in those with CD4 counts <100 vs. ≥100 cells/mm$^3$ (22/24 [92%] vs. 30/45 [67%]). However, the numbers in this analysis were small, and not all participants had a repeat Xpert test on the morning sample, potentially introducing bias. Limitations of this study include a case definition which included Xpert results and limits comparability with the aforementioned studies. The yield of a repeat Xpert could not be accurately estimated from this study because not all participants submitted the morning sputum sample on which the repeat Xpert was performed.

### *2.5.3. Studies undertaking repeat Xpert following an initial negative result*

There is only one published study reporting the yield of repeat Xpert testing following an initial negative test result.[188] This was undertaken in the context of screening PLHIV for TB prior to ART initiation in Mozambique. All participants underwent TB screening using the WHO tool, urine LF-LAM and Xpert on sputum. If the initial Xpert was negative then a second sample was collected after two to three days for a repeat Xpert test; this was undertaken irrespective of the presence of WHO tool symptoms. TB was diagnosed based on either a positive Xpert or LF-LAM result. Amongst 972 participants included in the analysis, representing 96% of those eligible, median CD4 count was 278 cells/mm$^3$; and 10.1% (98/972) were diagnosed with TB (positive Xpert, 90; positive LF-LAM, 34). The first Xpert was positive in 74/972 (7.6%) of participants. Repeat Xpert was undertaken in all 898 participants with a negative initial Xpert, and identified an additional 16 TB diagnoses. The sensitivity of Xpert testing of one vs. two samples was therefore 74/98 (76%) vs. 90/98 (92%). Limitations of this study include the inclusion of the Xpert result itself (and LF-LAM) in the reference standard and the absence of mycobacterial culture.

### *2.5.4. Factors improving the yield of Xpert from sputum*

Acuna-Villaorduna *et al* undertook a secondary analysis of data collected for a study evaluating a new TB microscopy method.[186] The authors enrolled adults attending outpatient clinics in Uganda who had symptoms of TB, defined as cough ≥ 2 weeks plus one other of the WHO tool symptoms, and reported convenience sampling to include more HIV-positive participants. Three sputa were collected (two spot and one morning), of which all underwent microscopy and TB culture on liquid media, and one spot sample was tested with Xpert. Amongst 860 participants in the analysis, 205 (24%) fulfilled the TB case

definition of positive mycobacterial culture on any sample; and 69% were HIV-positive.The authors reported lower Xpert sensitivity and greater specificity in mucosalivary vs. mucopurulent samples (sensitivity 82.5% [52/63]) vs. 95.8% [136/142]; specificity 95.6% [282/295] vs. 97.5% [350/359]).[186] Multivariate analysis was undertaken to investigate factors associated with discordant Xpert and culture results (both Xpert and culture positive [n=188] vs. Xpert-positive, culture-negative [n=22]). The final model was adjusted for age, sex, weight loss, fever, previous TB treatment, HIV infection, and sputum quality (salivary vs. purulent). Salivary sputum (adjusted odds ratio [aOR], 95% CI 4.1, 1.1-14.6), previous TB treatment (aOR 8.3, 2.1-32.0), and fever (aOR 0.23, 0.1-0.7) were independently associated with discordant results. The confidence intervals are wide, reflecting the small number with discordant results, and approach 1 for sputum quality. Limitations of this study include potential bias due to convenience sampling, and the strict definition of TB symptoms which is in contrast to the usual criteria of the presence of any WHO tool symptom in PLHIV and thus limits generalisability. The authors did not undertake any prospective follow up to confirm TB diagnoses in those with discordant results so may have missed TB diagnoses. In addition, the number of discordant results reported by the authors was small, reflected in the broad confidence intervals which almost approach unity for sputum quality. The findings suggest that Xpert might perform less well in salivary samples, but the authors also postulate that the discordant results might reflect worse yield of TB culture compared with Xpert from salivary samples.

Griesel *et al* report from their study which developed a clinical prediction model for TB in seriously ill HIV-positive inpatients in South Africa, a significantly greater yield of Xpert with sputum induction compared with spontaneous sputum samples (51.9% [162/312] vs. 41.7% [68/163]).[189] This may not be generalisable to PLHIV in outpatient settings attending for routine care.

### *2.5.5. Summary*

An increased yield of TB diagnoses from performing Xpert on multiple samples has been reported from studies where multiple samples have been taken at study enrolment for screening prior to ART initiation,[56] or investigation of HIV-positive and HIV-negative symptomatic individuals.[184] Increased yield was also reported from one further study screening PLHIV prior to ART initiation, in which Xpert was repeated on a further sputum sample collected after a few days if the initial sample was negative, irrespective of whether symptoms were reported. [188] Induction of sputum rather than spontaneous

expectoration, morning rather than spot specimens, and possibly also mucopurulent rather than salivary samples may improve the yield from Xpert testing. However, prior to the research undertaken in this thesis, the strategy of repeating Xpert testing on a fresh sputum sample had not been undertaken in the context of the Xpert-negative pathway after screening individuals established in HIV care for TB.

## 2.6. Causes of symptoms suggestive of TB amongst PLHIV

The aim of this section is to describe the findings from published studies which report the aetiology of symptoms suggestive of TB among PLHIV in LMIC settings, focussing on those conducted in outpatient settings, and in particular on chronic cough.

The Medline database was searched for publications in the English language up to 1st May 2019 using the search strategy detailed in **Table 2-9**.

**Table 2-9 Search terms used in MEDLINE to identify studies reporting causes of symptoms suggestive of TB**

| | |
|---|---|
| #1 | Cough OR fever OR sweats OR weight loss OR cachexia |
| #2 | chronic airflow obstruction OR post-TB OR tuberculosis-associated OR post-tuberculous OR post tuberculous |
| #3 | Lung Diseases, Obstructive[MeSH Major Topic]) OR Airway Obstruction [MeSH Major Topic]) OR Airways Obstruction OR Obstructive Airway Disease OR Obstructive Airways Disease OR Pulmonary Emphysema OR Emphysema OR asthma |
| #4 | "Africa South of the Sahara" [Mesh Terms] |
| #5 | "HIV"[MeSH Terms] OR acquired immune deficiency syndrome[MeSH Terms] OR HIV OR human immunodef* OR AIDS OR acquired immune def* OR acquired immunodef* |
| **Final search** | **(#1 OR #2 OR #3) AND (#4 AND #5)** |
| **Filters applied** | **Humans; English; Adult: 19+ years; Adolescent: 13-18 years** |

MeSH: Medical subject headings

957 publications were identified, and studies were excluded based on title or abstract. Seven relevant studies evaluating mainly ambulatory PLHIV with persistent cough or reporting diagnoses amongst individuals attending for routine HIV care are summarized in **Table 2.10**.

Four studies focussed on extensively investigating patients with chronic symptoms who were sputum smear negative or febrile, aiming to identify serious infectious causes for symptoms.[190-193] Amongst smear-negative patients with chronic cough, tuberculosis, bacterial pneumonia, lower respiratory tract infections, *Pneumocystis* pneumonia and pulmonary Kaposi's sarcoma were the most frequent diagnoses, with most participants having multiple aetiologies.[190, 192-194] Hargreaves *et al*, who performed bronchoscopy on patients with chronic cough reported that over half of TB cases were diagnosed on repeat sputum microscopy taken prior to bronchoscopy, although the proportion of these who were HIV-infected is not reported.[192] In this study no causative organism was identified in

7% of participants who made a full recovery and they were categorized as having a non-TB chest infection. Okwera *et al* also identified no causative organism in induced sputum samples from more than half of their study participants, who were smear-negative PLHIV with a previous history of tuberculosis undergoing investigation for chronic cough.[193] Serious blood stream infections, in particular, non-typhoidal Salmonellae and cryptococcosis were also identified as responsible for chronic symptoms suggestive of tuberculosis and acute fever amongst PLHIV.[190] One further study in which ART clinic attendees underwent limited (and not systematic) evaluation as part of routine care, with no access to mycobacterial culture, also reported tuberculosis as the most common diagnosis.[191] Limitations of these studies include the focus on investigating only for an infectious cause, selection bias,[190] and findings from studies which included only smear-negative individuals may not be generalisable to a population attending for routine HIV care.

Two studies investigated populations including PLHIV for non-communicable diseases (NCD) as causes of chronic cough.[194, 195] Munyati *et al* evaluated primary care attendees in Zimbabwe with chronic cough and unsuprisingly amongst 454 newly-diagnosed HIV-positive patients, the majority of diagnoses were infectious (TB 46%, lower respiratory tract infection 31%).[194] Munyati also identified a high proportion of NCD diagnoses, in particular post-tuberculous disease, asthma and heart failure.[194] Calligaro found that one-third of a cohort of patients on ART in South Africa, who had no features of acute respiratory disease, reported respiratory symptoms and that this was associated with current smoking. [195] In this cohort one-third of patients reported a smoking history and 7% had chronic airflow obstruction on lung function testing. If these findings are generalisable it is likely that smoking may contribute to respiratory symptoms amongst PLHIV,[195] although smoking itself is a recognised risk factor for TB disease.[196] One further study in which electronic and paper records of a consecutive sample of adults on ART were reviewed for NCD diagnoses, reported that 4% of study participants had a diagnosis of asthma and 2% of heart failure.[197] Both diagnoses can cause chronic cough, but a limitation of this study is reliance on documentation of diagnoses, rather than confirming the criteria used to assign diagnoses.

Post-tuberculous lung disease is increasingly recognised amongst PLHIV in LMIC settings. Allwood *et al* in a systematic review, largely comprising an HIV-negative population, reported an association between a past history of tuberculosis and the presence of spirometrically-confirmed chronic airflow obstruction (forced expiratory volume in 1 second [FEV1] / forced vital capacity [FVC] <0.70 or less than lower limit of normal [LLN]),

which was independent of cigarette smoking.[198] A systematic review of studies undertaken in South Africa, in community and occupational health settings, found an increased prevalence of respiratory symptoms amongst individuals who had previously been treated for TB.[199] In a prospective cohort of PLHIV in South Africa who underwent annual spirometry over a period of three years, at study enrolment prevalent spirometrically-confirmed obstructive lung disease (FEV1/FVC<0.70) was found to be associated with older age, current smoking, and higher C-reactive protein (CRP) levels. 25% of this cohort were on ART at enrolment. Amongst individuals with a previous history of tuberculosis, the authors reported a greater decline in lung function (FEV1 and FVC reductions of 35 ml/year and 57 ml/year respectively) compared to those with no previous tuberculosis. In multivariable analysis (adjusted for time-updated CD4 cell counts, viral load, ART use at enrolment) the authors reported that only ever having smoked and previous tuberculosis were independently associated with excess loss in FEV1.[200] Smoking and a previous history of TB are common among PLHIV, so it is likely that both contribute to the aetiology of chronic or recurrent cough in this population. However, it is also possible that asthma and chronic obstructive pulmonary disease (COPD) are underdiagnosed in busy primary health care settings in LMIC.

### *2.6.1. Summary*

Published studies focus mainly on identifying infectious aetiology for symptoms suggestive of tuberculosis, although a few report non-communicable diseases such as asthma and cardiac causes for respiratory symptoms. Some studies report multiple aetiologies in patients for these symptoms. In all studies where infectious aetiologies are sought, the most frequent diagnosis, where a cause is found, is active tuberculosis. Post-tuberculous lung disease is increasingly recognised in PLHIV in LMIC settings. Large-scale epidemiological studies are needed to describe this phenomenon better, and to provide better evidence to guide criteria to distinguish this from active TB and guide optimal management.

**Table 2-10 Diagnoses amongst PLHIV with symptoms suggestive of TB in LMIC or Sub-Saharan Africa**

| Author<br>Country<br>Design | Study population<br>Median CD4 count cells/mm$^3$<br>(N) | Inclusion criteria | Study procedures | Key findings & comments |
|---|---|---|---|---|
| Magoro 2016[197]<br>Zimbabwe<br>Cross-section | Adults attending HIV clinic<br>All on ART<br>Median CD4 191<br>(N=1033)<br>Consecutive sample | Adults on ART<br>Excluded if not already registered at clinic | Systematic review of all paper and electronic records for NCD diagnoses after patient had attended clinic | NCD identified from record review:<br>• Hypertension 106 (10%)<br>• Asthma 45 (4%)<br>• Type 2 diabetes mellitus 22 (2%)<br>• Cancer 19 (1.8%)<br>• Congestive cardiac failure 16 (2%)<br>• Stroke 10 (1%)<br>• Other 39 (4%)<br>Retrospective record review with no validation of diagnoses |
| Okwere 2013[193]<br>Uganda<br>Cross-sectional | Smear negative HIV-positive adults undergoing evaluation for recurrent PTB at TB clinic<br>47% on ART<br>Median CD4 261<br>(N=178)<br>Consecutive sample | Sputum smear negative & previous history of TB & cough > 2w<br>Excluded if other severe illness (cardiac disease or asthma) | Sputum (induced and spot) for TB culture + bacterial pathogens + *Pneumocystis jirovecii* PCR<br>FBC, CD4 | Pathogens identified in sputa:<br>• 95 (53%) no bacteria<br>• 33 (19%) bacteriologically confirmed TB<br>• 48 (27%) other bacteria (most commonly *S. pneumoniae* [10], *M. catarrhalis* [8]), *H. influenzae* [8])<br>• 12 (6.7%) *Pneumocystis jirovecii*<br>Not generalisable to routine HIV care settings<br>Authors looked only for infectious causes |
| Damtie 2013[191]<br>Ethiopia<br>Cross-sectional | Adults attending ART clinic<br>78% on ART<br>52% had CD4 >350 (median not reported)<br>(N=360)<br>Random sample | Adults on ART<br>Exclusion criteria not reported | Routine clinical investigation by clinician in accordance with clinic protocol, mainly clinical diagnoses<br>If cough>2w: sputum smear, + CXR if smear-negative<br>FNA if indicated for TB microscopy<br>Diarrhoea: stool microscopy | Diagnoses:<br>• 35 (10%) TB of which 30 PTB (22 smear-negative), 5 EPTB<br>• 18 (5%) Oral candidiasis<br>• 12 (3%) Diarrheal disease (*strongyloides* [2], *Schistosoma* [1])<br>• 6 (2%) Pneumonia<br>• 5 (1%) Skin fungal infection<br>• 12 (3%) Other<br>No systematic investigation of participants. Diagnoses were made during routine clinical care |

| Author<br>Country<br>Design | Study population<br>Median CD4 count cells/mm$^3$<br>(N) | Inclusion criteria | Study procedures | Key findings & comments |
|---|---|---|---|---|
| Bedell 2012[190]<br>Malawi<br>Prospective cohort | Ambulatory adults prior to ART initiation<br>Median CD4 129<br>(N=469)<br>Potential participants referred by clinicians undertaking routine clinical care | 3 negative sputum smears & unexplained weight loss and/or chronic fever / diarrhoea / unable to cough<br><br>Excluded if TB treatment in past month or pregnant | Blood culture (MTB + other pathogens)<br>CrAg<br>Sputum (induced): TB culture<br>FBC, CD4<br>CXR | Diagnoses:<br>52 (11%) bacteriologically confirmed TB<br>50 (11%) positive (non-TB) blood culture +/or CrAg-pos.<br>Non typhoidal Salmonellae most common blood culture pathogens (6% of participants and 52% bloodstream isolates).<br>Selection bias likely as referred to study by clinicians |
| Calligaro 2011[195]<br>South Africa<br>Cross-sectional | Cohort of patients on ART<br>Median CD4 380<br>On ART median 2.1 years<br>(N=152) | No features of acute respiratory disease<br>Stable on ART for at least 3 months | Respiratory questionnaire<br>Pulmonary function tests pre- and post- bronchodilator | 32.9% - history of smoking<br>31% - respiratory symptoms<br>Any respiratory symptom (cough, phlegm, wheeze or dyspnoea) associated with current smoking (OR 2.6, 95% CI 1.05-6.2)<br>CAO in 7% & associated with ever smoking (OR 6.4, 95% CI 1.6-25.9) |
| Munyati 2005[194]<br>Zimbabwe<br>Prospective cohort | Ambulatory adults with chronic cough<br>CD4 not reported<br>(N=544)<br>Systematically sampled | Cough ≥ 3 weeks<br><br>Excluded if danger signs requiring admission / on TB treatment | HIV test<br>Evaluation using standardised set of investigations including CXR, sputum (TB culture + bacterial pathogens) | 454/544 (83%) HIV-pos, (13% >1 diagnosis)<br>• >90% reported fever, NS and UWL<br>• 46% (207) TB diagnosed<br>  ○ microbiologically-confirmed (n=162)<br>  ○ smear and culture negative TB (n=45)<br>• 17% bacterial pneumonia<br>• 31% LRTI<br>• 7% fibrotic lung disease<br>• 3% heart failure<br>• 3% asthma<br>• 2% Pneumocystis pneumonia<br>• 1% Cryptococcosis |
| Hargreaves 2001[192]<br>Malawi<br>Prospective cohort | Ambulatory patients about to start treatment for smear-negative TB<br>N=352<br>Consecutive sample | Cough ≥ 3 weeks<br>Fulfilled national TB control programme criteria for diagnosis of smear-negative TB | HIV test<br>Clinical assessment<br>Sputum + blood for TB culture<br>Bronchoscopy and BAL examined for TB, *Pneumocystis jirovecii* and other fungi. | 278/313 (89%) of those tested HIV-pos (81% met the WHO case definition for AIDS)<br>Diagnoses:<br>• 137 (39%) microbiologically confirmed TB<br>• 17 (5%) Pneumocystis pneumonia<br>• 27 (7%) no organism identified but full recovery<br>• 10 (3%) pulmonary Kaposi's sarcoma |

CAO, chronic airflow obstruction; CrAg, Cryptococcal antigen test; CXR, chest radiograph; EPTB, extrapulmonary TB; FNA, fine needle aspiration; FBC, full blood count; LRTI, lower respiratory tract infection; NCD, non-communicable disease; NS, night sweats; UWL, unintentional weight loss; PTB, pulmonary TB

# 3) XPHACTOR study methods

The research undertaken for this PhD forms part of the XPHACTOR study. This chapter details the methods for XPHACTOR and how the research papers presented in this thesis flow from XPHACTOR (**Figure 3-1**).

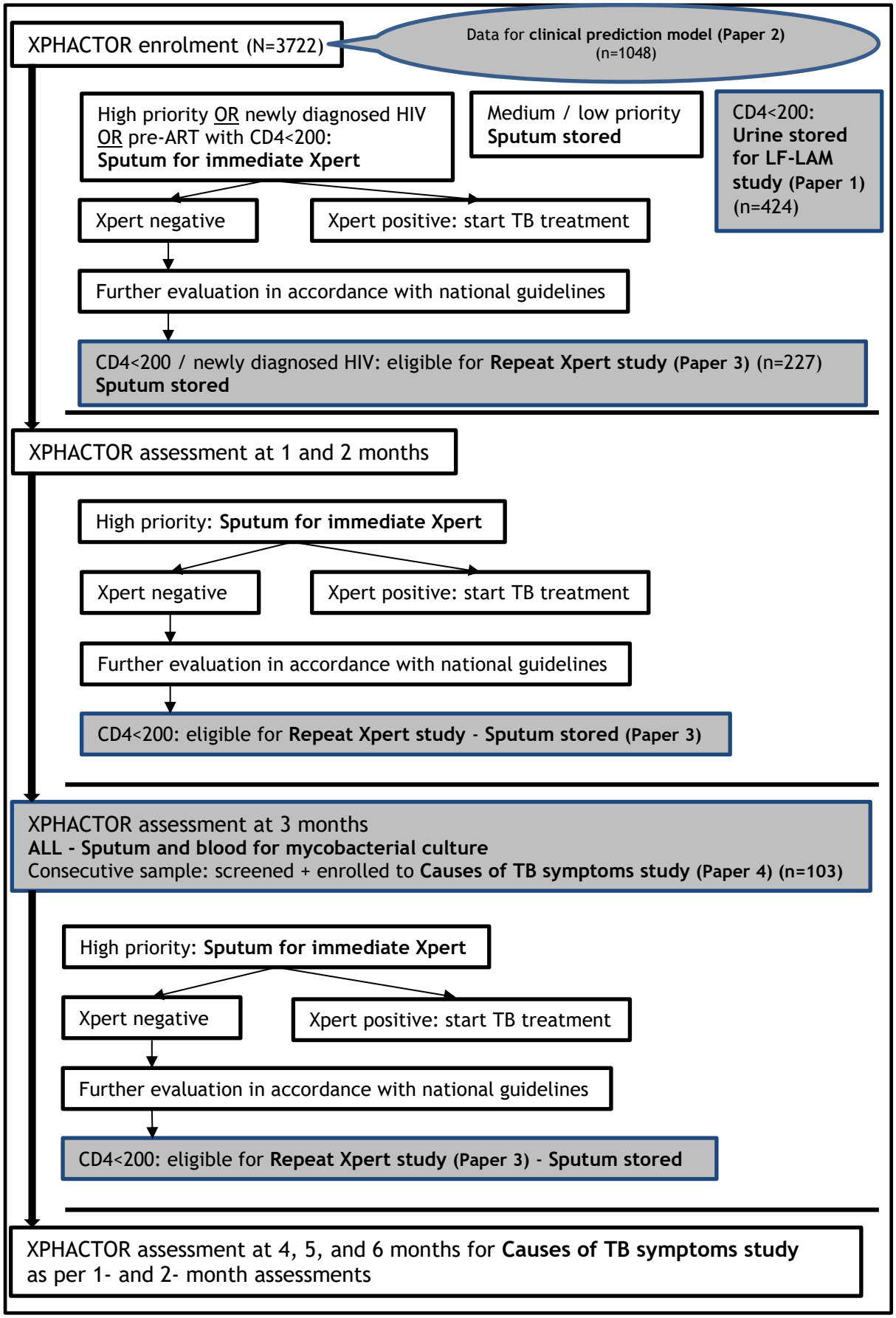
XPHACTOR evaluated an algorithm which was designed to identify, among HIV-positive clinic attendees, those deemed "high priority" for immediate investigation with Xpert MTB/RIF, and allowed watchful waiting for those assessed as lower priority. The study hypothesis was that an algorithm which prioritised immediate testing for the high priority group while allowing deferral of investigation for those assigned lower priority would reduce health service costs with minimal risk to patients. Investigation was prioritised for individuals at highest risk of death due to TB, and/or those at highest risk of transmitting TB to others, using markers readily available in primary care clinics in South Africa. The markers selected were body mass index (BMI) and CD4 count, which are known to be risk factors for TB and mortality,[140, 201-204] and cough as a clinical marker of smear positivity (as the best indicator of infectiousness).[27, 205, 206] The study algorithm is presented in **Figure 3-2**.

## 3.1. XPHACTOR study aims and objectives

The aims of the XPHACTOR study were:

- **Aim 1:** to determine the sensitivity and specificity of the study algorithm and to compare the outcomes (sensitivity of the algorithm, time to TB diagnosis) and costs of the strategy with modelled outcomes and costs assuming immediate testing with Xpert MTB/RIF for all symptomatic individuals, as defined by the WHO tool.
- **Aim 2:** to describe the diagnostic yield from two different strategies for investigating adults with HIV who are suspected of having TB, but whose first Xpert test is negative. **(Chapter 7 - Research Paper 3)**.
- **Aim 3:** to determine causes for persistent or recurrent symptoms suggestive of TB amongst ambulatory adults attending for HIV care who have negative initial TB investigations. (**Chapter 8 - Research Paper 4)**
- **Aim 4:** to determine the natural history of TB symptoms among individuals without a final diagnosis of TB, in order to estimate the likely demand for repeat Xpert testing among patients attending for HIV care. (**Chapter 4)**.

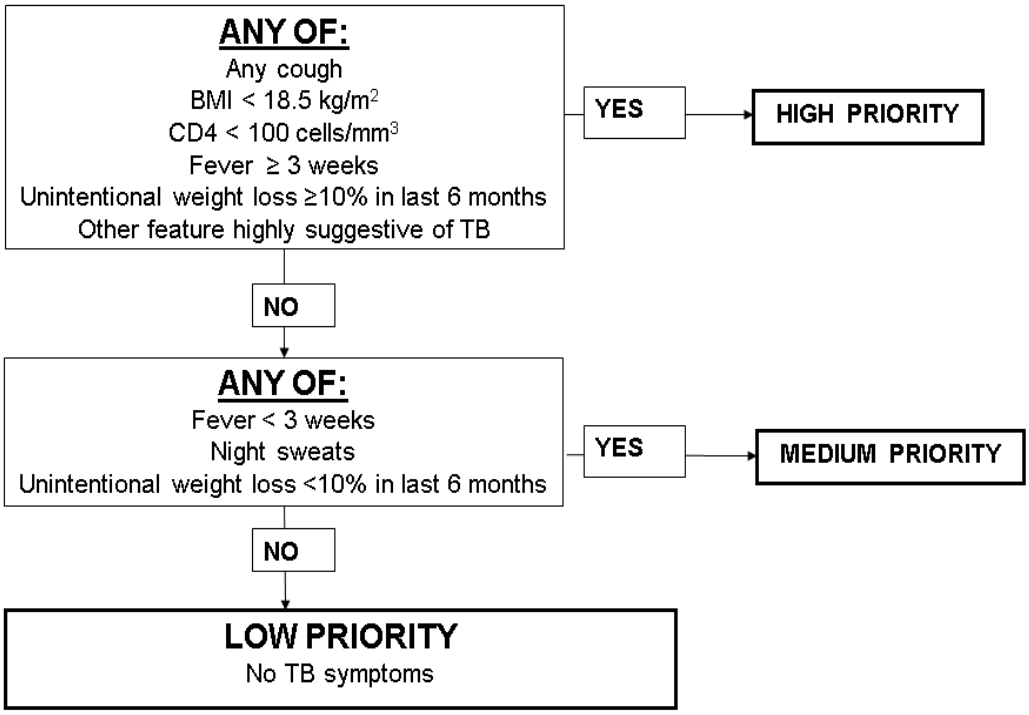
**Figure 3-1. XPHACTOR study flow and entry points for research papers in this thesis**



In addition, the opportunity was taken to evaluate the diagnostic accuracy of LF-LAM for TB at enrolment to XPHACTOR amongst participants with CD4 count <200 cells/mm$^3$ (**Chapter 5 – Research Paper 1**).

A secondary analysis of data collected for XPHACTOR was used to develop the clinical score for TB (**Chapter 6 – Research Paper 2**).

**Figure 3-2. XPHACTOR algorithm at enrolment**




BMI = Body mass index

## 3.2. XPHACTOR study setting

XPHACTOR was conducted in Gauteng province in South Africa, at two hospital-based and two community health centre (CHC) clinics. The two hospital-based clinics were at Chris Hani Baragwanath hospital, south of Johannesburg in Soweto, and Mamelodi hospital which is nearer Pretoria. The two community health clinics (CHC) were Ramokonopi and Jabulane Dumane CHCs, in Ekurhuleni district (**Figure 3-3**).

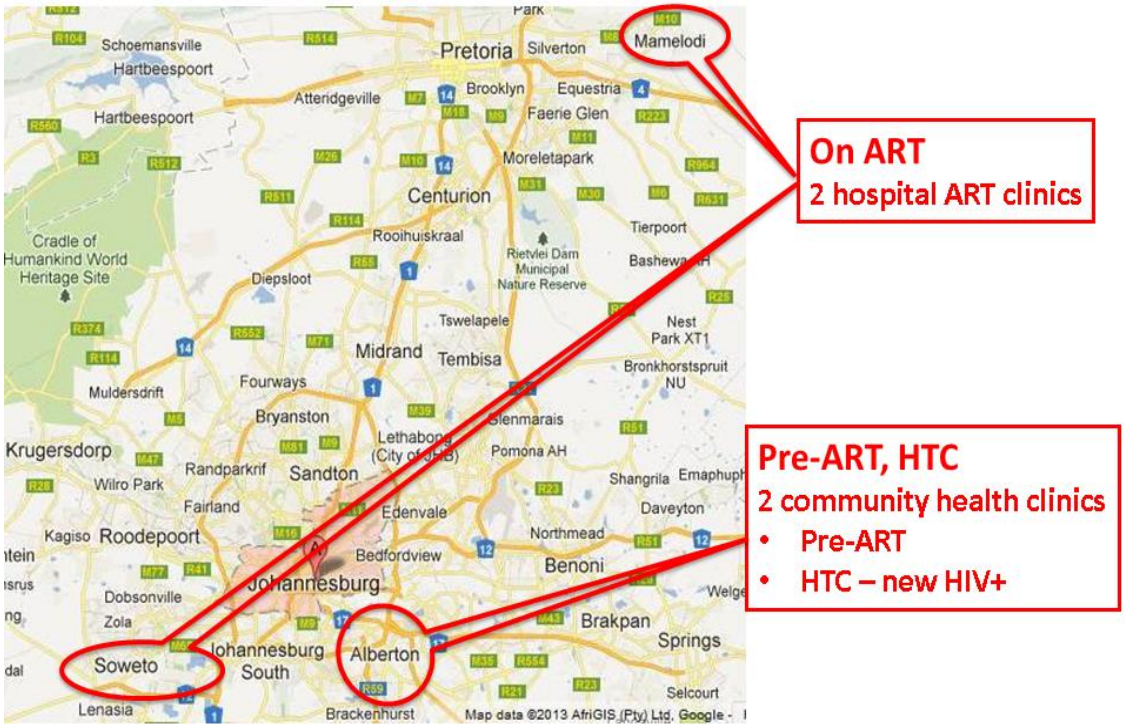
At the time the study was conducted, ART eligibility comprised CD4 ≤350 cells/mm$^3$ or WHO clinical stage ≥3. National guidelines for TB investigation during this time have already been described in **section 1.3** (**Figure 1-1)**.

## 3.3. XPHACTOR study population and recruitment

We enrolled a systematic sample of adults (aged ≥18 years) attending for HIV care, irrespective of the presence of symptoms suggestive of TB. Patients taking anti-tuberculosis treatment within the previous 3 months and those who were acutely unwell requiring urgent referral to higher level care were excluded. Patients were enrolled into three groups: "on antiretroviral therapy (ART)" (currently taking or ART-experienced) group; "pre-ART" (in HIV care but not yet taking ART) group; and "HIV Testing and Counselling (HTC)" (newly-diagnosed HIV-positive). We recruited to the on ART group from hospital clinics because their patient population solely comprised those ART-experienced; and pre-ART and HTC groups were recruited from CHCs.

The sampling strategy varied between study sites due to differences in clinic flow and numbers of patients. At the hospital-based clinics the numbers of patients attending were too large to invite consecutive patients to participate in our study. One of these sites had a clinic register which we used to systematically invite patients, at a predetermined frequency, to hear further information about the study. The other site had no register so we used simple random sampling; all patients in the waiting area were invited to select a stick or sweet hidden in a bag, and those who pulled a predetermined colour were invited to participate. The CHCs were smaller, and therefore consecutive patients attending the clinic were invited to participate.

**Figure 3-3. XPHACTOR study sites**




HTC = HIV Testing and Counselling

## 3.4. XPHACTOR procedures

### *3.4.1. Enrolment*

At enrolment, research staff administered a standardised questionnaire which incorporated the WHO tool, collected details of TB and HIV treatment, and basic demographic and socioeconomic information. Staff measured height and weight, MUAC, and recorded most recent clinic CD4 cell count. Further investigation was prioritised according to the XPHACTOR algorithm (**Figure 3-2**) with an immediate spot sputum sample sent for Xpert for individuals at *a priori* highest risk of active TB: (i) all assigned high priority; (ii) those in the pre-ART group with CD4 <200 cells/mm$^3$ at enrolment (iii) all in the HTC group (whose CD4 count was unknown) at enrolment. For all other participants, a spot sputum sample was collected at enrolment and frozen at -80 ºC within 24 hours, for smear microscopy and testing with Xpert at the end of the study. Testing of this sample enabled comparison of the sensitivity and specificity of the XPHACTOR study algorithm (Aim 1) to detect TB cases against the sensitivity and specificity if Xpert had been performed immediately for all with any WHO tool symptom; and to determine whether any smear-positive patients had been missed.

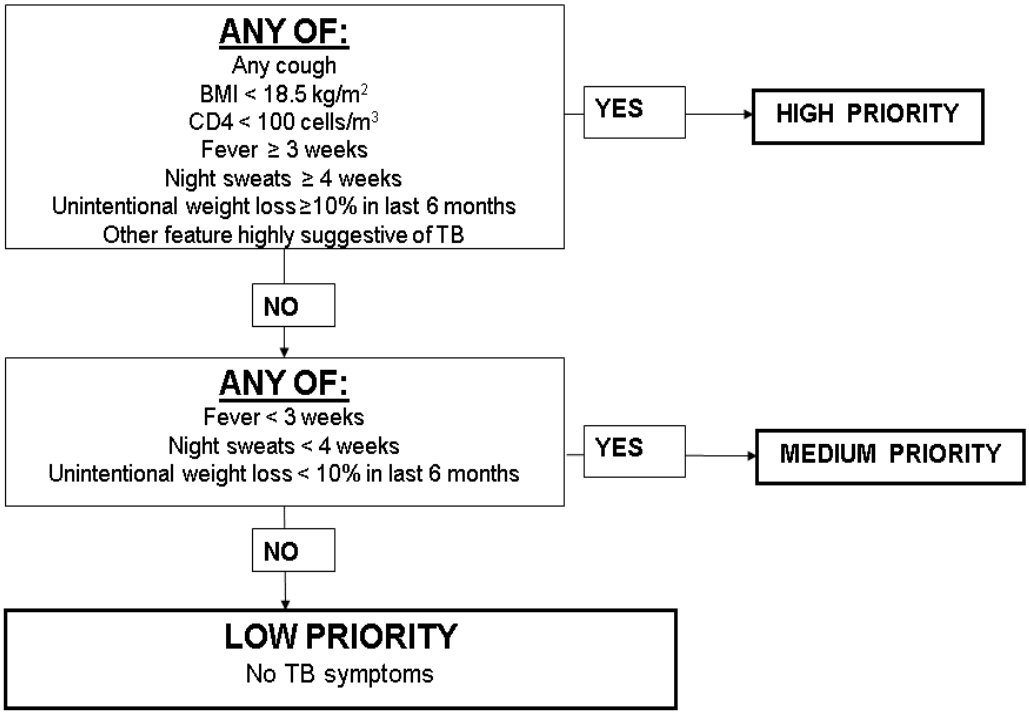
Individuals in the HTC group and those in the pre-ART group with CD4 <200 cells/mm$^3$ did not contribute to XPHACTOR aim 1 (evaluation of the study algorithm). This was because of *a priori* high risk of active TB, hence these participants underwent immediate testing with Xpert at enrolment and were not prioritised for testing using the study algorithm. These individuals contributed to XPHACTOR aim 2 **(Chapter 7 - Research Paper 3, "Investigating TB if initial Xpert is negative")**, if the immediate Xpert was negative.

### *3.4.2. Follow-up*

Participants were reviewed monthly to three months, with repeat WHO symptom screen and a spot sputum sample was requested for Xpert if high priority by the study algorithm at that visit (**Figure 3-4**), with the exception of those in the on ART group who were asymptomatic at enrolment, who were telephoned at 1 and 2 months to update locator information but were not asked about TB symptoms. The protocol was modified for these individuals, because we identified after enrolling around 1000 participants to the on ART group, that almost no TB diagnoses had been made at 1- and 2-month follow up in those assigned low priority at enrolment.

The study algorithm at follow up visits varied very slightly to ensure that participants who had persistent night sweats ≥4 weeks were investigated for TB, as night sweats of any duration were assigned medium priority at enrolment (**Figure 3-4**). At the 3-month visit sputum (induced if necessary) and blood were collected for mycobacterial culture on liquid media (Bactec MGIT 960 and 9240 systems, BD Diagnostics) from all study participants, regardless of symptoms. We allowed a broad window period around the scheduled 3-month visit, until around six months, in order to maximise study follow-up.

**Figure 3-4. XPHACTOR algorithm at monthly follow up**




BMI = Body mass index

Participants who submitted an Xpert sample were reviewed within one week. If Xpert-positive, TB treatment was initiated; if negative, research staff repeated the WHO symptom screen and facilitated the Xpert-negative algorithm which comprised chest radiograph, spot sputum for TB culture, and antibiotic trial if clinically appropriate. The Xpert-negative algorithm was also facilitated, because of *a priori* high risk of active TB, for all pre-ART participants with CD4 count <200 cells/mm$^3$ who had submitted sputum for immediate Xpert at enrolment to XPHACTOR.

Chest radiographs were reported by a single reader (consultant radiologist or physician), and data extracted onto a standardised form. Investigation results were returned to clinic staff, who were responsible for management decisions. Clinic records were reviewed at the end of the study to ascertain any additional relevant investigations and/or TB diagnoses. Deaths were identified through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants' South African identification (ID) numbers if they were South African citizens.

Methods relevant to specific research aims are detailed in the relevant papers (**chapters 5 to 8**).

## 3.5. Laboratory methods

### *3.5.1. Xpert MTB/RIF*

Xpert testing was undertaken at the routine National Health Laboratory Services (NHLS) for sputum samples for immediate Xpert requested at Chris Hani Baragwanath hospital. For all other sites (due to their resource limitations) and for all stored samples Xpert testing was undertaken at the research laboratory (Centre for Tuberculosis, which is a national reference laboratory) by experienced research laboratory technologists.

### *3.5.2. Mycobacterial culture*

Sputum for mycobacterial culture requested as part of the Xpert-negative algorithm was generally undertaken at the routine NHLS laboratories. Sputum samples collected for the XPHACTOR 3-month visit were processed at the research laboratory, by fluorochrome staining for acid-fast bacilli and fluorescence microscopy, and cultured using BACTEC™ Mycobacteria Growth Indicator Tube (MGIT) 960 (BD, Sparks, MD, USA). Line probe assay (LPA) was performed on smear-positive or cultured isolates (GenoType MTBDRplus, Hain Lifesciences) to identify MTB complex and resistance to isoniazid or rifampicin. If resistance was identified, then further drug susceptibility testing was undertaken. Mycobacterial culture on 3-month visit blood samples was performed using the BD Bactec™ 9240 system.

### *3.5.3. LF-LAM*

At the end of the study urine samples were thawed to ambient temperature and tested with LF-LAM by the research laboratory technologists in accordance with training provided by Alere representatives. The technologists did not have access to other bacteriological results when performing the LF-LAM tests. Each test was graded once, using the pre-January 2014 manufacturer's reference card comprising five grades of colour intensity

with the least intense band assigned grade 1, absence of a band graded negative, and absence of a control band deemed a failed test.[207]

## 3.6. Case Definitions

A 2005 community-based HIV and TB prevalence survey in South Africa reported a large burden of previously undiagnosed bacteriologically-confirmed pulmonary TB, mainly amongst those HIV-positive, of which two-thirds of cases were asymptomatic.[208] This study estimated mean time before initiation of TB treatment of around 1 year irrespective of smear or HIV status. Mathematical modelling estimates a nine month period of subclinical disease prior to a diagnosis of TB being made.[209] Studies amongst PLHIV with LTBI which have used highly sensitive imaging modalities,[210, 211] and the discovery of a blood biomarker which predicts the risk of active TB within 12 months,[212] provide evidence for a continuum of disease from infection with MTB to clinically active disease, and potentially a long infectious period.[213] The estimates for the duration of subclinical disease in the aforementioned study were considered when assigning case definitions for prevalent TB in XPHACTOR.[209]

### 3.6.1. *TB case definitions*

A diagnosis of **"confirmed TB"** was assigned to individuals with a positive result on i) Xpert (on sputum sample) or ii) LPA (GenoType MTBDR*plus*, Hain Lifesciences) performed on smear-positive or cultured isolate or iii) *M. tuberculosis* (MTB) culture, from any sample collected within six months of enrolment to the XPHACTOR study.

A diagnosis of **"clinical TB"** was assigned to individuals who commenced TB treatment within six months of enrolment to XPHACTOR in the absence of microbiological confirmation.

Participants who died within three months of enrolment without fulfilling TB case definitions or who were diagnosed with TB more than 6 months after enrolment were deemed to have **"unclassifiable"** TB outcome and excluded from all analyses.

For evaluating the accuracy of LF-LAM (**Chapter 5**, **Paper 1**), the XPHACTOR algorithm, and the WHO tool for TB screening in XPHACTOR participants, **"not TB"** was defined as

fulfilling all of the following: absence of criteria for confirmed or clinical TB; alive at least 3 months after enrolment; and no positive microbiology for MTB (at least 1 MTB culture or Xpert result) from any sample within 6 months of enrolment. Participants who did not fulfil the case definitions for TB or "not TB" were excluded from these analyses.

### 3.6.2. *Radiological definitions*

"Probable radiological TB" was defined as the presence of i) any of cavitation, predominantly upper lobe infiltrates, pleural or pericardial effusion, or clear miliary picture on chest radiograph or ii) any of abdominal lymphadenopathy, splenic microabscesses, pleural or pericardial effusion on ultrasound scan.

"Possible radiological TB" was defined as the presence of any of lymphadenopathy (hilar or mediastinal), pulmonary nodules or other infiltrates.

Participants with "probable" or "possible" radiological TB features, but without bacteriological confirmation, who started TB treatment within six months of enrolment (or within six months of the 3-month visit if participating in the "Causes of TB symptoms" aim) were assigned "clinical" TB.

## 3.7. Sample size

The sample size for XPHACTOR was based on estimating the sensitivity, with reasonable precision, of the study algorithm for undiagnosed TB amongst HIV-positive clinic attendees. The sample size calculation assumed a prevalence of bacteriologically-confirmed undiagnosed TB of 5% amongst HIV clinic attendees. If the sensitivity of the algorithm was 95%, 90%, and 85% respectively, then with 150 TB diagnoses, the sensitivity could be estimated with 95% confidence intervals respectively of 90.6-98.1%, 84.0-94.3%, and 78.6-90.6%. In order to identify 150 TB diagnoses, 3000 HIV-positive clinic attendees needed to be recruited, and assuming that 80% were followed up to 3-months, the total sample size required was 3750.

## 3.8. Ethical issues due to delaying diagnostic testing

Ethical issues were discussed with a member of the University of Cape Town (UCT) ethics committee when the protocol was being developed and were detailed in the study protocol which was approved by LSHTM and local ethics committees. Firstly, the study was conducted amongst those who would potentially benefit from the results, as individuals attending for HIV care are at high risk of both having undiagnosed TB and are at risk of acquiring TB from others with undiagnosed TB in the clinic. Secondly, although in theory the study withheld investigation from some individuals who, according to ICF guidelines, should have been investigated, experience from these clinics was that these guidelines were not being implemented, and were unlikely to be so in resource-limited settings because of the high cost. XPHACTOR was considered likely to promote effective screening, by generating an evidence-base for rational screening policy, which would ultimately benefit HIV clinic attendees.

There were potential issues around collecting sputum samples and storing them for later, rather than immediate testing with Xpert, for participants categorised as “medium” or “low” priority at enrolment. The strategy of storing for later testing was important in order to evaluate the study algorithm. Participants assigned “low priority” (no TB symptoms) at enrolment were highly unlikely to have had TB, so delayed testing of their sputum was unlikely to have delayed TB diagnosis in this group who would not have had sputum collected under routine circumstances. Delaying testing might have delayed TB diagnosis in participants assigned “medium priority”, but research staff always advised participants to return to the clinic (who were responsible for their care) if their symptoms worsened. Furthermore, these participants were reviewed at monthly intervals, and would have undergone investigation with Xpert if they became “high priority”. Any participant with cough was always assigned high priority, and therefore would not have had delayed testing, and therefore the risk to other patients at the clinic would be minimised.

# 4) XPHACTOR study key results

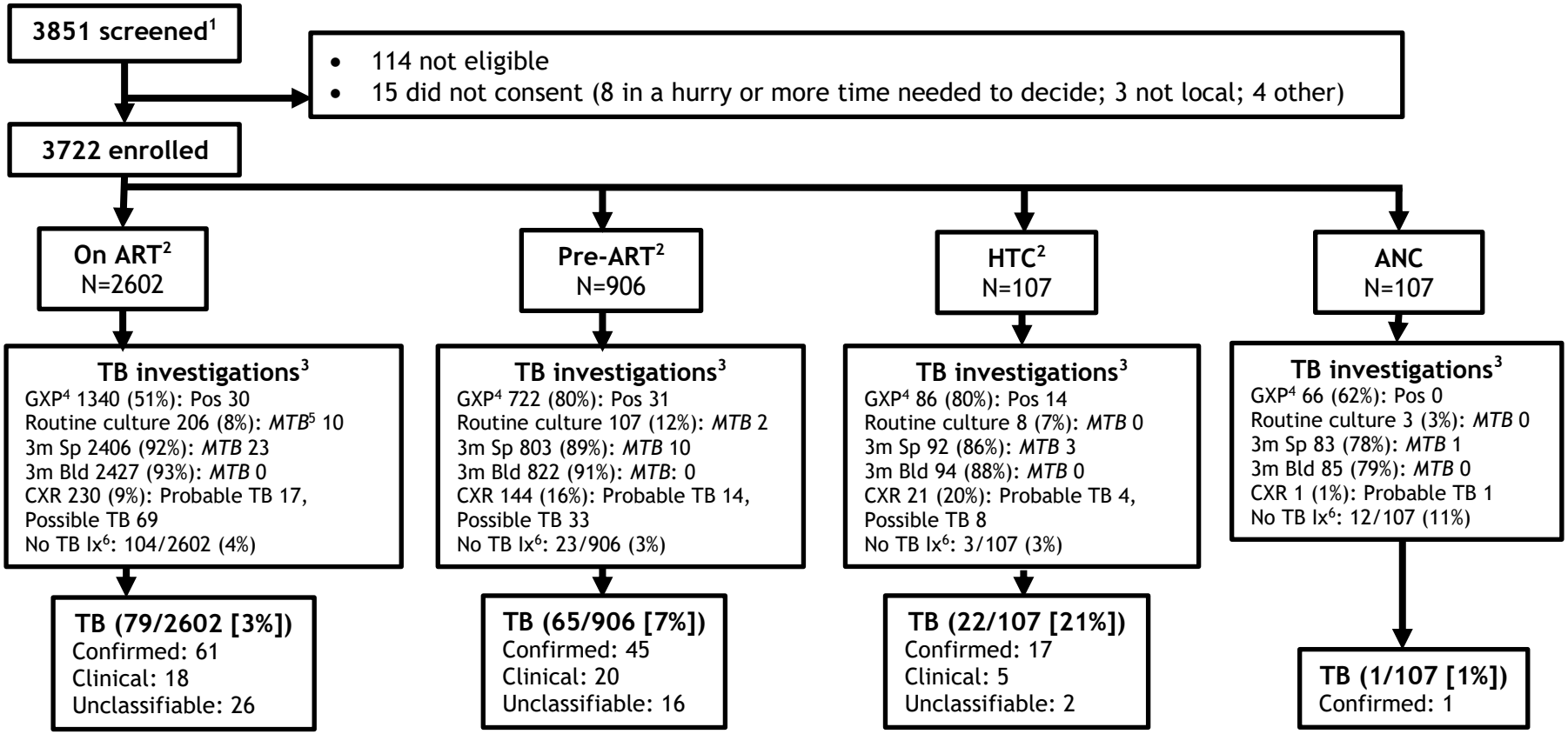
This chapter details key results from the XPHACTOR study which provide context for the findings of the research papers, and enable comparison with the published literature pertaining to TB screening in PLHIV in LMIC. The results presented in this section comprise the study profile and baseline characteristics of the participants, the prevalence of TB, the performance of the XPHACTOR study algorithm for TB screening, and the frequency of WHO tool symptoms.

## 4.1. Characteristics of study participants

From September 2012 to February 2014, 3722 participants were enrolled into XPHACTOR (2602 on ART, 906 pre-ART, and 214 from HTC services of whom 107 were enrolled from antenatal HTC services [ANC]) (**Figure 4-1**). 3473 (93%) of participants were followed to 3 months and all the 3-month visits were completed by May 2014.

**Table 4-1** summarises the baseline characteristics of XPHACTOR participants. The median CD4 cell counts amongst on ART vs. pre-ART vs. HTC vs. ANC groups were 436 vs. 402 vs. 248 vs. 379 cells/$mm^3$. In the on ART group the median duration on ART was 4 years (interquartile range [IQR] 2-6) and 74.7% had suppressed viral load. At enrolment 1213/3722 (32.6%) of all participants reported at least one WHO tool symptom (on ART 30.1%, pre-ART 38.6%, HTC 61.7%, ANC 13.1%). 1997/3722 (53.7%) of participants were able to produce a sputum sample at enrolment for testing with Xpert (either immediate testing or stored for testing at the end of the study). The most common WHO tool symptoms reported were cough 750/3722 (20.2%) and weight loss 544/3721 (14.7%).

**Figure 4-1. XPHACTOR profile**




[1] At on ART sites 4956 patients were approached & from one site data is available regarding reasons 1522/3186 declined to be screened (594 not interested; 567 no time;151 agreed to screen at next visit; 118 no reason; 92 other), at pre-ART sites all patients were referred by clinic staff and data is not available regarding those who declined screening; [2] 50 died within 6 months of enrolment (on ART = 23; pre-ART = 25; HTC = 2);
[3] Undertaken at enrolment or during follow-up to 3m visit & participants could have >1 sample positive for MTB;
[4] Routine or for study purposes; [5] 1 *MTB* in pleural fluid; [6] No CXR or TB microbiology
ANC, New HIV+ enrolled from antenatal services; HTC, New HIV+ enrolled from HIV testing and counselling services; GXP = sputum Xpert; 3m Sp cul = Sputum TB culture at 3-month visit; 3m bld cul = Blood TB culture at 3-month visit.

**Table 4-1 Baseline characteristics of XPHACTOR participants N=3722**

| Characteristic | On ART N=2602 | Pre-ART N=906 | HTC N=107 | ANC N=107 |
|---|---|---|---|---|
| **Demographics:** | | | | |
| Age, years - Median (IQR) | 41 (35-48) | 35 (29-42) | 35 (30-41) | 30 (25-33), N=105 |
| Female – N (%) | 1838 (70.6%) | 623 (68.8%) | 57 (53.3%) | 107 (100%) |
| Black African – N (%) | 2560 (98.4%), N=2601 | 904 (99.8%) | 105 (98.1%) | 107 (100%) |
| **HIV/TB history** | | | | |
| Duration since HIV diagnosed, months - Median (IQR) | 66 (38-99), N=2585 | 7 (1-30), N=899 | N/A | N/A |
| ART commenced during study follow-up n (%) | N/A | 396 (43.7%) | 57 (53.3%) | 8 (7.5%) |
| Duration on ART, months - Median (IQR) | 50 (28-79), N=2601 | N/A | N/A | N/A |
| Previous IPT – N (%) | 63 (2.4%), N=2601 | 167 (18.4%) | N/A | N/A |
| Current IPT – N (%) | 19 (0.7%) | 172 (19.0%) | N/A | N/A |
| Previous TB treatment – N (%) | 1028 (39.5%) | 71 (7.8%) | 10 (9.4%) | 4 (4.7%) |
| >1 previous episode of TB treatment – N (%) | 166 (6.3%) | 7 (0.8%) | 0 | 1 (0.9%) |
| **CD4 / Viral load / BMI at enrolment** | | | | |
| CD4, cells/mm$^3$ - Median (IQR) | 436 (278-621), N=2599 | 402 (224-555), N=905 | 248 (106-421), N=103 | 379 (234-556), N=104 |
| Viral load suppressed (<20 copies/ml) – N (%) | 1624 (74.7%), N=2174 | N/A | N/A | N/A |
| BMI, kg/m$^2$ - Median (IQR) | 25 (21.6-29.4), N=2598 | 24.6 (20.9-29.5) | 23.4 (20.2-28.6) | 29 (26.6-32.8), N=106 |
| **WHO tool positive at enrolment** – N (%) | **783 (30.1%)** | **350 (38.6%)** | **66 (61.7%)** | **14 (13.1%)** |
| Cough – N (%) | 500 (19.2%) | 200 (22.1%) | 40 (37.4%) | 10 (9.4%) |
| Unintentional weight loss – N (%) | 295 (11.3%), N=2601 | 206 (22.7%) | 41 (38.3%) | 2 (1.9%) |
| Night sweats – N (%) | 176 (6.8%) | 113 (12.5%) | 25 (23.4%) | 2 (1.9%) |
| Fever – N (%) | 121 (4.7%) | 74 (8.2%) | 19 (17.8%) | 3 (2.8%) |
| >1 WHO tool symptom reported – N (%) | 225 (8.6%) | 162 (17.9%) | 38 (35.5%) | 1 (0.9%) |
| **Reported history of smoking or respiratory disease** | | | | |
| Ex- or current smoker[1] – N (%) | 586 (22.5%) N=2600 | 268 (29.6%) | 33 (30.8%) | 13 (13.1%) |
| Chronic respiratory disease (asthma, COPD, silicosis) - N (%) | 121 (4.7%) N=2600 | 33 (3.6%) | 3 (2.8%) | 4 (3.7%) |

BMI, body mass index; COPD, chronic obstructive pulmonary disease; IPT, isoniazid preventive therapy; IQR, interquartile range; HTC, New HIV+ enrolled from HIV testing and counselling services; ANC, New HIV+ enrolled from antenatal services; N/A, not applicable; [1] Smoker defined as having ever smoked ≥ 100 cigarettes

## 4.2. Prevalence of TB

The prevalence of TB, overall and stratified by each group is shown in **Table 4-2** for 3678 participants, having excluding 44 participants with unclassifiable outcome (28 died within 3 months of enrolment without a TB diagnosis, 15 were diagnosed with TB > 6 months after enrolment, 1 participant did not attend for study follow up after enrolment). 167/3678 (4.5%) of participants fulfilled the study case definitions for TB, and for 153 the site of TB was recorded (pulmonary only 133/153 [86.9%], extrapulmonary only 15/153 [9.8%], and both 5/153 [3.3%]).

30/3678 (0.8%) of study participants who were diagnosed with TB did not report any WHO tool symptoms at enrolment. These comprised 27/124 (21.8%) with confirmed TB and 3/43 (7.0%) with clinical TB.

**Table 4-2 Prevalence of TB in XPHACTOR study**

| Group | Number | All TB<br>n/N<br>% (95% CI) | Confirmed TB<br>n/N<br>% (95% CI) | Clinical TB<br>n/N<br>% (95% CI) |
|---|---|---|---|---|
| On ART | 2576 | 79/2576<br>3.1% (2.4, 3.8) | 61/2576<br>2.4% (1.8, 3.0) | 18/2576<br>0.7% (0.4, 1.1) |
| Pre-ART | 890 | 65/890<br>7.3% (5.7, 9.2) | 45/890<br>5.1% (3.7, 6.7) | 20/890<br>2.3% (1.4, 3.4) |
| HTC | 105 | 22/105<br>21.0% (13.6, 30.0) | 17/105<br>16.2% (9.7, 24.6) | 5/105<br>4.8% (1.6, 10.7) |
| ANC | 107 | 1/107<br>0.9% (<0.001, 5.1) | 1/107<br>0.9% (<0.001, 5.1) | 0 |
| Overall | 3678 | 167/3678<br>4.5% (3.9, 5.3) | 124/3678<br>3.4% (2.8, 4.0) | 43/3678<br>1.2% (0.8, 1.6) |

ANC, New HIV+ enrolled from antenatal services; HTC, HIV testing and counselling services

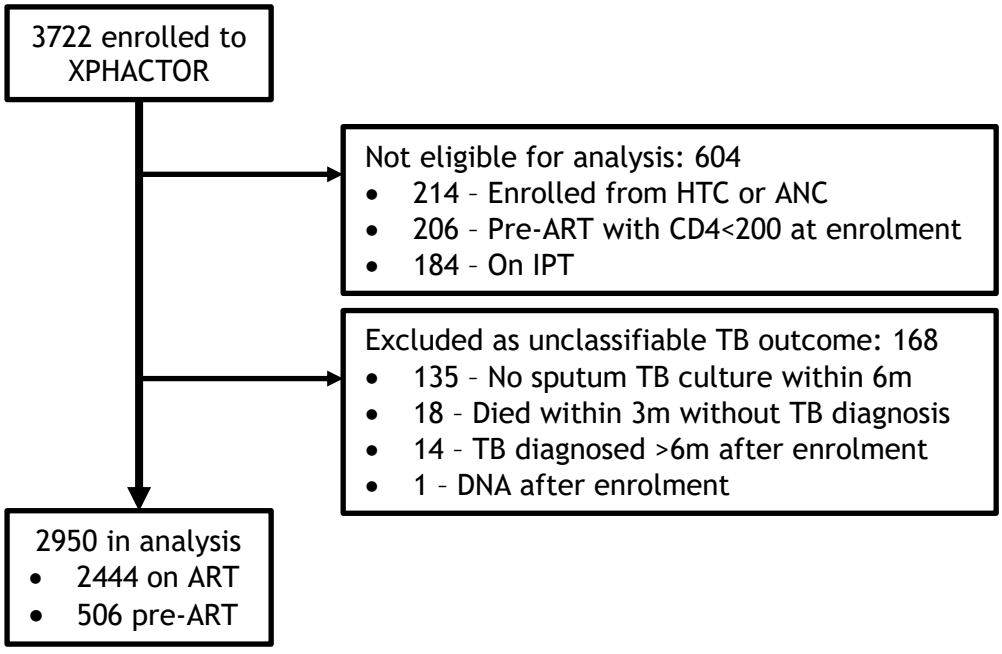
## 4.3. Performance of the XPHACTOR algorithm and the WHO tool

This analysis was undertaken using XPHACTOR enrolment data. Participants in the HTC and ANC groups, and those who were pre-ART with CD4 <200 cells/mm$^3$ at enrolment were excluded, because they were all investigated with immediate Xpert at enrolment due to their high risk of TB. Participants for whom we did not have microbiological confirmation of "not TB" from at least one sample, i.e. a negative TB culture or negative Xpert result;

and those currently on IPT were also excluded. The latter were excluded as they were likely to have recently undergone investigation for TB, and hence were effectively "pre-screened" for TB.

**Figure 4-2** details the flow of participants who were included in this analysis. Among 3722 participants enrolled to XPHACTOR, 604 were excluded as they were either enrolled through HTC or ANC (n=214), pre-ART with CD4<200 cells/$mm^3$ at enrolment (n=206), or were on IPT at enrolment (n=184). A further 168 participants were excluded due to unclassifiable TB outcome (no sputum result for MTB microbiology within 6 months of enrolment [135], died within 3 months of enrolment without a TB diagnosis [18], TB diagnosis from specimens taken more than 6 months after enrolment [14], did not attend for any study follow up [1]), leaving 2950 participants in the analysis (2444 on ART, 506 pre-ART).

**Figure 4-2. Flow chart of participants included in the evaluation of XPHACTOR algorithm**

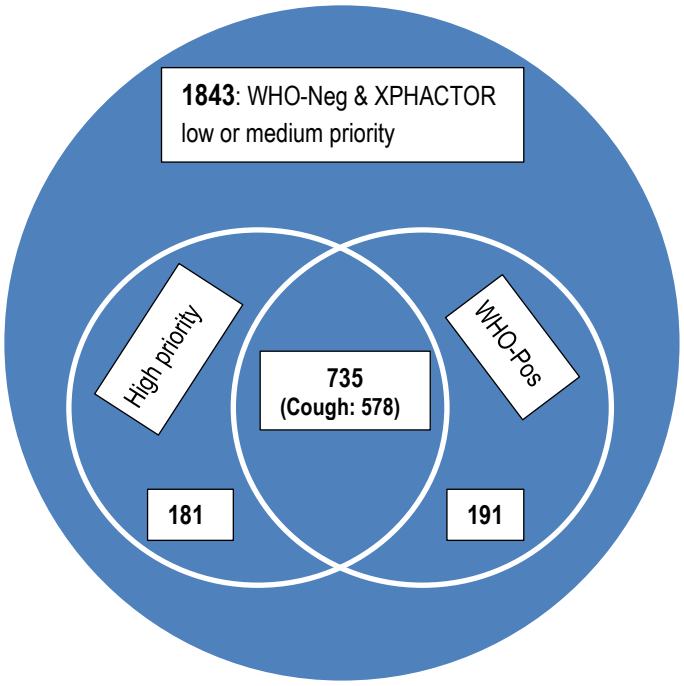



IPT, isoniazid preventive therapy; DNA, did not attend
ANC, New HIV+ enrolled from antenatal services; HTC, HIV testing and counselling services

916/2950 (31.1%) of participants fulfilled XPHACTOR high priority criteria, and 926/2950 (31.4%) reported WHO tool symptoms (**Figure 4-3**). 735/2950 (24.9%) of participants were

both XPHACTOR high priority and reported WHO tool symptoms, amongst whom the most commonly reported symptom was cough (n=578).

**Figure 4-3. Number of participants who were XPHACTOR high priority vs. number WHO tool positive (N=2950)**




98/2950 (3.3%; 95% CI 2.7, 4.0) of participants in this analysis fulfilled case definitions for TB (73 confirmed, 25 clinical). The sensitivity and specificity for TB (confirmed and clinical combined) was 69.4% and 70.3% for the XPHACTOR algorithm vs. 72.4% and 70.0% for the WHO tool (**Table 4-3**). The XPHACTOR algorithm had greater sensitivity for TB (confirmed and clinical combined) in the on ART vs. pre-ART group (70.9% vs. 63.2%), compared with the WHO tool which was less sensitive amongst those on ART vs. pre-ART (68.4% vs. 89.5%).

The performance of the study algorithm, in terms of overall sensitivity and specificity for TB, was therefore similar to that of the WHO tool in our study population, and this was largely because cough was common and the main driver of both algorithms (**Figure 4-3**).

The prevalence of TB, performance of the XPHACTOR algorithm and performance of the WHO tool in our study population are discussed and compared with the published literature in **Chapter 9.**

**Table 4-3 Performance of the XPHACTOR algorithm and WHO tool for TB screening at enrolment**

| *Confirmed and clinical TB (98/2950)* | Sensitivity<br>n/N<br>% (95% CI) | Specificity<br>n/N<br>% (95% CI) | NPV<br>n/N<br>% (95% CI) | PPV<br>n/N<br>% (95% CI) |
|---|---|---|---|---|
| **XPHACTOR high priority** | **68/98**<br>**69.4% (59.3, 78.3)** | **2004/2852**<br>**70.3% (68.6, 71.9)** | **2004/2034**<br>**98.5% (97.9, 99.0)** | **68/916**<br>**7.4% (5.8, 9.3)** |
| **On ART** | 56/79<br>70.9% (59.6, 80.6) | 1652/2365<br>69.9% (68.0, 71.7) | 1652/1675<br>98.6% (97.9, 99.1) | 56/769<br>7.3% (5.6, 9.4) |
| **Pre-ART** | 12/19<br>63.2% (38.4, 83.7) | 352/487<br>72.3% (68.1, 76.2) | 352/359<br>98.1% (96, 99.2) | 12/147<br>8.2% (4.3, 13.8) |
| **WHO tool positive** | **71/98**<br>**72.4% (62.5, 81.0)** | **1997/2852**<br>**70.0% (68.3, 71.7)** | **1997/2024**<br>**98.7% (98.1, 99.1)** | **71/926**<br>**7.7% (6.0, 9.6)** |
| **On ART** | 54/79<br>68.4% (56.9, 78.4) | 1673/2365<br>70.7% (68.9, 72.6) | 1673/1698<br>98.5% (97.8, 99.0) | 54/746<br>7.2% (5.5, 9.3) |
| **Pre-ART** | 17/19<br>89.5% (66.9, 98.7) | 324/487<br>66.5% (62.1, 70.7) | 324/326<br>99.4% (97.8, 99.9) | 17/180<br>9.4% (5.6, 14.7) |
| ***Confirmed TB[1] (73/2925)*** | | | | |
| **XPHACTOR high priority** | **48/73**<br>**65.8% (53.7, 76.5)** | **2004/2852**<br>**70.3% (68.6, 71.9)** | **2004/2029**<br>**98.8% (98.2, 99.2)** | **48/896**<br>**5.4% (4.0, 7.0)** |
| **On ART** | 39/61<br>63.9% (50.6, 75.8) | 1652/2365<br>69.9% (68.0, 71.7) | 1652/1674<br>98.7% (98.0, 99.2) | 39/752<br>5.2% (3.7, 7.0) |
| **Pre-ART** | 9/12<br>75.0% (42.8, 94.5) | 352/487<br>72.3% (68.1, 76.2) | 352/355<br>99.2% (97.6, 99.8) | 9/144<br>6.3% (2.9, 11.5) |
| **WHO tool positive** | **49/73**<br>**67.1% (55.1, 77.7)** | **1997/2852**<br>**70.0% (68.3, 71.7)** | **1997/2021**<br>**98.8% (98.2, 99.2)** | **49/904**<br>**5.4% (4.0, 7.1)** |
| **On ART** | 38/61<br>62.3% (49.0, 74.4) | 1673/2365<br>70.7% (68.9, 72.6) | 1673/1696<br>98.6% (98.0, 99.1) | 38/730<br>5.2% (3.7, 7.1) |
| **Pre-ART** | 11/12<br>91.7% (61.5, 99.8) | 324/487<br>66.5% (62.1, 70.7) | 324/325<br>99.7% (98.3, >99.9) | 11/174<br>6.3% (3.2,11.0) |

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value
[1]Clinical TB excluded from analysis

## 4.4. "Natural history" of symptoms suggestive of TB in XPHACTOR

### *4.4.1. Introduction, aim and objectives*

Published studies in which PLHIV have been systematically screened for TB prior to initiation of ART[54-56] or IPT[214] report a high proportion with symptoms suggestive of TB, although most do not have TB. At the time that the XPHACTOR study was commenced there was a paucity of published data regarding the prevalence of symptoms suggestive of TB amongst individuals established in HIV care.

The aim of this analysis was to determine the "natural history" of TB symptoms among individuals without a final diagnosis of TB, in order to estimate the likely demand for repeat diagnostic testing for TB among patients attending for HIV care.

The objective was, using data collected for the XPHACTOR study (from monthly follow-up visits during the entire study duration i.e. October 2012 to May 2014), to describe the frequency of WHO tool symptoms reported by individuals attending for HIV care at monthly intervals up to the 3-month visit.

### *4.4.2. Inclusion and exclusion criteria for this analysis*

This analysis was restricted to XPHACTOR study participants who had WHO tool symptom data available at both enrolment and 3-month visit. The following individuals were excluded from the analysis: i) those without sputum mycobacterial culture results from the 3-month visit, in order to ensure there was microbiological confirmation that TB had been excluded; ii) all those who fulfilled case definitions for TB at any point during study follow-up.

### *4.4.3. Statistical methods*

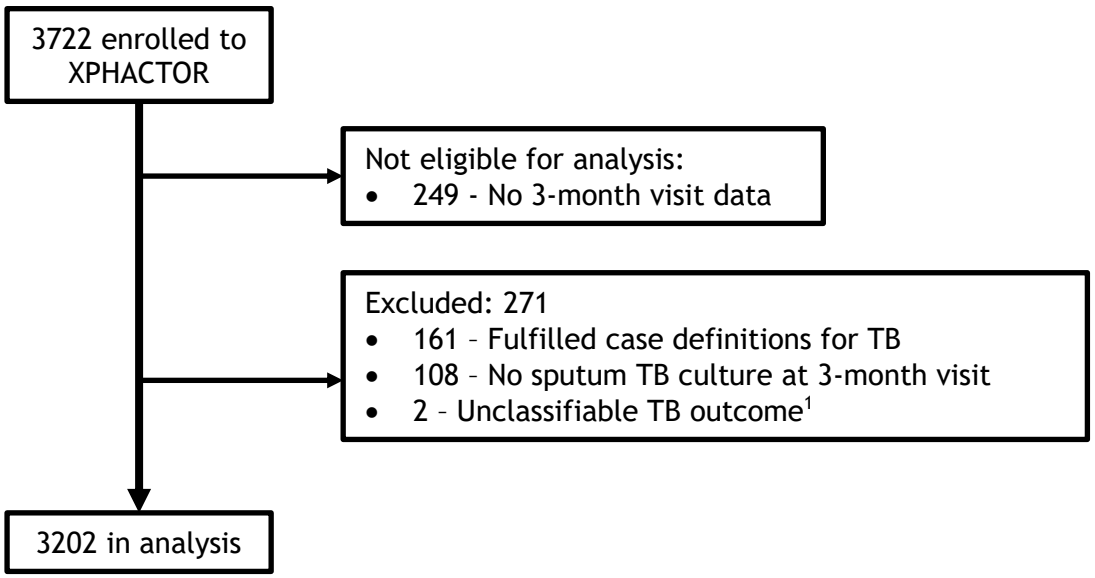
Positive WHO tool screen (any of self-reported cough, fever, night sweats or unintentional weight loss) and frequency of WHO tool symptoms were summarised at enrolment and at each monthly follow up visit. The same variables were summarised in a separate analysis restricted to those who reported WHO tool symptoms at enrolment to XPHACTOR.

The number of sputum samples collected as part of routine care or for study purposes because participants were: i) deemed at high risk of TB (pre-ART with CD4<200 or newly diagnosed HIV-positive at enrolment) or XPHACTOR high priority; or ii) WHO tool positive at enrolment was summarised.

### *4.4.4. Results*

Amongst 3722 participants enrolled into XPHACTOR (2602 on ART, 906 pre-ART, and 214 from HTC services of whom 107 were enrolled from antenatal services), 3-month visit data were available for 3473 (93%) of participants. 3202 XPHACTOR study participants fulfilled the criteria for this analysis (**Figure 4-4**), and their characteristics are summarized in **table 4-4**.

**Figure 4-4. Flow chart of participants in "frequency of symptoms suggesting TB"**




[1] Positive result on sputum Xpert or TB culture during follow-up, but reinvestigated by clinic as asymptomatic and repeat sputum mycobacteriology was negative so not treated for TB

**Table 4-4 Characteristics of participants in frequency of symptoms suggestive of TB analysis N=3202**

| Characteristic | On ART N=2306 | Pre-ART N=743 | HTC N=71 | ANC N=82 |
|---|---|---|---|---|
| **Demographics:** | | | | |
| Age, years - Median (IQR) | 41 (35-48) | 35 (29-42) | 34 (29-41) | 29 (25-32), N=81 |
| Female – N (%) | 1638 (71.0%) | 519 (69.9%) | 36 (50.7%) | 82 (100%) |
| Black African– N (%) | 2271 (98.5%) | 741 (92.5%) | 99 (96.1%) | 82 (100%) |
| **HIV/TB history** | | | | |
| Duration since HIV diagnosed, months - Median (IQR) | 66 (39-99), N=2290 | 9 (1-33), N=737 | N/A | N/A |
| ART commenced during study follow-up – N (%) | N/A | 307 (41.3%) | 42 (59.2%) | 76 (92.7%) |
| Duration on ART, months - Median (IQR) | 50 (28-79) | N/A | N/A | N/A |
| Previous IPT– N (%) | 51 (2.2%), N=2305 | 151 (20.3%) | N/A | N/A |
| Current IPT– N (%) | 17 (0.7%) | 144 (19.4%) | N/A | N/A |
| Previous TB treatment – N (%) | 900 (39.0%) | 56 (7.5%) | 4 (5.6%) | 5 (6.1%) |
| >1 previous episode of TB treatment – N (%) | 142 (6.2%) | 4 (0.5%) | 0 | 1 (1.2%) |
| **CD4 / Viral load / BMI at enrolment** | | | | |
| CD4, cells/mm$^3$ - Median (IQR) | 441 (285-626), N=2304 | 415 (252-561) | 271 (151-436), N=69 | 374 (217-530), N=81 |
| Viral load suppressed (<20 copies/ml) – N (%) | 1463 (75.1%), N=1947 | N/A | N/A | N/A |
| BMI, kg/m$^2$ - Median (IQR) | 25.2 (21.8-29.5), N=2303 | 25.3 (21.5- 30.0) | 24.7 (21.3-29.0) | 29.4 (26.6-32.8) |
| **WHO tool positive at enrolment** – N (%) | **653 (28.3%)** | **255 (34.3%)** | **37 (52.1%)** | **12 (14.6%)** |
| Cough – N (%) | 413 (17.9%) | 146 (19.7%) | 21 (29.6%) | 9 (11.0%) |
| Unintentional weight loss – N (%) | 226 (9.8%), N=2305 | 135 (18.2%) | 23 (32.4%) | 1 (1.2%) |
| Night sweats – N (%) | 141 (6.1%) | 76 (10.2%) | 11 (15.5%) | 1 (1.2%) |
| Fever – N (%) | 101 (4.4%) | 52 (7.0%) | 9 (12.7%) | 1 (1.2%) |
| >1 WHO tool symptom reported – N (%) | 168 (7.3%) | 105 (14.1%) | 18 (25.3%) | 0 |
| **Reported history of smoking or respiratory disease** | | | | |
| Ex- or current smoker[1] – N (%) | 522 (22.7%), N=2304 | 211 (28.4%) | 21 (29.6%) | 11 (13.4%) |
| Chronic respiratory disease (asthma, COPD, silicosis) – N (%) | 108 (4.7%), N=2305 | 23 (3.1%) | 1 (1.4%) | 3 (3.7%) |

BMI, body mass index; COPD, chronic obstructive pulmonary disease; IPT, isoniazid preventive therapy; IQR, interquartile range; HTC, New HIV+ enrolled from HIV testing and counselling services; ANC, New HIV+ enrolled from antenatal services; N/A, not applicable

[1] Smoker defined as having ever smoked ≥ 100 cigarettes

### *Characteristics of participants*

Amongst 3202 participants included in this analysis, at enrolment 2306 (72%) were on ART for a median of 4 years (interquartile range [IQR] 2-6), 743 (23%) were pre-ART, 71(2%) and 82 (3%) were newly diagnosed HIV-positive from HTC and ANC respectively. Overall 957/3202 (30%) were WHO tool positive at enrolment, of whom 291/957 (30%) reported more than one symptom. The most common WHO tool symptoms reported were cough 589/3202 (18%) and weight loss 385/3201 (12%).

### *Frequency of TB symptoms during study follow-up*

At 1, 2, and 3-month visits respectively, 325/2148 (15%) vs. 273/2017 (14%) vs. 325/3202 (10%) of participants reported at least one WHO tool symptom. The 3-month visit was undertaken at median 85 days (IQR 84-110; N=3196) from enrolment. At all visits the most commonly reported symptoms were cough and weight loss (**Figure 4-5**). A similar pattern was seen when the analysis was restricted to participants who were established on ART at enrolment (N=2306, **Figure 4-6**) or those not on ART at enrolment (N=896, **Figure 4-7**).

**Figure 4-5. Overall percentage with WHO tool symptoms during follow-up (N=3202)**

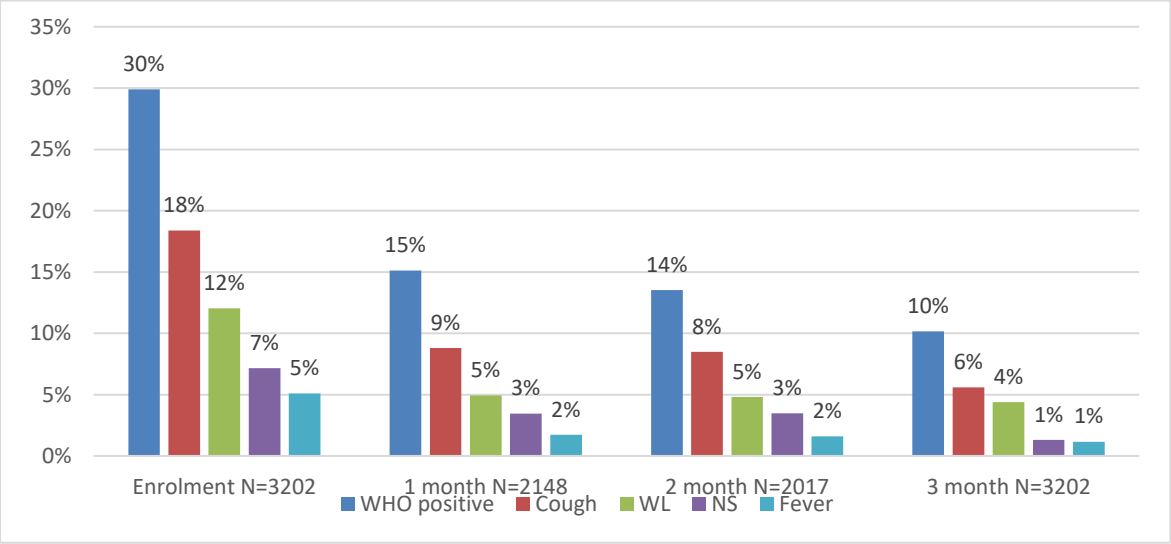


**Figure 4-6. WHO tool symptoms during follow-up amongst those on ART (N=2306)**

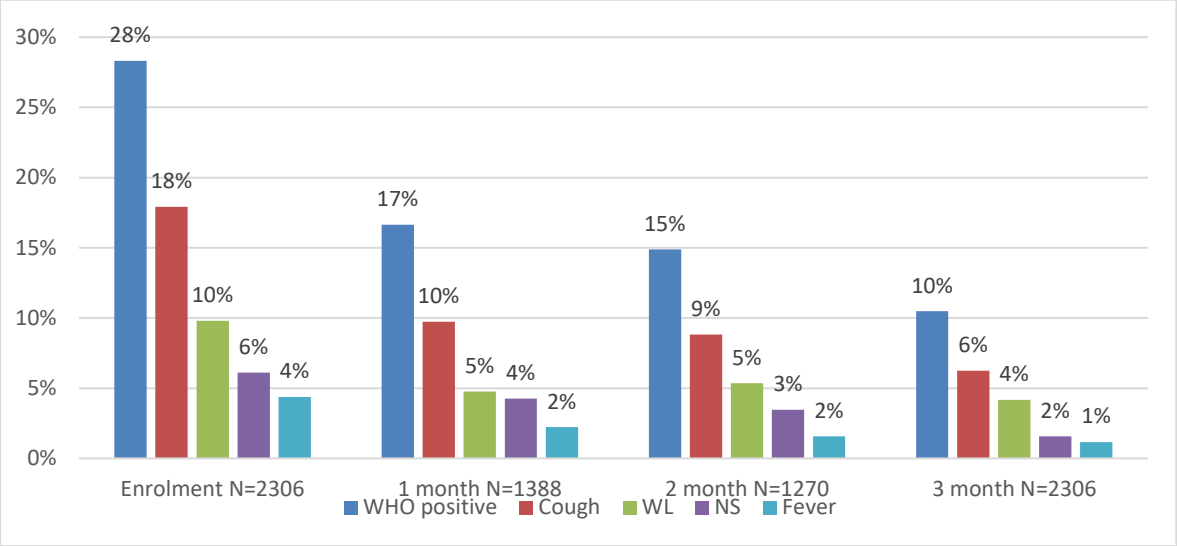
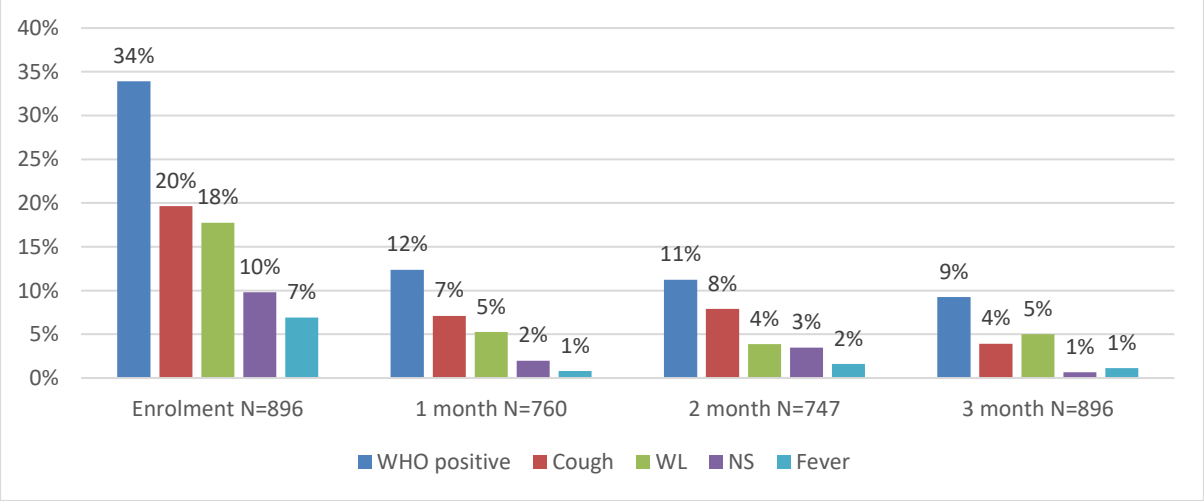
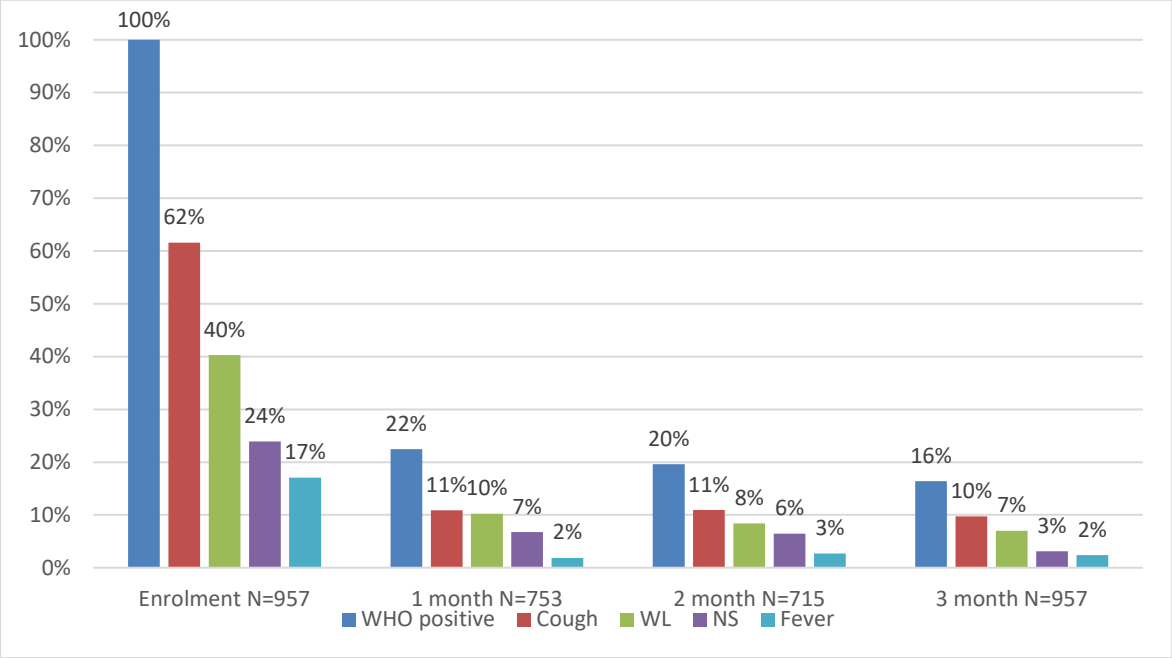



**Figure 4-7. WHO tool symptoms during follow-up amongst those not on ART (N=896)**




When the analysis was restricted to 957 participants who were symptomatic at enrolment, although the percentage reporting any WHO tool symptom reduced at each follow-up visit, 16% remained symptomatic at the 3-month visit, with again cough and weight loss the most commonly reported symptoms (**Figure 4-8**).

**Figure 4-8. Evolution of symptoms amongst those symptomatic at enrolment (N=957)**




***Sputum samples tested with Xpert***

1243/3202 (39%) of participants had an Xpert on sputum within 90 days of enrolment, either because they were WHO-tool positive at enrolment, XPHACTOR high priority, or as part of routine care. 316/3202 (10%) had more than one sample tested. Amongst those in the on ART vs. pre-ART vs. HTC vs. ANC groups respectively, 770/2306 (33%) vs. 364/743 (49%) vs. 55/71 (78%) vs. 54/82 (66%) had at least one sputum sample tested with Xpert, and 213/2306 (9%) vs. 95/743 (13%) vs. 7/71 (13%) vs. 1 (1.2%) had more than one sample tested.

Amongst participants who were reported WHO tool symptom(s) at enrolment, 786/957 (82%) had at least one sputum tested with Xpert during study follow-up. The proportions having one, two, three, or greater than four samples tested were 548/957 (57%), 155/957 (16%), 64/957 (7%), and 19/957 (2%) respectively.

### *4.4.5. Discussion*

This analysis, from which participants diagnosed with TB were excluded, demonstrates that individuals attending for HIV care were highly symptomatic (30% WHO tool positive at

enrolment). Cough and weight loss were the most commonly reported symptoms. Unsurprisingly we found that those newly diagnosed from HTC services were the most symptomatic; these individuals often present to services because they are unwell. The data presented span a duration of more than a year, i.e. are not confined to winter. We have previously shown, by linking symptom frequency to national sentinel influenza surveillance data that influenza-like illness (ILI) appears unlikely to be a major contributor to reported cough.[132] (**Appendix 10.6**) There are no other published data, to the best of my knowledge, which report the natural history of WHO tool symptoms on repeated screening amongst PLHIV who have had TB excluded. However, data from household TB prevalence surveys and studies which have screened clinic attendees for TB in sub-Saharan Africa are available, and provide comparitors in the general community and clinic settings for our reported frequency of WHO tool symptoms. These studies are discussed below.

***Comparison with studies reporting frequency of symptoms suggestive of TB***

In the 2013-2014 Zambian national TB prevalence survey adult (aged > 15 years) household members from 66 randomly-selected clusters across all provinces were systematically screened for TB, using both a symptom screen and chest radiography, and offered HTC.[215] Individuals with abnormal chest radiographs or those reporting ≥ 2 weeks duration of either cough, fever or chest pain underwent sputum smear and TB culture. Eighty-four percent of those eligible participated, around 46,000 individuals, amongst whom 10% reported symptoms suggestive of TB. Two-thirds of participants underwent HIV testing, amongst whom the prevalence of HIV was 7%. Most (92%) of the participants who fulfilled the criteria for requesting sputum submitted at least one sample, amongst whom 4% (265/6123) had bacteriologically-confirmed TB.

The 2016 Kenyan national TB prevalence survey was also a nationwide household survey of adults aged > 15 years from 100 randomly selected clusters.[216] All participants underwent symptom and chest radiograph screening, with sputum requested from those with cough > 2 weeks or abnormal chest radiography, or from those who did not undergo chest radiography. Sputum samples underwent microscopy, culture and testing with Xpert. Eighty-three percent of those eligible participated, around 63,000 individuals, amongst whom 38% reported symptoms suggestive of TB (cough [15%], night sweats [12%], fever [8%], weight loss [3%]; N= 63,050). Most (94%) of the participants who fulfilled criteria for requesting sputum submitted samples, amongst whom 305/9715 (3%) had bacteriologically-confirmed TB. HTC was undertaken only for participants with confirmed

TB, amongst whom 17% (41/245) were HIV-positive. However, half of all the prevalence survey participants knew their HIV status, amongst whom 5% (1627/32386) reported they were HIV-positive.[217] The aforementioned national prevalence surveys, in which very few participants had bacteriologically-confirmed TB, and the majority were HIV-negative, indicate that the prevalence of symptoms suggestive of TB in the community at large ranges from 10-38%.[215, 216] This range includes our finding that overall 30% of participants reported WHO tool symptom(s) at enrolment and the proportion reporting cough in the Kenyan national TB prevalence survey (15%)[216] was very similar to ours (18%). It is possible that some TB diagnoses were missed in both of the aforementioned national surveys as only those with persistent symptoms and/or abnormal chest radiographs underwent investigation, but the prevalence of TB in community-based surveys in which all participants have undergone mycobacterial culture on sputum is generally low.[134, 208] Therefore it appears that significant proportions of individuals in the general community also report symptoms suggestive of TB and in particular cough.

Ssemmondo *et al* undertook symptom-based TB screening in rural Uganda during mobile multidisease community health campaigns (CHC) which incorporated HIV testing.[218] Their study was undertaken between 2013-2014 in seven out of thirty-two previously enumerated communities participating in a cluster-randomised trial of universal HIV testing and treatment in Kenya and Uganda. CHCs were undertaken over a two-week period, one month after the baseline study census enumeration of all residents, at convenient locations within each community. TB screening comprised enquiring about current cough and sputum was requested for microscopy for AFB if cough had been present for > 2 weeks. The authors reported that 74% (27,214) of all adults (age ≥ 15 years old) enumerated in the baseline census attended the campaigns, of whom 99% underwent HIV testing and 3.5% (941/26813) were HIV-positive with median CD4 cell count of 474 cells/$mm^3$. Twenty-one percent of adults reported current cough and 11% reported cough > 2 weeks. The proportion of participants reporting prolonged cough increased with age and was greater in HIV-positive (17%) compared with HIV-negative (10%) adults. Only 38% (1099/2876) of participants with cough > 2 weeks were able to produce a sputum sample and ten had smear-positive TB, of whom three were HIV-positive. Individuals attending the campaigns are more likely to have attended because they were unwell and thus symptomatic. Therefore, the proportion of adults reporting cough is likely to be biased, and most likely an overestimation of the proportion of adults with cough in the community. It is also likely that some TB diagnoses were missed because investigation was restricted to those with prolonged cough and the majority of those requiring investigation

could not produce sputum. However their data also indicate that a significant proportion of adults participating in this rural community health campaign reported cough.

Owiti *et al* retrospectively analysed programme data collected from individuals attending for routine HIV care in Kenya between 2015 and 2016.[219] In this setting patients should have been screened for TB at every clinical encounter using a standardized form which was subsequently electronically captured. Amongst around 90,000 individuals, the majority (>75%) were aged over 19 years and on ART, median follow-up time was 1.5 years, and the median number of clinical encounters per individual was eight. The authors reported documentation of TB screening at almost 90% of all encounters, with 96% of PLHIV never reporting symptoms, and 3.6% and 0.4% of PLHIV reporting symptoms at only one encounter and at more than one encounter respectively. The most commonly reported symptom was cough, but only 7% of symptomatic individuals had documentation of investigation for TB (sputum microscopy or chest radiograph). The authors did not report the prevalence of TB. The proportion of PLHIV reporting symptoms in this study was much lower than amongst XPHACTOR study participants on ART. This may be due to the limitations of the retrospective design of the analysis which relied on routinely collected programme data. Forms might not have been completed fully due to lack of time in busy clinics, or the lack of symptoms might reflect a more mature population who had been on ART for a longer duration than those in XPHACTOR, although this was not ascertainable as the median CD4 cell count and duration on ART were not reported.

Adelman *et al*, as already discussed in the literature review (**Chapter 2**) systematically screened HIV clinic attendees in Ethiopia, of whom 90% were on ART, and reported the presence of WHO tool symptoms in 39% of attendees.[147] The authors did not investigate all participants with symptoms for TB and therefore could not exclude them from the proportion reporting symptoms, and this might explain the higher proportion reporting symptoms in their study compared with XPHACTOR. Chihota *et al* provide some data from primary health clinics (PHCs).[43] The authors screened consecutive adults leaving PHCs participating in the XTEND trial in order to ascertain the proportion of those reporting symptoms suggestive of TB who had sputum requested by HCW. The authors reported that about 50% (4098/8104) of those approached were eligible for their study, i.e. reported at least one of the WHO tool symptoms. This figure appears exceptionally high for PHC attendees and may reflect a heightened awareness of TB symptoms and willingness to report these at a research trial site, or perhaps those with no symptoms were missed.

Even amongst our participants who were established on ART, 28% reported WHO tool symptoms at enrolment, and 10% at the 3-month visit. Of note, in this group, amongst those for whom viral load data were available, only 75% had viral load suppression. The proportion of those on ART reporting symptoms at enrolment is slightly lower than from comparable studies screening individuals established in HIV care (33-39%).[142, 146-148] This probably reflects the exclusion of individuals diagnosed with TB from this analysis, whereas the aforementioned studies reported data from all enrolled.[142, 146-148] Furthermore 16% of our 957 participants who reported WHO tool symptoms at enrolment also reported symptoms at the 3-month follow-up visit. Amongst these participants 25% (238/957) had more than one sputum sample tested using Xpert during study follow-up. These data provide an indication of the volume of testing with Xpert that may arise as a result of regular screening in this population using the WHO tool. Our findings highlight the potential resource implications in LMIC settings of screening using a tool that lacks specificity and generates a large proportion of patients requiring a diagnostic test for TB. This might in part explain variations in adherence to TB screening guidelines.[68] There are no other published studies of the evolution of WHO tool symptoms at subsequent clinic visits amongst those symptomatic at enrolment who have had TB excluded.

### *Potential reasons for high frequency of reported cough*

Cough was the most frequently reported symptom overall, both at enrolment and follow-up visits. Potential reasons for reported cough other than TB include non-communicable diseases such as asthma and COPD, smoking, use of biomass fuels and post-tuberculous chronic lung disease. A significant proportion of our study participants were either ex- or current smokers, i.e. around 25% of those established in HIV care, 30% of those from HTC and 13% of those from ANC. This is comparable with data from South Africa's 2016 demographic and health survey (DHS) which reported amongst adult (> 15 years old) males 38% were current tobacco smokers and 6% ex-smokers compared with adult females amongst whom 7% were current smokers and 2% were ex-smokers.[220] Smoking itself may increase the risk of TB infection.[221] At the time the study was undertaken nicotine replacement therapy and other interventions to assist smoking cessation were not available in the public sector, the only option was advice and signposting to pharmacies. In resource-limitted settings, even if smoking cessation aids are now available in the public sector, it is unlikely that these are provided free-of-charge.

In the 2016 DHS asthma symptoms were reported by 3%-4% of adults and COPD symptoms by 2% of adults, which is comparable to our findings of 1-5% reporting chronic respiratory disease.[220] However less than 1% of DHS participants reported using any medications for these conditions. The use of wood as a cooking fuel was more common in rural vs. urban households (32% vs. 2%) in the DHS, but our study was conducted in an urban setting and therefore here the current use of biomass fuel is less likely to have been responsible for cough. The prevalence of previous TB treatment in our study participants was high, ranging from 6% in the HTC group to 39% in the on ART group (amongst whom 6% reported more than one previous episode of TB treatment). Therefore post-TB chronic lung disease may have been responsible for cough in some participants and better criteria are needed to identify this condition and to guide management. The large proportion of individuals reporting cough also highlights the need for better access to pulmonary function testing at PHC level or simpler tests such as the six-minute walk test, and if appropriate treatments such as inhalers or pulmonary rehabilitation.[222]

### *Strengths and limitations*

In keeping with other studies which enrolled ANC attendees, which report 16[145]-19%[141] with WHO tool symptoms, we found those newly diagnosed from ANC were less symptomatic (15% WHO tool positive), although the number in this group was small. As discussed in the literature review **(Chapter 2)** a limitation of the WHO tool is that pregnancy itself may impact on the presence of TB symptoms, and in particular on reported weight loss. In this population measured weight loss, failure to gain weight appropriate to the trimester of pregnancy, or MUAC require further evaluation.

A strength of our study was that the WHO tool was administered systematically by trained research staff, in the preferred language of the participant using standardized translations, thus ensuring that symptom screening questions were asked in a consistent manner.

### *Conclusions*

Given the burden of symptoms suggestive of TB clear guidelines for further evaluation and management of the underlying cause of these symptoms, providing TB is excluded, are needed. The high prevalence of previous TB in this population highlights the need for guidelines to assist with the indentification and optimal management of post-TB chronic

lung disease and to differentiate active TB from previous TB in those who have had treatment in the past. This is particularly important with task-shifting and differentiated models of ART delivery, and given that cough appears to predominate, consideration should be given to developing simpler tests of pulmonary function and better access to respiratory specialists in primary care. The Practical Approach to Lung Health in South Africa (PALSA) guideline was developed from the WHO Practical Approach to Lung Health strategy to assist management by primary care nurses of adults with respiratory symptoms.[223] The syndromic algorithms presented in the guideline equip primary care nurses, who are often the first port of call for patients and have limited access to doctors, to make diagnoses other than TB and enable them to manage common respiratory diseases. The Integrated Management of Adolescent and Adult Illness (IMAI) manuals provide similar but higher level guidance aimed at district level clinicians.[224] Sufficient time and human resources are needed to follow these guidelines, but both are too often lacking in resource-limited settings.

# 5) *Paper 1*: Diagnostic accuracy of lateral flow urine LAM assay for TB screening of adults with advanced immunosuppression attending routine HIV care in South Africa

## 5.1. Cover sheet

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

## RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

**SECTION A – Student Details**

| | | | |
|---|---|---|---|
| **Student ID Number** | 079810 | **Title** | Dr |
| **First Name(s)** | Yasmeen | | |
| **Surname/Family Name** | Hanifa | | |
| **Thesis Title** | Investigation pathways for tuberculosis among HIV-positive adults in South Africa | | |
| **Primary Supervisor** | Alison Grant | | |

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

**SECTION B – Paper already published**

| | | | |
|---|---|---|---|
| Where was the work published? | PLoS One | | |
| When was the work published? | June 2016 | | |
| If the work was published prior to registration for your research degree, give a brief rationale for its inclusion | N/A | | |
| Have you retained the copyright for the work?* | **Yes** | Was the work subject to academic peer review? | **Yes** |

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| Stage of publication | Choose an item. |

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| | |
|---|---|
| [redacted] For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | I managed the study, conducted the data analysis, and wrote the paper. |

**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
| **Date** | 28th May 2019 |

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|---|---|
| **Supervisor Signature** | [redacted] |
| **Date** | 28 May 2019 |

## 5.2. Research paper

PLOS | ONE

RESEARCH ARTICLE

# Diagnostic Accuracy of Lateral Flow Urine LAM Assay for TB Screening of Adults with Advanced Immunosuppression Attending Routine HIV Care in South Africa

**Yasmeen Hanifa[1]*, Katherine L. Fielding[1], Violet N. Chihota[2,3], Lungiswa Adonis[4], Salome Charalambous[2,3], Alan Karstaedt[3,5], Kerrigan McCarthy[2], Mark P. Nicol[6,7], Nontobeko T. Ndlovu[2], Faieza Sahid[3,5], Gavin J. Churchyard[1,2,3,8], Alison D. Grant[1,3]**

**1** London School of Hygiene & Tropical Medicine, London, United Kingdom, **2** Aurum Institute, Johannesburg, South Africa, **3** University of the Witwatersrand, Johannesburg, South Africa, **4** Mamelodi Hospital, Pretoria, South Africa, **5** Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa, **6** National Health Laboratory Service, Johannesburg, South Africa, **7** University of Cape Town, Cape Town, South Africa, **8** Advancing Care and Treatment (ACT) for TB/HIV, South African Medical Research Council Collaborating Centre for HIV and TB, Cape Town, South Africa

* yasmeen.hanifa@lshtm.ac.uk



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**Competing Interests:** The authors have declared that no competing interests exist.

## Abstract

### Background

We assessed the diagnostic accuracy of Determine TB-LAM (LF-LAM) to screen for tuberculosis among ambulatory adults established in HIV care in South Africa.

### Methods

A systematic sample of adults attending for HIV care, regardless of symptomatology, were enrolled in the XPHACTOR study, which tested a novel algorithm for prioritising investigation with Xpert MTB/RIF. In this substudy, restricted to participants with enrolment CD4<200x$10^6$/l, urine was stored at enrolment for later testing with LF-LAM. Sputum was sent for immediate Xpert MTB/RIF if any of: current cough, fever ≥3 weeks, body mass index (BMI)<18.5kg/$m^2$, CD4<100x$10^6$/l (or <200x$10^6$/l if pre-ART), weight loss ≥10% or strong clinical suspicion were present; otherwise, sputum was stored for Xpert testing at study completion. Participants were reviewed monthly, with reinvestigation if indicated, to 3 months, when sputum and blood were taken for mycobacterial culture. We defined tuberculosis as "confirmed" if Xpert, line probe assay or culture for *M. tuberculosis* within six months of enrolment were positive, and "clinical" if tuberculosis treatment started without microbiological confirmation.

### Results

Amongst 424 participants, 61% were female and 57% were taking ART (median duration 22 months); median age, CD4 and BMI were 39 years, 111x$10^6$/l, and 23 kg/$m^2$. 56/424 (13%) participants had tuberculosis (40 confirmed, 16 clinical). 24/424 (5.7%) vs. 8/424

(1.9%) were LAM-positive using grade 1 vs. grade 2 cut-off. Using grade 1 cut-off, sensitivity for confirmed TB (all clinical TB excluded) was 12.5% (95% CI 4.2%, 26.8%) and in CD4<100x10$^6$/l vs. CD4 ≥100x10$^6$/l was 16.7% (95% CI 4.7%, 37.4%) vs. 6.3% (95% CI 0.2%, 30.2%). Specificity was >95% irrespective of diagnostic reference standard, CD4 stratum, or whether grade 1 or grade 2 cut-off was used.

## Conclusion

Sensitivity of LF-LAM is too low to recommend as part of intensified case finding in ambulatory patients established in HIV care.

## Introduction

The global HIV-associated tuberculosis (TB) epidemic remains a huge public health challenge, with sub-Saharan Africa accounting for the vast majority of HIV-positive individuals diagnosed with and dying from TB. [1] Diagnosis of TB in people living with HIV (PLHIV) is complicated by limitations of available diagnostics and the effect of immunosuppression on clinical presentation of TB, e.g. reliance on sputum samples, the high proportion with smear-negative or extrapulmonary disease, [2] and slow turnaround time for mycobacterial culture. The World Health Organization recommends, as part of activities to address HIV-related TB, regular screening for active TB of all PLHIV followed by Xpert MTB/RIF (Cepheid, Sunnyvale, CA) as the primary diagnostic test. [3] Xpert MTB/RIF has far greater sensitivity than smear and provides results in under two hours, but like mycobacterial culture is expensive and laboratory-based, which presents challenges for resource-limited settings. [3]

Testing for lipoarabinomannan (LAM), a cell wall lipopolysaccharide specific to mycobacteria that is detectable in urine, is attractive as a screening tool for PLHIV, given a low-cost point-of-care lateral-flow LAM assay (LF-LAM) (Determine TB-LAM; Alere, USA), potential for rapid TB diagnosis, low biosafety risk, and ease of sample collection. Evaluations of LF-LAM as a screening tool for TB have been undertaken in ambulatory patients in Ethiopia and South Africa either prior to antiretroviral therapy (ART) initiation, [4, 5] or on receiving a positive HIV diagnosis at HIV counselling and testing services (HCT). [6, 7] In these groups LF-LAM sensitivity, compared to bacteriologically-confirmed TB, was inadequate as a stand-alone test, though improved at lower CD4 cell counts. When evaluated amongst hospitalised HIV-positive patients with TB symptoms in Uganda and South Africa sensitivity was much greater, particularly amongst those with advanced immunosuppression, suggesting utility as a rule-in test in this population. [8–10]

There are no published studies, to our knowledge, evaluating LF-LAM as a screening tool for TB as part of intensified case finding for ambulatory patients established in HIV care (rather than at their initial assessment). The aim of our study was to evaluate the diagnostic accuracy of LF-LAM among adults with advanced immunosuppression (CD4 <200x10$^6$/l) established in HIV care. Our study contributed data to a systematic review of LF-LAM for the diagnosis and screening of active TB in PLHIV, which informed the recently published World Health Organization (WHO) policy guidance.[11]

## Methods

This "LAM" study was part of XPHACTOR, a prospective cohort study evaluating a risk-based algorithm to prioritise Xpert MTB/RIF testing amongst adults attending for routine HIV care

in South Africa. Fig 1 depicts XPHACTOR study flow and how participants entered the LAM substudy.

## XPHACTOR study population and recruitment

We enrolled a systematic sample of adults (aged ≥18 years) attending four clinics in Gauteng province for HIV care, irrespective of presence of symptoms suggestive of TB. Patients taking anti-tuberculosis treatment within the previous 3 months were excluded. Patients were enrolled into "on ART" (currently taking ART) and "pre-ART" (in HIV care but not taking ART) groups. At the time of the study, ART eligibility comprised CD4 ≤350x$10^6$/l or WHO clinical stage ≥3.

## XPHACTOR procedures

**Enrolment.** At enrolment, research staff administered a standardised questionnaire incorporating the WHO TB screening tool (any of current cough, fever, night sweats or unintentional weight loss), measured height and weight, and recorded most recent clinic CD4 cell count. Further investigation was prioritised according to the XPHACTOR algorithm with an immediate spot sputum sample sent for Xpert MTB/RIF for (i) all assigned "high priority" (any of: current cough, fever ≥ 3 weeks, body mass index (BMI) <18.5 kg/$m^2$, CD4 <100x$10^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); (ii) those in the pre-ART group with CD4<200x$10^6$/l at enrolment because of *a priori* high risk of active TB. For all other participants a spot sputum sample was frozen at -80°C within 24 hours for testing with Xpert MTB/RIF at the end of the study.

**Follow-up.** Participants were reviewed monthly to three months, with repeat WHO symptom screen and a spot sputum requested for Xpert MTB/RIF if "high priority" by the study algorithm at that visit. Those in the "on ART" group who were asymptomatic at enrolment were telephoned at 1 and 2 months to update locator information but were not asked about TB symptoms. At the 3-month visit, sputum (induced if necessary) and blood were collected for mycobacterial culture in liquid media (Bactec MGIT 960 or 9240 systems) from all study participants. We allowed the 3-month visit to be undertaken more than three months post-enrolment in order to maximise study follow up.

Participants who submitted an Xpert sample were reviewed and if Xpert-positive, TB treatment was initiated; if negative, further investigation in accordance with national guidelines was facilitated (chest radiograph, sputum culture and trial of antibiotics).

Clinic medical records were reviewed at the end of the study to ascertain any additional TB diagnoses. We recorded deaths through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants' South African identification (ID) numbers, which enabled us to track vital status several months after final study visit for those with valid ID numbers.

## LAM substudy procedures

All participants with CD4<200x$10^6$/l were eligible for this substudy. Eligible participants were asked to provide a spot urine sample in a sterile container at enrolment, which was stored at 2–8°C prior to freezing at -80°C within 24 hours of collection. At the end of the study samples were thawed to ambient temperature and tested with LF-LAM by two trained laboratory technologists in accordance with training provided by Alere representatives. The technologists did not have access to other bacteriological results when performing LF-LAM tests. Each test was graded once, using the pre-January 2014 manufacturer's reference card comprising five grades

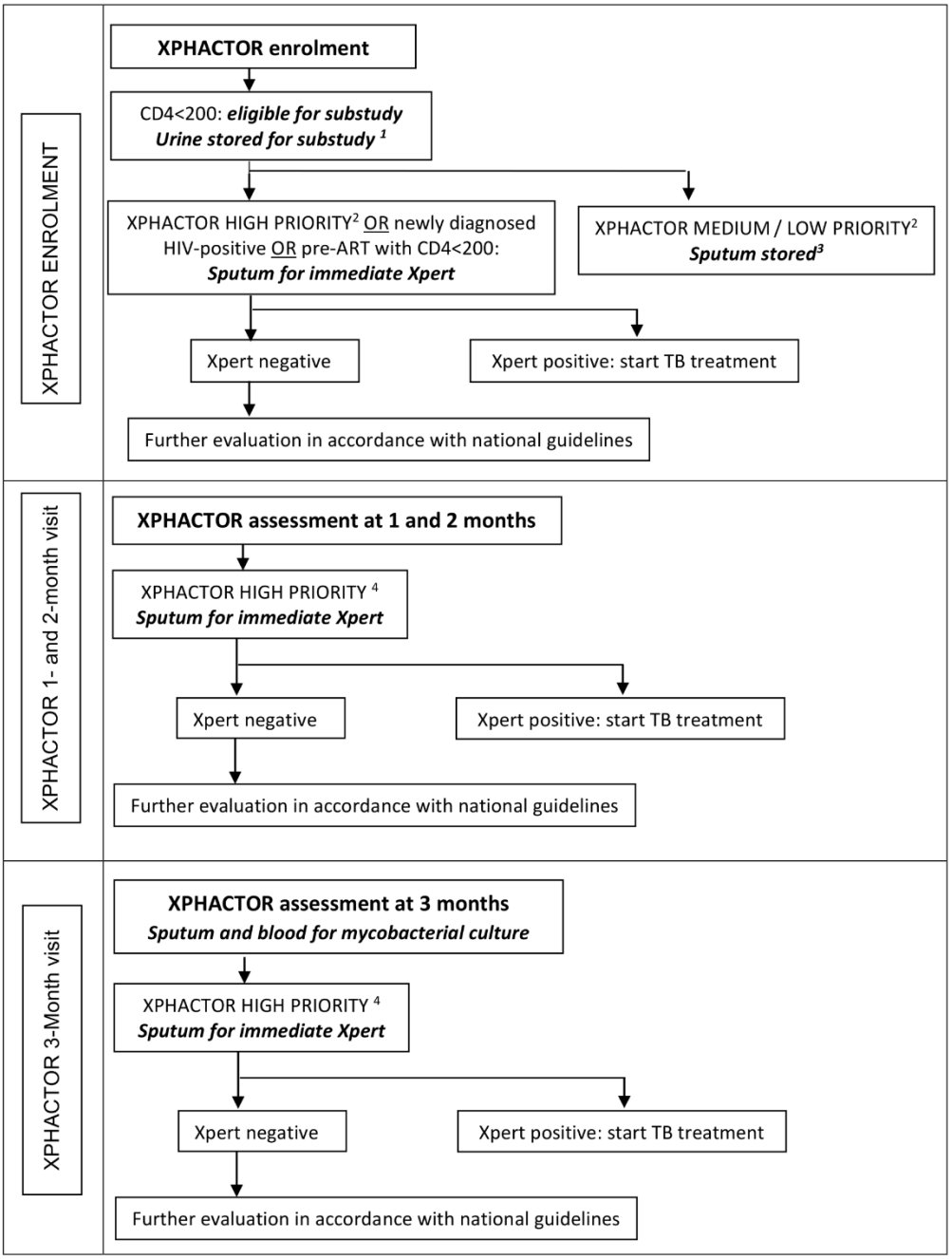


**Fig 1. XPHACTOR study flow and entry point to the LAM substudy.** [1] Samples tested with LF-LAM at the end of the study. [2] High priority (any of: current cough, fever ≥ 3 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of: fever < 3 weeks, night

sweats, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms. [3] Samples tested with Xpert MTB/RIF at the end of the study. [4] High priority (any of: current cough, fever ≥ 3 weeks, night sweats ≥ 4 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB).

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of colour intensity with the least intense band assigned grade 1, absence of a band graded negative, and absence of control band deemed a failed test. [12]

## TB Case Definitions

"Confirmed" TB was defined as a positive result on i) Xpert MTB/RIF or ii) line probe assay (LPA) performed on cultured isolate for identification and susceptibility or iii) *M. tuberculosis* (*Mtb*) culture, from any sample taken within six months of enrolment. Individuals who started TB treatment within six months of enrolment, in the absence of microbiological confirmation (including those with treatment starts reported at verbal autopsy) and those smear-positive in the absence of an associated positive culture or LPA result, were assigned "clinical" TB. This was based on the assumption that an HIV-positive adult with a positive test result or starting TB treatment within 6 months after enrolment likely had active TB at enrolment, supported by data from Zimbabwe which estimated the mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults to be 18–33 weeks. [13] Furthermore individuals diagnosed with clinical TB based on findings at the 3-month visit would only have started treatment after the 3-month visit.

"Not TB" was defined as fulfilling all of the following: absence of criteria for confirmed or clinical TB; alive at least 3 months after enrolment; and ≥1 MTB culture or Xpert result from any sample within 6 months of enrolment. Participants who did not fulfil the case definitions for TB or "not TB" were deemed "unclassifiable" and excluded from the main analysis.

## Statistical methods

Data were analysed using Stata 14 (Stata Corporation, College Station, TX, USA).

We did not undertake a formal sample size calculation for the LAM substudy as the sample size was all those eligible from the parent study. The target sample size for XPHACTOR was based on estimating the sensitivity of the study algorithm, the main aim of the study, with reasonable precision.

We calculated sensitivity, specificity and predictive values with 95% confidence intervals (CI) for LF-LAM using cut-offs of grade ≥1+ and ≥2+ to define LAM-positive against a diagnostic reference standard of i) confirmed plus clinical TB, and ii) confirmed TB with clinical TB excluded from numerator and denominator. We also calculated these parameters for grade ≥1+ cut-off for subgroups stratified by CD4<100x10$^6$/l and CD4 ≥100x10$^6$/l.

We undertook an exploratory assessment of mortality at six months in all participants who provided a urine specimen, assuming all without record of demise were alive: i) six months after enrolment if they had valid South African ID number, or ii) at latest study / clinic visit date (hereafter last visit) if no valid South African ID. Person-time was calculated from the date of study enrolment until: date of death if death recorded within six months of enrolment, and for all others six months from enrolment if valid South African ID or date of last visit if no valid South African ID. We constructed Kaplan-Meier curves of survival probability by LAM positivity using LAM grade ≥1+ to define positivity, and compared mortality using Cox regression.

## Ethics statement

The study was approved by the ethics committees at the University of the Witwatersrand, University of Cape Town, and the London School of Hygiene & Tropical Medicine. All participants gave written informed consent, or witnessed verbal consent if unable to write. For illiterate participants, an impartial witness was present during the consenting process, and signed the witness section of the consent form. All ethics committees approved this consent procedure. Consent and participation in the study was voluntary. Participants were able to refuse to take part, with no consequences to their healthcare or any other services as a result of refusal.

## Results

Between September 2012 and March 2014 we enrolled 3508 participants established in HIV care, of whom 586 had CD4 $<200x10^6$/l and were eligible for the LAM substudy. 80% (469/586) provided a urine sample, and the remaining 20% (117/586) did not, as unable (93) or reason not recorded (24) (Fig 2); 67% (395/586) provided a spot sputum sample at enrolment. 44 participants were excluded because unclassifiable: no TB diagnosed but absence of any TB microbiology results (26); death within three months of enrolment (14); and TB diagnosed >6 months after enrolment (4); and one sample could not be tested as damaged, leaving 424 eligible for evaluation of diagnostic accuracy of LF-LAM.

There was little difference in WHO-tool positivity or gender amongst those providing (N = 469) vs. not providing urine (N = 117): 52% vs. 44% WHO-tool positive (p = 0.1) and 61% vs. 62% female (p = 0.7). Median CD4 was lower in those providing urine compared with

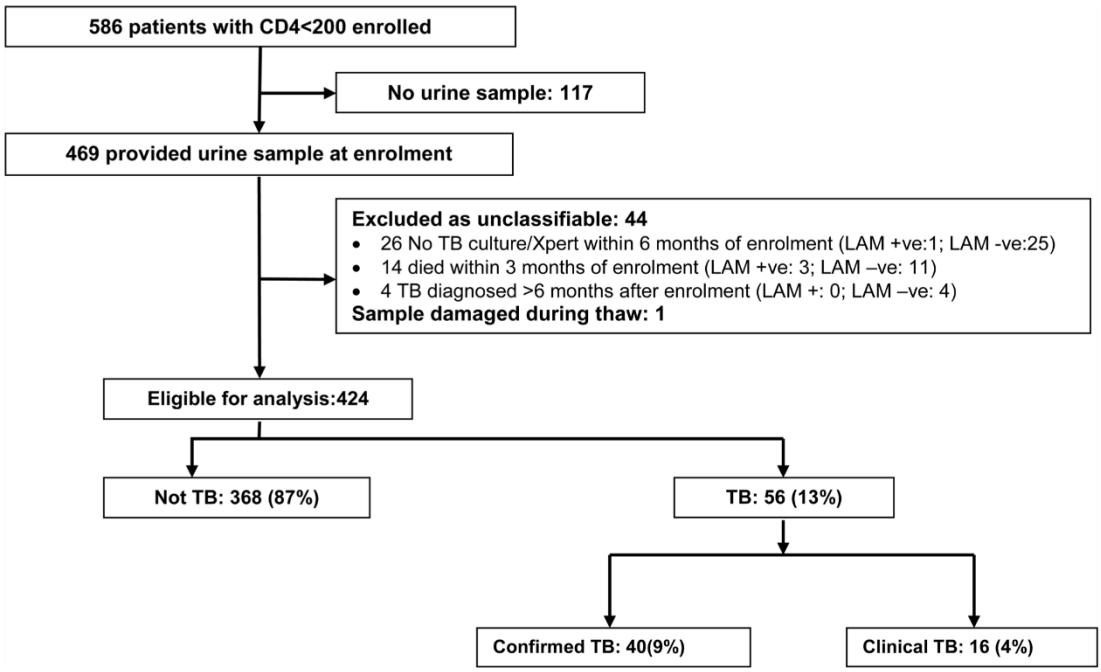


**Fig 2. Flow chart of study participants.** LAM+ defined as ≥ grade 1.

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**Table 1. Baseline characteristics of study participants.**

| | All participants N = 424 |
|---|---|
| **Age-years** | |
| Median (IQR) | 39 (32, 45) |
| **Sex** | |
| Female | 258 (60.8%) |
| **Ethnic group** | |
| Black/African | 421 (99.3%) |
| **Participant category** | |
| Pre-ART | 182 (42.9%) |
| On ART | 242 (57.1%) |
| ART duration for those on ART, months median (IQR) | 22 (6, 52) |
| **Previous TB treatment** | |
| Yes | 125 (29.5%) |
| **Ever had IPT** | |
| Yes | 18 (4.2%) |
| **Ever had CPT** | |
| Yes | 276 (65.1%) |
| **Current diuretic use** | |
| Yes | 10 (2.4%) |
| **Enrolment WHO symptom screen** | |
| Positive | 224 (52.8%) |
| **Enrolment BMI-kg/m$^2$ (N = 423)** | |
| Median (IQR) | 23 (20, 27) |
| **Enrolment CD4 x10$^6$/l [1]** | |
| Median (IQR) | 111 (56, 161) |

Numbers are median (Interquartile range [IQR]) or number (%)

ART = Antiretroviral therapy; CPT = Co-trimoxazole preventive therapy; IPT = Isoniazid preventive therapy; BMI = body mass index

WHO symptom screen positive = self-report of any of current cough, fever, night sweats or unintentional weight loss

[1] All participants had CD4<200x10$^6$/l

doi:10.1371/journal.pone.0156866.t001

those who did not (110 vs. 140x10$^6$/l [p = 0.01]); and a greater proportion also provided sputum at enrolment (74% vs. 42% [p<0.001]).

## Participant characteristics

Characteristics of the 424 LAM substudy participants are presented in Table 1. The majority of participants were female (61%), WHO-tool positive at enrolment (53%), and in the "on ART" group (57%) amongst whom median duration of ART was 22 months (interquartile range [IQR] 6, 52). Median age, CD4 and BMI at enrolment were respectively 39 years, 111x10$^6$/l, and 23 kg/m$^2$; and 30% had previously been treated for TB. In the "pre-ART" group, for 97% (176/182) participants with reported date of first positive HIV test recorded, median duration since HIV diagnosis was 20 days (IQR 10, 65). 94% (171/182) of "pre-ART" initiated ART within median 9 days (IQR 4, 24) from enrolment (initiation date available for 170/171).

The proportion reporting each WHO-tool symptom was cough, 32% (134/424) with median duration 14 days (interquartile range [IQR] 7,38); unintentional weight loss, 31% (131/

424); night sweats, 15% (65/424); fever, 10% (44/424); and 24% (102/424) reported more than one symptom.

## Tuberculosis diagnoses

13% (56/424) of participants fulfilled our case definitions for tuberculosis (7% [16/242] "on ART" and 22% [40/182] "pre-ART"), amongst whom treatment start date was available for 53/56, and was started at a median of 13 (IQR 5, 95) days from enrolment. 40/56 had confirmed TB (25 Xpert-positive, 7 Mtb culture-positive, 7 both Xpert and Mtb culture-positive, 1 pleural fluid cultured isolate LPA-positive) of whom 36/40 had pulmonary TB, 3/40 both pulmonary and extrapulmonary TB, and 1/40 extrapulmonary TB only. 16/56 had clinical TB for whom diagnosis was based on compatible chest radiograph or abdominal ultrasound (8), persistent cough and weight loss (1), positive sputum smear (1), and unknown (6) including TB treatment reported at verbal autopsy (2). Amongst those with clinical TB the site was pulmonary (10/16), extrapulmonary (4/16), and not recorded (2/16).

## Performance of urine LAM

LAM results were available for 424 participants. A positive LF-LAM result using grade 1 vs. grade 2 cut-off was observed in 5.7% (24) vs. 1.9% (8) of participants. The distribution of results was negative, 94% (400); grade 1, 3.8% (16); grade 2, 1.2% (5); grade 3, 0.5% (2); and grade 5, 0.2% (1).

Table 2 summarises the performance of LF-LAM in our study population. Sensitivity for all TB (clinical and confirmed) using grade 1 cut-off was 14.3% (95% CI 6.4%, 26.2%), similar if reference standard was confirmed TB with all clinical TB excluded (12.5% [95% CI 4.2%, 26.8%]), but lower if grade 2 cut-off utilised (5.4% [95% CI 1.1%, 14.9%] for all TB). Sensitivity was greater in participants with enrolment CD4$<$100 vs. CD4 $\geq$100x10$^6$/l: using grade 1 cut-off 17.1% (95% CI 6.6%, 33.6%) vs. 9.5% (95% CI 1.2%, 30.4%) for all TB, and 16.7% (95% CI 4.7%, 37.4%) vs. 6.3% (95% CI 0.2%, 30.2%) for confirmed TB. Specificity of the test was $>$95% irrespective of reference standard, CD4 stratum, or whether positivity was defined using grade 1 or grade 2 cut-off.

In a sensitivity analysis we included the 40 participants whom we deemed to have unclassifiable TB outcome, and considered those excluded because deceased within 3 months of enrolment to have TB (N = 14), and those excluded because of absence of microbiology to not have TB (N = 26). Against a reference standard for all TB (clinical and confirmed) and using grade 1 cut-off, the sensitivity and specificity of LF-LAM was 15.7% (95% CI 8.1, 26.4) and 95.7% (95% CI 93.2, 97.5) respectively. If a grade 2 cut-off was used the sensitivity and specificity of LF-LAM was 8.6% (95% CI 3.2, 17.7) and 98.7% (95% CI 97.1, 99.6) respectively.

There were five false positive LF-LAM rests using the grade 2 cut-off, of these one participant with CD4 of 4x10$^6$/l at enrolment had *M. avium* isolated from sputum culture but was not treated and was alive at six months. All of the remaining four participants with false positive LF-LAM had negative sputum mycobacteriology during follow up and were alive at six months.

## Mortality

Amongst 468 participants with evaluable urine samples, 6% (28/468) were LF-LAM positive using grade 1 cut-off, of whom 14% (4/28) died within six months of enrolment. Among the 440 who were LF-LAM negative, 5% (20/440) died (hazard ratio 3.6 [95%CI 1.2, 10.5], p = 0.04; Fig 3).

**Table 2. Diagnostic accuracy of LF-LAM among HIV clinic attendees with CD4<200.**

| | Prevalence of TB | | Prevalence of positive LAM | | Sensitivity | | Specificity | | PPV | | NPV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gold standard = confirmed* and clinical† TB** | **n/N** | **%** | **n/N** | **%** | **n/N** | **% (95% CI)** | **n/N** | **% (95% CI)** | **n/N** | **% (95% CI)** | **n/N** | **% (95% CI)** |
| ***Grade 1‡ cut-off*** | 56/424 | 13.2% | 24/424 | 5.7% | 8/56 | 14.3% (6.4, 26.2) | 352/368 | 95.7% (93.0, 97.5) | 8/24 | 33.3% (15.6, 55.3) | 352/400 | 88.0% (84.4, 91.0) |
| CD4 <100 | 35/187 | 18.7% | 12/187 | 6.4% | 6/35 | 17.1% (6.6, 33.6) | 146/152 | 96.1% (91.6, 98.5) | 6/12 | 50.0% (21.1, 78.9) | 146/175 | 83.4% (77.1, 88.6) |
| CD4 ≥100 | 21/237 | 8.9% | 12/237 | 5.1% | 2/21 | 9.5% (1.2, 30.4) | 206/216 | 95.4% (91.7, 97.8) | 2/12 | 16.7% (2.1, 48.4) | 206/225 | 91.6% (87.1, 94.8) |
| ***Grade 2§ cut-off*** | 56/424 | 13.2% | 8/424 | 1.9% | 3/56 | 5.4% (1.1, 14.9) | 363/368 | 98.6% (96.9, 99.6) | 3/8 | 37.5% (8.5, 75.5) | 363/416 | 87.3% (83.7, 90.3) |
| **Gold standard = confirmed* TB (all clinical† TB excluded) (N = 408)** | | | | | | | | | | | | |
| ***Grade 1‡ cut-off*** | 40/408 | 9.8% | 21/408 | 5.1% | 5/40 | 12.5% (4.2, 26.8) | 352/368 | 95.7% (93.0, 97.5) | 5/21 | 23.8% (8.2, 47.2) | 352/387 | 91.0% (87.6, 93.6) |
| CD4 <100 | 24/176 | 13.6% | 10/176 | 5.7% | 4/24 | 16.7% (4.7, 37.4) | 146/152 | 96.1% (91.6, 98.5) | 4/10 | 40.0% (12.2, 73.8) | 146/166 | 88.0% (82.0, 92.5) |
| CD4 ≥100 | 16/232 | 6.9% | 11/232 | 4.7% | 1/16 | 6.3% (0.2, 30.2) | 206/216 | 95.4% (91.7, 97.8) | 1/11 | 9.1% (0.2, 41.3) | 206/221 | 93.2% (89.1, 96.2) |
| ***Grade 2§ cut-off*** | 40/408 | 9.8% | 8/408 | 2.0% | 3/40 | 7.5% (1.6, 20.4) | 363/368 | 98.6% (96.9, 99.6) | 3/8 | 37.5% (8.5, 75.5) | 363/400 | 90.8% (87.5, 93.4) |

* Confirmed TB = positive on Xpert MTB/RIF or line probe assay or M. tuberculosis culture, from any sample taken within 6 months of enrolment

† Clinical TB = started TB treatment within 6 months of enrolment, in the absence of microbiological confirmation and those smear-positive in the absence of an associated culture

‡ Grade 1 positive: > = 1+

§ Grade 2 positive: > = 2+

NPV = Negative predictive value; PPV = Positive predictive value; CI = confidence interval

doi:10.1371/journal.pone.0156866.t002

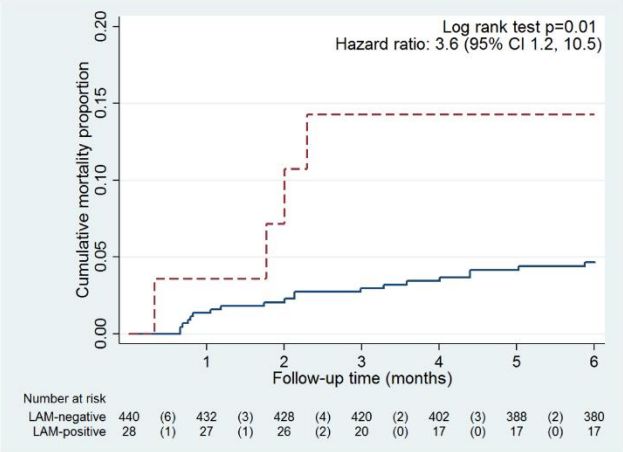


**Fig 3. Kaplan-Meier curve comparing mortality between LAM positive (dashed line) and LAM negative (solid line) participants using grade 1 cut-off.** Y-axis range for cumulative mortality is 0 to 0.2

doi:10.1371/journal.pone.0156866.g003

## Discussion

We found very low sensitivity of LF-LAM for TB (whether confirmed, or also including clinical diagnoses) among ambulatory outpatients established in HIV care with CD4<200x10$^6$/l, which does not support its use for TB screening in this study population. Recent WHO LF-LAM policy guidance recommends against use for TB screening, based on the systematic review to which our data contributed, which reported (using grade 2 cut-off and microbiological reference standard) a pooled sensitivity of only 23% for screening HIV-positive outpatients.[11] Our study is the first reporting performance of LF-LAM among outpatients established in HIV care. It adds to the published evaluations of LF-LAM amongst HIV-positive adult outpatients screened for TB, which to date have only been undertaken in those newly diagnosed [6, 7] or about to initiate ART, [4, 5] populations likely to be sicker than those already in HIV care.

In our study, LF-LAM had a sensitivity of 13% using the grade ≥1 cut-off (against confirmed TB as a gold standard) vs. reported 26–29% [4, 5] in those about to initiate ART, and 28–41% [6, 7] amongst those with a new HIV diagnosis. In those evaluations, sensitivity improved greatly at lower CD4 counts, e.g. for confirmed TB from around 20% if CD4≥100x10$^6$/l to >50% if CD4 <100x10$^6$/l; our improvement from 10% to 17% is in accord although still not useful for screening. [4–7] The sensitivity of LF-LAM is greater in those who are sicker, and this test is most useful as a rapid rule-in tool for TB when used for HIV-positive inpatients with symptoms suggestive of TB and advanced immunosuppression, a population with high mortality; and its use in this setting is supported by the recent WHO guidance.[11] In these populations sensitivity of 59–66% [9, 10] for culture-confirmed TB has been reported from evaluations in South Africa and Uganda, increasing to 85% [10] when combined with sputum Xpert MTB/RIF, thus potentially enabling rapid initiation of TB treatment. In contrast our participants were established in HIV care, and we were evaluating the usefulness of LF-LAM as part of a routine screening algorithm for intensified TB case finding in HIV clinic attendees, although restricted to those with CD4 counts below 200x10$^6$/l for whom LF-LAM was most likely to be useful. The median CD4 in our study population was 111x10$^6$/l which is lower than the 170–248x10$^6$/l reported in outpatient evaluations, [4–7] and our participants were frequently symptomatic, but in spite of this we found poor sensitivity for TB, possibly as patients were less ill.

An obvious advantage of a urine-based diagnostic test is ease of specimen collection, although privacy is clearly required. However, we found 20% of eligible patients did not provide a urine sample which, although less than the 33% unable to produce sputum spontaneously at enrolment, is still greater than 1–3% unable to produce urine in other outpatient studies. [5, 7] We found no indication that those unable to produce urine were more unwell than those who could, so this is unlikely to have affected our findings, although we acknowledge this as a limitation of our study. Our study procedures were fitted around routine appointments in busy public sector clinics, and we postulate that time limitations may have contributed, as these patients were also less likely to provide sputum at enrolment.

We found increased mortality amongst patients who were LF-LAM positive, consistent with studies of sicker inpatient HIV-positive populations reporting LAM-positivity as a predictor for mortality. [14, 15]

Strengths of our study include our systematic sampling and longitudinal follow-up, which minimised the number of TB diagnoses missed. All samples for mycobacteriology collected during the course of our study, many of which were sputum samples collected at enrolment, contributed to our reference standard of "confirmed" TB. We undertook all LF-LAM tests at study completion using frozen samples, but this is consistent with other published studies, and

all our samples were stored in accordance with manufacturer's recommendation with only one freeze-thaw cycle, and processed within one year from collection. [16] A limitation of our study is the exclusion of participants (N = 40) who were not diagnosed with TB but either did not have any TB microbiology results or died within three months of enrolment, but our sensitivity analysis shows that this made little difference to the performance of LF-LAM.

## Conclusion

Despite the appeal of LF-LAM as a cheap, non-sputum based, point-of-care TB screening tool, the low sensitivity in this population with advanced immunosuppression, of whom 13% had TB, precludes recommendation for its use to screen for TB in ambulatory patients established in HIV care in accordance with the recent WHO LF-LAM policy guidance.[11]

## Acknowledgments

We thank the study participants; the nursing and medical staff of Chris Hani Baragwanath and Mamelodi hospitals, Ramokonopi and Jabulani Dumane community health clinics, South Africa; the staff of National Health Laboratory Services, South Africa; and the staff of Aurum Institute for their essential contributions to this study.

## Author Contributions

Conceived and designed the experiments: YH KLF VNC LA SC AK KM MPN GJC ADG. Performed the experiments: YH VNC LA AK NTN FS ADG. Analyzed the data: YH KLF ADG. Wrote the paper: YH KLF VNC LA SC AK KM MPN NTN FS GJC ADG.

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# 6) *Paper 2*: A clinical scoring system to prioritise investigation for tuberculosis among adults attending HIV clinics in South Africa

## 6.1. Cover sheet

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

## RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

**SECTION A – Student Details**

| | | | |
|---|---|---|---|
| **Student ID Number** | 079810 | **Title** | Dr |
| **First Name(s)** | Yasmeen | | |
| **Surname/Family Name** | Hanifa | | |
| **Thesis Title** | Investigation pathways for tuberculosis among HIV-positive adults in South Africa | | |
| **Primary Supervisor** | Alison Grant | | |

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

**SECTION B – Paper already published**

| | | | |
|---|---|---|---|
| Where was the work published? | PLoS One | | |
| When was the work published? | August 2017 | | |
| If the work was published prior to registration for your research degree, give a brief rationale for its inclusion | N/A | | |
| Have you retained the copyright for the work?* | **Yes** | Was the work subject to academic peer review? | **Yes** |

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| Stage of publication | Choose an item. |

**SECTION D – Multi-authored work**

| | |
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| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | I managed the study, conducted the data analysis, and wrote the paper. |

**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
| **Date** | 28th May 2019 |

| | |
|---|---|
| **Supervisor Signature** | [redacted] |
| **Date** | 28 May 2019 |

## 6.2. Research paper

PLOS ONE

RESEARCH ARTICLE

# A clinical scoring system to prioritise investigation for tuberculosis among adults attending HIV clinics in South Africa

**Yasmeen Hanifa[1]*, Katherine L. Fielding[1], Violet N. Chihota[2,3], Lungiswa Adonis[4], Salome Charalambous[2,3], Nicola Foster[5], Alan Karstaedt[6,7], Kerrigan McCarthy[2], Mark P. Nicol[8,9], Nontobeko T. Ndlovu[2], Edina Sinanovic[5], Faieza Sahid[6,7], Wendy Stevens[9,10], Anna Vassall[1], Gavin J. Churchyard[1,2,3,11], Alison D. Grant[1,3,12]**

**1** London School of Hygiene & Tropical Medicine, London, United Kingdom, **2** The Aurum Institute, Johannesburg, South Africa, **3** School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, **4** Mamelodi Hospital, Pretoria, South Africa, **5** Health Economics Unit, School of public health and family medicine, University of Cape Town, Cape Town, South Africa, **6** Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa, **7** University of the Witwatersrand, Johannesburg, South Africa, **8** Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa, **9** National Health Laboratory Service, Johannesburg, South Africa, **10** Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, **11** Advancing Treatment and Care for TB/HIV, South African Medical Research Council Collaborating Centre for HIV and TB, Johannesburg, South Africa, **12** School of Nursing and Public Health, Africa Health Research Institute, University of KwaZulu-Natal, Durban, South Africa

* yasmeen.hanifa@lshtm.ac.uk



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**Data Availability Statement:** The XPHACTOR Clinical Score dataset has been uploaded to the LSHTM Data Compass repository at https://doi.org/10.17037/DATA.204 and is made available on request. Our study participants consented to the use of information collected from the XPHACTOR study for HIV and TB related research. Users of our data need to agree to this condition in order to fulfil the study's ethical obligations to research participants. The study team wish to avoid

## Abstract

### Background

The World Health Organization (WHO) recommendation for regular tuberculosis (TB) screening of HIV-positive individuals with Xpert MTB/RIF as the first diagnostic test has major resource implications.

### Objective

To develop a diagnostic prediction model for TB, for symptomatic adults attending for routine HIV care, to prioritise TB investigation.

### Design

Cohort study exploring a TB testing algorithm.

### Setting

HIV clinics, South Africa.

### Participants

Representative sample of adult HIV clinic attendees; data from participants reporting ≥1 symptom on the WHO screening tool were split 50:50 to derive, then internally validate, a prediction model.

unnecessary barriers to access and will seek to respond to data requests as quickly as possible.

**Funding:** GC received funding for the study from the Bill and Melinda Gates Foundation, grant number OPP1034523 (Churchyard). URL: http://www.gatesfoundation.org. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

## Outcome

TB, defined as "confirmed" if Xpert MTB/RIF, line probe assay or *M. tuberculosis* culture were positive; and "clinical" if TB treatment started without microbiological confirmation, within six months of enrolment.

## Results

Overall, 79/2602 (3.0%) participants on ART fulfilled TB case definitions, compared to 65/906 (7.2%) pre-ART. Among 1133/3508 (32.3%) participants screening positive on the WHO tool, 1048 met inclusion criteria for this analysis: 52/515 (10.1%) in the derivation and 58/533 (10.9%) in the validation dataset had TB. Our final model comprised ART status (on ART > 3 months vs. pre-ART or ART < 3 months); body mass index (continuous); CD4 (continuous); number of WHO symptoms (1 vs. >1 symptom). We converted this to a clinical score, using clinically-relevant CD4 and BMI categories. A cut-off score of $\geq$3 identified those with TB with sensitivity and specificity of 91.8% and 34.3% respectively. If investigation was prioritised for individuals with score of $\geq$3, 68% (717/1048) symptomatic individuals would be tested, among whom the prevalence of TB would be 14.1% (101/717); 32% (331/1048) of tests would be avoided, but 3% (9/331) with TB would be missed amongst those not tested.

## Conclusion

Our clinical score may help prioritise TB investigation among symptomatic individuals.

## Introduction

The World Health Organization (WHO) recommends, as part of activities to address the vast global burden of HIV-related tuberculosis (TB), regular screening for active TB of all people living with HIV (PLHIV) followed by Xpert MTB/RIF (Cepheid, Sunnyvale, CA) as the primary diagnostic test. [1] The recommended TB screening tool, which comprises any one of current cough, fever, weight loss or night sweats (subsequently referred to as the WHO tool), was developed for use in resource limited settings. [2] This simple tool, which was designed to rule out TB prior to the provision of isoniazid preventive therapy (IPT) to PLHIV, maximises sensitivity (78.9%) and negative predictive value (97.7% at TB prevalence of 5% in PLHIV), but has low specificity (49.6%) and positive predictive value (8% at TB prevalence of 5% in PLHIV). [2] South Africa, which is home to the world's largest HIV epidemic [3] and where 62% of individuals with TB are also HIV-positive, [4] has rolled out Xpert as the initial diagnostic test for all individuals with symptoms suggesting TB. [5] Regular TB screening of PLHIV with a tool that generates large numbers of patients requiring further investigation, of whom only a small proportion will have TB, combined with a diagnostic test that is currently far more expensive than smear microscopy, poses a huge challenge in resource constrained settings. In these settings prioritising testing for those at greatest risk of TB will help preserve resources.

Multivariable prediction models estimate the probability that an individual either has or will develop a particular condition. These models are increasingly abundant in the literature, with variable quality of construction as well as reporting, as highlighted by the recent TRIPOD

statement which presents a recommended reporting framework. [6, 7] Clinical scoring algorithms have been developed for PLHIV with symptoms suggestive of TB to prioritise investigation for those with greatest probability of having TB prior to antiretroviral therapy (ART) initiation, [8] and improve case finding, [9] but these algorithms have not been validated or applied to patients on ART.

The aim of our study was to develop a score, comprising elements readily available in primary care, to predict probability of TB in adults attending for routine HIV care screened for TB and found WHO tool positive. This score was used to develop a simple tool to help health care workers in resource limited settings decide whom to prioritise for TB investigation.

## Methods

We used data collected for "Xpert for people attending HIV/AIDS care: test or review?" (XPHACTOR), a prospective cohort study evaluating a risk-based algorithm to prioritise Xpert MTB/RIF testing amongst adults attending for routine HIV care in South Africa, to develop and validate our clinical score. Fig 1 depicts XPHACTOR study flow.

### XPHACTOR study population and recruitment

We enrolled a systematic sample of adults (aged ≥18 years) attending two hospital-based and two community health centre (CHC) clinics in Gauteng province, South Africa, for HIV care, irrespective of presence of symptoms suggestive of TB. Patients taking anti-tuberculosis treatment within the previous 3 months were excluded. Patients were enrolled into three groups: "on antiretroviral therapy (ART)" (currently taking or ART-experienced) group; "pre-ART" (in HIV care but not yet taking ART) group; and "HIV Testing and Counselling (HTC)" (newly-diagnosed HIV-positive). We recruited to the on ART group from hospital clinics because their patient population solely comprised those ART-experienced; and pre-ART and HTC groups were recruited from CHC clinics. At the time of the study, ART eligibility comprised CD4 ≤350 cells/$mm^3$ or WHO clinical stage ≥3.

### XPHACTOR procedures

**Enrolment.** At enrolment, research staff administered a standardised questionnaire incorporating the WHO TB screening tool (any of current cough, fever, night sweats or unintentional weight loss), measured height and weight, mid-upper arm circumference (MUAC), and recorded most recent clinic CD4 cell count. Further investigation was prioritised according to the XPHACTOR algorithm with an immediate spot sputum sample sent for Xpert MTB/RIF for (i) all assigned "high priority" (any of: current cough, fever ≥ 3 weeks, body mass index [BMI] <18.5 kg/$m^2$, CD4 <100x$10^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); (ii) those in pre-ART group with CD4< 200 x$10^6$/l at enrolment (iii) all in HTC group at enrolment, the latter two categories (who were recruited for XPHACTOR substudies) because of *a priori* high risk of active TB. For all other participants a spot sputum sample was frozen at -80˚C within 24 hours, for testing with Xpert at the end of the study.

**Follow-up.** Participants were reviewed monthly to three months, with repeat WHO symptom screen and a spot sputum requested for Xpert MTB/RIF if "high priority" by the study algorithm at that visit, with the exception of those in the "on ART" group who were asymptomatic at enrolment who were telephoned at 1 and 2 months to update locator information but were not asked about TB symptoms. At the 3-month visit sputum (induced if necessary) and blood were collected for mycobacterial culture on liquid media (Bactec MGIT 960

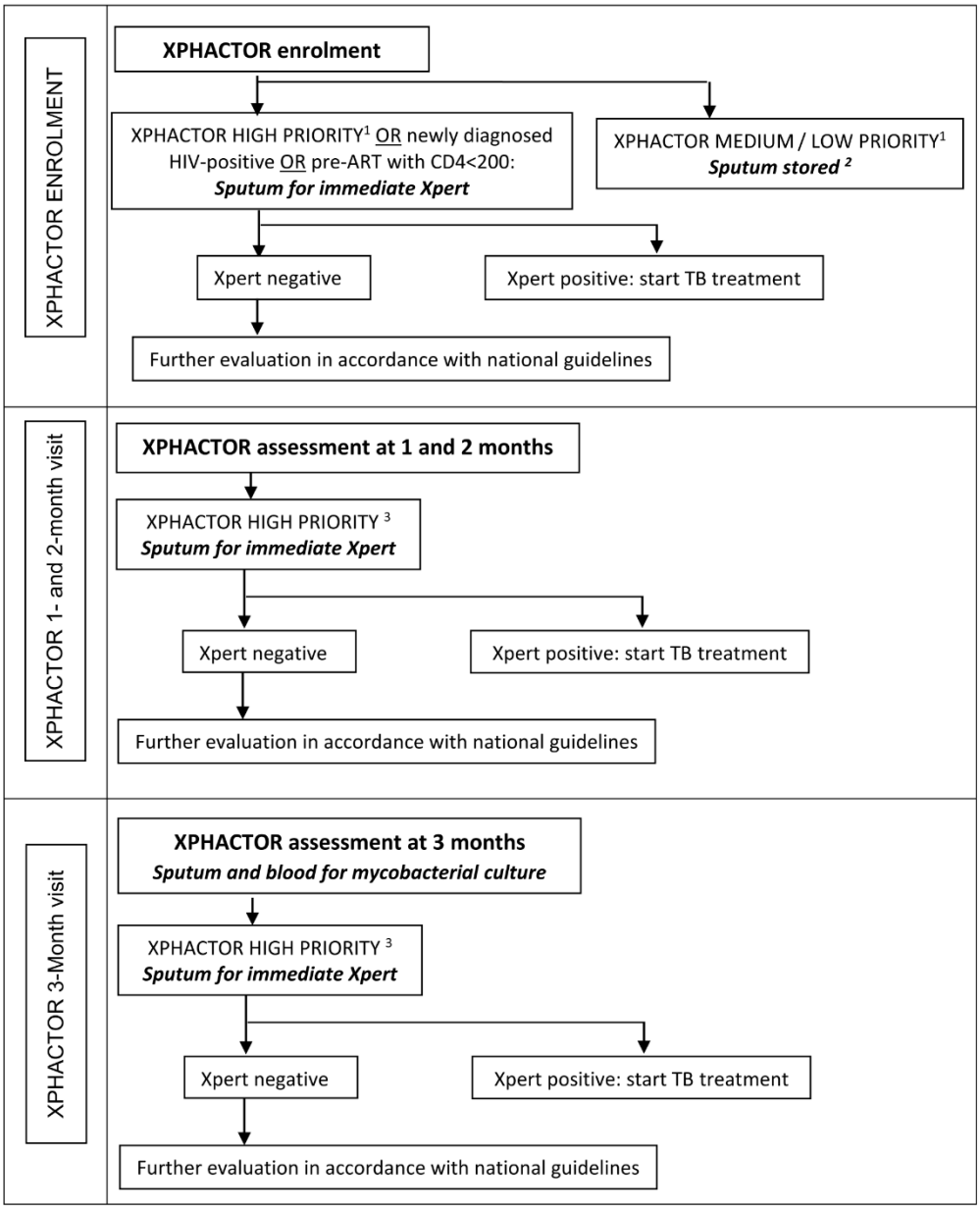


**Fig 1. XPHACTOR study flow.** [1] High priority (any of: current cough, fever ≥ 3 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of: fever < 3 weeks, night sweats, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms. [2] Samples tested with Xpert MTB/RIF at the end of the study. [3] High priority (any of: current cough, fever ≥ 3 weeks, night sweats ≥ 4 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of: fever < 3 weeks, night sweats <4 weeks, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms.

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and 9240 systems) from all study participants. We allowed a broad window period around the scheduled 3-month visit, till around six months, in order to maximise study follow-up.

Participants who submitted an Xpert sample were reviewed and if Xpert-positive, TB treatment was initiated; if negative, further investigation in accordance with national guidelines was facilitated (chest radiograph, sputum culture and trial of antibiotics).

Results of all investigations were fed back to clinic staff, who were responsible for management decisions. Clinic medical records were reviewed at the end of the study to ascertain any additional relevant investigations and/or TB diagnoses. Deaths were identified through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants' South African identification (ID) numbers.

## Development and validation of the prediction model

**Participants.** We restricted our analysis to all XPHACTOR participants who were WHO tool positive at enrolment and established in care (i.e. not newly testing HIV positive); and excluded those taking isoniazid preventive therapy (IPT) at enrolment, as those on IPT were likely to have recently undergone investigation for TB, and hence were effectively "pre-screened" for TB.

To be deemed clinically useful a prediction model should demonstrate accurate prediction of the outcome in data other than that in which the model was developed. We developed our prediction model using part of our dataset, and undertook internal validation of model performance using the remainder of the dataset. [10] Enrolment to XPHACTOR was staggered by site, commencing with hospital clinics; hence the dataset was stratified by site and split 50:50 by median date of enrolment within site. Data from the earlier half were used to derive our prediction model (derivation dataset), and from the latter half for validation (validation dataset). Data were analysed using Stata 14 (Stata Corporation, College Station, TX, USA), as detailed below.

**Outcome.** Our outcome was confirmed or clinical TB versus "not TB", ascertained within 6 months of enrolment to XPHACTOR, as defined below.

"Confirmed" TB was defined as a positive result on i) Xpert MTB/RIF or ii) line probe assay (LPA) performed on smear-positive or cultured isolate (GenoType MTBDR*plus*, Hain Lifesciences) or iii) *M. tuberculosis* (*Mtb*) culture, from any sample (including stored sputum and those requested by the health care provider) collected within six months of XPHACTOR enrolment. Individuals who started TB treatment within six months of enrolment (including those with treatment starts reported in the context of a separate verbal autopsy sub-study), in the absence of microbiological confirmation, were assigned "clinical" TB. This was based on the assumption that an HIV-positive adult with a positive bacteriological test result or starting TB treatment within six months after enrolment likely had active TB at enrolment, supported by data from Zimbabwe which estimated the mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults to be 18–33 weeks. [11]

"Not TB" was defined as fulfilling all of the following: absence of criteria for confirmed or clinical TB; and alive at least 3 months after enrolment. Participants who did not fulfil the case definitions for TB or "not TB" were deemed to have an unclassifiable outcome and excluded from the analyses.

Pulmonary and extrapulmonary TB were classified in accordance with WHO definitions. [12]

**Candidate predictor selection.** There is no consensus on the best method for selecting candidate variables, but suggested approaches include using literature review, clinical knowledge and studying the distribution of predictors in the study data. [6, 13, 14] It is

recommended, to ensure predictive accuracy, that the total number of candidate predictors is limited so that there are at least 10 outcomes for each candidate predictor studied. [6, 13] We considered predictors from data collected at enrolment to XPHACTOR known to be associated with prevalent and/or incident TB amongst PLHIV: age, sex, previous TB treatment, smoking, alcohol use, history of ART, duration on ART, previous IPT, previous cotrimoxazole preventive therapy (CPT), presence of individual WHO tool symptoms, duration of WHO tool symptoms, BMI, MUAC, CD4 count, haemoglobin, and viral load. [15–25] History of mining, [26] health care work, [27] and incarceration, [28] although established risk factors for TB were not considered as <10% participants fell into each category. The following variables were also excluded: MUAC, measured weight loss, haemoglobin and viral load, due to >20% missing data; and previous IPT, as there was only one outcome amongst participants with previous IPT.

*A priori* we combined history of ART with duration on ART to generate "ART status" categorised as: pre-ART or on ART <3 months *vs*. on ART for >3 months, as amongst patients on ART, duration of <3 months is a predictor for prevalent TB. [29] *A priori* we considered ART status, CD4 cell count, and BMI for our adjusted model, and used univariable screening to select additional candidate predictors with P-value (p)<0.25.

**Model building procedures in derivation dataset.** We undertook multivariable logistic regression of candidate predictors, sequentially removing the variable with the largest Wald *p*-value >0.05 (stepwise backward elimination), to generate our final model. [13] A complete-case analysis was undertaken, excluding participants with missing information relating to any of the candidate predictors. A model that categorised the number of WHO symptoms as 1 vs. >1 symptom (model A) was compared with one that included individual WHO tool symptoms (model B), aiming to select the simplest and most practical model to implement in primary care. We also considered a model without CD4 count for settings where this might not be easily available.

Transformations of continuous variables (BMI and CD4) were assessed using fractional polynomials. In our final selected model we tested for interactions between remaining variables and "ART status".

**Assessing model performance in derivation dataset.** We assessed model calibration, the agreement between probability of TB predicted by the model and observed probability of TB within quantiles of predicted risk, graphically in a calibration plot; and statistically using the Hosmer-Lemeshow test. We assumed p<0.05 from the Hosmer-Lemeshow test as indicating lack of model fit (poor calibration), although the test has limited statistical power to detect poor calibration unless the sample size is large and the outcome frequent. [13] We assessed discrimination, the ability of our model to differentiate patients with TB vs. those without, using the area under the receiver-operating characteristic curve (AUROC). AUROC 0.7 to 0.79, 0.8–0.89, ≥0.9 are respectively considered acceptable, excellent and outstanding discrimination. [30]

## Transformation from regression model to clinical score in derivation dataset

Continuous variables in the final model were categorised in a clinically meaningful manner based on their functional form, and each beta coefficient from this logistic regression model was divided by the smallest coefficient and rounded to the nearest integer to assign points to each variable. The total number of points was summed for each participant to calculate the clinical score.

**Internal validation.** We used the beta coefficients and intercept from the final regression models (before and after categorisation of continuous variables) generated from the derivation

dataset to calculate the risk score for each participant in our validation dataset. We converted the risk score into predicted risk using predicted risk = $1/(1+e^{-\text{risk score}})$, [13] and assessed performance of the regression model in the validation dataset by evaluating calibration and discrimination.

### Ethical approval

The study was approved by the ethics committees at the University of the Witwatersrand, University of Cape Town, and the London School of Hygiene & Tropical Medicine. All consenting participants gave written consent or, for illiterate participants, witnessed verbal consent. For illiterate participants, there was an impartial witness present during the consenting process, who then signed the relevant witness section of the consent form. All ethics committees approved the consent form, including the section on the use of witnessed oral consent for illiterate participants, at the beginning of the study. Principles expressed in the Declaration of Helsinki were followed in the conduct of this research.

## Results

We enrolled 3508 participants established in care (i.e. not newly testing HIV positive) to XPHACTOR. Overall, among patients taking ART, 783/2602 (30.1%) reported one or more symptom in the WHO tool and 79/2602 (3.0%) had TB. Among pre-ART patients 350/906 (38.6%) reported $\geq$1 symptom and 65/906 (7.2%) had TB. For this analysis, 2418/3508 were excluded because WHO tool negative (2227) or on IPT at enrolment (191), and a further 25 participants were excluded because of "unclassifiable" outcome leaving 1065 who were WHO tool positive and eligible for our analysis (Fig 2). We undertook a complete-case analysis and therefore excluded a further 17 participants with missing candidate predictor data (S1 Table), leaving 1048 for our analysis.

### Characteristics of study participants

Table 1 compares the characteristics of participants in the derivation and validation datasets. There were 515 participants in the derivation dataset, enrolled between September 2012 and September 2013, amongst whom 52 (10.1%) participants fulfilled case definitions for TB (36 confirmed, 16 clinical). In the validation dataset there were 533 participants enrolled between May 2013 and March 2014, amongst whom 58 (10.9%) participants fulfilled case definitions for TB (39 confirmed, 19 clinical). The proportion with pulmonary vs. extrapulmonary disease in derivation vs. validation datasets amongst those with confirmed TB was pulmonary (35/36 vs. 37/39) and extrapulmonary (1/36 vs. 2/39); and amongst those with clinical TB was pulmonary (9/16 vs. 7/19), extrapulmonary (4/16 vs. 5/19) and not recorded (3/16 vs. 7/19). [12] The median time from enrolment to earliest of positive TB test or date TB treatment was started amongst all participants diagnosed with TB (derivation and validation datasets combined) was 7 days (IQR 0, 63), with 90% of diagnoses made within 120 days of enrolment.

In derivation and validation datasets, median age was 41 years, 72% were in the on ART group, most participants were female (67% vs. 71%), and the most common WHO tool symptoms reported at enrolment were cough (59% vs. 66%) and weight loss (46% vs. 42%; Table 1). At enrolment median CD4 was greater in derivation compared with validation dataset (378 vs. 334 cells/$mm^3$), and median BMI was similar (24 $kg/m^2$). Participants in the derivation dataset were more likely to report previous IPT than those in the validation dataset (9.9% vs. 3.6%).

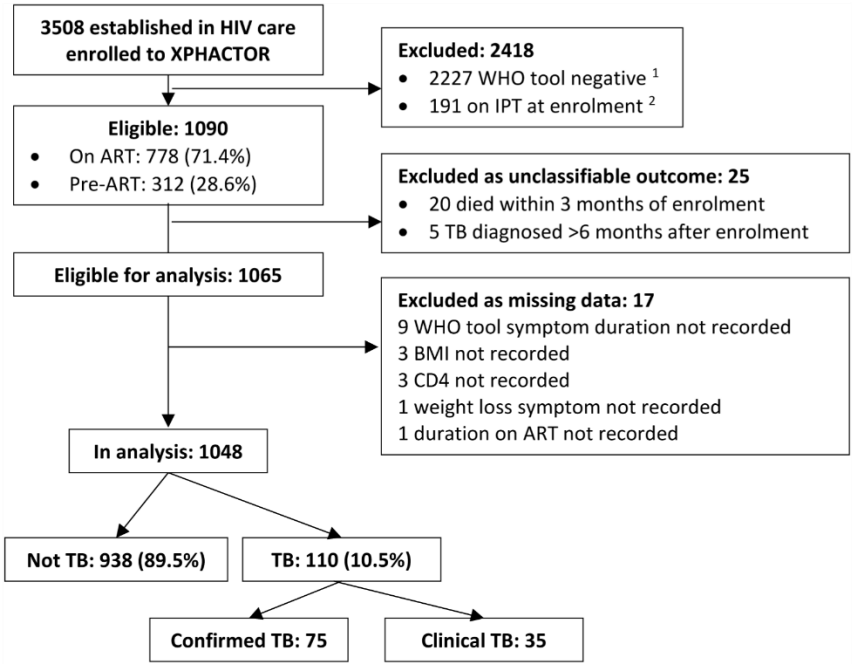


**Fig 2. Flow chart of study participants.** [1] 28/2227 TB diagnosed within six months of enrolment (25 confirmed TB and 3 clinical TB), of whom 25 on ART and 3 pre-ART. [2] 4/191 confirmed TB diagnosed within six months of enrolment, all pre-ART BMI = body mass index. IPT = Isoniazid preventive therapy. WHO tool negative = self-report of absence of all of: current cough, fever, night sweats and unintentional weight loss.

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## Development of regression model (derivation dataset)

Table 2 summarises the candidate predictors considered for model A, which categorised number of WHO symptoms reported (1 symptom vs. > 1 symptom), and the final multivariable model. We excluded age, alcohol status and previous history of TB as p>0.25 in univariable analysis. Our final model (model A) comprised: ART status (on ART > 3 months = 0 *v* pre-ART or ART <3 months = 1); BMI (continuous, linear); CD4 (continuous, linear); number of WHO symptoms (1 symptom = 0 *v* >1 symptom = 1). A linear relationship with log odds of the outcome was found to be adequate using fractional polynomials for both BMI and CD4 count. No evidence was found for statistical interactions between ART status and CD4 count, BMI, or number of WHO symptoms (Wald p-value ≥0.9). This model had Hosmer-Lemeshow statistic p = 0.65 and AUROC 0.79 (95% confidence intervals [CI] 0.73–0.86) indicating statistically adequate calibration and discrimination in the derivation dataset (Fig 3, S2 Table). In a sensitivity analysis where we excluded all clinical TB and used a gold standard of bacteriologically-confirmed TB, we obtained the same final multivariable model (S3 Table).

Univariable screening to select candidate predictors may result in the rejection of important predictors. [6, 13] When we repeated our multivariable analysis without univariable screening, and included all candidate predictors considered for model A, using stepwise backward elimination we obtained the same final model.

**Table 1. Characteristics of participants in derivation and validation datasets.**

| Characteristic | | Derivation dataset (N = 515) | Validation dataset (N = 533) |
|---|---|---|---|
| | | Value | Value |
| | | N (%) | N (%) |
| **Demographics** | | | |
| **Age, years** | Median (IQR) | 41 (34,48) | 41 (34,48) |
| **Sex** | Female | 345 (67.0%) | 377 (70.7%) |
| **Alcohol history** | Never [1] | 308 (59.8%) | 353 (66.2%) |
| **Smoking history** | Never [2] | 354 (68.7%) | 383 (71.9%) |
| **HIV/TB history** | | | |
| **Participant category** | On ART [3] | 370 (71.8%) | 381 (71.5%) |
| **Duration since HIV diagnosed, months** | Median (IQR) | 56 (21,95) (N = 505) | 51 (6,97) (N = 531) |
| **Duration on ART, months** | Median (IQR) | 55 (26,85) (N = 370) | 51 (28,83) (N = 381) |
| **Ever had IPT** | Yes | 51 (9.9%) | 19 (3.6%) |
| **Ever had CPT** | Yes | 370 (71.8%) | 355 (66.6%) |
| **Previous TB treatment** | Yes | 201 (39.0%) | 199 (37.3%) |
| **WHO symptoms at enrolment** | | | |
| | Cough | 304 (59.0%) | 350 (65.7%) |
| | Weight loss | 235 (45.6%) | 221 (41.5%) |
| | Night sweats | 131 (25.4%) | 130 (24.4%) |
| | Fever | 97 (18.8%) | 88 (16.5%) |
| **Number of symptoms** | 1 | 341 (66.2%) | 352 (66.0%) |
| | 2 | 114 (22.1%) | 122 (22.9%) |
| | 3 | 42 (8.2%) | 43 (8.1%) |
| | 4 | 18 (3.5%) | 16 (3.0%) |
| **Duration of WHO symptoms[4], days** | Median (IQR) | 30 (8,89) | 28 (7,84) |
| **CD4 / BMI at enrolment** | | | |
| **CD4, cells/mm$^3$** | Median (IQR) | 378 (228,543) | 334 (168,559) |
| | Range | 1–1630 | 2–1577 |
| **Time from CD4 to enrolment, days** | Median (IQR) | 147 (45,259) | 116 (27,267) (N = 528) |
| **BMI, kg/m$^2$** | Median (IQR) | 24.0 (20.6,28.4) | 24.0 (20.2,28.4) |
| | Range | 13.4–47.2 | 15.0–57.9 |
| **TB diagnoses over 6 months follow-up** | | | |
| | Total | 52 (10.1%) | 58 (10.9%) |
| | Confirmed TB | 36 (7.0%) | 39 (7.3%) |
| | Clinical TB | 16 (3.1%) | 19 (3.6%) |
| **Time from enrolment to TB diagnosis [5], days** | Median (IQR) | 7 (0,31) (N = 52) | 8 (0,83) (N = 57) |
| **Follow up** | | | |
| **Time from enrolment to most recent of last study / clinic [6] visit, days** | Median (IQR) | 280 (203,350) (N = 514) | 179 (133,231) (N = 532) |
| **Alive 6 months after enrolment [7]** | Yes | 98% (477/487) | 98% (463/473) |

[1] compared with any alcohol in last 1 year

[2] compared with ever/ex-smoker

[3] compared with pre-ART group

[4] duration WHO tool positive

[5] defined as earliest of positive TB test or date TB treatment started

[6] Most recent clinic visit at time of clinic file review

[7] Amongst participants with most recent study/clinic visit <6 months from enrolment, if participant had valid South African ID number and demise not reported by Department of home affairs / participant-nominated contacts / clinic staff within 6 months of enrolment, participant assumed to be alive at 6 months after enrolment.

IPT = isoniazid preventive therapy; CPT = cotrimoxazole preventive therapy; IQR = interquartile range

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**Table 2. Univariable and multivariable logistic regression analysis in the derivation dataset (N = 515).**

| Predictor | | Patients with TB | Unadjusted | P value | Adjusted [3] | P value | Adjusted β |
|---|---|---|---|---|---|---|---|
| | | N = 52/515 | odds ratio | (Wald) | odds ratio | | coefficient |
| | | n/N (%) | (95% CI) | | (95% CI) Model A | | (log [adjusted OR]) |
| | | | | | | | (95% CI) |
| **Age [1], years** | | | 1.00 (0.97, 1.03) | 0.96 | | | |
| **Sex** | Male | 23/170 (13.5%) | 1 | | | | |
| | Female | 29/345 (8.4%) | 0.59 (0.32, 1.05) | ***0.07*** | | | |
| **Smoking status** | Never smoked | 28/354 (7.9%) | 1 | | | | |
| | Current or ex-smoker | 24/161 (14.9%) | 2.04 (1.14, 3.64) | ***0.02*** | | | |
| **Alcohol status** | Current | 23/207 (11.1%) | 1 | | | | |
| | None in last 1 year | 29/308 (9.4%) | 0.83 (0.47, 1.48) | 0.53 | | | |
| **ART status** | On ART ≥ 3 months | 24/347 (6.9%) | 1 | | 1 | | 0 |
| | Pre-ART / ART <3 months | 28/168 (16.7%) | 2.69 (1.51, 4.80) | ***0.001*** | 2.22 (1.17, 4.22) | ***0.01*** | 0.80 (0.16, 1.44) |
| **Ever had CPT** | No / don't know | 19/145 (13.1%) | 1 | | | | |
| | Yes | 33/370 (8.9%) | 0.65 (0.36, 1.18) | ***0.16*** | | | |
| **Previous history of TB** | No | 33/314 (10.5%) | 1 | | | | |
| | Yes | 19/201 (9.5%) | 0.89 (0.49, 1.61) | 0.70 | | | |
| **Number of WHO symptoms** | 1 symptom | 18/341 (5.3%) | 1 | | 1 | | 0 |
| | > 1 symptom | 34/174 (19.5%) | 4.36 (2.38, 7.98) | ***<0.001*** | **3.45 (1.83, 6.49)** | ***<0.001*** | 1.24 (0.60, 1.87) |
| **Duration of WHO tool symptoms** | <1 week | 3/97 (3.1%) | 1 | | | | |
| | ≥ 1 week | 49/418 (11.7%) | 4.16 (1.27, 13.64) | ***0.02*** | | | |
| **BMI [1,2], kg/m²** | | | 0.88 (0.82, 0.94) | ***<0.001*** | 0.89 (0.83, 0.95) | ***0.001*** | -0.12 (-0.19, -0.05) |
| **CD4 [1,2], cells/mm³** | | | 0.997 (0.995, 0.998) | ***<0.001*** | 0.998 (0.996, 0.999) | ***0.006*** | -0.002 (-0.004, -0.0006) |

[1] Age, BMI and CD4 count were modelled as continuous variables

[2] In the multivariable analysis BMI and CD4 count were modelled as continuous variables, a linear relationship with log odds of outcome was found to be adequate after modelling using fractional polynomials.

[3] Adjusted for all variables shown in column. 100 unit increase in CD4 corresponds to reduction in adjusted odds ratio (aOR) of TB of 0.80 (95% CI 0.68, 0.94); 5 unit increase in BMI corresponds to reduction in aOR of TB of 0.56 (95% CI 0.39, 0.79).

Intercept (log odds) for multivariable model is 0.39.

In the validation dataset the risk score was calculated using the formula: risk score = 0.39 + 0.80 (if pre-ART / ART< 3months)–(0.002 x CD4 count)–(0.12 x BMI) + 1.24 (if > 1 symptom)

https://doi.org/10.1371/journal.pone.0181519.t002

## Internal validation of final regression model

The risk score and predicted risk were calculated for the validation dataset using model A and showed that calibration and discrimination were adequate (Hosmer-Lemeshow p = 0.31 [S2 Table], AUROC 0.75 [95% CI 0.68–0.82]), though the calibration plot demonstrates over-prediction at higher deciles of risk (Fig 3).

## Alternative prediction models

S4 Table presents an alternative multivariable model developed using individual WHO tool symptoms rather than total number of symptoms (model B). Model B comprised ART status, BMI, cough, night sweats and unintentional weight loss. In the derivation dataset there was evidence that presence of cough was modified by ART status (p = 0.03 for interaction term); and the model had adequate calibration and discrimination (Hosmer-Lemeshow statistic

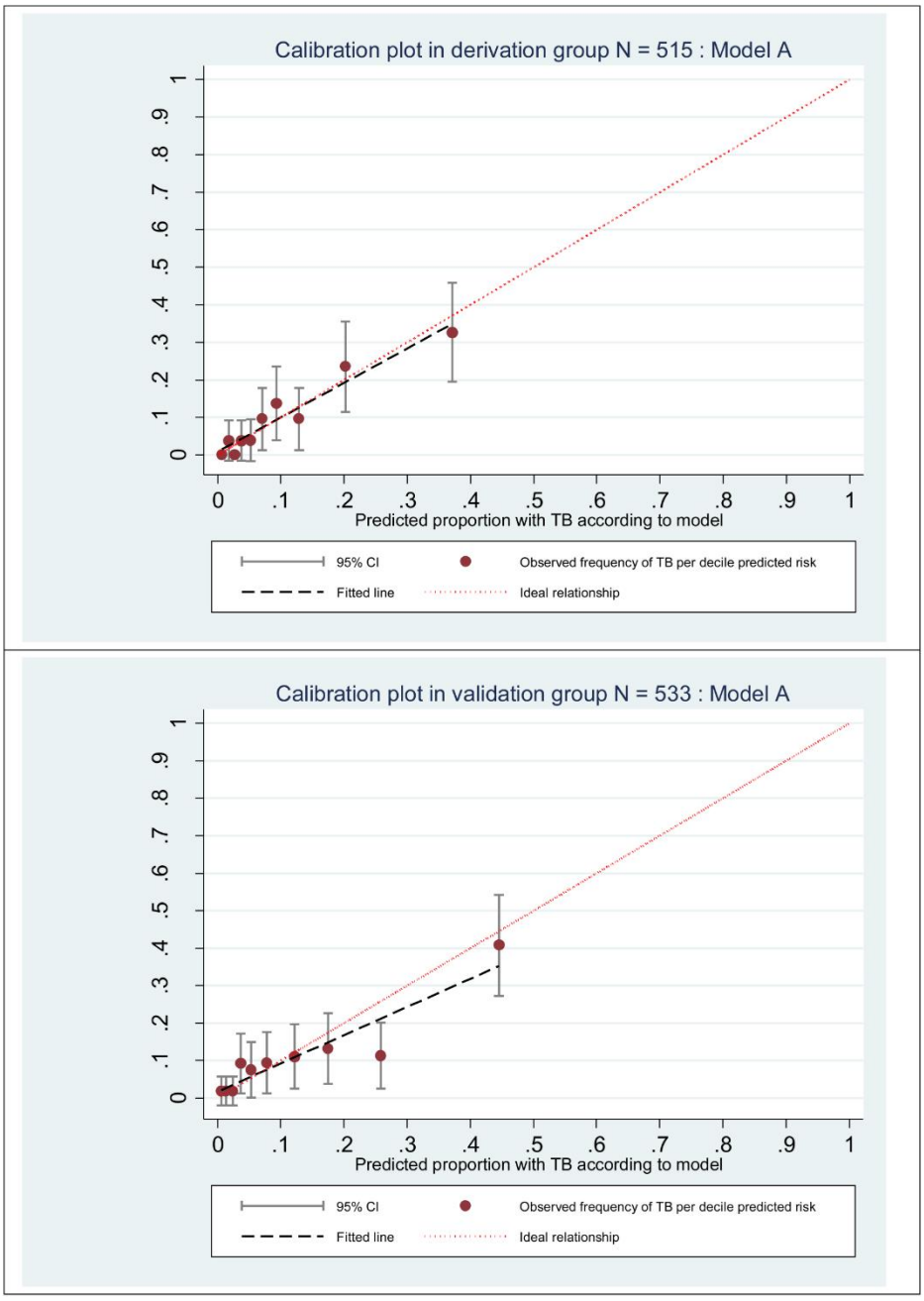


**Fig 3. Calibration plot of final prediction model in derivation and validation datasets.**

https://doi.org/10.1371/journal.pone.0181519.g003

p = 0.81, AUROC 0.82 [95% CI 0.76–0.88]). In the validation dataset this model had poor calibration (Hosmer-Lemeshow statistic p = 0.01) although discrimination was acceptable (AUROC 0.75 [95% CI 0.69–0.82]).

We repeated our multivariable analysis using all candidate predictors considered for model A removing CD4 count, for use in a setting where CD4 count is not easily obtainable. This model containing ART status, BMI and number of WHO symptoms (data not shown), performed adequately in the derivation dataset (Hosmer-Lemeshow statistic p = 0.54, AUROC 0.77 [95% CI 0.71–0.84]). In the validation dataset this model had poor calibration (Hosmer-Lemeshow statistic p = 0.02) although discrimination was acceptable (AUROC 0.70 [95% CI 0.63–0.77]).

We selected model A as our final model to develop the risk score because it was simpler and performed better in the validation dataset.

## Transformation from regression model to clinical score

We used WHO BMI categorisation of $<18.5$ kg/m$^2$ as underweight, 18.5–24.9 kg/m$^2$ as normal weight, and $\geq 25$ kg/m$^2$ as overweight. CD4 count was categorised as $<200$ cells/mm$^3$, 200–349 cells/mm$^3$ and $\geq 350$ cells/mm$^3$ to reflect clinically relevant cut-offs and the skewed CD4 count distribution amongst HIV-infected patients with TB. [31] The multivariable model with categorisation of these continuous variables in the derivation dataset is presented in Table 3. This model had statistically adequate discrimination in both derivation (AUROC 0.79 [95% CI 0.73, 0.86]) and validation datasets (AUROC 0.72 [95% CI 0.65, 0.79]). The Hosmer-Lemeshow statistic *p*-value was 0.89 in the derivation dataset but 0.02 in the validation dataset indicating poor calibration in the validation dataset.

The clinical score for each predictor was generated and the possible range for the total score was 0 to 16 (Table 3).

Table 4 shows the percentage of patients diagnosed with TB at each value of clinical score in derivation and validation datasets, and S1 Fig boxplot illustrates the distribution of clinical score, stratified by dataset, amongst those diagnosed with TB vs. those not diagnosed with TB.

**Selection of cut-off for clinical score.** Fig 4 shows the performance of the clinical score at different cut-offs, in terms of sensitivity, specificity, negative predictive value and AUROC in the entire dataset. A cut-off of clinical score of $\geq 3$ to trigger TB investigation had sensitivity of 91.8% (95% CI 85, 96.2), specificity 34.3% (95% CI 31.3, 37.5), negative predictive value 97.3% (94.9, 98.7) and AUROC 63.1% (95% CI 60.1, 66.1). Increasing the cut-off to $\geq 7$, where

**Table 3. Multivariable model and clinical score in the derivation dataset (N = 515).**

| Predictor | | Adjusted odds ratio (95% CI) | β coefficient | P value | Score* |
|---|---|---|---|---|---|
| **ART status** | On ART ≥ 3 months | 1 | 0 | | 0 |
| | Pre-ART / ART < 3 months | 2.34 (1.22,4.46) | 0.85 | 0.01 | 3 |
| **BMI, kg/m$^2$** | ≥ 25 | 1 | 0 | | 0 |
| | 18.5–24.9 | 2.23 (1.05,4.74) | 0.80 | 0.04 | 2 |
| | <18.5 | 6.79 (2.61,17.62) | 1.91 | <0.0001 | 6 |
| **CD4, cells/mm$^3$** | ≥ 350 | 1 | 0 | | 0 |
| | 200–349 | 1.40 (0.63,3.11) | 0.34 | 0.4 | 1 |
| | < 200 | 2.55 (1.23,5.30) | 0.94 | 0.01 | 3 |
| **Number of WHO symptoms reported** | 1 symptom | 1 | 0 | | 0 |
| | > 1 symptom | 3.59 (1.90,6.80) | 1.28 | <0.0001 | 4 |
| **Intercept (log odds)** | -4.23 | | | | |

* Each coefficient was divided by 0.335 (the smallest coefficient in our model, CD4 200–349 cells/mm$^3$) and rounded to the nearest integer to form the score for that predictor

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**Table 4. Performance of clinical score in derivation and validation datasets.**

| Clinical score | Derivation dataset | | Validation dataset | |
|---|---|---|---|---|
| | Total with score | Number diagnosed with TB (%) [1] | Total with score | Number diagnosed with TB (%) [1] |
| **0** | 69 | 1 (1.5) | 90 | 2 (2.2) |
| **1** | 24 | 0 | 32 | 1 (3.1) |
| **2** | 62 | 2 (3.2) | 54 | 3 (5.6) |
| **3** | 74 | 3 (4.1) | 50 | 7 (14) |
| **4** | 33 | 3 (9.1) | 37 | 3 (8.1) |
| **5** | 52 | 3 (5.8) | 42 | 2 (4.8) |
| **6** | 54 | 4 (7.4) | 54 | 2 (3.7) |
| **7** | 41 | 6 (14.6) | 21 | 4 (19.1) |
| **8** | 23 | 6 (26.1) | 33 | 6 (18.2) |
| **9** | 21 | 2 (9.5) | 25 | 2 (8.0) |
| **10** | 27 | 7 (25.9) | 34 | 4 (11.8) |
| **11** | 7 | 1 (14.3) | 4 | 1 (25) |
| **12** | 18 | 8 (44.4) | 41 | 18 (43.9) |
| **13** | 7 | 4 (57.1) | 6 | 0 |
| **14** | 0 | | 2 | 0 |
| **16** | 3 | 2 (66.7) | 8 | 3 (37.5) |
| **TOTAL** | **515** | **52** | **533** | **58** |

[1] Row percentages shown

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sensitivity and specificity were closest offered the best discrimination (AUROC 70.1% [95% CI 65.8, 75.1]), with improvement in specificity to 73.7% (95% CI 70.7, 76.5), but sensitivity was only 67.3% (95% CI 57.7, 75.9) although negative predictive value was maintained at 95% (95% CI 93.2, 96.5).

We selected a cut-off of clinical score $\geq 3$ to trigger TB investigation as we deemed that in this population, in order to avoid missing TB diagnoses, maintaining a higher sensitivity was more important than optimising discrimination. Investigating patients who had a clinical score of $\geq 3$ would have resulted in no further investigation of 30% (155/515) patients in the derivation dataset, and missed 6% (3/52) of TB diagnoses. The same cut-off for investigation in the validation dataset would have resulted in no further investigation of 33% (176/533) patients and missed 10% (6/58) of TB diagnoses. Amongst the nine patients with clinical score $< 3$ and TB diagnosed (4 confirmed TB, 5 clinical TB) all had had been on ART for $\geq 3$ months and all reported only one symptom which was cough; median BMI was 24.3 kg/m$^2$ (range 20.3–30.8) and median CD4 was 429 cells/mm$^3$ (range 241–1183).

Fig 5 presents a proforma of how this scoring system, using combined data from both derivation and validation datasets to demonstrate the prevalence of TB by clinical score group, could be used in practice (combined data sets, N = 1048). If investigation was prioritised for individuals with a score of $\geq 3$, 68% (717/1048) of symptomatic individuals would be tested, among whom the prevalence of TB would be 14.1% (101/717). 32% (331/1048) of tests would be avoided using this strategy, at the cost of missing 8% (9/110) individuals with TB or 3% (9/331) with TB amongst those not tested.

## Discussion

Our study is the first to derive and internally validate a clinical score for patients attending for routine HIV care, both ART-experienced and pre-ART, for use as a second step after TB

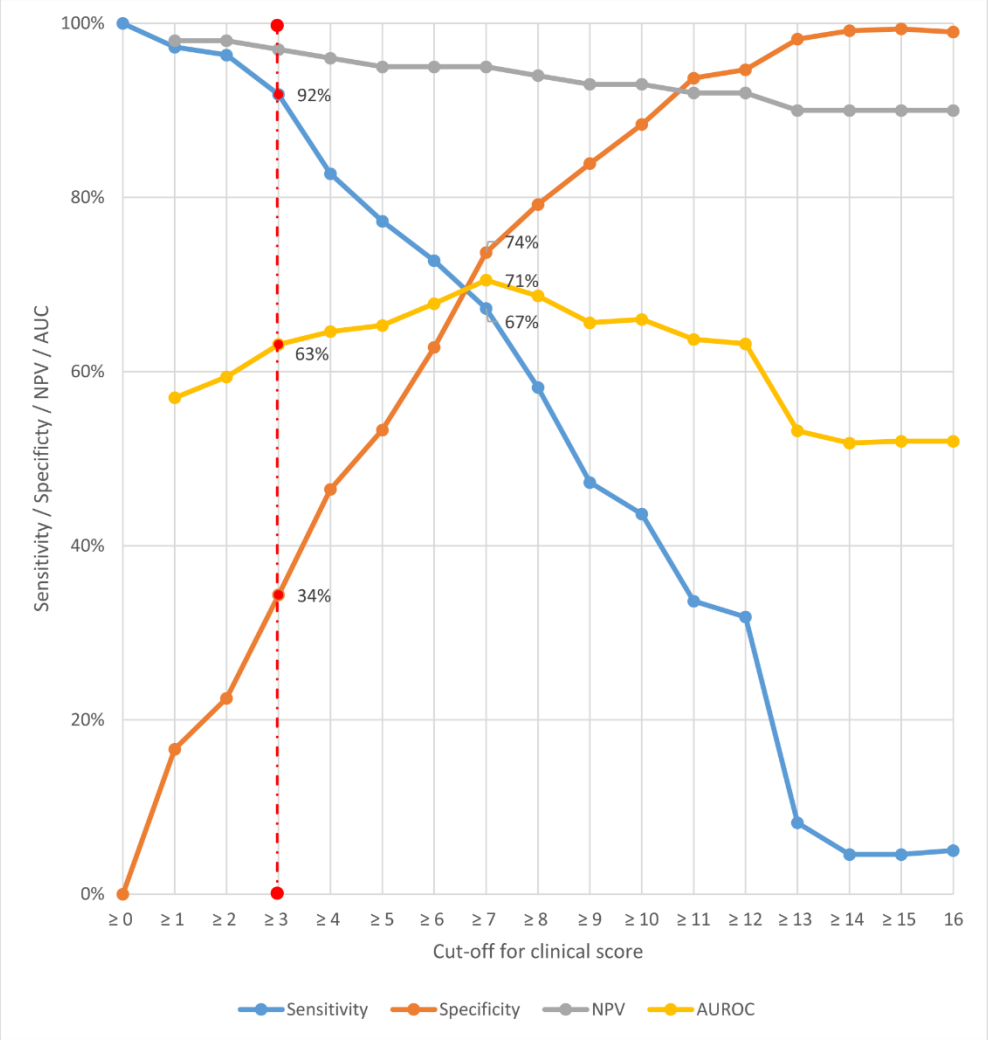


**Fig 4. Performance of clinical score at different cut-offs offs (derivation and validation datasets combined; N = 1048).** NPV = negative predictive value. AUROC = area under the receiver-operating characteristic curve.

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screening with the WHO tool. The score is designed to assist health care workers in resource limited settings to identify whom to prioritise for TB investigation. Our score uses elements which should be readily available at any level of health care and is simple to use, highlighting to less experienced clinicians those at greatest risk of TB, and providing a useful tool for other cadres of health care worker. In our study population, not investigating those who have a clinical score <3, amongst whom the prevalence of TB is 3% (9/331), would avoid investigation of 32% (331/1048) of those reporting WHO symptom(s), whilst missing only 8% (9/110) of TB diagnoses. We hypothesise that the WHO tool positive patients with clinical score <3 who had

| Risk factor | Category | Select one | Associated points | Assigned score |
|---|---|---|---|---|
| ART status | Pre-ART | ☐ | 3 | |
| | ART < 3 months | ☐ | 3 | |
| | ART ≥ 3 months | ☐ | 0 | ⊕ |
| BMI | < 18.5 | ☐ | 6 | |
| | 18.5-24.9 | ☐ | 2 | |
| | ≥ 25 | ☐ | 0 | ⊕ |
| CD4 | <200 | ☐ | 3 | |
| | 200-349 | ☐ | 1 | |
| | ≥ 350 | ☐ | 0 | ⊕ |
| Number of WHO symptoms | >1 | ☐ | 4 | |
| | 1 | ☐ | 0 | ⊖ |
| TOTAL SCORE | | | | |

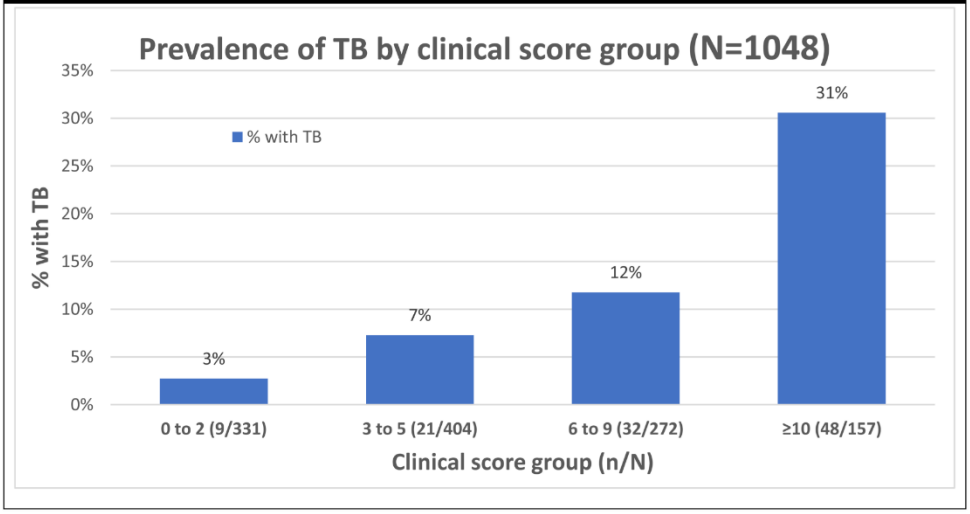


**Fig 5. Clinical score and prevalence of TB.**

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TB diagnoses missed were more likely to have less advanced disease and more favourable prognosis. This is suggested by their clinical characteristics (all on ART with normal weight and CD4 count >240 cells/mm$^3$), and consistent with findings from other studies. [32–34] In the broader context of the original XPHACTOR study population, not investigating the 2227 who were WHO tool negative at enrolment would have missed the 28 TB diagnoses in this group (Fig 2). The overall risk of TB in those who were WHO tool negative or had clinical score <3 was 1% (37/2558), and the two step strategy (WHO tool followed by clinical score) would have avoided investigating 78% (2558/3275) of clinic attendees (Fig 2). The TB diagnoses missed using this strategy, 27% (37/138) of TB diagnoses, were mainly amongst those who were WHO tool negative.

Our clinical scoring system compares favourably, in terms of simplicity and ability to identify patients with lowest prevalence of TB, with that derived by Balcha et al, also as a second step after WHO symptom screen, for ART naïve patients attending for HIV care in Ethiopia. [8] In this smaller and as yet unvalidated (internally or externally) study, amongst 569 WHO tool positive patients, a more complex score which included Karnofsky status, MUAC, peripheral lymphadenopathy and anaemia, using a cut off of ≥2 was able to avoid investigation of 45% (255/569) of whom 8% (20/255) had culture confirmed TB. Rudolf et al derived TBscore II from a population in Bissau who were seeking care for symptoms suggestive of TB, of whom only 164 were HIV-positive. [9] Their score is also more complex than our score, incorporating physical signs in addition to symptoms and also requires both internal and external validation.[9]

The majority of our study population were established on ART in contrast to those in the meta-analysis which derived the WHO tool who were largely pre-ART, [2] and the populations used to derive clinical scores by Balcha [8] and Rudolf. [9] Thus our study addresses a key question concerning operationalisation of TB screening among the increasingly large population of adults on ART. Our study participants were established in HIV care and thus likely had had previous screening, which is known to reduce sensitivity of the WHO tool for bacteriologically confirmed TB, [2] as also is ART use. [35] Rangaka et al evaluated the utility of the WHO tool to rule out TB prior to IPT in a population similar to ours, i.e. both pre-ART and on ART although duration on ART was shorter (median 12 months), against a gold standard of culture-confirmed TB. [29] Their study suggested that amongst those on ART addition of BMI and CD4 to the tool could be considered, but recommended sputum culture first for all prior to IPT. [29] We ensured that in our clinical score we included BMI, CD4 and a measure of ART status which incorporated duration on ART, and believe that our score therefore will prove useful for all patients screened for TB during routine HIV care. Our score obviates the need for a separate tool for those pre-ART vs. on ART, although people with newly diagnosed HIV have such a high TB prevalence that investigation for all may be justified. [36]

In contrast with other studies deriving clinical algorithms or evaluating performance of the WHO rule, [2, 8, 29, 33, 35] our case definition for TB included clinical TB. This reflects the real life scenario of high TB burden resource-limited settings, and is a strength of our study. Most of our TB diagnoses were bacteriologically-confirmed pulmonary TB, which is what the WHO tool was largely designed to rule out prior to provision of IPT. [2] In sensitivity analysis restricted to bacteriologically-confirmed TB we obtained the same final multivariable model (S3 Table).

We assumed that all participants starting TB treatment or with a sample which was bacteriologically confirmed collected within six months of enrolment were likely to have had active TB at enrolment. We based this decision on data from a community survey and TB notification data in Zimbabwe, estimating a mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults of 18–33 weeks. [11] In actual fact 90% of our study participants

who started TB treatment commenced within four months of enrolment. In the derivation vs. validation dataset the interquartile range for time from enrolment to TB diagnosis is shorter (0–31 vs. 0–83 days) and this may reflect implementation of a substudy later in the course of XPHACTOR evaluating causes for persistent TB symptoms in patients without TB diagnosis by the 3-month visit. There were 47 participants (with 10 TB diagnoses) in this substudy in the validation dataset compared with 7 in the prediction dataset (with 1 TB diagnosis). We undertook the majority of our case notes reviews towards the end of the XPHACTOR study and this is reflected in the longer duration of follow up in the derivation vs. validation dataset, which may have resulted in ascertainment bias in terms of TB diagnoses made in the derivation dataset, although the total number of TB diagnoses was similar in both groups. Differences between the derivation and validation datasets represent a strength in terms of evaluating our predictive model, as non-random splitting which reduces the similarity of the two datasets is preferred for internal validation.[6]

We developed our score in accordance with TRIPOD recommendations [6] and internal validation of our final multivariable model (model A) demonstrated adequate calibration and discrimination in our validation dataset. The multivariable model resulting from our categorisation of BMI and CD4 in a clinically meaningful manner, also showed acceptable and clinically useful discrimination in the validation dataset. Our model requires external validation in order to confirm that it predicts well in individuals outside of our dataset [37] and, following this, impact studies to assess patient outcomes and cost effectiveness of this strategy. [38] Assuming external validity, our suggested threshold for investigation (clinical score $\geq$3), could be varied depending on available resources. We have suggestions for updating our prediction model, which we were unable to evaluate due to insufficient data: MUAC, which is simpler to measure than BMI; haemoglobin, because anaemia is a strong independent predictor of TB amongst those poised to initiate ART; [39] and viral load. [19] Recent WHO guidelines recommend ART initiation for all PLHIV at any CD4 count suggesting that in settings where viral load monitoring can be assured that CD4 count for monitoring purposes may be reduced or stopped. [40] CD4 count itself is not always easily available, and in these settings viral load monitoring is also unlikely to be easily available, but given this new guidance models without CD4 should be considered.

Strengths of our study include systematic evaluation of a representative sample of adults attending for routine HIV care who underwent rigorous assessment for TB and longitudinal follow-up which minimised the number of TB diagnoses missed, and model development and validation in accordance with TRIPOD guidelines. [6]

## Conclusions

We have developed and internally validated a simple clinical score comprising ART status, BMI, CD4 count and number of WHO symptoms, for patients attending for routine HIV care in resource limited settings. Our score is designed to identify, amongst those reporting WHO tool symptom(s), whom should be prioritised for TB investigation. Our findings are highly relevant given the national roll out of Xpert MTB/RIF in South Africa.

## Supporting information

**S1 Table. Characteristics of eligible participants and missing values (N = 1065).**
(PDF)

**S2 Table. Hosmer-Lemeshow test for calibration of final model (model A).**
(PDF)

**S3 Table. Model A Multivariable logistic regression analysis in derivation dataset after exclusion of all clinical TB (N = 499).**
(PDF)

**S4 Table. Model B: Multivariable logistic regression analysis in derivation dataset (N = 515).**
(PDF)

**S1 Fig. Boxplot illustrating distribution of clinical score in individuals with and without TB.**
(PDF)

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## Author Contributions

**Conceptualization:** YH KLF VNC LA SC NF AK KM MPN ES WS AV GJC ADG.

**Data curation:** KLF VNC SC NTN GJC ADG.

**Formal analysis:** YH KLF ADG.

**Funding acquisition:** GJC ADG.

**Investigation:** YH VNC AK NTN FS.

**Methodology:** YH KLF VNC LA SC AK KM MPN WS GJC ADG.

**Project administration:** YH VNC NTN.

**Resources:** KLF VNC LA SC AK FS GJC ADG.

**Supervision:** KLF SC GJC ADG.

**Visualization:** YH KLF AV ADG.

**Writing – original draft:** YH KLF ADG.

**Writing – review & editing:** YH KLF VNC LA SC NF AK KM MPN NTN ES FS WS AV GJC ADG.

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## 6.3. Material provided as supplementary online appendices

S1 Table. Characteristics of eligible participants and missing values (N=1065)

| **Characteristic** | | Derivation dataset (N=525) | | Validation dataset (N=540) | |
|---|---|---|---|---|---|
| | | Value<br>N (%) | Missing values<br>N (%) | Value<br>N (%) | Missing values<br>N (%) |
| **Demographics** | | | | | |
| **Age, years** | Median (IQR) | 41 (34,48) | 0 | 41 (34,48) | 0 |
| **Sex** | Female | 353 (67.2) | 0 | 382 (70.7) | 0 |
| **Alcohol history** | Never [1] | 315 (60) | 0 | 360 (66.7) | 0 |
| **Smoking history** | Never [2] | 361 (68.8) | 0 | 389 (72.0) | 0 |
| **HIV/TB history** | | | | | |
| **Participant category** | On ART [3] | 377 (71.8) | 0 | 387 (71.7) | 0 |
| **Duration since HIV diagnosed, months** | Median (IQR) | 56 (21,95) | 10 (2) | 51 (6,97) | 2 (0.4) |
| **Duration on ART, months** | Median (IQR) | 55 (26,85) | 1/377(0.3) | 51 (28,83) | 0 |
| **Ever had IPT** | Yes | 51 (9.7) | 0 | 19 (3.5) | 0 |
| **Ever had CPT** | Yes | 378 (72.0) | 0 | 360 (66.7) | 0 |
| **Previous TB treatment** | Yes | 205 (39.1) | 0 | 202 (37.4) | 0 |
| **WHO symptoms at enrolment** | | | | | |
| | Cough | 308 (58.7) | 0 | 354 (65.6) | 0 |
| | Weight loss | 238/524(45.4) | 1 (0.2) | 224 (41.5) | 0 |
| | Night sweats | 133 (25.3) | 0 | 132 (24.4) | 0 |
| | Fever | 99 (18.9) | 0 | 89 (16.5) | 0 |
| **Number of symptoms** | | 1 (1,2) | 0 | 1 (1,2) | 0 |
| **Duration of WHO symptoms[4], days** | Median (IQR) | 30 (8,94) | 5 (1) | 28 (7,84) | 4 (0.7) |
| **CD4 / BMI at enrolment** | | | | | |
| **CD4, cells/mm$^3$** | Median (IQR) | 379 (228,543) | 2 (0.4) | 335(168,559) | 1 (0.2) |
| **Time from CD4 to enrolment, days** | Median (IQR) | 147 (43,259) | 2 (0.4) | 118 (27,267) | 6 (1) |
| **BMI, kg/m$^2$** | Median (IQR) | 24.0(20.6,28.5) | 1 (0.2) | 24.1(20.3,28.4) | 2 (0.4) |
| **TB diagnoses** | | | | | |
| | Total | 52 (9.9) | 0 | 60 (11.1) | 0 |
| | Confirmed TB | 36 (6.9) | 0 | 41 (7.6) | 0 |
| | Clinical TB | 16 (3.1) | 0 | 19 (3.5) | 0 |
| **Time from enrolment to TB diagnosis [5], days** | Median (IQR) | 7 (0,31) | 0 | 13 (0,83) | 1 (0.2) |
| **Follow up** | | | | | |
| **Time from enrolment to most recent of last study / clinic [6] visit, days** | Median (IQR) | 281 (203,347) | 1 (0.2) | 181 (133,231) | 1 (0.2) |
| **Alive 6 months after enrolment [7]** | Yes | 487 (98) (N=497) | 28 (5.3) | 469 (98) (N=479) | 61(11.3) |

[1] compared with any alcohol in last 1 year; [2] compared with ever/ex-smoker; [3] compared with pre-ART group; [4] duration WHO tool positive;[5] defined as earliest of positive TB test or date TB treatment started; [6] Most recent clinic visit at time of clinic file review;
[7] Amongst participants with most recent study/clinic visit <6 months from enrolment, if participant had valid South African ID number and demise not reported by Department of home affairs / participant-nominated contacts / clinic staff within 6 months of enrolment, participant assumed to be alive at 6 months after enrolment.

IPT=isoniazid preventive therapy; CPT=cotrimoxazole preventive therapy

S2 Table. Hosmer-Lemeshow test for calibration of final model (model A)

| | Derivation dataset [1] | | | | Validation dataset [2] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TB | | | | TB | |
| **Decile** | ***N*** | **Cut off[3]** | **Observed** | **Predicted** | ***N*** | **Cut off[3]** | **Observed** | **Predicted** |
| **1** | 52 | 0.0126 | 0 | 0.4 | 54 | 0.0098 | 1 | 0.3 |
| **2** | 51 | 0.0220 | 2 | 0.9 | 53 | 0.0186 | 1 | 0.7 |
| **3** | 52 | 0.0308 | 0 | 1.4 | 53 | 0.0288 | 2 | 1.3 |
| **4** | 51 | 0.0448 | 2 | 1.9 | 54 | 0.0450 | 4 | 2.0 |
| **5** | 52 | 0.0611 | 2 | 2.7 | 53 | 0.0627 | 4 | 2.9 |
| **6** | 51 | 0.0805 | 5 | 3.6 | 53 | 0.1024 | 5 | 4.2 |
| **7** | 52 | 0.1078 | 7 | 4.9 | 54 | 0.1478 | 6 | 6.6 |
| **8** | 51 | 0.1604 | 5 | 6.6 | 53 | 0.2044 | 8 | 9.3 |
| **9** | 52 | 0.2681 | 12 | 10.6 | 53 | 0.3346 | 6 | 13.8 |
| **10** | 51 | 0.5963 | 17 | 19.0 | 53 | 0.6479 | 21 | 23.7 |
| | **515** | | **52** | **52** | **533** | | **58** | **64.8** |

[1] Hosmer-Lemeshow p=0.65

[2] Hosmer-Lemeshow p=0.31

[3] Upper boundary of predicted risk

Observed = observed number with TB

Predicted = expected number with TB predicted by model

S3 Table: Model A Multivariable logistic regression analysis in derivation dataset after exclusion of all clinical TB (N=499)

| Predictor | | Patients with TB N=36/499 n/N (%) | Unadjusted odds ratio (95% CI) | P value (Wald) | Adjusted [3] odds ratio (95% CI) | P value | Adjusted β coefficient (log [adjusted OR]) (95% CI) |
|---|---|---|---|---|---|---|---|
| **Age [1], years** | | | 1.00 (0.96, 1.03) | 0.89 | | | |
| **Sex** | Male | 16/163 (9.8%) | 1 | | | | |
| | Female | 20/336 (6.0%) | 0.59 (0.29, 1.15) | ***0.12*** | | | |
| **Smoking status** | Never smoked | 20/346 (5.8%) | 1 | | | | |
| | Current or ex-smoker | 16/153 (10.5%) | 1.90 (0.96, 3.78) | ***0.07*** | | | |
| **Alcohol status** | Current | 15/199 (7.5%) | 1 | | | | |
| | None in last 1 year | 21/300 (7.0%) | 0.92 (0.46, 1.84) | 0.82 | | | |
| **ART status** | On ART ≥ 3 months | 18/341 (5.3%) | 1 | | 1 | | 0 |
| | Pre-ART / ART <3 months | 18/158 (11.4%) | 2.31 (1.17, 4.57) | ***0.02*** | 1.84 (0.87, 3.89) | 0.11 | 0.61 (-0.14, 1.36) |
| **Ever had CPT** | No / don't know | 12/138 (8.7%) | 1 | | | | |
| | Yes | 24/361 (6.7%) | 0.75 (0.36, 1.54) | 0.43 | | | |
| **Previous history of TB** | No | 25/306 (8.2%) | 1 | | | | |
| | Yes | 11/193 (7.7%) | 0.68 (0.33, 1.41) | 0.30 | | | |
| **Number of WHO symptoms** | 1 symptom | 11/334 (3.3%) | 1 | | 1 | | 0 |
| | > 1 symptom | 25/165 (15.2%) | 5.24 (2.51, 11.00) | ***<0.001*** | 4.33 (2.02, 9.23) | ***<0.001*** | 1.46 (0.70, 2.23) |
| **Duration of WHO tool symptoms** | <1 week | 2/96 (2.1%) | 1 | | | | |
| | ≥ 1 week | 34/403 (8.4%) | 4.33 (1.02, 18.35) | ***0.05*** | | | |
| **BMI [1,2], kg/m²** | | | 0.87 (0.80, 0.94) | ***0.001*** | 0.88 (0.81, 0.96) | ***0.004*** | -0.12 (-0.21, -0.04) |
| **CD4 [1,2], cells/mm³** | | | 0.997 (0.995, 0.998) | ***<0.001*** | 0.998 (0.996, 0.999) | ***0.012*** | -0.002 (-0.004, -0.0005) |

[1] Age, BMI and CD4 count were modelled as continuous variables

[2] In the multivariable analysis BMI and CD4 count were modelled as continuous variables, a linear relationship with the outcome was found to be adequate after modelling using fractional polynomials.

[3] Adjusted for all variables shown. 100 unit increase in CD4 corresponds to reduction in adjusted odds ratio (aOR) of TB of 0.78 (95% CI 0.64, 0.95); 5 unit increase in BMI corresponds to reduction in aOR of TB of 0.54 (95% CI 0.35, 0.82).
Intercept (log odds) for multivariable model is 0.21. In the multivariable model we found no statistically significant interaction between remaining variables and "ART status".

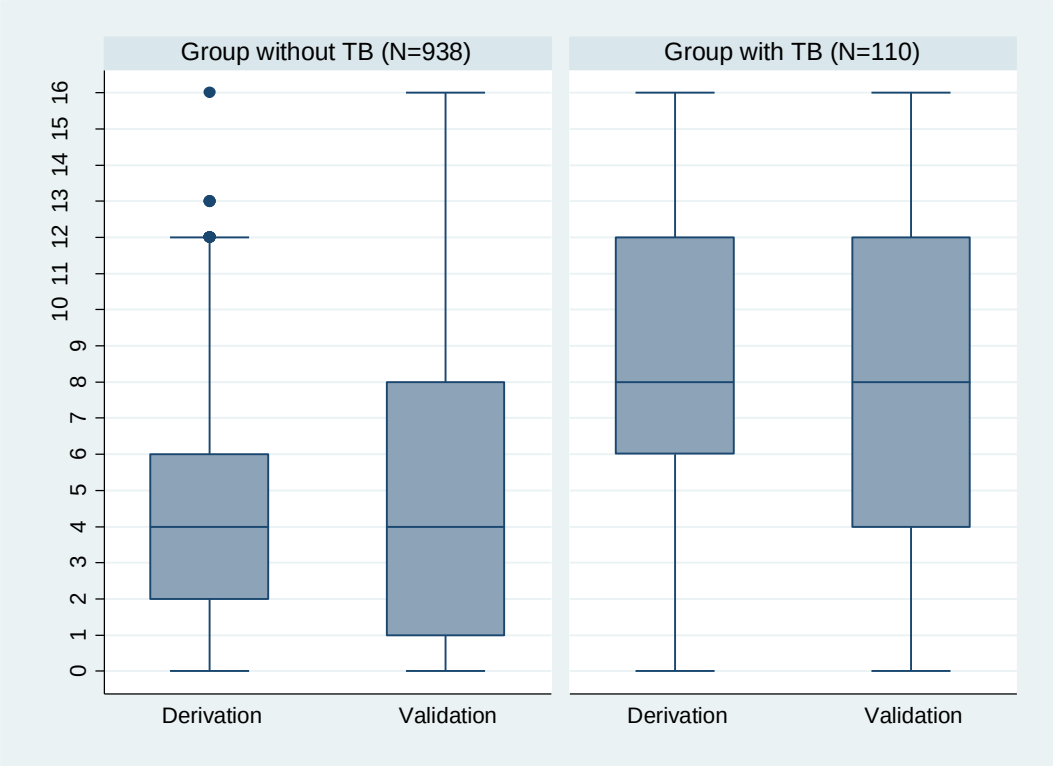
S4 Table. Model B: Multivariable logistic regression analysis in derivation dataset (N=515)

| Predictor | | Patients with TB N=52/515 n/N (%) | Unadjusted odds ratio (95% CI) | P value (Wald) | Adjusted [3] odds ratio Model B (95% CI) | P value (Wald) | Adjusted β coefficient (log [adjusted OR]) (95% CI) |
|---|---|---|---|---|---|---|---|
| **Age [1], years** | | | 1.00 (0.97, 1.03) | 0.96 | | | |
| **Sex** | Male | 23/170 (13.5%) | 1 | | | | |
| | Female | 29/345 (8.4%) | 0.59 (0.32, 1.05) | ***0.07*** | | | |
| **Smoking status** | Never smoked | 28/354 (7.9%) | 1 | | | | |
| | Current or ex-smoker | 24/161 (14.9%) | 2.04 (1.14, 3.64) | ***0.02*** | | | |
| **Alcohol status** | Current | 23/207 (11.1%) | 1 | | | | |
| | None in last 1 year | 29/308 (9.4%) | 0.83 (0.47, 1.48) | 0.53 | | | |
| **ART status** | On ART ≥ 3 months | 24/347 (6.9%) | 1 | | 1 | | 0 |
| | Pre-ART / ART <3 months | 28/168 (16.7%) | 2.69 (1.51, 4.80) | ***0.001*** | 2.07 (1.07, 4.01) | ***0.03*** | 0.73 (0.06, 1.39) |
| **Ever had CPT** | No / don't know | 19/145 (13.1%) | 1 | | | | |
| | Yes | 33/370 (8.9%) | 0.65 (0.36, 1.18) | ***0.16*** | | | |
| **Previous history of TB** | No | 33/314 (10.5%) | 1 | | | | |
| | Yes | 19/201 (9.5%) | 0.89 (0.49, 1.61) | 0.70 | | | |
| **Cough** | No | 16/211 (7.6%) | 1 | | 1 | | 0 |
| | Yes | 36/304 (11.8%) | 1.64 (0.88-3.03) | ***0.12*** | 2.96 (1.50, 5.85) | ***0.002*** | 1.08 (0.40, 1.77) |
| **Fever** | No | 38/418 (9.1%) | 1 | | | | |
| | Yes | 14/97 (14.4%) | 1.69 (0.87-3.25) | ***0.12*** | | | |
| **Night sweats** | No | 31/384 (8.1%) | 1 | | 1 | | 0 |
| | Yes | 21/131 (16.0%) | 2.17 (1.20-3.94) | ***0.01*** | 1.99 (1.02, 3.89) | ***0.04*** | 0.69 (0.02, 1.36) |
| **Unintentional weight loss** | No | 12/280 (4.3%) | 1 | | 1 | | 0 |
| | Yes | 40/235 (17.0%) | 4.58 (2.34-8.96) | ***<0.001*** | 4.08 (1.96, 8.49) | ***<0.001*** | 1.41 (0.67, 2.14) |
| **BMI [1,2], kg/m$^2$** | | | 0.88 (0.82, 0.94) | ***<0.001*** | 0.90 (0.84, 0.97) | ***0.005*** | -0.10 (-0.17, -0.03) |
| **CD4 [1,2], cells/mm$^3$** | | | 0.997 (0.995, 0.998) | ***<0.001*** | 0.997 (0.996, 0.999) | ***0.009*** | -0.002 (-0.004, -0.0005) |

[1] Age, BMI and CD4 count were modelled as continuous variables
[2] BMI and CD4 count were modelled as continuous variables, a linear relationship with the outcome was found to be a good approximation after assessment of nonlinearity using fractional polynomials.
[3] Adjusted for all variables shown. 100 unit increase in CD4 corresponds to reduction in adjusted odds ratio (aOR) of TB of 0.81 (95% CI 0.69, 0.95); 5 unit increase in BMI corresponds to reduction in aOR of TB of 0.61 (95% CI 0.43, 0.86).
In the multivariable model we tested for interactions between "ART status" and CD4 cell count, "ART status" and BMI, "ART status" and cough, "ART status" and night sweats, "ART status" and weight loss. Interaction term with p<0.05: ART status and cough.
Intercept (log odds) for multivariable model is 0.32
In derivation vs. validation datasets: Hosmer-Lemeshow statistic p=0.81 vs. p=0.01, AUROC 0.82 (95% CI 0.76-0.88) vs. AUROC 0.75 (95% CI 0.69-0.82)

S1 Fig. Boxplot illustrating distribution of clinical score in individuals with and without TB

N=515 in derivation dataset with 52 TB diagnoses; N=535 in validation dataset with 58 TB diagnoses



# 7) *Paper 3*: The utility of repeat Xpert MTB/RIF testing to diagnose tuberculosis in HIV-positive adults with initial negative result

## 7.1. Cover sheet

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

## RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

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| | | | |
|---|---|---|---|
| **Student ID Number** | 079810 | **Title** | Dr |
| **First Name(s)** | Yasmeen | | |
| **Surname/Family Name** | Hanifa | | |
| **Thesis Title** | Investigation pathways for tuberculosis among HIV-positive adults in South Africa | | |
| **Primary Supervisor** | Alison Grant | | |

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

**SECTION B – Paper already published**

| | | | |
|---|---|---|---|
| Where was the work published? | Gates Open Research. It has been reviewed by two reviewers, one of whom has requested revisions. | | |
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**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
| **Date** | 28th May 2019 |

| | |
|---|---|
| **Supervisor Signature** | [redacted] |
| **Date** | 28 May 2019 |

## 7.2. Research paper

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RESEARCH ARTICLE

# The utility of repeat Xpert MTB/RIF testing to diagnose tuberculosis in HIV-positive adults with initial negative result [version 1; peer review: 1 approved, 1 approved with reservations]

Yasmeen Hanifa [1], Katherine L. Fielding[1], Violet N. Chihota[2,3], Lungiswa Adonis[4], Salome Charalambous[2,3], Nicola Foster[5], Alan Karstaedt[6,7], Kerrigan McCarthy[2], Mark P. Nicol[8,9], Nontobeko T. Ndlovu[2], Edina Sinanovic[5], Faieza Sahid[6,7], Wendy Stevens[9,10], Anna Vassall[1], Gavin J. Churchyard[1-3,11], Alison D. Grant[1,3,12]

[1]TB Centre, London School of Hygiene & Tropical Medicine, London, UK
[2]The Aurum Institute, Johannesburg, South Africa
[3]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[4]Mamelodi Hospital, Pretoria, South Africa
[5]Health Economics Unit, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa
[6]Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa
[7]University of the Witwatersrand, Johannesburg, South Africa
[8]Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
[9]National Health Laboratory Service, Johannesburg, South Africa
[10]Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[11]Advancing Care and Treatment for TB/HIV, South African Medical Research Council Collaborating Centre for HIV and TB, Johannesburg, South Africa
[12]Africa Health Research Institute, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa

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**Abstract**

**Background:** Amongst HIV-positive adults in South Africa with initial negative Xpert results, we compared the yield from repeating Xpert MTB/RIF ("Xpert") on sputum to guideline-recommended investigation for tuberculosis (TB).

**Methods:** A systematic sample of adults attending for HIV care were enrolled in a cohort exploring TB investigation pathways. This substudy was restricted to those at highest risk of TB (CD4<200 cells/mm$^3$ or unknown) who had a negative initial Xpert result.

At attendance for the Xpert result, a repeat sputum sample was stored, and further investigations facilitated per national guidelines. Participants were reviewed monthly, with reinvestigation if indicated, for at least three months, when sputum and blood were cultured for mycobacteria, and the stored sputum tested using Xpert. We defined TB as "confirmed" if Xpert, line probe assay or *Mycobacterium tuberculosis* culture within six months of

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| **version 1** published 26 Apr 2018 | ? report | ✓ report |

1 **Colleen F. Hanrahan**, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

2 **Tow Keang Lim**, National University Hospital, Singapore, Singapore

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enrolment were positive, and "clinical" if TB treatment was started without microbiological confirmation.
**Results:** Amongst 227 participants with an initial negative Xpert result (63% female, median age 37 years, median CD4 count 100 cells/mm$^3$), 28 (12%) participants had TB diagnosed during study follow-up (16 confirmed, 12 clinical); stored sputum tested positive on Xpert in 5/227 (2%). Amongst 27 participants who started TB treatment, the basis was bacteriological confirmation 11/27 (41%); compatible imaging 11/27 (41%); compatible symptoms 2/27 (7%); and unknown 3/27 (11%).
**Conclusions:** Amongst HIV-positive individuals at high risk of active TB with a negative Xpert result, further investigation using appropriate diagnostic modalities is more likely to lead to TB treatment than immediately repeating sputum for Xpert. TB diagnostic tests with improved sensitivity are needed.

**Keywords**
Tuberculosis, Diagnostic Test, HIV infection, South Africa

Any reports and responses or comments on the article can be found at the end of the article.

**Corresponding author:** Yasmeen Hanifa (yasmeen.hanifa@lshtm.ac.uk)

**Author roles: Hanifa Y**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Fielding KL**: Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Supervision, Visualization, Writing – Review & Editing; **Chihota VN**: Conceptualization, Methodology, Project Administration, Resources, Writing – Review & Editing; **Adonis L**: Resources, Writing – Review & Editing; **Charalambous S**: Conceptualization, Methodology, Project Administration, Resources, Writing – Review & Editing; **Foster N**: Conceptualization, Writing – Review & Editing; **Karstaedt A**: Conceptualization, Resources, Writing – Review & Editing; **McCarthy K**: Conceptualization, Writing – Review & Editing; **Nicol MP**: Conceptualization, Writing – Review & Editing; **Ndlovu NT**: Investigation, Project Administration, Writing – Review & Editing; **Sinanovic E**: Conceptualization, Writing – Review & Editing; **Sahid F**: Resources, Writing – Review & Editing; **Stevens W**: Conceptualization, Writing – Review & Editing; **Vassall A**: Conceptualization, Writing – Review & Editing; **Churchyard GJ**: Conceptualization, Resources, Writing – Review & Editing; **Grant AD**: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – Review & Editing

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## Introduction

Since 2011 the World Health Organization (WHO) has recommended Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA) as the initial diagnostic test for individuals being investigated for HIV-associated tuberculosis (TB)[1]. TB diagnosis in people living with HIV (PLHIV) is complicated by the high proportion who are smear-negative and/or have extrapulmonary disease[2]. Although Xpert has superior sensitivity to sputum microscopy, it is less sensitive than culture, with a pooled sensitivity of 61% for smear-negative, culture-positive TB among PLHIV[3].

South Africa replaced smear microscopy with Xpert starting in 2011, for all individuals with symptoms suggesting TB[4]. Further evaluation of those who are HIV-positive and Xpert-negative comprises clinical reassessment, chest radiograph if available, sputum for mycobacterial culture, and treatment with antibiotic if clinically indicated[4]. In a South African study of 394 patients investigated for TB (irrespective of presence of symptoms) prior to antiretroviral therapy (ART) initiation, the sensitivity of Xpert for smear-negative, culture-positive TB increased from 43% to 62% when a second sample collected at the first visit was tested[5]. Mathematical modelling using a decision model from South Africa[6] suggested that replacing sputum culture with the cheaper option of a second Xpert would reduce loss to follow-up so 1% more patients would start TB treatment[7], and save an estimated US$17.4 million per year[7]. This model assumed, based on limited data, the same sensitivity for the second Xpert test as for the first[7], guidelines would be correctly followed[7], and only 1% of those with TB symptoms start TB treatment based on a clinical diagnosis[6]. The strategy of sending a repeat Xpert for HIV-positive individuals whose initial Xpert result is negative has not been evaluated empirically.

The aim of our study was, amongst HIV-positive adults being investigated for TB whose initial Xpert result is negative, to describe the diagnostic yield from an immediate repeat sputum tested with Xpert, compared to sequential further investigation guided by South African recommendations, reflecting pragmatic clinical practice.

## Methods

This "repeat Xpert" substudy was part of "Xpert for people attending HIV/AIDS care: test or review?" (XPHACTOR), a prospective cohort study evaluating a risk-based algorithm to prioritise Xpert testing amongst adults attending for routine HIV care in South Africa[8].

### XPHACTOR study population, recruitment and procedures

XPHACTOR study flow, procedures and algorithm are described in detail in Supplementary File 1. In summary, we enrolled a systematic sample of adults (aged ≥18 years) attending four HIV clinics in Gauteng province, irrespective of presence of symptoms suggestive of TB, in the XPHACTOR study. Patients taking anti-tuberculosis treatment within the previous three months were excluded. Patients were enrolled into three groups: "on ART" (ART-experienced); "pre-ART" (in HIV care but not taking ART); and "HIV Testing and Counselling (HTC)" (newly-diagnosed HIV-positive). At the time of the study, ART eligibility comprised CD4 ≤350 cells/mm$^3$ or WHO clinical stage ≥3. Research staff screened participants for TB at monthly intervals to three months, using a standardised questionnaire which incorporated the WHO symptom screen (any one of current self-reported cough, fever, weight loss or night sweats, hereafter the WHO tool). A spot sputum sample was collected for Xpert for individuals at *a priori* highest risk of active TB according to the study algorithm, which prioritised testing for those with any of: current cough, fever ≥ 3 weeks, night sweats ≥ 4 weeks, BMI <18.5 kg/m$^2$, CD4 <100 cells/mm$^3$, or weight loss ≥10%; and at enrolment from all in HTC group or pre-ART with CD4 <200 cells/mm$^3$ (Supplementary File 1). At enrolment all participants with CD4 <200 cells/mm$^3$ were asked to provide a spot urine sample, which was stored at 2–8°C prior to freezing at -80°C within 24 hours of collection. At the end of the study samples were thawed to ambient temperature and tested with lateral-flow LAM assay (LF-LAM; Determine TB-LAM; Alere, USA), and graded using the pre-January 2014 manufacturer's reference card comprising five grades of colour intensity with the least intense band assigned grade 1, absence of a band graded negative, and absence of control band deemed a failed test.

At enrolment and follow-up visits, participants who submitted an Xpert sample were reviewed within one week, and if Xpert-positive, TB treatment was initiated. If Xpert was negative, research staff repeated WHO symptom screen and facilitated the Xpert-negative algorithm for all who were WHO tool positive, which comprised chest radiograph, spot sputum for TB culture, and/or antibiotic trial as clinically appropriate. The Xpert-negative algorithm was also facilitated, because of *a priori* high risk of active TB, for all pre-ART participants with CD4<200x10$^6$/l who submitted sputum for immediate Xpert at enrolment to XPHACTOR.

At the three-month visit all participants had sputum and blood cultured for mycobacteria (Bactec MGIT 960 and 9240 systems). We allowed a broad window period around the three-month XPHACTOR main study final visit, until around six months, to maximise follow-up.

### Repeat Xpert substudy procedures

XPHACTOR participants who were Xpert-negative with i) CD4 count<200 cells/mm$^3$ , or ii) new HIV diagnosis (HTC group) were eligible for this substudy, irrespective of presence of WHO tool symptoms; these restrictions aimed to minimise unnecessary testing of individuals at lower risk of active TB. If a participant had more than one negative Xpert result during follow-up, only the first episode was included.

At attendance for Xpert result review, eligible participants were asked for an additional spot sputum sample for "repeat" Xpert, which was frozen at -80°C within 24 hours of collection. All stored samples were thawed and tested with Xpert at the end of the study to evaluate the diagnostic yield that could have been achieved if an immediate repeat Xpert had been sent at the Xpert result review visit. We decided *a priori* not to induce sputum for this substudy in order to reflect what would be achievable in routine practice.

### Definitions

***Repeat Xpert substudy entry and exit dates.*** Repeat Xpert substudy cohort entry date was defined as the date that the Xpert result review was conducted and sputum was collected for storage. Cohort exit date was defined as the last XPHACTOR study visit date.

***TB case definitions.*** "Confirmed" TB was defined as a positive result on i) Xpert (on sputum sample) or ii) line probe assay (LPA) performed on smear-positive or cultured isolate (GenoType MTBDR*plus*, Hain Lifesciences) or iii) *Mycobacterium tuberculosis (Mtb)* culture, from any sample (including stored sputum and those requested by health care providers) collected within six months of XPHACTOR enrolment. Clinical TB was defined as TB treatment started within six months of enrolment ascertained from clinical records, self or family report, or reported in the context of a separate verbal autopsy sub-study, in the absence of microbiological confirmation. Six months was chosen because TB disease evolves gradually;[9,10] data from Zimbabwe estimated the mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults at 18–33 weeks[11].

"Not TB" was defined as absence of criteria for confirmed or clinical TB, and alive at least 3 months (the minimum follow-up period) after enrolment. Participants who did not fulfil the case definitions for TB or "not TB" were deemed to have unclassifiable outcome and excluded from analyses.

Pulmonary and extrapulmonary TB were classified in accordance with WHO definitions[12].

***Radiological definitions.*** "Probable radiological TB" was defined as presence of any of cavitation, predominantly upper lobe infiltrates, pleural or pericardial effusion, or clear miliary picture on chest radiograph. "Possible radiological TB" was defined as presence of any of lymphadenopathy (hilar or mediastinal), pulmonary nodules or other infiltrates. Participants with "probable" or "possible" radiological TB features, but without bacteriological confirmation, who started TB treatment within six months of substudy enrolment were assigned as having "clinical" TB.

### Statistical methods

Data were analysed using Stata 14 (Stata Corporation, College Station, TX, USA).

We did not undertake formal sample size calculation for this substudy as the sample size was all those eligible from the parent study.

We compared TB diagnoses made by Xpert using the sample stored at substudy enrolment, with all TB diagnoses fulfilling our case definitions during follow-up. We chose this pragmatic comparison because in real life, individuals with smear or Xpert-negative TB have sequential investigation, rather than all tests performed simultaneously. Our research staff facilitated the Xpert-negative algorithm when participants attended for Xpert result review, and therefore investigations are likely to have been initiated faster than in a routine setting. The proportion of TB diagnoses made by Xpert using the stored sputum was compared with TB diagnoses made during follow-up using McNemar's test.

In a sensitivity analysis restricted to participants who had at least one component of the Xpert-negative algorithm (chest radiograph, sputum for TB culture, or antibiotic trial) within a two-week window of providing the stored repeat Xpert sample, we compared the proportion of TB diagnoses made by Xpert using the stored sputum with TB diagnoses made by the Xpert-negative algorithm using McNemar's test.

We calculated sensitivity and specificity with 95% confidence intervals (CI) for LF-LAM using a cut-off of grade =2+ to define LAM-positive against a diagnostic reference standard of confirmed plus clinical TB. We used the grade 2 cut-off as this corresponds with the grade 1 band in the current LF-LAM reference card, which is deemed a positive result in accordance with manufacturer's recommendations[13].

### Ethical approval

The study was approved by the ethics committees at the University of the Witwatersrand (approval # M120343), University of Cape Town (approval # 106/2012), and the London School of Hygiene & Tropical Medicine (approval # 6165). All consenting participants gave written consent or, witnessed verbal consent if unable to read or write. All ethics committees approved the consent form. Principles expressed in the Declaration of Helsinki were followed in the conduct of this research.

## Results

Between September 2012 and March 2014, 235/410 (57.3%) potentially eligible participants were able to provide a sputum sample, stored for testing at study completion with Xpert (Figure 1). Eight participants with "unclassifiable" outcome were excluded, leaving 227 participants for analysis.

### Participant characteristics

Characteristics of the 227 substudy participants and comparison with the 175 excluded because they were unable to produce sputum are presented in Table 1. The majority of participants were female (63%), median age was 37 years (interquartile range [IQR] 31,44), median CD4 count was 100 cells/mm$^3$ (IQR 51,147), and 26% had previously been treated for TB. 78/227 (34%) of participants reported a TB symptom, most often cough (23%, 52/227) or weight loss (19%, 43/227) (Table 1). Amongst the remaining 149/227 (66%) of participants who reported no WHO-tool symptoms at attendance for Xpert result, sputum was collected for repeat Xpert due to *a priori* high risk of active TB because newly-diagnosed HIV-positive (42); pre-ART with CD4 count <200 cells/mm$^3$ (42); CD4 count <100 cells/mm$^3$ (33); on ART with CD4 count 100–199 cells/mm$^3$ (17); BMI <18.5 kg/m$^2$ or weight loss ≥10%, (15). Enrolment to the repeat Xpert study was at median 7 days (IQR 7,8) from collection of the initial sputum sample for Xpert.

### Tuberculosis diagnoses

12% (28/227) of substudy participants fulfilled case definitions for TB, of which 16 were confirmed and 12 were clinical

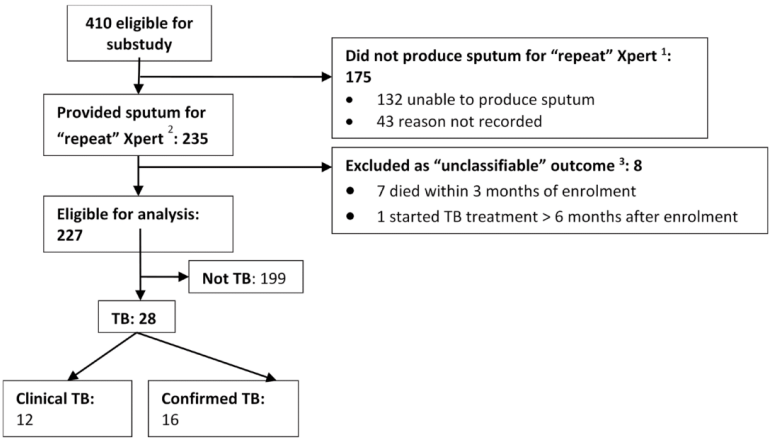


**Figure 1. Flow chart of repeat Xpert substudy participants.** [1] 15/175 who were excluded because they did not produce sputum fulfilled case definitions for TB (clinical TB [9/15], confirmed TB [6/15]) and a further 3/175 had unclassifiable outcome [2] For 22/235 participants who provided more than one "repeat" sample (all Xpert-negative) only the result of the first sample and data from the associated review visit were used in the analysis [3] All had negative "repeat" Xpert.

(Table 2). One participant died before TB treatment could be commenced, and for one, the treatment start date was unknown. The remaining 26 started TB treatment at a median 49 days (IQR 0,108) after substudy entry. The range for time from substudy entry to earliest of positive TB investigation (including chest radiograph) or date TB treatment was started (amongst all fulfilling our case definitions for TB) was 0–118 days. 24% (19/78) participants who were WHO tool positive when the sample for stored repeat Xpert was collected fulfilled TB case definitions (confirmed 11, clinical 8).

***Basis for commencement of TB treatment.*** Eleven participants started treatment based on a bacteriologically-confirmed TB result (Xpert [7]; *Mtb* isolated from sputum [3] or blood [1]) (Table 2).

Eleven participants started TB treatment because of compatible imaging. Nine had compatible chest radiographs, of whom four were subsequently bacteriologically confirmed (Xpert 2, pleural fluid cultured isolate LPA-positive 1, positive *Mtb* sputum culture 1). Two participants started treatment based on ultrasound scans, one compatible with abdominal TB (subsequently confirmed by positive *Mtb* from sputum culture); and the other showing pericardial effusion (Table 2).

Two participants started treatment because of compatible symptoms and positive sputum mycobacterial culture (later identified as non-tuberculous mycobacteria [NTM]) with symptomatic improvement on standard TB treatment. One participant started TB treatment solely based on stored repeat Xpert sample. The basis for starting TB treatment was not clear for the remaining three participants (Table 2).

***Diagnoses made by repeat Xpert on stored sputum samples.*** The stored sputum sample was positive by Xpert at the end of the study for five participants (sensitivity of repeat Xpert 31.3% [5/16; 95% CI 11.0–58.7%] *vs.* gold standard of confirmed TB and 18% [5/28, 95% CI: 6.1%-36.9%] *vs.* gold standard of confirmed / clinical TB combined) (Figure 2). In a matched analysis the odds of TB diagnosis was much greater by other modalities during follow-up than by the repeat Xpert, odds ratio 24.0 (95% CI: 3.9–986.9; p<0.0001, McNemar's test). Amongst the five participants with positive repeat Xpert, three were in the pre-ART group, and two in the on ART group. We were unable to undertake multivariable analysis to look at independent predictors of positive repeat Xpert on the stored sample because only five were positive.

In a sensitivity analysis restricted to 123 participants who had at least one component of the Xpert-negative algorithm within a two-week window of providing the stored repeat Xpert sample, 23 participants fulfilled our TB case definitions (13/23 confirmed, 10/23 clinical). The stored sputum sample was positive by Xpert for four participants (sensitivity of repeat Xpert for confirmed and clinical TB combined 17% [4/23]; for sputum culture-confirmed TB 20% [1/5]). Ten participants started TB treatment because of evaluation by the Xpert-negative algorithm (four confirmed, six clinical), of whom two also had positive stored repeat Xpert. Eleven other participants fulfilled TB case definitions during study follow-up (eight confirmed, three clinical). We did a matched analysis, classifying as "not TB" for the purpose of this analysis, 11 participants who fulfilled our TB case definitions but were not identified by either the Xpert-negative algorithm or stored repeat Xpert. The odds of TB diagnosis by the Xpert-negative algorithm was greater than by

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**Table 1. Characteristics of substudy participants (n=227) vs. eligible non-productive of sputum (n=175).**

| Characteristic | Study participants (N=227) | Did not provide sputum for "repeat" Xpert (N=175) |
|---|---|---|
| | N (%) | N (%) |
| **Demographics** | | |
| Age, years - Median (IQR) | 37 (31-44) (N=226) | 36 (30-43) |
| Female | 144 (63.4%) | 109 (62.3%) |
| Black African | 222 (97.8%) | 175 (100%) |
| **Participant category** | | |
| On ART | 99 (43.6%) | 67 (38.3%) |
| Pre-ART | 75 (33.0%) | 60 (34.3%) |
| HTC | 53 (23.4%) | 48 (27.4%) |
| **HIV/TB history** | | |
| Previous TB treatment | 59 (26.0%) | 37 (21.1%) |
| Ever had IPT | 18 (7.9%) | 6 (3.4%) |
| Ever had CPT | 122 (53.7%) | 84 (48.0%) |
| **BMI / CD4 when immediate Xpert was requested** | | |
| BMI, $kg/m^2$ - Median (IQR) | 23.3 (20.1-27.4) (N=226) | 23.4 (20.1-28.1) |
| CD4[1], $cells/mm^4$ - Median (IQR) | 100 (51-147) (N=188) | 113 (56-169) (N=148) |
| **WHO tool symptoms when sample for "repeat" Xpert was requested** | | |
| WHO-positive | 78 (34.4%) | 44 (25.1%) |
| Cough | 52 (22.9%) | 23 (13.1%) |
| Weight loss | 43 (18.9%) | 32 (18.3%) |
| Night sweats | 16 (7.0%) | 9 (5.1%) |
| Fever | 7 (3.1%) | 2 (1.1%) |
| **TB diagnoses over 6 months follow-up** | | |
| Total | 28 (12.3%) | 18 (10.3%) |
| Confirmed TB | 16 (7.1%) | 7 (4.0%) |
| Clinical TB | 12 (5.3%) | 11 (6.3%) |
| **Follow-up** | | |
| **Time from XPHACTOR enrolment to 3-month" study visit, days** - Median (IQR) | 84 (84,95) (N=220) | 86 (84,106) (N=169) |
| **Accuracy of LF-LAM for confirmed and clinical TB combined using Grade 2 cut-off** | | |
| Prevalence of positive LAM, n/N (%) | 2/142 (1.4%) | 1/100 (1.0%) |
| Sensitivity n/N | 0/18 | 0/9 |
| Specificity n/N % (95% CI) | 122/124 98.4% (94.3, 99.8) | 90/91 98.9% (94.0, >99.9) |

IPT= Isoniazid preventive therapy; BMI = body mass index; CPT= Cotrimoxazole preventive therapy; HTC= Enrolled from HIV testing and counselling service; WHO positive = self-report of any of current cough, fever, night sweats or unintentional weight loss.

[1] Most recent clinic CD4 cell count when participant attended for Xpert result review. CD4 available for 188/227 participants enrolled (99/99 on ART, 75/75 pre-ART, 14/54 HTC); and 148/175 who did not provide sputum for repeat Xpert" (67/67 on ART, 60/60 pre-ART, 21/48 HTC)

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**Table 2. Basis for TB diagnoses in repeat Xpert substudy (N=28).**

| Characteristic | Participants diagnosed with TB N=28 N (%) |
|---|---|
| **Case definition** | |
| **Bacteriologically confirmed TB:** | **16 (57%)** |
| Sputum Xpert positive[1] | 6 (21%) |
| Sputum *Mtb* culture positive | 4 (14%) |
| Sputum both Xpert and *Mtb* culture-positive | 4 (14%) |
| Blood *Mtb* culture-positive | 1 (4%) |
| Pleural fluid cultured isolate LPA-positive | 1 (4%) |
| **Clinical TB:** | **12 (43%)** |
| **Site of TB** | |
| Pulmonary TB only | 18 (64%) |
| Extrapulmonary TB only[2] | 5 (18%) |
| Both pulmonary and extrapulmonary TB[3] | 2 (7%) |
| Not recorded | 3 (11%) |
| **TB treatment commenced** | **27 (96%)** |
| **Basis upon which TB treatment commenced:** | |
| Bacteriologically-confirmed *Mtb*[4] | 11 (41%) |
| Compatible imaging[5] | 11 (41%) |
| Compatible symptoms and positive sputum mycobacterial culture (later identified as NTM)[6] | 2 (7%) |
| Not known[7] | 3 (11%) |
| **Time from substudy entry to treatment start (n=26), days -** Median (IQR) | 49 (0, 108) |
| **Repeat Xpert on stored sputum** | |
| **Xpert positive** | 5 (18%) |

CXR = chest radiograph; LPA = line probe assay; NTM = Non tuberculous mycobacteria; USS = ultrasound scan

[1] Includes two participants for whom bacteriological confirmation was provided by stored sputum which was Xpert-positive; one of whom started treatment based on this result, and the other had already started TB treatment because of compatible chest radiograph (miliary TB).

[2] Pleural effusion (3); positive mycobacterial blood culture (1); pericardial effusion (1)

[3] Compatible abdominal ultrasound and sputum *Mtb* culture-positive (1), pleural effusion and sputum Xpert positive (1)

[4] Sputum Xpert positive (7) of which one was the sample stored for repeat Xpert and samples were collected at median 97 days (IQR 79, 118) after substudy entry; sputum *Mtb* culture-positive (3); blood *Mtb* culture-positive (1)

[5] Compatible CXR (9) of which four subsequently bacteriologically confirmed, compatible USS (2)

- Case definitions fulfilled for CXR reporting:
  - 7 Probable radiological TB (pleural effusion [4], miliary TB [1], cavitation and infiltrates [2]).
  - 2 Possible radiological TB
- USS: Pericardial effusion (1); abdominal TB with subsequent sputum *Mtb* culture-positive (1)

One participant categorised as probable TB had bilateral pleural effusions and cardiomegaly and was reported at verbal autopsy as having started TB treatment based on CXR

[6] Started on basis of compatible symptoms and positive sputum culture later identified as *M. avium* (1) and *M. intracellulare* (1); both had improvement in symptoms after treatment was initiated.

[7] Identified as having started TB treatment at verbal autopsy (2), started by clinic doctor (1)

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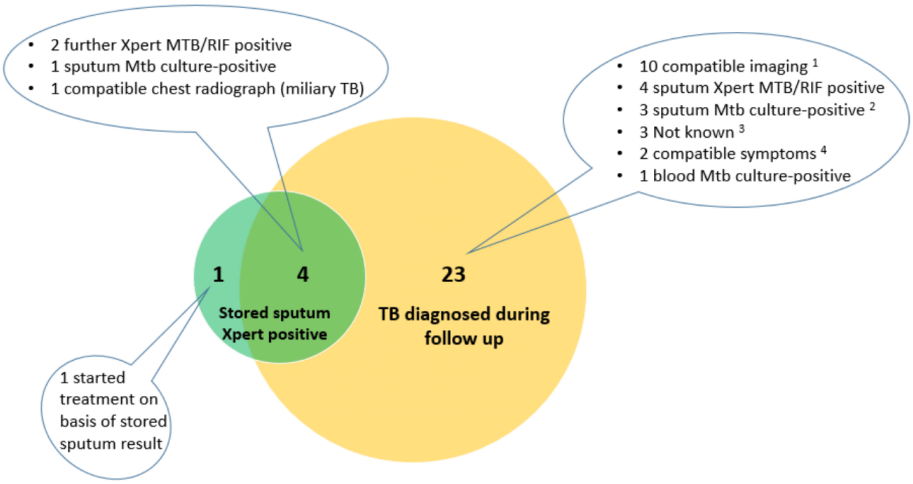


**Figure 2. Number of participants diagnosed with TB by "repeat" Xpert vs. number diagnosed during follow-up (N=28).** CXR = chest radiograph; USS = ultrasound scan [1] CXR features compatible with "Probable TB" (pleural effusion [4], cavitation and infiltrates [2]); CXR features compatible with "Possible TB" (2); USS features compatible with TB (Pericardial effusion [1]; abdominal TB [1]) [2] One participant died before treatment commenced [3] Two identified as having started TB treatment at verbal autopsy. One had *M. xenopi* identified in sputum culture prior to commencement of empiric TB treatment. [4] Started on basis of compatible symptoms and positive sputum culture later identified as *M. avium* (1) and *M. intracellulare* (1); both had improvement in symptoms after treatment was initiated.

repeat Xpert, but did not attain statistical significance, odds ratio 4.0 (95% CI: 0.8–38.7; p=0.11, McNemar's test).

The participant who started TB treatment solely based on the stored repeat Xpert sample was in the pre-ART group with a CD4 cell count of 113 cells/mm$^3$ at substudy enrolment, had no previous history of TB treatment, and had a five week history of cough and fever when the initial sputum sample for Xpert was collected. The sputum culture, the only component of the Xpert-negative algorithm arranged at the Xpert review visit, was contaminated. This participant initiated ART on the day of entry to the substudy, was WHO-tool negative at all subsequent study visits, and had negative sputum and blood for mycobacterial culture at the 3-month visit. The remaining four participants with positive stored repeat Xpert sample started TB treatment before the stored sample was processed, based on further evaluation during follow-up: sputum Xpert-positive (2, one with rifampicin resistance); sputum *Mtb* culture-positive (1); and compatible chest radiograph (1).

### Further evaluation of substudy participants undertaken during substudy follow-up

Figure 3 summarises all evaluations undertaken for TB during substudy follow-up, aside from 3-month visit mycobacterial cultures and Xpert on stored sputum samples. As part of routine care or facilitated by research staff for the Xpert-negative algorithm, 97/227 (43%) had a chest radiograph (38/97 [39%] fulfilled criteria for radiological TB), and 100/227 (44%) had mycobacterial culture on sputum (3/100 [3%] *Mtb* positive). 34/227 (15%) of participants were prescribed an antibiotic trial at the Xpert result review, and 14/21 (67%) of those reviewed reported resolution of symptoms.

89 participants submitted sputum specimens for Xpert as part of routine care or because they fulfilled XPHACTOR algorithm criteria at monthly follow-up visits, for whom 6/89 (7%) were positive. An additional four participants had positive Xpert, of which three were stored sputum samples for repeat Xpert (bacterial confirmation provided solely by stored sample [2], also positive *Mtb* sputum culture [1]), and one was collected after the 3-month visit (Table 2).

The mycobacterial cultures performed routinely at the 3-month visit yielded *Mtb* isolates in 2% (5/219) of sputum and 0/220 blood samples.

### Performance of urine LAM

LAM results were available for 142/227 (63%) of study participants, with a positive result (grade 2 cut-off) observed in 2/142 (1%). 18/142 (13%) fulfilled case definitions for TB (clinical and confirmed). The sensitivity of LF-LAM for TB (clinical and confirmed) was 0% and specificity was 98.4% (95% CI 94.3, 99.8). Sensitivity and specificity were similar in those 175 excluded because they were unable to produce sputum (Table 1).

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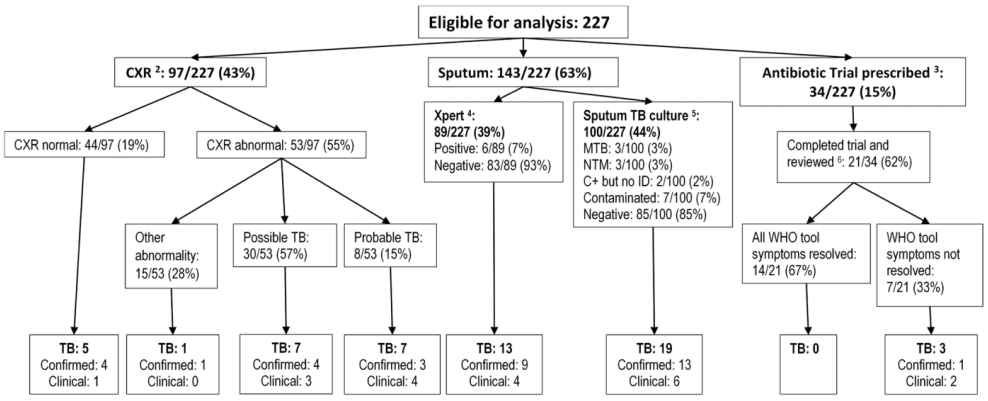


**Figure 3. Evaluation of participants undertaken from repeat Xpert substudy entry to 3-month visit.** Categories are not mutually exclusive as each participant could undergo >1 mode of evaluation for TB C+ = culture positive; CXR = chest radiograph; ID = final identification; *MTB = M. tuberculosis*; NTM = non-tuberculous mycobacteria. [1] Excludes stored sputum samples for repeat Xpert, and sputum and blood samples for mycobacterial culture collected at 3-month visit [2] As part of routine care or facilitated by research staff for GXP-negative algorithm. 97 participants had 100 CXRs, for those with multiple CXR most recent CXR is reported. [3] Facilitated by research staff for GXP-negative algorithm [4] As part of routine care or facilitated by research staff as high priority by XPHACTOR study algorithm. 89 participants had 137 sputum samples tested with Xpert, for those with multiple samples most recent sample reported. [5] As part of routine care or facilitated by research staff for GXP-negative algorithm. 99 participants had 109 sputum samples processed for mycobacterial culture, for those with multiple samples most recent sample reported. [6] Reviewed and reported completing at least 5 days of antibiotics

## Discussion

Among HIV-positive individuals at high risk of active TB, with a negative sputum Xpert result, very few TB diagnoses would have been made in this study by immediately repeating Xpert. We limited our study to HIV-positive individuals at highest risk of active TB, i.e. those who were WHO tool positive with CD4<200x10$^6$/l, or pre-ART with CD4<200x10$^6$/l, or newly diagnosed, to minimise unnecessary testing of individuals at lower risk of active TB. The low yield from repeat Xpert in those with negative initial Xpert is likely due to paucibacillary or extrapulmonary disease. These TB diagnoses may be better identified by alternative diagnostic modalities, such as chest radiography. A South African study of patients with sputum screened for TB by Xpert and mycobacterial culture prior to ART initiation, using a gold standard of culture-confirmed TB (N=85), found that those who were Xpert-negative had higher CD4 cell counts and lower viral loads than those who were Xpert-positive[14]. We did not have enough positive stored Xpert results to undertake a similar analysis.

Our study illustrates the realities of implementing the test negative algorithm in HIV-positive individuals. Despite research staff facilitating the algorithm, less than half (100/227) of participants produced sputum for mycobacterial culture during follow-up (*vs.* 100% assumed by Schnippel)[7]. We found sensitivity of the repeat Xpert was only 18% (5/28) for all TB or 31% (5/16) for bacteriologically-confirmed TB *vs.* 79% assumed by Schnippel[6,7]. Data from South Africa demonstrate poor adherence in routine care settings to TB diagnostic algorithms amongst HIV-positive individuals with initial negative Xpert test[15,16]. The aforementioned model[6,7] assumes 1% of patients with TB symptoms start TB treatment based on a clinical diagnosis, but we found this to be far greater; and the model does not consider extrapulmonary TB (one-fifth of our participants diagnosed with TB had only extrapulmonary disease). An economic evaluation of repeat sputum Xpert *vs.* the Xpert-negative algorithm for HIV-positive individuals using assumptions that are more realistic is needed.

Evaluation of the 2007 WHO algorithm for smear-negative TB (comprising chest radiograph, single sputum for mycobacterial culture, and antibiotic trial), in HIV-positive individuals being investigated for TB in Cambodia[17], against a gold standard of culture-confirmed TB based on multiple specimens, demonstrated sensitivity of 60%[17]. Sensitivity of this algorithm is imperfect, and there is a risk of overtreatment when only clinical-radiological features are used to start TB treatment. 40% (11/27) of our study participants who started TB treatment did so because of compatible imaging, of whom almost half were subsequently bacteriologically confirmed, highlighting its value to support rapid initiation of TB treatment. Our findings are in accord with data from the XTEND trial, which found that compatible chest radiograph was the main reason for initiating empiric TB treatment in a cohort of patients investigated for TB in primary care in South Africa, amongst whom microbiological confirmation was subsequently obtained for 13%[18]. South

African national guidelines now recommend chest radiography for all individuals with symptoms suggestive of TB who cannot produce a sputum sample, but limited access to radiography facilities may limit implementation[4]. Amongst our study participants who provided sputum for mycobacterial culture prior to their 3-month visit there was a low yield of *Mtb* (3/100 [3%]), and the yield from further Xpert during follow-up was 7% (6/89), representing just over half (9/16) of all confirmed TB diagnoses. Our findings highlight the need for more sensitive diagnostic tests, and for repeating TB investigation using all available modalities, in HIV-positive individuals with initial negative sputum test result who remain symptomatic or have advanced immunosuppression. Current WHO guidance supports the use of urine LF-LAM to assist TB diagnosis in symptomatic HIV-positive adult in- or out-patients with CD4 cell counts ≤100 cells/mm$^3$, or those who are seriously ill irrespective of CD4 count[13]. Data from the STAMP trial showed that systematic screening with LF-LAM of hospitalised HIV-positive adults increased overall TB diagnosis and in certain subgroups of patients reduced mortality[19]. We have previously reported the low sensitivity of LF-LAM in the broader XPHACTOR study population[20]. In this substudy we found that LF-LAM would not have helped make earlier diagnoses of TB.

Our study has some limitations. In the parent XPHACTOR study, Xpert testing was prioritised in people with BMI<18.5kg/m$^2$ or CD4<100, those newly diagnosed HIV-positive or pre-ART with CD4<200, as well as those with TB symptoms[8]. Thus the population in this substudy did not all have classic "TB symptoms" at the time of collection of either initial or repeat sputum samples for testing with Xpert. However, TB prevalence in our substudy population was high, and we anticipate our results to be relevant at least to these high-risk groups. We froze all our raw sputum samples within 24 hours of collection, and all were thawed and tested within 6 months of collection, in line with other studies[21]. We assumed that all participants starting TB treatment or with a sample which was bacteriologically confirmed collected within six months of enrolment were likely to have had active TB at enrolment, regardless of whether it was diagnosable using sputum based tests at the time of enrolment. In fact, our study participants who started TB treatment commenced within a median of seven weeks from collection of the "repeat" Xpert sample. Some sputum samples for mycobacterial culture and chest radiographs were taken at an interval after participants returned for their initial Xpert test result, reflecting real-life investigation practice; we cannot be certain of the same result if they had been performed at the same time as sample collection for repeat Xpert. However, our findings suggest that following the Xpert-negative algorithm is more likely to lead to TB diagnosis than immediate repeat Xpert test.

Strengths of our study include systematic evaluation of participants and longitudinal follow-up which minimised the number of TB diagnoses missed, and the pragmatic nature of the study which reflected as far as possible real-life conditions, albeit with optimised implementation of TB diagnostic algorithms.

## Conclusions

Amongst ambulatory HIV-positive individuals at high risk of active TB, if an initial Xpert is negative, the Xpert-negative pathway should be implemented and there should be a low threshold for investigating those who remain at high risk using all clinically appropriate diagnostic modalities. In addition, those for whom no TB diagnosis is made must be made aware of the importance of returning for review if symptoms persist or recur. Our findings do not support sending an immediate repeat Xpert and highlight the need for more sensitive diagnostic tests capable of detecting pulmonary and extrapulmonary TB.

## Data availability

The XPHACTOR "Investigating TB if initial Xpert is negative" dataset, which includes data underlying this substudy, has been uploaded to the LSHTM Data Compass repository: https://doi.org/10.17037/DATA.284[22].

The reader will need to request the dataset from LSHTM (request access is provided within the data record) with a brief summary of how the dataset will be utilised. On request, a data sharing agreement will be made available which will first need to be signed, prior to provision of the dataset. This enables LSHTM to confirm that the reader is using the data for HIV or TB-related research, which is required because study participants consented to use of their data for HIV or TB-related research only.

The data is shared under a Data Sharing Agreement license (see above).

The study team wish to avoid unnecessary barriers to access and will seek to respond to data requests as quickly as possible.

### Competing interests

No competing interests were disclosed.

### Grant information

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**Supplementary material**

Supplementary File 1: XPHACTOR study flow, procedures and algorithm.

Click here to access the data.

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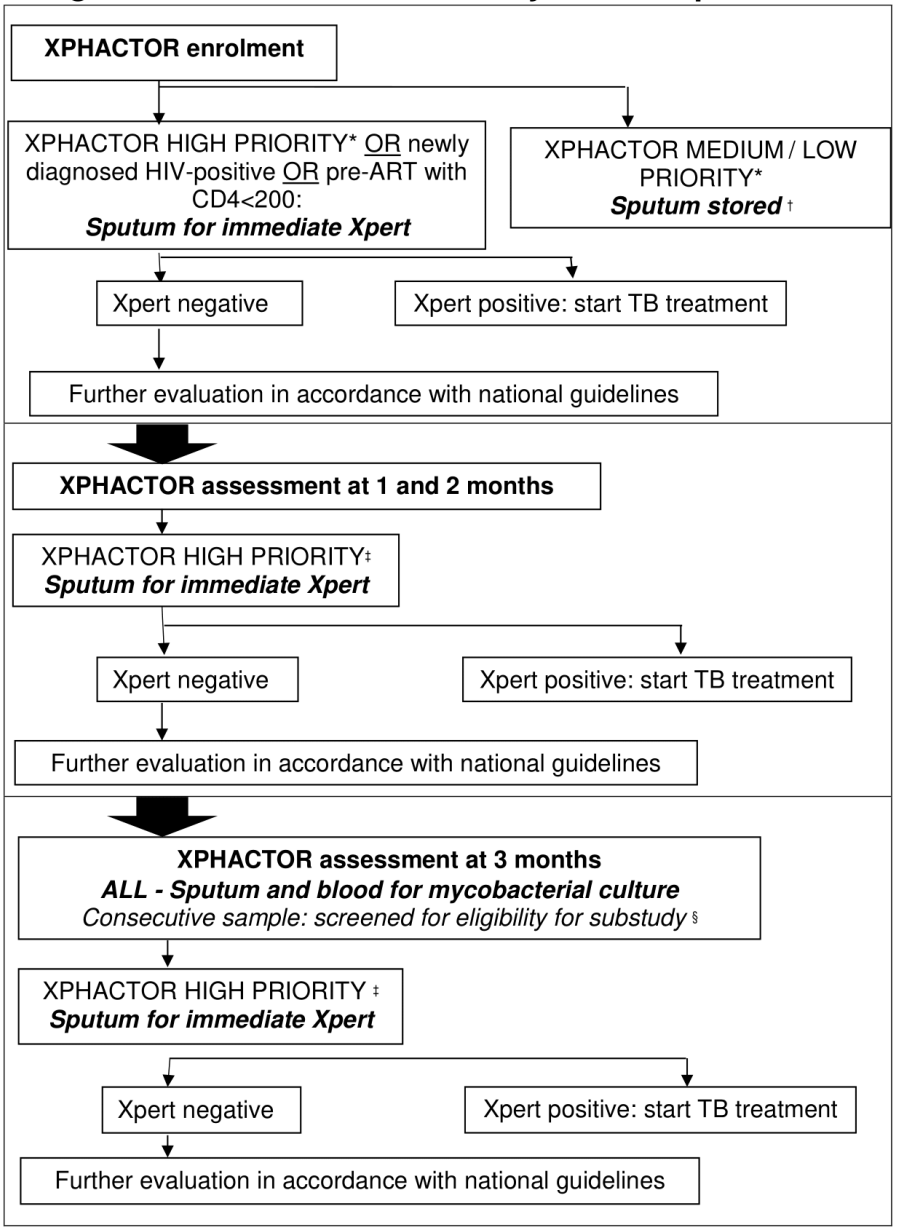
Data Source

## 7.3. Material provided as supplementary online appendices

# SUPPLEMENTARY MATERIAL INDEX

## I. Figure 1. XPHACTOR main study flow and procedures




* XPHACTOR algorithm at enrolment: high priority (any of: current cough, fever ≥ 3 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of: fever < 3 weeks, night sweats, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms.

† Samples tested with Xpert at the end of the study to enable comparison of sensitivity and specificity of the XPHACTOR study algorithm to detect TB cases against sensitivity and specificity if Xpert had been performed immediately for all with any WHO tool symptom.

‡ XPHACTOR algorithm at monthly follow up: high priority (any of: current cough, fever ≥ 3 weeks, night sweats ≥ 4 weeks, BMI <18.5 kg/$m^2$, CD4 <100x$10^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of: fever < 3 weeks, night sweats <4 weeks, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms.

§ Screened by research nurse between October 2013 and April 2014. Eligible if not on TB treatment **&** persistent TB symptoms, defined as: (i) any of cough, fever, or night sweats reported at enrolment and at 3-month visit; OR (ii) ≥5% measured weight loss at 3-month visit and reported unintentional weight loss.

# II. XPHACTOR main study procedures

### *Enrolment*

At enrolment, research staff administered a standardised questionnaire which incorporated the WHO tool, collected details of TB and HIV treatment, and basic demographic and socioeconomic information. Further investigation was prioritised according to the XPHACTOR algorithm with an immediate spot sputum sample sent for Xpert for individuals at *a priori* highest risk of active TB: (i) all assigned "high priority" (any of: current cough, fever ≥ 3 weeks, BMI <18.5 kg/m$^2$, CD4 <100x10$^6$/l, measured weight loss ≥10% in preceding 6 months, or other feature raising high clinical suspicion of TB); (ii) those in pre-ART group with CD4<200x10$^6$/l at enrolment (iii) all in HTC group (whose CD4 count was unknown) at enrolment. For all other participants, a spot sputum sample was collected at enrolment and frozen at -80 ºC within 24 hours, for testing with Xpert at the end of the study (figure 1). This enabled comparison of sensitivity and specificity of the XPHACTOR study algorithm to detect TB cases against sensitivity and specificity if Xpert had been performed immediately for all with any WHO tool symptom.

All participants with CD4<200x10$^6$/l were asked to provide a spot urine sample in a sterile container at enrolment, which was stored at 2-8 ºC prior to freezing at -80 ºC within 24 hours of collection. At the end of the study samples were thawed to ambient temperature and tested with lateral-flow LAM assay (LF-LAM) (Determine TB-LAM; Alere, USA) by two trained laboratory technologists in accordance with training provided by Alere representatives. The technologists did not have access to other bacteriological results when performing LF-LAM tests. Each test was graded once, using the pre-January 2014 manufacturer's reference card comprising five grades of colour intensity with the least intense band assigned grade 1, absence of a band graded negative, and absence of control band deemed a failed test.

### *Follow-up*

Participants were reviewed monthly to three months, with repeat WHO symptom screen and a spot sputum requested for Xpert if "high priority" by the study algorithm at that visit, with the exception of those in the "on ART" group who were asymptomatic at enrolment who were telephoned at 1 and 2 months to update locator information but were not asked about TB symptoms. At the 3-month visit sputum (induced if necessary) and blood were collected for mycobacterial culture on liquid media (Bactec MGIT 960 and 9240 systems) from all study participants, regardless of symptoms (figure 1). We allowed a broad window period around the scheduled 3-month visit, until around six months, in order to maximise study follow-up.

Participants who submitted an Xpert sample were reviewed within one week. If Xpert-positive, TB treatment was initiated; if negative, research staff repeated the WHO symptom screen and facilitated

the Xpert-negative algorithm which comprised chest radiograph, spot sputum for TB culture, and/or antibiotic trial as clinically appropriate (Figure 1).

Investigation results were returned to clinic staff, who were responsible for management decisions. Clinic records were reviewed at the end of the study to ascertain any additional relevant investigations and/or TB diagnoses. Deaths were identified through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants' South African identification numbers.

## 7.4. Peer reviewers' reports

Gates Open Research

Gates Open Research 2018, 2:22 Last updated: 15 MAY 2019

# Open Peer Review

## Current Peer Review Status: ? ✓

**Version 1**

Reviewer Report 01 June 2018

https://doi.org/10.21956/gatesopenres.13882.r26494



✓ **Tow Keang Lim**
Division of Respiratory and Critical Care Medicine, National University Hospital, Singapore, Singapore

This study examined the sensitivity of repeating an expectorated sputum specimen for Xpert testing for TB in a group of high risk immuno-compromised patients who had returned negative tests initially. negative tests. The repeat Xpert test had lower than expected sensitivity (~20%) and contributed very little to treatment timeliness. However the overall incidence of TB was low and it was mostly pauci-bacillary disease. These results many not be applicable to patients with higher disease burden or for induced sputa.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

***Competing Interests:*** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Gates Open Research

Gates Open Research 2018, 2:22 Last updated: 15 MAY 2019

Reviewer Report 31 May 2018

https://doi.org/10.21956/gatesopenres.13882.r26447



?

**Colleen F. Hanrahan**
Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Overall

- This study provides some interesting data on how useful a 2nd Xpert is to follow-up on those initially negative by Xpert. The addition of LAM to this study needs to be clearly and explicitly explained. Currently it reads as a very extraneous add-on.
- There are too many tables/figures for the amount of actual data. I find the presentation of data currently to be excessive and unnecessarily complicated and duplicative.

Introduction:

- Aim is awkwardly stated. Possibly reword with study population at the end, and try to make it more succinct.
- LAM needs to be included as an aim from the start. Otherwise it feels like a very tangential add on.

Methods:

- I would like a formal definition of the primary outcome and any secondary outcomes. They are probably in there, but not explicitly stated enough.
- I think a flow diagram of testing would be helpful to flesh out the methods.
- It's not clear to me where LAM fits in. An Xpert negative pt is re-screened for symptoms, has a chest x-ray and sputum for culture, and/or antibiotic trial is given. But then suddenly under statistical methods, you mention LAM for the first time. LAM is not part of the SA algorithm. We need to know when this was done, and even why it is relevant to this study.

Results:

- Not sure why 1 person who started TB treatment >6 months after enrollment would be excluded? This gets back to my previous point that you need to explicitly state the primary outcome, so we can understand why you would have excluded these 7 individuals.
- I'm confused by figure 1. What i'd like to know is what is the additional yield of Xpert, but I can't tell that from this figure. The way it looks is that all the people who had confirmed TB were not diagnosed with clinical TB. However, I can't believe that is true. I would prefer to see who was diagnosed how up front (clinical, microbiological) and who wasn't ("not tb"). Then from those boxes, show us how many in each were Xpert positive from the 2nd sputum.

- I'm confused by the section on TB diagnosis. Those diagnosed by Xpert don't appear to be by the 2nd Xpert that was taken and stored, because lower down you detail those individuals. But a 2nd Xpert is not part of the SA algorithm. Something needs to be done to make this clearer.
- Table 2 confuses me for the same reason that figure 1 confuses me.
- If you change figure 1 as suggested above, then you don't need a venn diagram. That diagram conveys very little information.
- Figure 3 is totally confusing because people can be in more than one of the top boxes...I'm not sure what this adds, particularly how it speaks to your primary outcome.

Discussion:

- Although the authors note that their population was not limited to symptomatic individuals as a potential weakness, they do not explicitly state that an asymptomatic population is likely to have paucibacillary or subclinical disease, for which Xpert would not have good sensitivity. The SA algorithm for TB investigation starts with a positive symptom screen, not merely for being HIV positive with a low CD4 despite having no symptoms. So these findings are not necessarily generalizeable to actual practice.
- I would also note that the prevalence of clinical diagnosis is not necessarily generalizeable. Its unclear the level of care that was given to these participants- was this routine care available in a primary public health clinic, or is this specialized care as part of a research study? These details could help the reader evaluate how generalizeable the findings are.

Conclusions:

- The reference to the "Xpert negative pathway" is vague - authors should be specific about what kinds of followup are most useful. Authors should also include something about LAM.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

***Competing Interests:*** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

# 8) *Paper 4*: What causes symptoms suggesting TB in HIV-positive people with negative initial investigations?

## 8.1. Cover sheet

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

## RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

**SECTION A – Student Details**

| | | | |
|---|---|---|---|
| **Student ID Number** | 079810 | **Title** | Dr |
| **First Name(s)** | Yasmeen | | |
| **Surname/Family Name** | Hanifa | | |
| **Thesis Title** | Investigation pathways for tuberculosis among HIV-positive adults in South Africa | | |
| **Primary Supervisor** | Alison Grant | | |

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

**SECTION B – Paper already published**

| | | | |
|---|---|---|---|
| Where was the work published? | International Journal of Tuberculosis and Lung Disease | | |
| When was the work published? | February 2019 | | |
| If the work was published prior to registration for your research degree, give a brief rationale for its inclusion | N/A | | |
| Have you retained the copyright for the work?* | **Yes** | Was the work subject to academic peer review? | **Yes** |

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

**SECTION C – Prepared for publication, but not yet published**

| | |
|---|---|
| Where is the work intended to be published? | |
| Please list the paper's authors in the intended authorship order: | |
| Stage of publication | Choose an item. |

**SECTION D – Multi-authored work**

| | |
|---|---|
| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | I managed the study, performed clinical evaluation of participants, conducted all analyses and wrote the paper |

**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
| **Date** | 28th May 2019 |

| | |
|---|---|
| **Supervisor Signature** | [redacted] |
| **Date** | 28 May 2019 |

## 8.2. Research paper

INT J TUBERC LUNG DIS 23(2):157–165

http://dx.doi.org/10.5588/ijtld.18.0251
E-published ahead of print 24 January 2019

# What causes symptoms suggestive of tuberculosis in HIV-positive people with negative initial investigations?

Y. Hanifa,* S. Toro Silva,* A. Karstaedt,†‡ F. Sahid,†‡ S. Charalambous,§¶ V. N. Chihota,§¶ G. J. Churchyard,*§¶# A. von Gottberg,**†† K. McCarthy,§ M. P. Nicol,‡‡§§ N. T. Ndlovu,§ W. Stevens,§§¶¶ K. L. Fielding,* A. D. Grant*¶##

*TB Centre, London School of Hygiene & Tropical Medicine, London, UK; †Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, ‡University of the Witwatersrand, Johannesburg, §The Aurum Institute, Johannesburg, ¶ School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, #Advancing Care and Treatment for TB-HIV, South African Medical Research Council Collaborating Centre for HIV and TB, Tygerberg, **Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Johannesburg, ††School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, ‡‡Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, §§National Health Laboratory Service, Johannesburg, ¶¶Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, ##Africa Health Research Institute, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa

SUMMARY

OBJECTIVE: To identify the causes of symptoms suggestive of tuberculosis (TB) among people living with the human immunodeficiency virus (PLHIV) in South Africa.

METHODS: A consecutive sample of HIV clinic attendees with symptoms suggestive of TB (⩾1 of cough, weight loss, fever or night sweats) at enrolment and at 3 months, and negative initial TB investigations, were systematically evaluated with standard protocols and diagnoses assigned using standard criteria. TB was 'confirmed' if *Mycobacterium tuberculosis* was identified within 6 months of enrolment, and 'clinical' if treatment started without microbiological confirmation.

RESULTS: Among 103 participants, 50/103 were pre-antiretroviral therapy (ART) and 53/103 were on ART; respectively 68% vs. 79% were female; the median age was 35 vs. 45 years; the median CD4 count was 311 vs. 508 cells/mm$^3$. Seventy-two (70%) had ⩾5% measured weight loss and 50 (49%) had cough. The most common final diagnoses were weight loss due to severe food insecurity ($n = 20$, 19%), TB ($n = 14$, 14%: confirmed $n = 7$; clinical $n = 7$), other respiratory tract infection ($n = 14$, 14%) and post-TB lung disease ($n = 9$, 9%). The basis for TB diagnosis was imaging ($n = 7$), bacteriological confirmation from sputum ($n = 4$), histology, lumbar puncture and other ($n = 1$ each).

CONCLUSION: PLHIV with persistent TB symptoms require further evaluation for TB using all available modalities, and for food insecurity in those with weight loss.

KEY WORDS: South Africa; Xpert® MTB/RIF; TB symptoms; human immunodeficiency virus

THE WORLD HEALTH ORGANIZATION (WHO) recommends regular screening of people living with the human immunodeficiency virus (PLHIV) for tuberculosis (TB) using a symptom screen comprising any one of current self-reported cough, fever, weight loss or night sweats (hereafter termed the 'WHO tool'), as an essential part of the HIV care package.[1] Although people attending for HIV care in sub-Saharan Africa are highly symptomatic,[2] most of those reporting WHO tool symptoms have negative TB investigations,[2–4] and a proportion continue to report symptoms. Early identification of people with active TB among PLHIV is a priority; however, the evidence underpinning investigation pathways after an initial sputum test is weak.[5–14]

The aim of our study was to determine the causes of persistent or recurrent symptoms suggestive of TB among ambulatory adults attending for HIV care who had negative initial TB investigations.

## METHODS

This sub-study was part of a prospective cohort study evaluating a risk-based algorithm to prioritise Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing among adults attending for routine HIV care in South

Correspondence to: Yasmeen Hanifa, Department of Clinical Research, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK. e-mail: yasmeen.hanifa@lshtm.ac.uk
*Article submitted 30 March 2018. Final version accepted 29 July 2018.*

Africa: 'Xpert for people attending HIV/AIDS care: test or review?' (XPHACTOR).[15]

*XPHACTOR study population, recruitment and procedures*

XPHACTOR study flow, procedures and algorithm are described in detail in the Online Appendix* (section on 'Main study procedures', Appendix Figure A.1). Briefly, we enrolled a systematic sample (using a predetermined system designed to minimise the risk of researcher selection bias) of adults (aged ⩾18 years) attending four HIV clinics in Gauteng Province, South Africa, irrespective of the presence of symptoms suggestive of TB. Patients taking anti-tuberculosis treatment within the previous 3 months were excluded. Patients were enrolled into 'on antiretroviral therapy (ART)' 'ART-experienced' and 'pre-ART' (in HIV care or newly diagnosed HIV-positive, not taking ART) groups. At the time of the study, ART eligibility comprised CD4 count ⩽350 cells/mm$^3$ or WHO clinical stage ⩾3. Research staff screened participants for TB at monthly intervals up to 3 months using a standardised questionnaire that incorporated the WHO tool. The study algorithm defined individuals as a priori at highest risk of active TB if they had any of the following: current cough, fever ⩾ 3 weeks, night sweats ⩾ 4 weeks, body mass index (BMI) <18.5 kg/m$^2$, CD4 count <100 cells/mm$^3$ or weight loss ⩾10%. A spot sputum sample was collected from these individuals if possible for Xpert testing. At the 3-month visit, all participants underwent sputum (induced if necessary) and blood cultures for mycobacteria (BACTEC MGIT™ 960™ and 9240™ systems; BD, Sparks, MD, USA). We allowed a broad window period around the 3-month XPHACTOR main study visit until around 6 months to maximise follow-up.

*Sub-study eligibility and enrolment*

Between October 2013 and April 2014 at the XPHACTOR 3-month visit, consecutive participants who were not on anti-tuberculosis treatment and who had persistent or recurrent symptoms suggestive of TB were invited to participate in this sub-study. Persistent or recurrent TB symptoms were defined as 1) self-report of any of cough, fever or night sweats at enrolment, and self-report of any of the aforementioned symptoms at 3-month visit; or 2) self-report of unintentional weight loss and ⩾5% measured weight loss since XPHACTOR enrolment.

Figure 1 shows the sub-study flow and procedures. A chest radiograph (CXR) was requested if there was no film available for the previous 6 weeks, and all were asked to bring samples (stool, early-morning urine and sputum) for mycobacterial culture when they attended for research physician assessment. Further procedures were determined by symptoms (Figure 1); if cough was reported, the research nurse collected an additional sputum sample for bacterial culture (induced if necessary), two nasopharyngeal swabs and one oropharyngeal swab. Sputum samples were tested using routine bacterial microscopy and culture, and polymerase chain reaction (PCR) for bacteria, including *Bordetella pertussis*. One nasopharyngeal swab was inserted directly into Regan Lowe transport media for *Bordetella* spp. culture, and the remaining swabs were placed in Primestore medium for PCR detection of *B. pertussis* and other pathogens (Figure 1). All samples were transported within 24 h of collection to the research laboratory. PCR for *B. pertussis* was performed in accordance with the method described by Tatti et al.[16] An abdominal ultrasound scan was requested for those with weight loss. Participants reporting fever or night sweats were given a digital thermometer to record oral temperature (morning, evening, and if any fever or sweats) for 1 week.

*Research physician assessment*

Around 1 week after enrolment, sub-study participants underwent systematic clinical evaluation, including examination by a research physician who arranged a standard set of investigations according to the participant's symptoms (Figure 1 and Appendix Figure A.2, Appendix section on 'Sub-study research physician assessment').

First-line evaluation for cough was spirometry if cough ⩾8 weeks or features suggestive of chronic obstructive pulmonary disease (COPD) or asthma; if clinically appropriate, blood samples were collected for C-reactive protein (CRP) testing to help distinguish the likelihood of bacterial infection and, if cardiac failure was suspected, for serum β-natriuretic peptide.

Second-line evaluation for cough comprised a trial of appropriate treatment for those with clinical features suggestive of cough due to upper airways disease, angiotensin-converting enzyme (ACE) inhibitors or gastro-oesophageal reflux disease (GORD). All participants were screened using validated tools for depression (Patient Health Questionnaire 9 [PHQ-9]),[17] household food insecurity (household food insecurity access score [HFIAS]),[18] and alcohol misuse (Fast Alcohol Screening Test [FAST] score),[19] and were asked about use of tobacco, snuff and wood-burning stoves. Using a standardised form, the physician abstracted information from clinic records relevant to assigning final diagnoses, such as chronic disease diagnoses, results of recent investigations in particular for TB, and history of HIV, ART and TB.

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2019/00000023/00000002/art000 .....

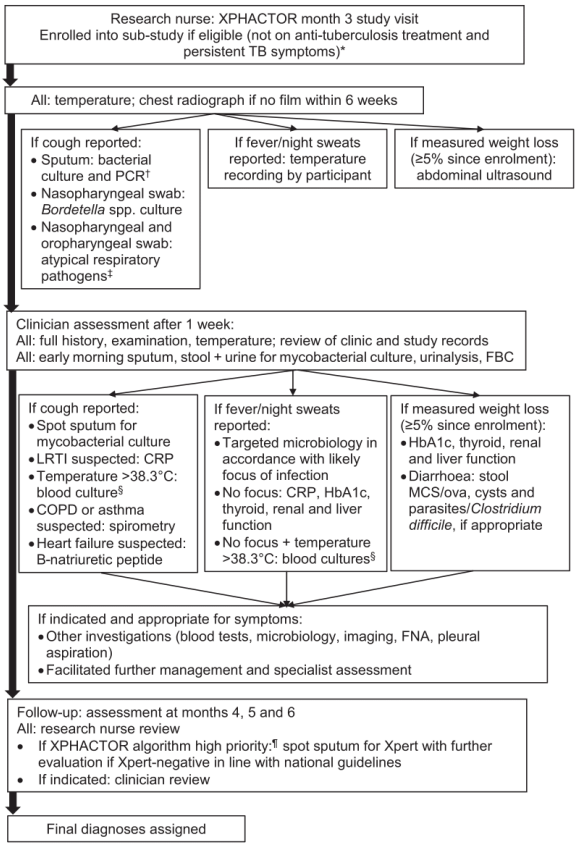



**Figure 1** Sub-study procedures. * Eligible if not on anti-tuberculosis treatment and persistent or recurrent TB symptoms, defined as: 1) any of cough, fever, or night sweats at enrolment and 3-month visit; or 2) ⩾5% measured weight loss at 3-month visit and reported unintentional weight loss. † Sputum samples underwent macroscopic and microscopic evaluation; culture on 5% horse blood agar (routine respiratory pathogens), Regan Lowe (*Bordetella* spp.), buffered charcoal yeast extract (*Legionella* spp.); PCR for *Bordetella* spp., *M. pneumoniae, C. pneumoniae* and *Legionella* spp. ‡ PCR for *Bordetella* spp., *M. pneumoniae, C. pneumoniae* and *Legionella* spp. § Aerobic and anaerobic bacterial cultures. ¶ High priority (any of current cough, fever ⩾ 3 weeks, night sweats ⩾ 4 weeks, body mass index < 18.5 kg/m$^2$, CD4 < 100×10$^6$/l, measured weight loss ⩾ 10% in preceding 6 months, or other feature raising high clinical suspicion of TB); medium priority (any of fever < 3 weeks, night sweats < 4 weeks, measured weight loss < 10% in preceding 6 months); low priority = no TB symptoms. XPHACTOR = Xpert for people attending HIV/AIDS care: test or review? TB = tuberculosis; PCR polymerase chain reaction; FBC = full blood count; LRTI = lower respiratory tract infection; CRP = C-reactive protein; COPD = chronic obstructive pulmonary disease; HbA1c = glycated haemoglobin; MC&S = microscopy, culture and sensitivities; FNA = fine-needle aspiration; HIV = human immunodeficiency virus; AIDS = acquired immune-deficiency syndrome.

*Sub-study follow-up*

Sub-study participants were followed for a further 3 months and screened for TB at each visit by research staff using a standardised questionnaire incorporating the WHO tool, with further investigation for TB in accordance with the XPHACTOR study algorithm (Figure 1). The research physician reviewed participants at these visits if required to assign final diagnoses.

*Definitions*

Final diagnoses were assigned by the research

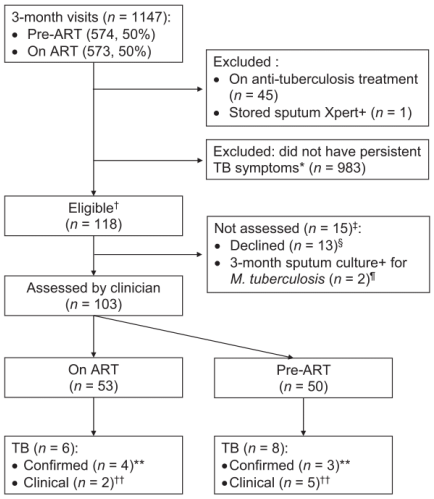



**Figure 2** Study flow. * Persistent TB symptoms, defined as: 1) self-report of any of cough, fever, or night sweats at enrolment and at 3-month visit; or 2) self-report of unintentional weight loss and ⩾5% measured weight loss. † 60/540 (11%) pre-ART and 58/561 (10%) on ART/eligible. ‡ Pre-ART ($n = 10$); on ART ($n = 5$). § No time ($n = 12$); too unwell ($n = 1$). ¶ Both pre-ART: started anti-tuberculosis treatment before assessment ($n = 1$); died before assessment ($n = 1$). ** Confirmed: Xpert+ or LPA+ from smear-positive/cultured isolate OR culture + *M. tuberculosis* from any sample collected within 6 months from enrolment to sub-study. ††Clinical: started treatment in the absence of microbiological confirmation within 6 months from enrolment to sub-study. ART = antiretroviral therapy; + = positive; TB = tuberculosis; LPA = line-probe assay.

physician using pre-set criteria (Appendix Table A), including the case definitions for TB detailed below.

### *TB case definitions*

'Confirmed' TB was defined as a positive result on 1) Xpert (on sputum sample), 2) line-probe assay (LPA) performed on smear-positive or cultured isolate (GenoType MTBDR*plus*, Hain Lifesciences, Nehren, Germany) or 3) *M. tuberculosis* culture, from any sample collected within 6 months of sub-study enrolment. 'Clinical TB' was defined as anti-tuberculosis treatment started within 6 months of sub-study enrolment in the absence of microbiological confirmation.

### *Radiological definitions*

'Probable radiological TB' was defined as the presence of 1) any of cavitation, predominantly upper lobe infiltrates, pleural or pericardial effusion, or clear miliary picture on CXR, or 2) any of abdominal lymphadenopathy, splenic microabscesses, pleural or pericardial effusion on ultrasound scan. 'Possible radiological TB' was defined as the presence of any of lymphadenopathy (hilar or mediastinal), pulmonary nodules or other infiltrates. Participants with 'probable' or 'possible' radiological TB features but without bacteriological confirmation who started anti-tuberculosis treatment within 6 months of sub-study enrolment were assigned 'clinical' TB.

### *Ethical approval*

The study protocol was approved by the ethics committees of the University of the Witwatersrand, Johannesburg; University of Cape Town, Cape Town, South Africa; and the London School of Hygiene & Tropical Medicine, London, UK. All participants provided written informed consent or, if unable to write, witnessed verbal consent.

## RESULTS

### *Sub-study enrolment and eligibility*

A total of 1147 XPHACTOR study participants were screened for the sub-study, 45 of whom were excluded because they were currently taking anti-tuberculosis treatment (Figure 2). One further participant was excluded because a stored sputum sample collected for the main study was Xpert-positive when tested after the 3-month visit. Among the remaining 1101 participants, 118 (11%) were eligible, and 103/118 (87%) underwent physician assessment (53/103 [51%] on ART, 50/103 [49%] pre-ART), at a median of 126 days (interquartile range [IQR] 96–175) after enrolment in the parent study. Among 15/118 (13%) participants who did not undergo physician assessment (10 pre-ART, 5 on ART), all had only one symptom (11/15 [73%] ⩾5% measured weight loss, 4/15 [27%] cough), and two subsequently had *M. tuberculosis* isolated from the 3-month sputum sample.

### *Participant characteristics*

Table 1 presents the participants' characteristics; 30/50 (60%) pre-ART participants initiated ART during study follow-up, and in the on-ART group, 28/51 (55%) were virologically suppressed. Overall, 40/102 (39%) had PHQ-9 scores suggestive of moderate depression and 53/103 (51%) had HFIAS scores indicating households with severe food insecurity.

The most common WHO tool symptoms reported were weight loss (83/103, 81%), with 72/103 (70%) having ⩾5% measured weight loss and cough (50/103, 49%); 57/103 (55%) had one WHO tool symptom (45 [44%] weight loss, 9 [9%] cough, 3 [3%] night sweats), and 46/103 (45%) had multiple symptoms (25 [24%] had two, 18 [17%] three, 3 [3%] four symptoms). Among participants reporting cough, 20/50 (40%) had previously received anti-tuberculosis treatment at a median of 4 years (IQR 2–6) before sub-study enrolment (7/50 [14%] more than one course), 29/50 (58%) were current or ex-smokers

**Table 1** Characteristics of study participants

| Characteristic | Pre-ART ($n = 50$) $n$ (%) | On ART ($n = 53$) $n$ (%) | Total ($n = 103$) $n$ (%) |
|---|---|---|---|
| Demographics | | | |
| Age, years, median [IQR] | 35 [28–41] | 45 [39–49] | 40 [31–46] |
| Female | 34 (68) | 42 (79.3) | 76 (73.8) |
| Black African | 50 (100) | 49 (92.5) | 99 (96.1) |
| Completed secondary education (grade 12) | 15 (30.0) | 11 (20.8) | 26 (25.2) |
| Number of people in household, median [IQR] | 4 [3–5] | 4 [2–5] | 4 [2–5] |
| Monthly household income <2000 ZAR | 29 (58.0) | 30 (56.6) | 59 (57.3) |
| HIV-TB history | | | |
| Duration since HIV diagnosed, months, median [IQR] | 6 [4–17] | 88 [52–118] | 38 [6–99] |
| ART commenced after enrolment into main study* | 25 (50.0) | NA | 25 (24.3) |
| Duration on ART, months, median [IQR] | 3 [2–4] ($n = 21$) | 76 [43–100] | 47 [5–88] ($n = 74$) |
| Previous anti-tuberculosis treatment | 4 (8.0) | 29 (54.7) | 33 (32.0) |
| >1 previous episode of anti-tuberculosis treatment | 0 | 11 (20.8) | 11 (10.7) |
| CD4†/viral load†/BMI | | | |
| CD4, cells/mm$^3$, median [IQR] | 311 [162–445] ($n = 49$) | 508 [354–673] | 401 [226–588] ($n = 102$) |
| Viral load suppressed (<20 copies/ml) | NA | 28 (54.9) ($n = 51$) | 29 (53.7) ($n = 54$) |
| BMI at enrolment to sub-study, kg/m$^2$, median [IQR] | 22.7 [19.3–26.0] | 23.6 [19.6–26.1] | 23 [19.4–26.1] |
| WHO tool symptoms reported | | | |
| Unintentional weight loss‡ ⩾5% | 42 (84.0) | 30 (56.6) | 72 (69.9) |
| Cough | 15 (30.0) | 35 (66.0) | 50 (48.5) |
| Night sweats | 10 (20.0) | 19 (35.9) | 29 (28.2) |
| Fever | 4 (8.0) | 18 (34.0) | 22 (21.4) |
| >1 of the above symptoms | 17 (34.0) | 29 (54.7) | 46 (44.7) |
| Screening for food insecurity and depression | | | |
| HFIAS score: severe food insecurity | 23 (46.0) | 30 (56.6) | 53 (51.5) |
| HFIAS score: moderate food insecurity | 11 (22.0) | 8 (15.1) | 19 (18.5) |
| PHQ-9 score ⩾10 (moderate depression) | 18 (36.0) | 22 (42.3) ($n = 52$) | 40 (39.2) ($n = 102$) |
| Tobacco/alcohol/drug use§ | | | |
| Ex- or current smoker | 19 (38.0) | 26 (49.1) | 45 (43.7) |
| >15 pack years cigarette smoking | 3/19 (15.8) | 6/26 (23.1) | 9/45 (20.0) |
| Current snuff user | 7 (14.0) | 15 (28.3) | 22 (21.4) |
| Current harmful alcohol intake (FAST score ⩾3) | 9 (18.0) | 7 (13.2) | 16 (15.5) |
| Recreational drug use | 0 ($n = 49$) | 2 (3.8) | 2 (2.0) |

* A further 5 pre-ART participants initiated ART following clinician assessment.
† Most recent of any result available within 1 year before, or within 6 weeks following clinician assessment.
‡ ⩾5% measured weight loss and reported unintentional weight loss at sub-study enrolment.
§ 'Current' defined as use within past 1 year, and 'smoker' defined as having ever smoked ⩾100 cigarettes.
ART = antiretroviral therapy; IQR = interquartile range; ZAR = South African rand; HIV = human immunodeficiency virus; TB = tuberculosis; NA = not applicable; BMI = body mass index; WHO = World Health Organization; HFIAS = Household Food Insecurity Access Score; PHQ-9 = Patient Health Questionnaire 9; FAST = Fast Alcohol Screening Test.

(7 [14%] had >15 pack years), 8/50 (16%) used snuff, 5/50 (10%) used paraffin stoves and none used wood-burning stoves. A further 18/50 (35%) reported wheeze and 26/50 (52%) dyspnoea.

Among 72 participants with ⩾5% measured weight loss, the median BMI, weight loss and percentage weight loss at physician assessment were respectively 23 kg/m$^2$ (IQR 18.9–25.9), 4.4 kg (IQR 3.6–6) and 6.8% (IQR 5.5–9.4). Of these 72 patients, 32 (50%) had HFIAS scores indicating severely food insecure households, 29/71 (41%) had PHQ-9 scores suggestive of moderate depression and 53/72 (74%) had a monthly household income of <2000 South African rand; 67/72 (93%) had follow-up weight measurements, among whom 42/67 (63%) gained weight and in 12/67 (18%) weight was stabilised. Among the 42 participants who gained weight during follow-up, 16/42 (38%) had initiated ART, three of whom had also started anti-tuberculosis treatment. Among 36 participants reporting fever or night sweats, 3/36 (8%) had measured fever >38.3°C at physician assessment or from home measurement.

### *Final diagnoses*

Table 2 summarises the final diagnoses assigned over a median of 100 days (IQR 89–144) of follow-up. For nine participants (measured weight loss only, $n = 8$; measured weight loss and night sweats, $n = 1$), we were unable to determine any final diagnosis; these patients were assigned a final diagnosis of 'unexplained' or 'unexplained—symptom resolved spontaneously'. One hundred and twenty-one diagnoses were assigned for the remaining 94/103 (91%) participants. The most common diagnoses were

**Table 2** Final diagnoses of patients*

| Final diagnoses assigned | (*n* = 103) *n* (%) |
|---|---|
| Weight loss due to severe food insecurity | 20 (19.4) |
| TB | 14 (13.6) |
| Confirmed | 7 (6.8) |
| Clinical | 7 (6.8) |
| Upper respiratory tract infection | 12 (11.7) |
| Post-TB chronic lung disease | 9 (8.7) |
| Bronchiectasis | 2 (1.9) |
| Chronic loculated pleural effusion | 1 (1.0) |
| Likely | 6 (5.8) |
| Weight loss due to loss of appetite | 9 (8.7) |
| Treatment-related† | 4 (3.9) |
| Stress-related | 4 (3.9) |
| Unexplained | 1 (1.0) |
| Asthma | 7 (6.8) |
| Confirmed | 2 (1.9) |
| Likely | 5 (4.9) |
| COPD‡ | 5 (4.9) |
| Confirmed | 3 (2.1) |
| Likely | 2 (1.9) |
| Weight loss due to depression | 4 (3.9) |
| Upper airway cough syndrome | 4 (3.9) |
| Confirmed | 3 (2.9) |
| Likely | 1 (1.0) |
| Perimenopausal vasomotor symptoms | 4 (3.9) |
| Diarrhoeal illness§ | 4 (3.9) |
| Other infection¶ | 4 (3.9) |
| Malignancy# | 3 (2.9) |
| Alcohol misuse** | 3 (2.9) |
| Lower respiratory tract infection | 2 (1.9) |
| Pertussis | 2 (1.9) |
| Confirmed | 1 (1.0) |
| Likely | 1 (1.0) |
| Weight loss due to previously undiagnosed type 2 diabetes | 2 (1.9) |
| Weight loss due to end-stage renal disease | 2 (1.9) |
| Other†† | 11 (10.7) |
| Unexplained | 10 (9.7) |
| Unexplained - symptom resolved spontaneously | 9 (8.7) |

* 140 diagnoses were assigned for 103 participants: 70 (68%) had one final diagnosis, 29 (28%) two diagnoses and 4 (4%) had three diagnoses.
† Attributed to new ART regimen (*n* = 2), radiotherapy for Kaposi's sarcoma (*n* = 1), dental extraction (*n* = 1).
‡ One participant with likely COPD had a previous addiction to nyaope (a street drug that is smoked and reported to contain heroin, cannabis and antiretrovirals); one participant with confirmed COPD had clinical cor pulmonale.
§ Microbiological confirmation of isospora (*n* = 1), giardia (*n* = 1); cause not known (*n* = 2).
¶ *Escherichia coli* urinary tract infection (*n* = 2), chronic skin infection (*n* = 1), likely chronic pelvic infection (*n* = 1).
# Newly diagnosed Hodgkin's lymphoma (*n* = 1), progression of previously diagnosed malignancy (renal cell carcinoma, *n* = 1; cervical cancer, *n* = 1).
** Hazardous alcohol intake and weight loss after stopping (*n* = 1), no other cause identified for night sweats (*n* = 2).
†† Diagnoses for cough, *n* = 5 (ACE inhibitor-related, *n* = 1; GORD-related, *n* = 1; post-thoracic surgery for benign lung mass, *n* = 1; post-infectious, *n* = 1; smoking-related, *n* = 1). Diagnoses for weight loss, *n* = 6 (endoscopy-confirmed gastritis, *n* = 1; recurrent small bowel obstruction, *n* = 1; confirmed heart failure, *n* = 1; subclinical hyperthyroidism, *n* = 1; increased exercise, *n* = 1; chronic unexplained gastrointestinal symptoms resolved by end of study, *n* = 1).
TB = tuberculosis; COPD = chronic obstructive pulmonary disease; ART = antiretroviral therapy; ACE = angiotensin-converting enzyme; GORD = gastroesophageal reflux disease.

weight loss due to severe food insecurity (20/103, 19%), TB (14, 14%), upper respiratory tract infection (12, 12%) and post-TB chronic lung disease (9, 9%).

Table 3 summarises the final diagnoses for the most common symptoms reported: cough and ⩾5% measured weight loss. Among 50 participants reporting cough, the most common diagnoses were upper or lower respiratory tract infection (11/50, 22%), post-tuberculous chronic lung disease (9/50, 18%), TB (7/50, 14%: pulmonary only, *n* = 4; extra-pulmonary only, *n* = 1; both, *n* = 2), asthma (7/50, 14%), COPD (5/50, 10%) and upper airways cough syndrome (4/50, 8%). Samples collected from 40 participants for respiratory pathogens yielded only one positive sample that was PCR-positive for *B. pertussis*.

Among the 72 participants with measured weight loss, the most common diagnoses were weight loss due to severe food insecurity (20/72, 28%), TB (10/72, 14%: pulmonary only, *n* = 3; extra-pulmonary only, *n* = 4; both, *n* = 3) and prolonged loss of appetite (8/72, 11%: 4 due to medical treatment and 4 stress-related). The most common diagnosis among 45 participants with weight loss in the absence of other symptoms was severe food insecurity (13/45, 29%). Of the 72 patients, 11 (15%) had weight loss unexplained by study investigations, among whom four gained weight after ART initiation. Nine of 68 participants (13%) with available samples had glycated haemoglobin ⩾ 6.5%; in two of these weight loss was attributed to newly diagnosed type 2 diabetes mellitus. Thyroid function test abnormalities were newly identified in 18/67 (27%); one participant with biochemically subclinical hyperthyroidism (high thyroxine, normal thyroid stimulating hormone) and marked weight loss (18 kg) also had severe food insecurity. Abdominal ultrasound scans were abnormal for 22/65 (34%), five of whom had features compatible with probable radiological TB and 17 other abnormalities (gallstones, *n* = 5; hepatomegaly, *n* = 4; echogenic kidney or liver, *n* = 3; splenomegaly, *n* = 2; metastatic disease, *n* = 2; fatty liver, *n* = 1).

### *Tuberculosis diagnoses*

Appendix Figure A.2 gives the results of the mycobacteriology and radiology requested for all sub-study participants. All participants had at least one sample subjected to mycobacterial culture; culture was positive for *M. tuberculosis* in 5/103 (5%) participants (5/176, 3% sputum samples [one multidrug-resistant]; 0/103 blood, 0/83 urine and 0/57 stool). Of 98 CXRs, 17 (17%) fulfilled the criteria for radiological TB (probable radiological TB, *n* = 12; possible radiological TB, *n* = 5); 6/17 (35%) participants whose CXRs fulfilled the criteria for radiological TB also fulfilled TB case definitions (3 confirmed, 3 clinical). Of 65 abdominal ultrasound scans, 7 (11%) fulfilled the criteria for probable radiological TB (abdominal only, *n* = 4; abdominal and possible renal, *n* = 1; pericardial effusion and abdominal, *n* = 1; pleural and pericardial effusions and abdominal TB, *n* = 1); 6/7 (86%) participants

**Table 3** Final diagnoses of participants with cough and/or weight loss ($n = 100$)

| | Cough only ($n = 28$) | Cough and weight loss ($n = 22$)* | Weight loss only ($n = 50$) |
|---|---|---|---|
| Severe food insecurity | — | 6 | 14 |
| Asthma or COPD | 10 | 2 | — |
| Unexplained weight loss | — | 2 | 9 |
| Upper or lower respiratory tract infection | 5 | 6 | — |
| TB | 2 | 5 | 5 |
| Pulmonary | 2 | 2 | 2 |
| Extra-pulmonary | — | 1 | 2 |
| Both | — | 2 | 1 |
| Post-TB chronic lung disease | 4 | 5 | — |
| Weight loss due to loss of appetite† | — | 3 | 5 |
| Weight loss due to depression | — | 2 | 1 |
| Diarrhoeal illness | — | — | 3 |
| Malignancy‡ | — | — | 3 |
| Upper airway cough syndrome | 2 | 2 | — |
| Pertussis§ | 2 | — | — |
| Weight loss due to previously undiagnosed type 2 diabetes | — | 1 | 1 |
| End-stage renal disease | — | — | 2 |
| Other¶ | 3 | 4 | 7 |

* 38 final diagnoses made for 22 participants with both cough and weight loss
† Weight loss only, $n = 3$: stress-related, $n = 2$; unexplained, $n = 1$; both cough and weight loss, $n = 5$: treatment-related, $n = 3$; stress-related, $n = 2$.
‡ Newly diagnosed Hodgkin's lymphoma, $n = 1$; progression in previously diagnosed malignancy (renal cell carcinoma, $n = 1$; cervical cancer, $n = 1$).
§ One confirmed, and one likely.
¶ 1) Cough only, $n = 3$: GORD-related, $n = 1$; post-thoracic surgery for benign lung mass, $n = 1$; smoking-related, $n = 1$. 2). Weight loss only, $n = 7$: endoscopy-confirmed gastritis, $n = 1$; confirmed heart failure, $n = 1$; subclinical hyperthyroidism, $n = 1$; increased exercise, $n = 1$; chronic unexplained gastrointestinal symptoms resolving by end of study, $n = 1$; likely chronic pelvic infection, $n = 1$; hazardous alcohol intake and weight loss after stopping, $n = 1$. 3). Both cough and weight loss, $n = 4$: ACE inhibitor-related cough, $n = 1$; post-infectious cough, $n = 1$; weight loss likely due to recurrent small bowel obstruction, $n = 1$; weight loss due to unexplained loss of appetite, $n = 1$.
COPD = chronic obstructive pulmonary disease; TB = tuberculosis; GORD = gastro-oesophageal reflux disease; ACE = angiotensin-converting enzyme.

whose abdominal ultrasound scans fulfilled the criteria for radiological TB also fulfilled TB case definitions (3 confirmed, 3 clinical).

Of 103 sub-study participants, 14 (14%) (6 on ART, 8 pre-ART) fulfilled TB case definitions (7 confirmed, 7 clinical). Eight participants started treatment due to compatible imaging (4 ultrasound, 2 abdominal ultrasound and CXR, and 2 CXR), of whom 3 were subsequently bacteriologically confirmed on sputum (1 Xpert + culture, 2 culture). Four participants started anti-tuberculosis treatment based on a positive sputum result (2 Xpert, 2 culture). One participant started treatment based on histology following fine-needle lymph node aspiration, and one based on lumbar puncture.

The median time from enrolment to start of anti-tuberculosis treatment was 21 days (range 1–137) for 13 participants with a documented anti-tuberculosis treatment start date. One further participant had positive Xpert on sputum 149 days after enrolment but an unknown treatment start date. Among the 8 pre-ART participants, 4 started anti-tuberculosis treatment after ART initiation (3 within 3 months, 1 within 6 months). A further two participants who were enrolled but who did not undergo physician assessment fulfilled case definitions for confirmed TB, of whom one died before anti-tuberculosis treatment was initiated (Figure 2).

## DISCUSSION

In this representative sample of HIV clinic attendees in South Africa reporting persistent or recurrent WHO tool symptoms 3 months after a negative initial investigation for TB, among those able to produce sputum, 14/103 (14%) had TB. Half started anti-tuberculosis treatment based on imaging, mainly abdominal ultrasound, which illustrated the limitations of sputum-based diagnostics for detecting extra-pulmonary TB. With an estimated 40% shortfall globally between notified cases and estimated incidence of TB in 2016, and with South Africa one of the 10 countries accounting for most of this gap,[20] we recommend using multiple diagnostic modalities, particularly imaging, to help identify these missing TB patients.

Our study is the first to systemically evaluate patients established in HIV care with persistent or recurrent symptoms suggestive of TB, and with an initial negative Xpert result among those able to produce sputum, for a broad spectrum of diagnoses. Previous studies have investigated patients with persistent symptom(s) for specific infectious[5,7] or non-communicable causes,[6,8,14] or evaluated chronic cough in smear-negative patients before the roll-out of Xpert.[9,12] Munyati et al. evaluated primary care attendees in Zimbabwe with chronic cough and, unsurprisingly, among 454 newly diagnosed HIV-positive patients, the majority of the diagnoses were

infectious (TB, 46%; lower respiratory tract infection, 31%).[21] Munyati et al. also identified a high proportion of non-communicable disease diagnoses, in particular post-tuberculous disease, asthma and heart failure.[21] We also found post-TB chronic lung disease to be a relatively common diagnosis; better criteria to distinguish it from active TB and optimal management are needed.[22–24] Our data support Chakaya et al.'s call for large-scale epidemiological studies of post-TB lung disease.[22]

Severe food insecurity was the most common cause of weight loss. Food insecurity has not previously been evaluated as a possible cause for weight loss in the context of TB screening, although it is well described as a barrier to adherence to ART.[25] We only assigned this diagnosis after searching for other, more likely diagnoses, and chose severe (rather than moderate) food insecurity as a more specific marker. Clinicians should consider screening for food insecurity among people with weight loss, particularly if not associated with other symptoms, and ensure patients are linked to social support where available. Forty per cent of our study participants screened positive for significant depression, and almost one fifth had harmful alcohol use, comparable with estimates of 31% and 7–31%, respectively, from a systematic review in sub-Saharan Africa of HIV-positive people on ART by Nakimuli-Mpungu et al.[26] In their pooled analysis, individuals with significant depression were less likely to adhere to ART.[26] Screening for depression with provision of appropriate care should be part of the HIV care package in lower-income settings to help optimise ART adherence and treatment outcomes.

The WHO tool was developed for use in resource-limited settings to provide a simple clinical algorithm to reliably rule out TB before providing isoniazid preventive therapy to PLHIV. As the tool was designed to maximise sensitivity (78.9%) and minimise the negative likelihood ratio for TB, it has low specificity (49.6%). At a TB prevalence of 5% in PLHIV, it has a negative predictive value of 97.7%, but a very low positive predictive value (8%).[27] Individuals who screen positive, the majority of whom will not have TB, require further evaluation for TB using Xpert, which has been recommended as the initial diagnostic test.[1] This poses a huge challenge in resource-constrained settings when it is used, as recommended, for active TB case finding in PLHIV at every clinical encounter.[1] Simple, low-cost strategies to prioritise those with WHO tool symptoms for TB investigation, such as 'second-step' clinical algorithms,[15,28] or point-of-care CRP testing (also suggested as an alternative TB screening tool),[29] are potential solutions that merit further evaluation.

Strengths of our study included our systematic physician evaluation of a representative sample of HIV clinic attendees in a clinically relevant manner with a standardised set of investigations and longitudinal follow-up of participants. We cannot rule out that additional diagnosis of TB and other specific diagnoses might have been made if further investigations had been undertaken. Weight loss was commonly reported by our study participants, but we restricted our study to those with measured weight loss to make this criterion more objective.

## CONCLUSIONS

TB, post-TB chronic lung disease and food insecurity were the main diagnoses for symptoms suggestive of TB in our population of HIV clinic attendees who had previously undergone systematic screening and investigation for TB, and we were able to assign diagnoses for more than 90% of participants. Our study highlights the need to continue to investigate for TB using multiple modalities among HIV-positive people with persistent symptoms, as well as evaluation for food insecurity, and for further studies to guide the identification and management of the sequelae of pulmonary TB.

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## 8.3. Material provided as supplementary online appendices



### APPENDIX

### MAIN STUDY PROCEDURES FOR XPHACTOR

*Enrolment*

At enrolment, research staff administered a standardised questionnaire, which incorporated the World Health Organization (WHO) tool, collected details of tuberculosis (TB) and human immunodeficiency virus (HIV) treatment, and basic demographic and socio-economic information. Further investigation was prioritised according to the XPHACTOR ('Xpert for people attending HIV/AIDS care: test or review?') algorithm with an immediate spot sputum sample sent for Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing for individuals at a priori highest risk of active TB: 1) all assigned 'high priority' (any of the following: current cough, fever $\geqslant$ 3 weeks, body mass index [BMI] $<$18.5 kg/m$^2$, CD4 count $<$ 100$\times$10$^6$/l, measured weight loss $\geqslant$10% in preceding 6 months or other feature raising high clinical suspicion of TB); 2) those in the pre-ART group with CD4 count of $<$200$\times$10$^6$/l at enrolment; 3) all those in the HIV testing and counselling (HTC) group (whose CD4 count was unknown) at enrolment. For all other participants, a spot sputum sample was collected at enrolment and frozen at –80°C within 24 h for testing with Xpert at the end of the study (Figure A.1). This enabled comparison of sensitivity and specificity of the XPHACTOR study algorithm to detect TB cases against sensitivity and specificity if Xpert had been performed immediately for all with any WHO tool symptom.

*Follow-up*

Participants were reviewed monthly to 3 months, with repeat WHO symptom screen and a spot sputum requested for Xpert if 'high priority' by the study algorithm at that visit, with the exception of those in the 'on ART' group who were asymptomatic at enrolment, who were telephoned at 1 and 2 months to update locator information but were not asked about TB symptoms. At the 3-month visit, sputum (induced if necessary) and blood were collected for mycobacterial culture on liquid media (BACTEC MGIT™ [Mycobacterium Growth Indicator Tubes; BD, Sparks, MD, USA] 960™ and 9240™ systems) from all study participants, regardless of symptoms (Figure A.1). We allowed a broad window period around the scheduled 3-month visit until around 6 months to maximise study follow-up.

Participants who submitted an Xpert sample were reviewed within 1 week. If Xpert-positive, anti-tuberculosis treatment was initiated; if negative, research staff repeated WHO symptom screening and facilitated the Xpert-negative algorithm, which comprised chest radiograph (CXR), spot sputum for TB culture and/or antibiotic trial as clinically appropriate (Figure A.1).

Investigation results were returned to clinic staff, who were responsible for management decisions. Clinic records were reviewed at the end of the study to ascertain any additional relevant investigations and/or TB diagnoses. Deaths were identified through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants' South African identification (ID) numbers.

*Sub-study research physician assessment*

The research physician administered a standardised questionnaire which had targeted questions to systematically identify the cause for WHO tool symptom(s) reported. All participants were asked about past medical history, current medications, and investigations and treatment undertaken to date. For example, those with cough were asked about cough duration and triggers, associated symptoms and any preceding respiratory tract infection. Results for all previous investigations undertaken for the study were reviewed by the research physician, for example, CXR and abdominal ultrasound scan; Xpert and mycobacterial culture on sputum; and sputum, nasopharyngeal and oropharyngeal samples for respiratory pathogens. Participants with pleural effusions or lymphadenopathy were referred to the clinic physician for consideration for aspiration or fine-needle aspiration, as appropriate. Symptom-specific evaluation undertaken by the research physician is summarised below.

### EVALUATION OF COUGH

*First-line evaluation*

Blood samples were collected from all participants with cough for full blood count and, where appropriate, C-reactive protein (CRP), to help distinguish the likelihood of bacterial respiratory infection and, if febrile (>38.3°C), aerobic and anaerobic bacterial blood cultures. If bacterial infection was suspected, oral antibiotics or hospital admission were facilitated when clinically appropriate.

A trained research physician performed spirometry in accordance with American Thoracic Society (ATS) and European Respiratory Society (ERS) standards[1] for any participant with cough $\geqslant$ 8 weeks, or features suggestive of chronic obstructive pulmonary disease (COPD) or asthma, unless respiratory clinic spirometry results were already available. The Advanced Medical Engineering spirometer (AME, Cape Town, South Africa) was used, with calibration checks performed in accordance with the manufacturer's recommendations. Up to eight seated readings were taken, and post-bronchodilator (5 mg nebulised salbutamol) spirometry performed if a spirometry

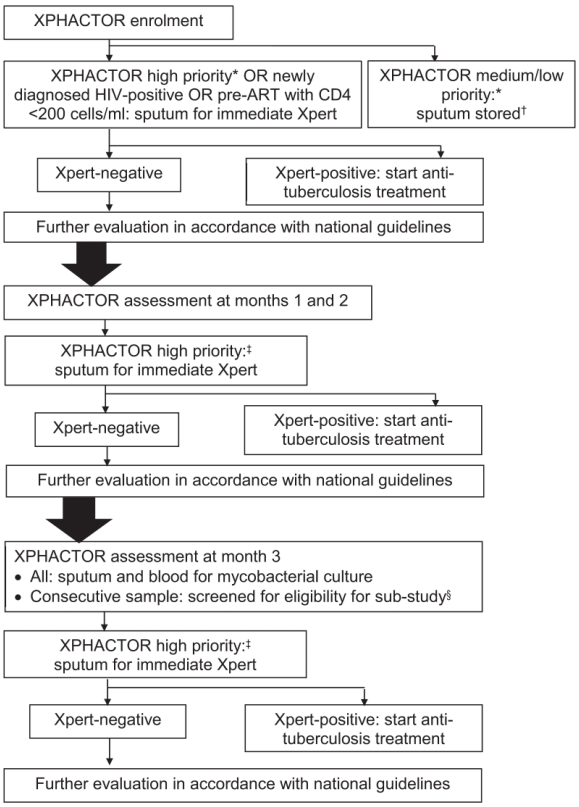


**Figure A.1** XPHACTOR main study flow and procedures. * XPHACTOR algorithm at enrolment: high priority (any of current cough, fever ⩾ 3 weeks, BMI < 18.5 kg/m$^2$, CD4 < 100×10$^6$/l, measured weight loss ⩾ 10% in preceding 6 months or other feature raising high clinical suspicion of TB); medium priority (any of fever <3 weeks, night sweats, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms. † Samples tested using Xpert at the end of the study to enable comparison of sensitivity and specificity of the XPHACTOR study algorithm to detect TB cases against sensitivity and specificity if Xpert had been performed immediately for all with any WHO tool symptom. ‡ XPHACTOR algorithm at monthly follow-up: high priority (any of current cough, fever ⩾3 weeks, night sweats ⩾4 weeks, BMI < 18.5 kg/m$^2$, CD4 < 100x10$^6$/l, measured weight loss ⩾10% in preceding 6 months or other feature raising high clinical suspicion of TB); medium priority (any of fever <3 weeks, night sweats <4 weeks, measured weight loss <10% in preceding 6 months); low priority = no TB symptoms. § Screened by research nurse between October 2013 and April 2014. Eligible if not on anti-tuberculosis treatment and persistent or recurrent TB symptoms, defined as: 1) any of cough, fever or night sweats reported at enrolment and any of aforementioned symptoms reported at 3-month visit; OR 2) ⩾5% measured weight loss at 3-month visit and reported unintentional weight loss. XPHACTOR = Xpert for people attending HIV/AIDS care: test or review? HIV = human immunodeficiency virus; ART = antiretroviral therapy; BMI = body mass index; TB = tuberculosis; WHO = World Health Organization.

abnormality was found. If ATS/ERS within- and between-manoeuvre acceptability criteria were not met, we reported usable curves (good start and satisfactory exhalation). Post-bronchodilator spirometry data were used to confirm airflow obstruction, defined using Global Lung Initiative (GLI) 2012 equations (forced expiratory volume in 1 s [$FEV_1$]/forced vital capacity [FVC] < lower limit of normal at the 5$^{th}$ centile).[2] Post-bronchodilator increase in $FEV_1$ >12% of predicted and >200 ml was used to confirm asthma. Participants were referred to the clinic physician for further management if spirometry confirmed asthma or COPD, or if spirometry was normal but obstructive airways disease likely, or if another spirometry abnormality was identified. Response to any treatment provided was assessed at 4–12 weeks.

If participants had clinical features suggestive of cardiac failure, serum β-natriuretic peptide (BNP) was measured and if levels were >100 pg/ml, further

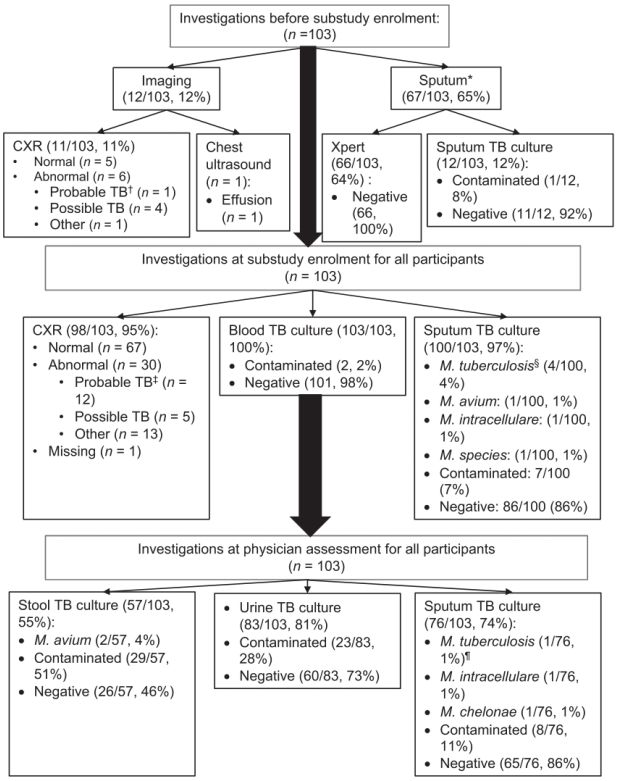


**Figure A.2** Evaluation for TB undertaken for all study participants. * At least one sputum for Xpert or mycobacterial culture; 11 participants had both. † Pleural effusion ($n = 1$). ‡ Pleural effusion ($n = 5$), upper lobe infiltrates ($n = 4$), cardiomegaly with ultrasound confirmed pericardial effusion and pleural effusion ($n = 1$), cardiomegaly with ultrasound confirmed pericardial effusion ($n = 1$) cavitation and pleural effusion ($n = 1$). § Three of the four participants with *M. tuberculosis* on sputum culture had previously had sputum negative for *M. tuberculosis* on culture and/or Xpert before sub-study enrolment. ¶ Participant had previously had negative sputum for mycobacterial culture at enrolment to sub-study. CXR = chest radiograph; TB = tuberculosis.

cardiological evaluation and management was facilitated. If participants had symptoms compatible with *Pneumocystis jiroveci* pneumonia (fever/exertional dyspnoea/tachypnoea) and CD4 count <200 cells/μl, exercise oximetry was undertaken and the participant referred to the responsible clinic physician for further management.

Any suspicious CXR features were discussed with the clinic physician to facilitate further appropriate evaluation or treatment as deemed appropriate, for example, pleural aspiration, computed tomography (CT) imaging, endoscopy or bronchoscopy or presumptive anti-tuberculosis treatment.

### *Second-line evaluation of cough*

If diagnosis for cough was not identified by first-line evaluation, a trial of appropriate treatment was arranged for those with clinical features suggestive of cough due to upper airways disease, angiotensin-converting enzyme (ACE) inhibitors or gastro-oesophageal reflux (GORD). This comprised corticosteroid nasal spray and/or antihistamine, or ear, nose, throat referral if upper airways disease was suspected; switching ACE inhibitor to a suitable alternative; lifestyle advice and trial of a proton pump inhibitor if GORD was suspected. Response to treatment was reviewed at 4–12 weeks. Smoking cessation advice was given to all current smokers, and improvement in cough evaluated at 4–12 weeks post-cessation, if applicable. If no likely cause for cough was identified after second-line evaluation, referral to a respiratory physician was facilitated.

### *Evaluation of ⩾5% unintentional weight loss*

Evaluation aimed to identify a broad spectrum of causes of weight loss, including TB, endocrine

**Table A** Criteria for diagnoses assigned for WHO tool symptoms

| Diagnosis | Criteria |
|---|---|
| Cough | |
| Asthma | Likely: compatible symptoms (recurrent or chronic cough,* wheeze, dyspnoea, chest tightness) without spirometry confirmation;<br>Confirmed: compatible symptoms confirmed using spirometry (GLI criteria,† and improvement in $FEV_1$ after bronchodilator, >12% predicted and >200 ml); OR documented diagnosis |
| COPD | Age >35 years and risk factor (smoking or snuff use or exposure to biomass fuel); AND<br>Likely: compatible symptoms (chronic cough,* wheeze, dyspnoea, sputum production, frequent bronchitis) and either borderline $FEV_1$/FVC on spirometry or absence of spirometry confirmation<br>Confirmed: compatible symptoms confirmed using spirometry;† OR documented diagnosis<br>Not COPD: compatible features but normal spirometry |
| Post-TB CLD | Post-TB bronchiectasis: chronic* productive cough with compatible chest CT scan; OR documented diagnosis<br>Chronic loculated pleural effusion post-TB: documented diagnosis with compatible chest ultrasound or CT scan<br>Likely post-TB CLD: chronic cough* or recurrent respiratory tract infections‡ in patient previously treated for TB, and/or CXR abnormality compatible with previous pulmonary TB (fibrosis, hyperinflation, bronchovascular distortion, bronchiectasis) |
| Lower respiratory tract infection | Symptoms for ⩽3 week duration, and:<br>Cough and ⩾1 lower respiratory tract symptom (fever, sputum production, breathlessness, wheeze, chest discomfort or pain) |
| Upper respiratory tract infection | Symptoms for ⩽3 weeks' duration, and includes:<br>Cold: ⩾1 of cough, nasal symptoms, sneezing, sore throat;<br>Influenza: fever and ⩾1 of headache, myalgia, cough or sore throat |
| Pertussis | Acute cough ⩾2 weeks and symptomatic (⩾1 of: paroxysms of cough, post-tussive vomiting or inspiratory whoop)<br>Likely: no microbiological confirmation and no other likely cause<br>Confirmed: respiratory sample positive for *Bordetella pertussis* |
| Post-infectious cough | Cough duration 3–8 weeks preceded by acute respiratory tract infection and no other likely cause |
| Gastro-oesophageal reflux disease | Compatible symptoms (chronic cough worse with or after meals associated with heartburn or regurgitation, dysphonia, cough resolves during sleep) improving within 3 months with appropriate treatment |
| Upper airway cough syndrome | Chronic* or recurrent cough and compatible symptoms (post-nasal drip, nasal discharge or congestion, catarrh) responding to appropriate treatment; OR documented diagnosis<br>Likely: symptoms as above but no trial of treatment, and no other likely cause |
| ACE inhibitor cough | Chronic* dry cough resolving within 12 weeks of stopping ACE |
| Tobacco-related cough | Current smoker or snuff user and no other likely cause for cough |
| Unintentional weight loss | |
| Malignancy | Compatible clinical features with confirmatory imaging or histology; OR documented diagnosis |
| Food insecurity | Compatible HFIAS score (severe food insecurity) |
| Type 2 diabetes | HbA1c ⩾ 48 mmol/mol (6.5%) and compatible symptoms |
| Hyperthyroidism | Low TSH with raised free thyroxine level<br>Subclinical/biochemical: low TSH with normal free thyroxine level |
| Depression | Compatible history and PHQ-9 score ⩾10; OR documented diagnosis |
| Stress-related | History of loss of appetite triggered by acute stressful event |
| Treatment-related | History of loss of appetite/reduced food intake due to side effects of medical treatment (e.g., switch to second-line ART) |
| Fever/night sweats | |
| Infection | Likely: compatible symptoms responding to appropriate treatment or resolving spontaneously<br>Confirmed: compatible symptoms with microbiological confirmation |
| Perimenopause | Compatible symptoms, confirmed with FSH if age <45 years, and no other likely cause |
| Diagnoses relevant to >1 WHO tool symptom | |
| Confirmed TB | Positive result on 1) Xpert or 2) line-probe assay or 3) *M. tuberculosis* culture, from any sample collected within 6 months of sub-study enrolment |
| Clinical TB | Anti-tuberculosis treatment started within 6 months of sub-study enrolment in the absence of microbiological confirmation |
| Heart failure§ | Likely: compatible symptoms (orthopnoea, dyspnoea, peripheral oedema) and serum BNP > 100 pg/ml<br>Confirmed: compatible symptoms and echocardiogram; OR documented diagnosis |
| Alcohol misuse¶ | Hazardous alcohol consumption (FAST score ⩾3) and no other likely cause |
| Unexplained, symptom resolved spontaneously | No cause for symptom identified, and symptoms resolved by final study visit |
| Unexplained | No cause identified |

* Defined as cough ⩾8 weeks.
† Defined as no improvement in $FEV_1$ after bronchodilator and either 1) GLI[2] equation criterion $FEV_1$/FVC <lower limit of normal, or 2) GOLD $FEV_1$/FVC < 0.7. GOLD criteria were used in spirometry results from medical records.
‡ Defined as ⩾2 episodes in preceding 6 months.
§ Considered as possible cause of chronic cough or weight loss.
¶ Considered as possible cause of weight loss or sweats on withdrawal.
WHO = World Health Organization; GLI = Global Lung Initiative; $FEV_1$ = forced expiratory volume in 1 s; COPD = Chronic Obstructive Lung Disease; FVC = forced vital capacity; TB = tuberculosis; CLD = chronic lung disease; CT = computed tomography; CXR = chest radiograph; ACE = angiotensin-converting enzyme; HFIAS = Household Food Insecurity Access Score; HbA1c = glycated haemoglobin; TSH = thyroid stimulating hormone; PHQ9 = Patient Health Questionnaire 9; ART = antiretroviral therapy; FSH = follicle stimulating hormone; BNP = brain natriuretic peptide; FAST = Fast Alcohol Screening Test; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

disorders, malignancy, depression, inadequate access to food and drug misuse.

Blood samples were collected from all participants with weight loss for renal, liver and thyroid function, full blood count and glycated haemoglobin (HbA1c; to identify type 2 diabetes mellitus), and clinically significant results were reported to the clinic physician for further management. If participants reported diarrhoea, stool samples were collected for microscopy, bacterial culture, parasitology and for *Clostridium difficile*, if antibiotics had been taken in the preceding 12 weeks.

The Patient Health Questionnaire-9 (PHQ-9)[3] was used to screen all sub-study participants for depression. This score categorises depression as 0–4 (none or minimal), 5–9 (mild), 10–14 (moderate), 15–19 (moderately severe) and 20–27 (severe). Participants with scores of $\geqslant$10 were evaluated further by the research physician, and referred to the clinic physician or psychology service if deemed clinically depressed. The Fast Alcohol Screening Test (FAST)[4] was used to screen for hazardous alcohol consumption (FAST score $\geqslant$ 3) and all participants were asked about drug misuse. If FAST score was $\geqslant$3, brief intervention was provided and, if appropriate, participants were referred to drug services.

We used the Household Food Insecurity Access Scale (HFIAS)[5] to measure food access, categorising the scale as 1) 'food secure', 2) 'mildly food insecure access', 3) 'moderately food insecure access' or 4) 'severely food insecure access'. A HFIAS score of 4 was deemed a cause of unintentional weight loss. Participants with food insecurity were referred to the clinic dietician.

Clinical features suggestive of possible malignancy were discussed with the clinic physician to facilitate further appropriate evaluation or treatment as deemed appropriate.

### *Evaluation of fever or night sweats*

Self-recorded participant temperature measurements were reviewed. If a likely focus of infection was identified, then relevant samples were submitted for appropriate microbiological evaluation such as midstream urine (bacterial culture), stool (bacterial culture, parasitology, *Clostridium difficile*), sputum (bacterial culture), blood (malaria film) or swabs (bacterial culture). If deemed clinically appropriate, antibiotics were provided and the participant reviewed to assess response to treatment.

If no likely focus infection was identified, blood was collected for renal, liver and thyroid function, full blood count, CRP, HbA1c, aerobic and anaerobic culture if documented fever >38.3°C; and urine for microscopy and culture. Abdominal ultrasound was arranged and CXR, if either no recent film or no film since onset of symptoms.

A clinical diagnosis was made if no other more likely cause was identified, and symptoms were suggestive of perimenopausal vasomotor symptoms in females aged $\geqslant$45 years; in younger cases, blood samples were collected to determine follicle-stimulating hormone (FSH) levels.

If, during in-patient treatment, specialist referral or further evaluation such as lumbar puncture, CT imaging (abdomen, chest, sinus), fine-needle aspiration, bone-marrow aspiration or pleural aspiration were deemed necessary, this was facilitated by the clinic physician.

### *References*

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4 Hodgson R, Alwyn T, John B, Thom B, Smith A. The FAST Alcohol Screening Test. Alcohol Alcohol 2002; 37: 61–66.
5 Coates J, Swindale A, Bilinsky P. Household Food Insecurity Access Scale (HFIAS) for measurement of household food access: indicator guide (v 3). Washington, DC, USA: Food and Nutrition Technical Assistance Project, Academy for Educational Development, 2007.

RÉSUMÉ

**OBJECTIF** : Identifier les causes des symptômes suggestifs de tuberculose (TB) parmi les personnes vivant avec le virus de l'immunodéficience humaine (PVVIH) en Afrique du Sud.

**MÉTHODE** : Un échantillon consécutif de patients d'un dispensaire VIH ayant des symptômes suggestifs de TB ($\geqslant$1 parmi toux, perte de poids, fièvre ou sueurs nocturnes) lors de l'enrôlement et à 3 mois, mais des premières investigations à la recherche de TB négatives, a été systématiquement évalué grâce à des protocoles standard et des diagnostics assignés grâce aux critères standard. La TB a été « confirmée » lorsque *Mycobacterium tuberculosis* a été identifié dans les 6 mois de l'enrôlement, et « clinique » si le traitement a démarré sans confirmation microbiologique.

**RÉSULTATS** : Parmi 103 participants, 50/103 pré ART et 53/103 sous ART, respectivement ; 68% contre 79% ont été des femmes ; d'âge médian 35 contre 45 ans, et le taux médian de CD4 de 311 contre 508 cellules/mm$^3$. Soixante-douze patients (70%) ont eu une perte de poids mesurée $\geqslant$5% et 50 (49%) une toux. Les diagnostics finaux les plus fréquents ont été une perte de poids due à une insécurité alimentaire majeure ($n = 20$, 19%), une TB ($n = 14$, 14% : confirmée, $n = 7$ ; clinique, $n = 7$), d'autres infections des voies respiratoires ($n = 14$, 14%), une affection pulmonaire post-TB ($n = 9$, 9%). La base du diagnostic de TB a été l'imagerie ($n = 7$), la confirmation bactériologique des crachats ($n = 4$), l'histologie, la ponction lombaire et autres ($n = 1$ chacun).

**CONCLUSION** : Les PVVIH ayant des symptômes de TB persistants requièrent davantage d'évaluation à la recherche de TB avec toutes les modalités disponibles et à la recherche de problèmes de sécurité alimentaire chez ceux qui ont perdu du poids.



RESUMEN

**MARCO DE REFERENCIA**: El estudio tuvo por objeto reconocer las causas de los síntomas indicativos de tuberculosis (TB) en las personas infectadas por el virus de la inmunodeficiencia humana (PLVIH) en Suráfrica.

**MÉTODO**: Una muestra consecutiva de pacientes que acudían a la consulta del VIH y presentaban síntomas indicativos de TB (uno o varios síntomas como tos, pérdida de peso, fiebre o sudoración nocturna) en el momento de la inscripción y a los 3 meses y cuyas investigaciones iniciales de la TB habían sido negativas se evaluó de manera sistemática mediante protocolos normalizados y los diagnósticos se asignaron según criterios definidos. El diagnóstico de TB se consideró 'confirmado' cuando se detectaba el *Mycobacterium tuberculosis* en los primeros 6 meses después de la inscripción en la consulta y 'clínico' cuando se iniciaba tratamiento sin confirmación microbiológica.

**RESULTADOS**: De los 103 participantes, 50 no recibían aun tratamiento antirretrovírico y 53 ya lo recibían; respectivamente, el 68% y el 79% eran de sexo femenino, la mediana de la edad era 35 y 45 años; la mediana de la cifra de linfocitos CD4 fue 311 y 508 células/mm$^3$. Setenta y dos pacientes (70%) presentaron una pérdida de peso cuantificada $\geqslant$5% y 50 presentaron tos (49%). Los diagnósticos definitivos más frecuentes fueron pérdida de peso debida a una inseguridad alimentaria grave en pacientes ($n = 20$, 19%), TB ($n = 14$, 14%: 7 diagnósticos confirmados y 7 clínicos), otras infecciones de las vías respiratorias ($n = 14$, 14%) y enfermedad pulmonar crónica posterior a la TB ($n = 9$, 9%). El diagnóstico de TB se fundamentó en las imágenes ($n = 7$), la confirmación bacteriológica en el esputo ($n = 4$), el examen histológico, la punción lumbar y otros medios ($n = 1$, en cada caso).

**CONCLUSIÓN**: Es necesario investigar la presencia de TB en las PLVIH y síntomas persistentes indicativos de TB, mediante todos los medios al alcance y la inseguridad alimentaria en las personas que presentan pérdida de peso.

# 9) Discussion and conclusions

## 9.1. Introduction

This thesis examined TB screening and investigation strategies for adults attending routine HIV care in a LMIC setting with an HIV-associated TB epidemic. The research was undertaken at a time when the TB diagnostic landscape was rapidly evolving and CD4 count determined eligibility for ART. The rollout of new diagnostic tools in the "real world" raised questions around resource prioritisation and how to use them most efficiently. Contrary to expectations, the advent of Xpert has not resulted in greater numbers of individuals starting TB treatment, but the proportion of bacteriologically-confirmed diagnoses has increased, and when Xpert is positioned at point-of-care TB treatment is started faster.[101, 181, 225-227] The role of LF-LAM is very limited.[112]

Since this research was undertaken the gap between the number of TB diagnoses notified to national programmes and the number estimated by the WHO each year remains large.[2] Now, more sensitive versions of the diagnostic tests used in this research are either available,[90] or being developed;[114, 228, 229] immediate ART is recommended for all PLHIV; and different models of ART delivery are in place. The recommended screening tool for intensified TB case finding in PLHIV remains the same, developed using data largely from the pre-ART era, in order to enable rapid scale-up of IPT. The performance of a screening test will differ when it is used in different settings and different populations from that in which it was originally developed. This is illustrated by the performance of the WHO tool for TB screening in individuals on ART amongst whom the tool is less sensitive but more specific for TB.[42] In the future if more PLHIV are on ART and for longer periods of time, consequently undergoing repeated rounds of TB screening, which is also known to reduce the sensitivity of the tool, then more TB diagnoses will be missed by the tool. The improved specificity will however generate fewer false-positives on screening, thus reducing the numbers undergoing unnecessary diagnostic testing. The hoped-for decline in the burden of HIV-related TB will also reduce the PPV of the WHO tool, so that an even smaller proportion of those who report symptoms will actually have TB. Nevertheless, screening PLHIV for TB remains an important strategy in the goal of ending the global TB epidemic, as well as on an individual level to reduce suffering. World leaders pledged only last year to regularly screen all PLHIV and to finding the missing people with TB.[38]

This chapter summarises the main findings from the research undertaken, making comparison with the published literature; discusses the implications arising from this

work, its strengths and limitations; and makes recommendations for HIV programmes and future research. It also considers the possible impact on these research findings of the aforementioned changes in HIV care and "next-generation" TB diagnostics. Now that ART is started irrespective of CD4 cell count, the entity of pre-ART care for those waiting to reach a predetermined CD4 threshold for ART initiation should no longer exist. Therefore, the discussion will focus more on the findings in the on ART group, as this is of more relevance to HIV programmes.

The flow of the discussion will follow the pathway depicted in **Figure 1-2** (the aims of the thesis), which reflects the journey PLHIV attending for routine care may undergo following TB screening.

## 9.2. Summary of findings and comparison with published studies

### *9.2.1. Options for screening for TB among people attending for HIV care*

#### *WHO 4-symptom TB screening tool*

The XPHACTOR study enabled a prospective evaluation of the performance of the WHO tool in the era of ART, uniquely, and of particular relevance since the advent of treat-all, in a large cohort established on ART. At enrolment, almost one-third of those on ART reported WHO tool symptom(s), most commonly cough and weight loss, and 3.1% (95% CI 2.4, 3.8) fulfilled our case definitions for clinical and confirmed TB combined. In the on ART group the sensitivity and specificity of the WHO tool for clinical and confirmed TB combined was 68.4% (95% CI 56.9, 78.4) and 70.7% (95% CI 68.9, 72.6) respectively. The sensitivity was slightly lower for bacteriologically-confirmed TB (62.3%, 95% CI 49.0, 74.4), but the specificity was unchanged.

Most published studies which have investigated the performance of the WHO tool for TB screening have focussed on populations comprising those newly diagnosed HIV-positive,[73, 79] those preparing to initiate ART,[54, 56, 72] or those screened prior to IPT.[75, 77] Studies which have screened individuals on ART using the WHO tool have excluded large numbers of participants because they could not produce sputum or were missing data,[138, 143] or included individuals who had already been extensively pre-screened for TB.[143] These factors would have generated biased estimates of sensitivity (probably underestimations) and limit the generalisability of their findings to routine HIV care settings. Furthermore, compared to XPHACTOR, PLHIV in these aforementioned studies had been on ART for a

shorter length of time (1-2 years), and had lower median CD4 cell counts. Ours was a more "mature" population, reflected by their longer duration on ART (median 4 years) and higher median CD4 count (436 cells/$mm^3$). This also explains the lower prevalence of confirmed TB in our on ART group of 2.4%, compared with 5.4-5.9% in comparable studies.[138, 143] Our study findings are likely to provide a better indication of the workload associated with, and the diagnostic yield from intensified TB case-finding in the future, among HIV-positive individuals attending for routine care. These individuals will probably have higher CD4 cell counts at ART initiation, have been taking ART for longer, and should have been repeatedly screened for TB.

### Prevalence of WHO tool symptoms in XPHACTOR

In XPHACTOR, one-third of those on ART reported WHO tool symptoms at enrolment, and this reduced to just over one-quarter if those diagnosed with TB were excluded. This is in accordance with studies conducted in HIV clinic or MCH settings, in which 33-39% of participants who were on ART reported WHO tool symptoms at study enrolment.[142, 147, 148] TB screening studies which have excluded individuals unable to expectorate sputum are likely to have overestimated the prevalence of cough.[138, 143, 154] We did not exclude participants who were unable to produce sputum at enrolment, and therefore our findings are more generalisable to HIV care settings. We also found that cough was the most commonly reported symptom. Even when the same participants were screened over a 3-month period using the WHO tool at monthly intervals, and having excluded those who were diagnosed with TB, cough remained the most common symptom reported on screening. Recurrent or persistent cough will impact on the quality of life of sufferers, and therefore capacity is needed at all levels of healthcare to enable timely evaluation, diagnosis, and provision of appropriate treatment for these patients.

We found that 10% of XPHACTOR participants in the on ART group reported WHO tool symptoms on screening at the 3-month visit, and this was after excluding individuals who had been diagnosed with TB during the course of the study. Extrapolating our findings to ART programmes suggests that a large volume of confirmatory diagnostic testing will be required when the WHO tool is used as intended, to screen the same population at every clinical encounter. To the best of my knowledge there are no other published data which report the evolution of WHO tool symptoms when the same individuals are repeatedly screened.

**Performance of the WHO tool in XPHACTOR dataset**

We found that the WHO tool was less sensitive (62.3% vs. 91.7%) but more specific (70.7% vs. 66.5%) for confirmed TB in the on ART compared with the pre-ART group. NPV was high (>98%) for both groups at confirmed TB prevalence of 3.1%, and at prevalence of confirmed and clinical TB combined of 7.3%, but PPV was <10%. Therefore the WHO tool worked well, as designed, for ruling out TB in both groups, but the vast majority of those reporting symptoms did not have TB and would have required unnecessary diagnostic testing for TB. (**Table 4-3**) If the TB prevalence falls in HIV clinic settings, as is hoped for with PLHIV initiating ART at higher CD4 cell counts, the PPV of the WHO tool will be further reduced. Therefore, in resource-constrained settings the rollout of treat-all may require alternative TB screening and diagnostic strategies to the WHO tool followed by a diagnostic test. Failing this, HCWs may be even less likely to adhere to TB screening and investigation algorithms in heavily pressurised LMIC settings.[68] PLHIV may tire of repeatedly undergoing investigation after screening with a tool that lacks specificity. One could speculate that they might even prefer not to disclose the presence of WHO tool symptoms when repeatedly screened, to avoid the associated obligatory reattendances for test results and follow-up.

In our pre-ART group the XPHACTOR algorithm was less sensitive than the WHO tool (75.0% [95% CI 42.8, 94.5] vs. 91.7% [95% CI 61.5, 99.8]) and more specific (72.3% [95% CI 68.1, 76.2] vs. 66.5% [95% CI 62.1, 70.7]) for confirmed TB. Sensitivity and specificity were similar for both the WHO tool and the XPHACTOR algorithm in the on ART group. These findings are unsurprising given that the XPHACTOR algorithm was intended to improve the specificity for TB by "tightening" WHO criteria, i.e. requiring duration of fever, or evidence of weight loss; and as expected this was at the expense of sensitivity. The WHO tool was designed to maximise sensitivity and developed in a dataset comprising a pre-ART population.

XPHACTOR data afforded the opportunity to prospectively evaluate the performance of the WHO tool in a population attending for HIV care who had been previously screened for TB. In the original meta-analysis which developed the WHO tool, the sensitivity of the WHO tool amongst those previously screened for TB was 40.5% (95% CI 16.6, 69.9), the wide confidence intervals reflecting the small numbers in that group.[80] When combining our pre- and on ART groups, who were established in care and therefore should have been previously screened for TB, we found the sensitivity of the WHO tool for confirmed TB was

greater, 67.1% (95% CI 55.1, 77.7). This reflects our study population being more representative of HIV clinic attendees, who are more likely to be symptomatic and at higher risk of TB than participants in the studies providing this data for the meta-analysis, who were mainly enrolled from community-based surveys.[134, 135] It therefore provides a more accurate reflection of WHO tool performance amongst those previously screened for TB in clinic settings.

## Limitations of the WHO tool

Strategies to find the missing millions with TB also need to address how best to identify asymptomatic individuals with microbiologically-confirmed TB, and individuals with extrapulmonary TB. A symptom-based TB screening tool will obviously not identify asymptomatic TB, and extrapulmonary TB is less likely to be identified by a screening tool which was developed using a reference standard of culture-confirmed TB from sputum samples. These issues are discussed below.

### Asymptomatic Tuberculosis

Two studies in South Africa, which systematically screened PLHIV for pulmonary TB just prior to ART initiation[230] or as part of pre-ART care,[231] reported prevalences of asymptomatic TB of 4% in the larger study (28/654)[230] and 8.5% (18/213) in the smaller study.[231] In these studies, individuals with asymptomatic TB had an intermediate degree of immunosuppression, as suggested by median CD4 counts of 136-249 cells/$mm^3$ being in between those of PHLIV with active TB (68-148 cells/$mm^3$) and those with negative TB microbiology who did not require TB treatment (249-322 cells/$mm^3$).[230, 231] 56% of these individuals developed TB symptoms within a median of 28 days. When compared with a group of PLHIV with symptomatic TB enrolled from a TB clinic in the same setting, those with asymptomatic TB were more likely to be smear-negative.[231] The numbers with asymptomatic TB in both of these studies were small, but the phenomenon of asymptomatic bacteriologically-confirmed TB is well described when PLHIV are systematically screened using sensitive diagnostic tests.[56, 232, 233]

In XPHACTOR, all study participants were asked to provide a sputum sample at enrolment, which was tested with Xpert immediately or stored for later testing. 0.7% (27/3678) of our participants fulfilled the case definition for confirmed TB but reported no WHO tool symptoms at enrolment, suggesting that asymptomatic TB might be less common in populations established in HIV care. The proportion of individuals with asymptomatic TB

may change over time as the characteristics of people attending for HIV care changes, hopefully shifting towards people with higher CD4 cell counts, whom the aforementioned studies indicate are less likely to have asymptomatic TB. [230, 231]

A model of TB as a continuum of disease from infection with MTB to clinically active disease is currently considered more appropriate than the traditional binary concept of an individual switching directly from latent TB infection to active TB disease.[234] Alternative screening modalities to the WHO tool are needed to identify asymptomatic disease. However, one could postulate that repeatedly screening PLHIV using a symptom screen, should first identify (and treat) those who are most unwell. Those with asymptomatic TB will hopefully passively present for care if they do develop symptoms, or if disease has progressed, will be identified at the next round of screening. There is currently insufficient data on survival outcomes in PLHIV with asymptomatic TB who do not receive TB treatment to suggest that they fare any worse than those without TB, so this phenomenon might not be clinically important.[230]

**Extrapulmonary Tuberculosis**

20/153 (13%) of XPHACTOR study participants who fulfilled the case definitions for TB and had the site of disease recorded had evidence of extrapulmonary disease, of whom 15 only had extrapulmonary disease. In **Research Paper 4 (“Causes of TB symptoms in HIV-positive adults”)** over half (8/14) of participants initiating TB treatment did so based on the results of investigations for extrapulmonary TB (abdominal ultrasound, 6; LNA; 1; lumbar puncture, 1). Although the number of participants investigated in this study was small, most (65%) had submitted sputum samples for mycobacteriology prior to enrolment. In actual fact, all should already have undergone investigation as part of the XPHACTOR main study procedures, because everyone was asked to provide sputum for testing with Xpert (immediately or stored for later testing) at enrolment, and a further sample was requested at the 3-month visit for mycobacterial culture. We found that only 1997/3722 (53.7%) of study participants were able to produce a sputum sample at enrolment. This highlights the limitation of sputum-based diagnostics, in terms of difficulties in collecting sputum and for diagnosing extrapulmonary TB, and the need for access to other investigation modalities.

The studies included in the WHO metanalysis mainly collected sputum samples for mycobacterial culture, i.e. the WHO tool was developed using a reference standard of bacteriologically-confirmed pulmonary TB.[80] This reflects the reality of investigating TB in LMIC and the data that were available at the time the meta-analysis was undertaken,

when there was an urgent need for a simple TB screening tool to facilitate rollout of IPT. Individuals with extrapulmonary disease are also likely to present with cough, fever, unintentional weight loss and night sweats. However, it may be that extrapulmonary TB is more likely to be missed when screening is undertaken using the WHO tool because of the reference standard used to develop the tool. Extrapulmonary disease is more common in individuals with advanced HIV disease, and may become less common as PLHIV initiate ART at higher CD4 counts, although those who drop out of HIV care may present at a later stage with advanced immunosuppression.

### *Alternative TB screening and diagnostic algorithms examined in this thesis*

This thesis looked at alternatives to the recommended algorithm of WHO symptom screen, followed by Xpert if WHO tool symptoms were reported; and if Xpert-negative but symptomatic, then further investigation in line with the WHO smear-negative pathway (mycobacterial culture, chest x-ray, and if indicated a trial of antibiotic). This research, uniquely, was undertaken in the context of active case finding for TB amongst PLHIV established in HIV care, in contrast to most other published studies that have focussed on screening individuals prior to ART initiation or at new HIV-positive diagnosis.

In summary we found that the sensitivity of LF-LAM, when used to screen study participants with CD4 cell count < 200 cells/$mm^3$, was too low to be useful as a screening test and certainly could not be recommended to replace the WHO tool (**Chapter 5 - Research Paper 1, "TB Screening with LAM in an HIV Clinic"**). When a grade 2 cut-off was used, which is equivalent to the current recommended designation for a positive test, sensitivity was only 5.4% (95% CI 1.1, 14.9) for confirmed and clinical TB combined. There were only three positive LF-LAM results using this cut-off amongst 56 individuals who fulfilled these TB case definitions, so we could not explore the sensitivity of LF-LAM in those with CD4 <100 cells/$mm^3$.

In **Research Paper 2 (Chapter 6 - "Clinical Score for TB in HIV-positive adults in South Africa")** a triage tool was developed to prioritise individuals reporting WHO tool symptoms for diagnostic testing for TB. The score was designed to be simple, using information readily available at primary healthcare level; and was derived from a clinical prediction model developed using multivariate analysis. The clinical score comprised ART status (categorised as on ART > 3 months vs. pre-ART or ART < 3 months), BMI (<18.5 vs. 18.5-24.9 vs. ≥25 kg/$m^2$), CD4 cell count (<200 vs. 200-349 vs. ≥350 cells/$mm^3$), and number of WHO

tool symptoms (1 vs. >1 symptom). Prioritising a diagnostic test for symptomatic individuals with a cut-off score of ≥3 would have avoided one-third of the volume of Xpert tests required in our study population, at the expense of missing 3% of TB diagnoses in those not tested.

**Research Paper 3 (Chapter 7 - "Investigating TB if initial Xpert is negative")** looked at the diagnostic yield from undertaking a repeat Xpert test on a fresh sputum sample amongst individuals with an initial negative test result. The sputum sample was collected at attendance for the result of the initial Xpert. This study was restricted to individuals at highest risk of TB, defined by CD4 <200 cells/mm$^3$ or those newly diagnosed HIV-positive, in order to ensure sufficient TB diagnoses to enable comparison with the Xpert-negative algorithm. Amongst 27/227 TB diagnoses in this study, only five were identified by the repeat Xpert test, and the remainder started TB treatment during study follow-up mainly on the basis of compatible imaging (10) or mycobacteriology (culture-positive for *MTB* [4], further Xpert positive [4]). This highlights the need for good access to imaging, both chest radiograph and ultrasound scan. Furthermore, in those at high risk of TB, further investigation for TB should not be halted following an initial negative Xpert result.

### *Published studies reporting other TB screening options*

Alternative TB screening options can be subdivided into i) methods to replace the WHO tool; ii) a second step to triage WHO-tool-positive individuals for investigation with Xpert (sequential screening); and iii) methods which include the WHO tool in order to improve algorithm sensitivity (parallel screening).[235] Sequential screening strategies, by virtue of further screening only those who test positive by the initial test, will lose sensitivity but improve upon both the specificity and PPV compared to the first screening test. Parallel screening strategies, whereby all individuals have multiple screening tests, and screen negative only if all tests are negative, lose specificity but improve upon sensitivity compared with the individual screening tests.[71]

Screening tests perform differently in different settings and the selected screening strategy is determined not just by financial constraints, but also by the prevalence of TB, the setting and the potential risks of failing to identify TB. In a community-level setting, where the prevalence of TB is lower, individuals are also less likely to be symptomatic and less likely to have TB. A screening test designed in a hospital setting, where the prevalence of TB is likely to be higher and attendees are also more likely to be unwell and

more symptomatic, will have lower specificity in a community based setting. This will result in large numbers of individuals undergoing unnecessary diagnostic tests for TB.

**Table 9-1** summarises alternative screening strategies pertinent to the research undertaken for this thesis, i.e. replacements for the WHO tool and triage tools for those reporting WHO tool symptoms. The studies presented in table 9-1 have been evaluated in ambulatory PLHIV, or are currently used instead of the WHO tool, and largely published either following the 2010 recommendation to use the WHO tool or after this research was commenced.

### *WHO high priority target product profile (TPP) for a triage test[84]*

The need for a triage test has been identified, ideally for use at community-level or as a minimum at primary or higher level healthcare, to reduce the volume of diagnostic testing required for TB. The role of the triage test is to identify, amongst symptomatic individuals, those most likely to have TB who should therefore be prioritised for diagnostic testing.[84] A test that needed minimal training and infrastructure, and required sputum or non-sputum based samples was envisaged. Minimum product requirements were agreed, by consensus: sensitivity (>90%) and specificity (>70%) for bacteriologically confirmed pulmonary TB; cost <US$2; availability of results within 30 minutes; and with ease of access to a confirmatory test deemed a prerequisite. According to the WHO report,[84] this product was not intended to be used as a TB screening tool, but rather to triage individuals attending because of symptoms suggestive of TB for diagnostic testing, or for anyone attending for care with a risk factor for TB such as HIV. The latter is confusing as it suggests that the triage test could be used as a TB screening tool for PLHIV attending for care, even though the report stipulates that separate TPPs for a screening test (which requires higher sensitivity) still need to be agreed upon. Investigators have applied these minimum criteria of sensitivity and specificity when evaluating alternative screening algorithms to the WHO tool; to date only POC CRP, as discussed below, fits these criteria.[236]

**Table 9-1 Alternative TB screening and investigation options for ambulatory PLHIV in LMIC**

| Role | Method | Population<br>Median CD4<br>Author year | TB case definition | TB prevalence | Sensitivity | Specificity | Comment |
|---|---|---|---|---|---|---|---|
| **Replacement for WHO tool** | LF-LAM | HTC<br>CD4 213<br>Drain[151] 2016 | Sputum C+ | 123/675 (18.2%) | 31% | 92.0% | Pre-2014 Gd 1 cut off used, overestimates sensitivity + underestimates specificity<br>Sensitivity better in CD4<100[107] |
| | | Prior to ART<br>CD4 211<br>Balcha[104] 2014 | Sputum or LNA C+ or GXP+; or clinical TB | 128/757 (16.9%) confirmed<br>148/757 (19.6%) clinical + confirmed | 25.8% for confirmed TB | 92.8% | Pre-2014 Gd 1 cut off<br>Sensitivity better in CD4<100<br>"Not TB" required negative TB culture & no TB Rx<br>Followed to 6 months<br>Bias and not generalisable as did not enrol those unable to produce sputum |
| | | Prior to ART<br>CD4 170<br>Lawn[105] 2012 | Sputum C+ | 85/516 (16.4%) | 28.2% | 98.6% | Pre-2014 Gd 1 cut off<br>Sensitivity better in CD4<100 |
| | | ANC<br>CD4 437<br>(54% on ART)<br>LaCourse[141] 2016 | Sputum C+ | 7/288 (2.4%) | 0/7 | 95.1% | Pre-2014 Gd 1 cut off used<br>Possible selection bias as high refusal rate for participation |
| | CRP | HTC<br>CD4 306<br>Shapiro[237] 2018 | Sputum C+ | 42/425 (10%) | CRP >5: 90.5%<br>CRP >10: 78.6% | CRP >5: 58.5%<br>CRP >10: 72.3% | Specificity greater if CD4>200<br>Lab based CRP<br>Retrospective design, verification bias |
| | | Prior to ART<br>CD4 171<br>Lawn[238] 2013 | Sputum C+ | 81/496 (16.3%) | CRP ≥5: 90.1%<br>CRP ≥10: 85.2% | CRP ≥5: 43.9%<br>CRP ≥10: 57.6% | Lab based CRP |
| | | Prior to ART<br>CD4 165<br>Yoon[236] 2017 | Sputum C+ | 163/1177 (13.8%) | CRP ≥5: 92·6%<br>CRP ≥8: 90.2%<br>CRP ≥10: 89·0% | CRP ≥5: 59·7%<br>CRP ≥8: 69·6%<br>CRP ≥10: 72·1% | GXP+ C-ve deemed not TB<br>"Not TB" required negative TB culture<br>POC CRP |
| | CXR | New enrolees to HIV clinic<br>58% on ART<br>CD4 336<br>Nguyen[142] 2016 | Sputum C+ | 28/397 (7.1%) | CXR suggestive of active PTB: 71% | 71% | Excluded those who did not complete all evaluations – selection bias |

| Role | Method | Population<br>Median CD4<br>Author year | TB case definition | TB prevalence | Sensitivity | Specificity | Comment |
|---|---|---|---|---|---|---|---|
| | | Attending PHC for HIV care<br>50% on ART<br>CD4 215<br>Gounder[239] 2011 | C+ or SM+ or histology on sputum / LNA / blood | 30/422 (7%) | CXR suggestive of active PTB: 95% | 47% | Convenience sample[140] – selection bias, overestimates sensitivity, not generalisable |
| | | Prior to ART<br>CD4 100<br>Bassett[54] 2010 | Sputum C+ | 158/825 (19%) | Any abnormality on CXR: 83% | 35% | |
| | | Prior to ART<br>CD4 120<br>Hanifa[55] 2012 | Sputum C+ | 64/300 (21%) | Any abnormality on CXR: 85.5%<br>CXR suggestive of active PTB: 77.4% | 48.1%<br><br>63.4% | Followed to 3-6 months |
| | Alternative symptom screen<br>ID-TB/HIV | Prior to ART<br>CD4 242<br>Cain[72] 2010 | C+ on sputum / urine / blood / stool / LNA | 267/1748 (15%) | 93% | 36% | Contributed to WHO meta-analysis.<br>Age > 6yrs<br>Used in SE. Asia<br>In preceding 4 wks: Any cough or fever or NS >3w |
| **Triage test if WHO-tool positive** | Clinical Score | Prior to ART<br>625 WHO tool positive<br>CD4 212 (N=791)<br>Balcha[176] 2014 | Sputum or LNA C+ or GXP+; | 115/625 (18.6%) | Score ≥2: 96/116 (83%)<br><br>Score ≥4: 19/116 (16%) | 291/509 (57%)<br><br>494/509 (97%) | Followed to 6 months<br>"Not TB" required negative TB culture & no TB Rx<br>Score (each assigned 1 point): cough, Karnofsky score ≤80, MUAC <20 cm, lymphadenopathy, HB <10<br>Not enrolled if unable to produce sputum (same population as [104]) |
| | CRP | HTC<br>All WHO tool positive<br>CD4 268<br>Shapiro[237] 2018 | Probable TB = TB Rx / GXP+ / C+ / Sm+ within 3 m | 78/749 (10.4%) | CRP ≥5: 98·7% | CRP ≥5: 48.3% | Retrospective analysis<br>Case definition applied retrospectively<br>TB investigation only if clinician requested – verification bias<br>Lab based CRP on stored samples |

ANC, antenatal clinic; ART, antiretroviral therapy; C+, culture-positive MTB; C-, culture-negative for MTB; CRP, C-reactive protein mg/L; CXR, chest radiograph; GXP+, Xpert-positive; HB, haemoglobin g/dL; HTC, HIV testing and counselling services; LNA, lymph node aspirate; MUAC, mid upper arm circumference; PHC, Primary health clinic; POC, point of care; SM, TB microscopy; TB Rx, TB treatment

### *Alternative screening methods to replace the WHO tool*

Methods replacing the WHO tool include LF-LAM, POC CRP, chest radiograph, and the ID-TB/HIV algorithm which is used in Southeast Asia.[72] In addition, screening using sputum Xpert or mycobacterial culture has been examined and advocated in all PLHIV prior to ART initiation in settings with HIV-associated TB epidemics.[54, 56, 188] 2017 South African HIV clinicians society guidelines recommend, if feasible, sputum mycobacterial culture for all individuals with CD4 count <200 cells/mm$^3$ as part of TB screening prior to IPT initiation.[62] I will not discuss further the strategy of "investigating all", as it is impractical and too expensive to use for the regular TB screening of PLHIV established in care in LMIC. The research undertaken in this thesis sought to prioritise use of limited resources in a population established in HIV care.

Published studies examining alternative screening methods have in general been undertaken at very specific milestones in an HIV-positive individual's journey through care, i.e. at new HIV diagnosis, or prior to ART or IPT initiation, when the likelihood of active TB and the negative impact of a missed TB diagnosis are greatest. These studies are summarised in **Table 9-1** and discussed in further detail below.

#### LF-LAM

We found LF-LAM was too insensitive for use as a TB screening tool for individuals attending for routine HIV care, even when restricted to those with CD4 count <200 cells/mm$^3$. Sensitivity was only 7.5% and specificity was 98.6% for bacteriologically confirmed TB using a grade 2 cut-off on the pre-January 2014 manufacturer's reference card (equivalent to the grade 1 cut-off on the current reference card). The urine samples in our study were frozen, but stored and processed in accordance with manufacturer's recommendations and other studies.[105] The WHO guidance (to which our data contributed) and a recent Cochrane review advise against the use of LF-LAM for TB screening, as already discussed in the literature review.[103]

Studies presented in **Table 9-1** report a higher sensitivity of LF-LAM for TB than we found. This is likely due to the different populations investigated in these studies, i.e. individuals preparing to initiate ART or those newly diagnosed HIV-positive.[137] Their study participants would have been more unwell and at greater risk of TB than our participants, who were established in HIV care, as reflected by the much higher prevalence of TB reported in these studies. These studies also used the more sensitive, but less specific, grade 1 cut-off

in the pre-2014 reference card to define a positive LF-LAM result. The only study which did include individuals on ART enrolled participants from antenatal care, so is not generalisable to routine HIV care settings.[141] 20% of those approached declined to participate in the aforementioned study, and selection bias is likely to have impacted on the sensitivity of LF-LAM, which was negative for all seven participants who fulfilled the study case definitions for TB.

Urine is generally considered to be a relatively easy sample to collect, but in XPHACTOR, 20% of those eligible for the LF-LAM study did not produce a urine sample. In contrast other studies which have evaluated LF-LAM as a screening test for TB in PLHIV in outpatient settings reported that very few individuals were unable to produce urine (**Table 9-1**).[104, 105, 151] It is possible that individuals who were unable to produce urine declined to participate in these studies in the first place, or were not enrolled. Inpatient studies report ease in collection of samples and this is to be expected with a "captive" population. In crowded HIV clinics adequate access to a toilet might not always be feasible. Even in higher income countries with better access to outpatient toilet facilities on-demand urine samples are not always forthcoming. Newer diagnostic tests therefore need to use samples that a healthcare worker can collect and complete all testing on during the consultation itself, e.g. finger prick blood or saliva.

### Point-of-care CRP

POC CRP currently appears the most attractive option for replacing the WHO tool, because of superior sensitivity and specificity for TB, but it has only been evaluated in a population preparing to initiate ART, i.e. at high risk of TB.[236] It costs less than $2 per test and provides results within three minutes. Yoon *et al* evaluated the diagnostic accuracy of POC CRP for culture-confirmed pulmonary TB at ART initiation in a hospital-based ART clinic in Uganda.[236] The study was undertaken at a time when ART was provided when the CD4 count was <350 cells/mm$^3$.[236] In this study consecutive adults were prospectively enrolled and all were systematically screened for TB using the WHO tool, POC CRP and two sputum samples were collected (one induced if necessary) for one Xpert and two TB cultures on solid and liquid media. The reference standard used for analyses was culture-confirmed TB, but those who were Xpert-positive but culture-negative were designated as not TB. A diagnosis of not TB was assigned if there were at least two negative sputum cultures, as is appropriate for a diagnostic accuracy study. However, excluding all individuals who were unable to produce two sputum samples will bias the sensitivity estimates for POC CRP. A

large number of individuals were excluded from this study because of missing sputum culture results (5% of those enrolled, and more so amongst those with CRP <10 mg/L).

TB prevalence in this population with a median CD4 count of 165 cells/mm$^3$ was 14%.[236] The great majority (87%) of participants reported WHO tool symptoms, reflecting an unusual definition of WHO tool positivity which allowed for symptoms reported within the 30 days prior to enrolment. Positive POC CRP defined as ≥8 mg/L had sensitivity and specificity of 90% and 70% respectively, compared with 96% and 14% for the WHO tool, thus fitting the TPP criteria for a triage test for pulmonary TB. The very high sensitivity and poor specificity of the WHO tool arise from the expanded definition for WHO tool symptoms. The sensitivity of POC CRP is likely to be biased and overestimated because of the large number of participants who were excluded due to missing results or failure to produce two sputum samples. Individuals who could produce two sputa are more likely to have been unwell, probably because they had TB. TB diagnoses may have been missed due to the failure to collect extrapulmonary samples, the lack of prospective follow-up, and Xpert-positive culture-negative TB being deemed "not TB", the rationale for which is not provided. The authors' findings cannot be generalised to HIV-positive individuals attending for routine care, as 63% of their participants were new to HIV care. CRP is likely to be less sensitive for TB screening in individuals stable on ART, who are less likely to be unwell or to have TB.[137]

In a later study, which included some of those enrolled in the above study, Yoon *et al* compared POC CRP-based screening algorithms to the WHO tool followed by Xpert prior to ART initiation.[240] In this study enrolment procedures were identical to those detailed above, but in addition a urine sample was collected for testing with LF-LAM. The authors compared the yield from algorithms which started with CRP-based screening (instead of the WHO tool) and were followed by confirmatory testing with 1) urine LF-LAM if CD4 count < 100 cells/mm$^3$, and Xpert on sputum if CD4 ≥ 100 cells/mm$^3$ or LF-LAM negative; or 2) as per 1) but followed by sputum mycobacterial culture if the Xpert result was negative. Amongst 1245 participants 88% were WHO tool positive, 40% fulfilled CRP screening criteria (≥8 mg/L), median CD4 count was 153 cells/mm$^3$, and TB prevalence was 16%. CRP ≥8 mg/L followed by Xpert had a comparable yield to WHO tool followed by Xpert (sensitivity 59% vs. 56% respectively), but fewer Xpert tests were required per TB diagnosis. A large number of those enrolled (15%) were excluded from analyses because of incomplete sputum culture results for purposes of assigning TB case definitions. The sensitivity of this algorithm is therefore also likely to be biased and again probably overestimated. For the reasons already discussed above, the findings from this study may

not translate into improved diagnostic yield when used for intensified case finding in a population stable in HIV care.

### Chest x-ray and ID-TB/HIV algorithm

Screening for TB using chest radiography at every clinical encounter is clearly not practical, feasible, or even desirable because of the amount of radiation exposure entailed. Studies undertaken in which at least 50% of enrolees were on ART have reported sensitivities of chest radiograph findings suggestive of active pulmonary TB ranging from 71%[142] to 95%.[239] The latter reported sensitivity was from a study which enrolled a convenience sample and participants were highly symptomatic. The estimate is therefore biased and it is highly likely that those who participated did so because they were unwell and more likely to have had TB. Khan *et al* reported improved sensitivity for bacteriologically-confirmed TB in individuals on ART using an algorithm comprising either WHO tool positive or any abnormality on chest x-ray vs. WHO tool alone (52% vs. 77%).[138] The limitations of this study have been detailed in the literature review, in particular a large number of exclusions from the study which will bias the estimate of sensitivity.

The ID-TB/HIV algorithm has already been discussed in the literature review.[72] It is used in Southeast Asia prior to ART initiation, and its performance has been evaluated in Kenya in a population newly enrolling in HIV care. Their symptom screen had a sensitivity of 72.5% for bacteriologically confirmed TB, which was similar to the performance of the WHO tool in this setting.

#### *Triage test to prioritise PLHIV with WHO tool symptoms for Xpert*

Two methods have been investigated to prioritise PLHIV reporting WHO tool symptoms for confirmatory diagnostic testing. Shapiro *et al*[237] investigated the diagnostic accuracy of CRP amongst individuals at HIV-positive diagnosis in South Africa who reported WHO tool symptom(s).[237] The authors used a composite reference standard of clinical and bacteriologically-confirmed TB. A cut-off of ≥5 mg/L attained the TPP minimum target for sensitivity (99%), but lacked specificity (48%). Limitations of this study include its retrospective design, and verification bias because participants did not undergo standardised investigation for TB at enrolment, which was instead dependant on a

clinician's assessment at enrolment. Hence the sensitivity reported is likely to be biased and probably overestimated.

Balcha *et al*[176] derived a clinical score to determine the risk of bacteriologically-confirmed TB, using data collected as part of a prospective cohort study screening clinic attendees in Ethiopia prior to ART initiation. Their final model is discussed in the literature review (**Table 2-7**). Although their score is intended to be simple to use, the inclusion of the Karnofsky score and haemoglobin levels do not make for ease of use in busy primary care settings. The parent study from which the data were derived enrolled only participants who could produce sputum, greatly limiting generalisability, and probably resulted in an overestimation of the sensitivity of the score. Those who could produce sputum were probably more unwell, and therefore more likely to have had TB. A clinical score of ≥2 (maximum score 5) to trigger diagnostic testing in individuals with WHO symptoms, enabled 53% (414/784) of all participants vs. 20% (159/784) if only the WHO tool had been used to avoid a diagnostic test. This strategy missed 7.2% (30/414) of TB diagnoses in those who were not investigated, compared with using the WHO tool alone, which missed 6.3% (10/159). Their prediction model has not yet undergone any form of validation and does not fulfil the minimum TPP for a triage test (**Table 9-1**).

The clinical score derived using XPHACTOR study data **(Research Paper 2, Chapter 6)** was designed for the same purpose as that of the aforementioned study by Balcha *et al*.[176] Our tool is simpler to use and has been internally validated. A cut-off score of ≥3 (maximum score 16) did exceed the 90% minimum sensitivity for a triage test, but it lacked specificity. It did, however, miss a smaller proportion of TB diagnoses in those who were not tested (3% [9/331]) compared with Balcha *et al*.[176] However, the study populations are not comparable, ours was a population established in HIV care, with a much lower prevalence of confirmed TB (3.4%), compared with their participants who were screened for TB prior to ART initiation and had a much higher prevalence of TB (16.9%).

***Summary***

Alternative options to the WHO tool followed by confirmatory testing with Xpert have been investigated for PLHIV prior to ART initiation and at new HIV diagnosis. These are LF-LAM, CRP, chest radiography, an alternative clinical algorithm, and a clinical score to triage symptomatic individuals for confirmatory testing. Only POC CRP at cut-off ≥8 mg/L

attains the minimum sensitivity and specificity requirements stipulated by the WHO for a triage test, or for use in anyone attending for care with a risk factor for TB such as HIV. This test has only been evaluated in a population at very high risk of TB, those newly diagnosed HIV-positive, who should arguably all be investigated for TB. These studies are limited by a large number of exclusions, which is likely to have resulted in biased estimates of sensitivity, and their findings cannot be generalised to other patient populations. Other than the clinical score derived in this thesis, no other algorithms have been evaluated for TB screening in a population established in HIV care.

### *9.2.2. Alternative pathways following a negative initial Xpert result*

In **Research Paper 3**, we reported limited utility from repeating Xpert on a fresh sputum sample for individuals with an initial negative test result. We found that only 5/28 participants with an initial negative Xpert result who fulfilled our study case definitions for TB were identified by the repeat Xpert. Studies evaluating the incremental yield from repeating Xpert suggest that testing more than one sample does improve the diagnostic yield of Xpert. Studies investigating factors that impact on the yield of Xpert from sputum suggest that early morning,[187] induced,[189] and mucopurulent[186] sputum provide a better diagnostic yield. These studies, which are discussed in the literature review, collected samples from participants attending because of TB symptoms or prior to ART initiation, and mainly collected multiple samples at enrolment. The increased yield may simply reflect the much greater proportion of participants in these studies who did actually have TB, compared with that reported in research paper 3. Our repeat sputum sample was collected from participants who had already had an initial Xpert result, so if they did have TB they were more likely to have paucibacillary disease which would be harder to detect.

We did not induce sputum, and collected a spot rather than a morning sample for the repeat sputum, to better reflect what happens in real life, and this might have impacted on our reported yield from the repeat Xpert test. We froze the repeat sputum sample for later testing with Xpert, but the storage, testing and processing are all in accordance with that reported from other published studies.[56]

The study population in research paper 3 was at high risk of TB, but not typical of those who would traditionally follow this pathway, i.e. firstly identified by the WHO tool as symptomatic and subsequently following the Xpert-negative pathway because they remained symptomatic. We included participants from whom we collected sputum

samples, irrespective of the presence of WHO tool symptoms, because they were deemed at high risk of TB, i.e. those pre-ART with CD4 count <200 cells/$mm^3$, and all enrolled from HTC services. This was in order to ensure sufficient TB diagnoses to enable a comparison to be made. These individuals, if asymptomatic but later diagnosed with TB, are likely to have been less unwell at the time that the initial and repeat samples were collected, and had paucibacillary disease which would have been missed by Xpert.

Irrespective of the strategy used, repeat Xpert or Xpert-negative pathway, both require multiple clinic attendances for patients. In XPHACTOR we found considerable drop out along the cascade of care for this pathway, in terms of attendance for chest radiograph or review of response to antibiotic trial, and more efficient strategies need to be considered. One potential strategy is the upfront collection of two sputum samples from all symptomatic individuals, with testing of the second sample determined by the result of the initial Xpert. Another strategy is the diagnostic algorithm derived using CART analysis by Cain *et al* in the pre-Xpert era, in which empiric TB treatment is commenced based on chest radiography and CD4 cell counts whilst awaiting sputum TB culture results.[72]

### *9.2.3. Other causes for TB symptoms*

**Research Paper 4** describes a small number of individuals with persistent symptoms suggestive of TB whom we extensively evaluated using simple tests which should generally be available within primary or secondary level settings in LMIC. The most common criteria for entry to this study were measured weight loss and cough. The most common final diagnoses were weight loss due to severe food insecurity, TB, other respiratory tract infections and post-TB lung disease.

This study is the first to systematically evaluate patients established in HIV care with persistent or recurrent symptoms suggestive of TB, and with an initial negative Xpert result among those able to produce sputum, for a broad spectrum of diagnoses. The only other comparable study is from Munyati *et al* who investigated primary care attendees with chronic cough and also identified a high proportion of non-communicable disease diagnoses, in particular post-tuberculous disease, asthma and heart failure. We also found post-TB chronic lung disease to be a relatively common diagnosis; better criteria to distinguish it from active TB and to guide optimal management are needed.

## 9.3. Implications of this research

The journey an HIV-positive individual takes through care can be divided into the initial diagnosis, ART initiation with or followed by IPT, and subsequent routine attendances for HIV care (to monitor treatment success and collect medication), all interspersed with attendances for other medical conditions, which may or may not be HIV-related. Differentiated care delivery models are encouraged with task-shifting to enable ART pick-up for stable patients at more convenient times, locations and frequencies. The definition of "stable" individuals can vary from the WHO criteria of someone who has received ART for at least one year with evidence of treatment success (based on viral load suppression, or if not available, on rising CD4 count); in South Africa viral load suppression after 3-6 months suffices.[241] Since 2010 HIV care in South Africa has been routinely provided by nurses in primary health clinics, rather than at hospital-based clinics. The rollout of treat-all risks overloading these clinics and strategies to reduce workload, such as reducing the frequency of visits, are required.

Ongoing heightened risk of TB in PLHIV mandates screening at each of the aforementioned encounters. However, the risk of TB and/or negative consequences of missing TB are arguably greatest at initial HIV diagnosis, prior to ART initiation, during the first few months of ART, and prior to IPT initiation. Further investigation and the exclusion of TB in individuals who are WHO tool positive should enable the diagnosis and treatment of the actual disorder responsible for these symptoms. Other considerations arising from the TB screening pathway which cannot be ignored are the negative sequelae and impact of a false-positive diagnosis resulting in unnecessary TB treatment,[242] and the inconvenience of repeated visits for further investigations and test results in those false-positives identified on screening.

We have shown that the WHO tool is less sensitive but more specific for screening individuals on ART for TB, and in our study the prevalence of confirmed TB in the on ART group was half (2.4%) that of the pre-ART group (5.1%). Screening a population established on ART using the WHO tool will therefore miss more TB diagnoses than if the tool were used in a pre-ART population. The higher specificity in those on ART will result in fewer people undergoing unnecessary diagnostic testing, but one-third of this group (who should all have been previously screened for TB) reported WHO tool symptoms at enrolment and therefore required investigation for TB. The PPV of the WHO tool in the on ART group for

confirmed TB was very low (5%), so the vast majority of those identified by the tool will not have TB. The NPV was very high, enabling TB to be reliably ruled out and IPT provided.

The volume of confirmatory diagnostic testing needed as a result of using the WHO tool at every clinical encounter for individuals established on ART, which will become the case for all PLHIV as treat-all is implemented, will be large (potentially one-third of all attendees). Individuals who are stable on ART should have previously been screened for TB and if indicated investigated for TB; hence if they are later diagnosed with TB they are more likely to have been less unwell at previous screening, and probably had paucibacillary disease. Repeated rounds of screening should identify (and treat) first those who are most symptomatic and those with the highest bacillary load. Therefore, in a resource-limited setting individuals who are stable on ART, particularly those with higher CD4 cell counts or those on IPT who are likely to have been recently investigated for TB, could be screened at less frequent intervals. This is probably inevitable in the future if longer supplies of ART are provided and to reduce the workload at overstretched clinics, and PLHIV need to be aware of the importance of attending for investigation if they become unwell in between scheduled clinic attendances. Additionally, a different screening and investigation algorithm could be considered, such as a triage test to prioritise investigation amongst those who are WHO tool positive, or POC CRP instead of the WHO tool. These strategies require further evaluation in populations established in HIV care, and different investigation pathways for different groups of PLHIV may prove too complicated to implement. Alternative algorithms to the Xpert-negative pathway are also needed, as there is a high potential for drop out due to the number of visits and investigations needed. Simply repeating the Xpert does not appear useful, in contrast with WHO recommendations.[127]

This thesis focussed on individuals established in HIV care at a time when a division was present between those in pre-ART care, who received CD4 monitoring and TB preventive therapy, and those on ART. Most of our on ART group had been so for more than one year, so are likely to have fulfilled the criteria for “stable”, although we do not have the data to confirm this. Therefore, the findings from this large cohort of patients stable on ART, one-third of whom reported WHO tool symptom(s) is of particular relevance in the era of treat-all to HIV care programmes. There is a great need for a better tool for TB screening in the context of active case finding for those stable in HIV care, and alternatives to the onerous Xpert-negative pathway. Diagnostic tools are not the only answer, as the rollout of Xpert has proven, and good health systems infrastructure is also needed.

### *Impact of recent changes in HIV care and TB diagnostics on this research*

This research commenced in 2012 and subsequent changes in HIV care, in particular treat-all, the recommendation for viral load rather than CD4 count monitoring, and next-generation diagnostics will impact upon our findings. Treat-all should realise ART initiation at higher CD4 counts, so PLHIV should become less symptomatic, the prevalence of TB should become lower in this population, and there should be less extrapulmonary disease. The characteristics of individuals attending for HIV care are likely to change over time with treat-all, and therefore the performance of the WHO tool in this population will differ from the pre-ART population in which it was originally developed; it is likely to be less sensitive and more specific. The PPV of any TB screening tool will reduce as the prevalence of TB declines. A lower PPV will generate a greater number of false-positives, and a larger number of people to be screened in order to identify one TB diagnosis. Differentiated ART delivery models may result in fewer clinic visits for routine HIV care and therefore a longer interval between rounds of TB screening, or screening by alternative cadres of healthcare worker e.g. pharmacists or lay community health care or peer group workers. The clinical score derived in research paper 2 requires ART status and CD4 cell count; in the future viral load should also be considered for incorporation in this model, as CD4 counts may be measured less frequently.

The arrival of Ultra, which is more sensitive but less specific than Xpert, may reduce the proportion of indivduals requiring chest radiograph and sputum culture along the Xpert-negative pathway. However, a negative Ultra or Xpert result in a PLHIV who remains symptomatic should not halt further evaluation along the Xpert-negative pathway, as this risks missing TB diagnoses. On the other hand, false-positive diagnoses arising from the lower specificity of Ultra may result in unnecessary TB treatment being provided, particularly in those previously treated for TB; and strategies to address this phenomenon are required. The next-generation LAM test, which improves on sensitivity, may make it more suitable as a screening test, providing it remains a simple test suitable for point-of-care use.

## 9.4. Limitations and strengths

### *9.4.1. Limitations*

The main limitations of this study are in the selection of participants for research papers 3 and 4, which limit the generalisability of study findings, and in the development of the clinical prediction model. These are discussed below in greater detail.

In retrospect, the dataset used for developing the clinical prediction model was too small to use the split sample method for derivation then validation. It would have been better to use the entire dataset to develop the prediction model. Imputing missing values might have enabled development of a model more relevant to the era of treat-all, which could include viral load. Internal validation should have been undertaken using a resampling technique such as bootstrapping. Univariate analysis was used to preselect some of the candidate predictors, and the EPV was less than 10, i.e. the model is likely to have been overfitted; statistical methods for developing prediction models when there are few events should have been considered.[243] The model requires external validation before it can be utilised in practice.

We enrolled participants to the repeat Xpert study **(Research Paper 3)** based on risk of TB, rather than on symptoms, and in fact some participants were asymptomatic at collection of both the initial and the "repeat" sputum samples. In reality patients would only follow the Xpert-negative pathway if they still had symptoms following an initial negative Xpert. Our comparator to the repeat Xpert was pragmatic and in line with routine clinical practice. The components of the Xpert-negative pathway could not always be performed on the same day as collection of the sputum sample for repeat Xpert, but rather in a sequential manner reflecting "real-life". One could argue that it was unfair to compare Xpert on a sputum sample collected one week following the initial Xpert test with potentially multiple different investigations during the course of study follow-up. The negative repeat Xpert in this scenario might just reflect paucibacillary disease, and indeed we did have participants who were diagnosed with TB by Xpert on a sputum sample collected later during study follow-up.

For **Research Paper 4**, if we had required the presence of the same symptom(s) reported both at enrolment to XPHACTOR and at the 3-month visit for inclusion in this study, we would have enrolled very few participants. Therefore, in order to ensure sufficient participants, we defined persistent weight loss as objectively measured significant weight loss, which was subjectively confirmed as unintentional weight loss by the patient at the

3-month visit. Consequently, weight loss and cough were the most common symptoms based on which we enrolled to this substudy, and our final diagnoses may not be representative of findings from other HIV care settings. We were also limited in terms of the extent of investigation that we were able to undertake, but these do reflect investigations commonly available at primary or secondary care level in LMIC.

Our study population was mainly individuals established in HIV care, but in reality, amongst those in the pre-ART group who were exclusively enrolled from CHCs, a proportion had only received their HIV-positive diagnosis a few weeks previously. This is reflected in the IQR for the median time in HIV care for this group of 7 months (IQR 1-30).

In our assessment of the diagnostic accuracy of the XPHACTOR algorithm and the WHO tool we excluded all unclassifiable TB outcomes. Although this did not entail many exclusions, it might have been better to either impute these values, or present "best" or "worst" case scenarios, by computing diagnostic accuracy after including the individuals as firstly having TB and then subsequently as not having TB.

### *9.4.2. Strengths*

Strengths of this study include its prospective design, collection of sputum from all participants at enrolment (irrespective of symptoms) for testing with Xpert (immediate or stored for later testing). All participants were followed for a period of around three months, with repeat TB screening and investigation if indicated. At the 3-month visit all participants had sputum and blood collected for mycobacterial culture; our study retention rates were high. We also facilitated the Xpert-negative pathway in those with an initial negative Xpert. Thus, we are unlikely to have missed many TB diagnoses. We did not exclude participants who were unable to produce sputum, limiting bias and ensuring generalisability.

As already discussed, our study is unique in its characterisation of a large cohort of individuals established in HIV care, with a large proportion on ART; and therefore relevant in the era of treat-all. This study population provides a good indication of the frequency of reporting WHO tool symptoms and the likely need for confirmatory diagnostic testing if the WHO tool is used as recommended, at every clinical encounter.

## 9.5. Conclusions and recommendations

For a population established in HIV care, the current screening and diagnostic algorithm generates a large number of individuals who require a diagnostic test for TB. This is not feasible in resource limiting settings, and may impede intensified TB case finding. There is evidence that TB screening and investigation algorithms are not adhered to in these settings, and particularly with the rollout of treat-all there is a great need for alternative strategies. Amongst those at highest risk of TB or negative sequelae of missing TB, i.e. those newly initiating ART or prior to IPT, screening with the WHO tool followed by Xpert probably remains the best strategy; if resources allow then all should be investigated with mycobacterial culture or Xpert. In those stable on ART, who are less likely to have TB than those prior to ART or prior to IPT, perhaps a clinical score to triage individuals who have WHO tool symptoms, a biomarker such as CRP used as a POC screening test, or simply screening at less frequent intervals could be considered to preserve limited resources. This would rely on patients having a high level of awareness to present passively if they developed any symptoms of TB. At present no published tools, except for POC CRP, fulfil the WHO TPP for a triage test, and all strategies require further evaluation in a population established on ART, as most studies were undertaken prior to ART initiation or IPT.

The Xpert negative pathway is onerous on both patients and healthcare workers, with patients being lost along the diagnostic cascade, and alternative strategies are needed. However, it is important to keep looking for TB in individuals with symptoms, using all available modalities. With the advent of treat-all, and PLHIV established on ART for longer periods of time, it is also important to identify non-communicable disease related causes if they have persistent symptoms suggestive of TB but negative TB investigations. Post-tuberculous lung disease requires better criteria to distinguish it from active TB, and respiratory physicians need to be more easily accessible at primary care level to help better diagnose and manage this condition and also cough which we found was the most commonly reported WHO tool symptom.

Future research is needed to externally validate our clinical score for TB; evaluate POC CRP as an alternative to screening using the WHO tool in populations established on ART (although even <US$2 per test may be too expensive if a large volume of testing is required); and derive speedier options to the Xpert-negative pathway.

# 10) Appendices

## 10.1. Ethical approvals

**London School of Hygiene & Tropical Medicine**
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

**www.lshtm.ac.uk**

**Observational / Interventions Research Ethics Committee**

Alison Grant
Professor
CRD/ITD
LSHTM

10 December 2012

Dear Professor Grant,

| | |
|---|---|
| **Study Title:** | **Xpert MTB/RIF for people attending HIV care: an interventional cohort study to guide rational implementation ("XPHACTOR")** |
| **LSHTM ethics ref:** | **6165** |
| **LSHTM amend no:** | **A374** |

Thank you for your application of 15 November 2012 for the amendment above to the existing ethically approved study and submitting revised documentation. The amendment application has been considered by the Interventions Committee.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

**Conditions of the favourable opinion**

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

**Approved documents**

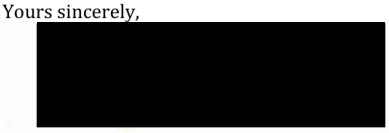
The final list of documents reviewed and approved by the Committee is as follows:

| Document | Version | Date |
|---|---|---|
| LSHTM amendment application | n/a | |
| XPHACTOR_protocol_v3_15Nov12_changesmarked | 3.0 | 15 nov 2012 |
| [the protocol includes Main study PIS/ICF v3 in appendix 4, pilot study PIS/ICF v3 in appendix 5 and PIS/ICF v1 for Pre-ART with CD4<200 in appendix 6] | | |

**After ethical review**

Any further changes to the application must be submitted to the Committee via an E2 amendment form. The Principal Investigator is reminded that all studies are also required to notify the ethics committee of any serious adverse events which occur during the project via form E4. At the end of the study, please notify the committee via form E5.

Yours sincerely,



**Professor Andrew J Hall**
**Chair**
ethics@lshtm.ac.uk
http://intra.lshtm.ac.uk/management/committees/ethics/

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

26 November 2012

Dr Violet Chihota
The Aurum Institute
Private Bag X30500
Houghton
2041

Sent by e-mail to: vchihota@auruminstitute.org

Dear Dr Chihota

**RE:** **Protocol M120343: 'XPERT MTB/RIF For People Attending HIV Care: an International Cohort study to Guide Rational Implementation'**
**Protocol amendment: version 3.0, 15 November 2012**

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved the following amendments to the abovementioned protocol as detailed in your letter dated 16 November 2012:

- Recruit an additional group of eligible patients as detailed
- Study procedures: pg 17: 3.4.1 as detailed
- Modified selection criteria pg 16: 3.2
- Travel reimbursement as detailed
- Revised Participant Information sheet
- Page 18: section 3.4.2 as detailed
- Page 20: 3.6 as detailed
- Page 21: 3.9 as detailed
- Revised time require for enrolment as detailed
- Page 20: 3.8 as detailed
- Page 26 8.3 as detailed
- Page 21: section 4 as detailed
- Updated contact for Ms N Foster as detailed Pages 6, 26 and all information sheet and consent forms
- Page 1
- Protocol version 3.0, 15/11/2012
- Main Study Information Sheet and Consent Form version 3.0, 15/11/2012
- Pilot Study Information and Consent Form version 3.0, 15/11/2012
- Pre-ART with CD4<200 Information Sheet and Consent form version1.0, 15/11/2012

Thank you for keeping us informed and updated.

[redacted]
Anisa Keshav
Secretary
Human Research Ethics Committee (Medical)

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

**FACULTY OF HEALTH SCIENCES**
Human Research Ethics Committee

# Form FHS006: Protocol Amendment

**Note:** All amendments should include a Synopsis for the amendment (please see notice dated 23 April 2012)

| **HREC office use only (FWA00001637; IRB00001938)** | | |
|---|---|---|
| ☑ Approved | ☐ Type of review: Expedited | ☐ Full committee |
| This serves as notification that all changes and documentation described below are approved. | | |
| Signature Chairperson of the HREC | [redacted] | Date 21/11/2012 |

**Principal Investigator to complete the following:**

**1. Protocol information**

| | |
|---|---|
| Date | 20th November 2012 |
| HREC REF Number | 106/2012 |
| Protocol title | Xpert MTB/RIF for people attending HIV care: an interventional cohort study to guide rational implementation ("XPHACTOR") |
| Protocol number (if applicable) | 3 |
| Principal Investigator | Prof Gavin Churchyard (and Dr Edina Sinanovic) |
| Department / Office Internal Mail Address | Health Economics Unit, School of Public Health and Family Medicine, Falmouth Annex, University of Cape Town, Observatory, 7925 |

| | | |
|---|---|---|
| 1.1 Does this protocol receive US Federal funding? | ☐ Yes | ☒ No |
| 1.2 Is this a major or a minor amendment (see FHS006hlp)? | ☐ Major | ☒ Minor |

**2. List of Proposed Amendments with Revised Version Numbers and Dates**

**Please itemise on the page below, all amendments with revised version numbers and dates, which need approval.** This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.

**The substantive changes vs. the original protocol are:**

1. We propose to recruit an additional group of patients first presenting to clinic and ART eligible with CD4 <200 cells/µl to a substudy contributing to Aim 2 (protocol section 3.3.1, page 17; section 3.5, page 19), which will enable timely completion of Aim 2. These patients are at high risk of having active TB, and thus all (regardless of symptoms or algorithm categorisation) will be asked to give a sputum sample for immediate testing with Xpert MTB/RIF. For those whose result is negative, if they remain symptomatic, study staff will facilitate initiation of further management as per National Department of Health guidelines (sputum for mycobacterial culture, chest radiograph, trial of antibiotics) and will obtain a second spot sputum which will be stored for study purposes. Participants in this group will be followed in the same way as the main study cohort. A separate information sheet and consent form has been developed for these individuals (Pre-ART with CD4<200 Information Sheet and Consent Form).
2. Originally, the economics component was only going to enrol symptomatic individuals to measure patient costs, but we would now like to include a sample of all participants regardless of symptoms. Protocol section 3.4.1, page 17; and section 6.1, page 23 have been amended accordingly.
3. We have modified our clinic selection criteria to enable recruitment from smaller clinics, as we found that are very few clinics of the size we had originally stipulated (Protocol section 3.2, page 16). In addition, we may need to work at more than three clinics to recruit the numbers needed, so we have

adjusted all references to the number of clinics to read "about 3" accordingly.

4. From our experience to date, we have found that participants find it difficult to attend for the results of investigations due to financial and time constraints. We therefore propose the option to do these telephonically where appropriate, and to compensate those who do attend for their travel costs (ZAR 20) and time (ZAR 30), i.e. total of ZAR 50 which is in keeping with the 1-, 2-, 4-, and 5-month visits. The protocol (section 8.2, page 25) and participant information sheet (Main Study Information Sheet pages 2-4) have been amended accordingly.

**We have also made some changes for clarification or reflecting administrative or logistic issues, or correcting typing errors:**

5. TB suspects who are Xpert or smear negative but asymptomatic when reviewed with the results, would not as part of routine clinical care be subjected to further investigation. We have amended the protocol to clarify that only those who are symptomatic on review will be investigated further (protocol section 3.4.2, page 18; section 3.5, page 19). This is also clarified in the participant information sheet (Main Study Information Sheet pages 2-3).
6. We have clarified that we will only enrol participants with persistent TB symptoms at the month 3 visit to study aim 3. (Protocol section 3.6, page 20)
7. We will allow flexibility in the time point when we commence collection of costs from each clinic implementing the study algorithm for the economics component. (Protocol section 3.9, page 21)
8. We have amended the estimated time required for enrolment to 40 minutes (plus additional 20 minutes if taking part in the economics component), based on our experience to date. (Main Study Information Sheet pages 2, 4)
9. The turnaround time for GeneXpert testing is likely to vary between sites, hence we have removed "expected two working days" from the participant information sheet (Main Study Information Sheet page 2).
10. We would like to allow flexibility in when we may perform the LAM assay on urine taken for research purposes, i.e. immediate LAM assay at the research laboratory or freezing the sample to afford the opportunity to test at the end of the study. (Protocol section 3.8, page 20). We also clarify in the participant information sheet (Main Study Information Sheet, page 2) that the result, if LAM test is performed, is not fed back to the clinic.
11. We prefer not to stipulate the languages into which we will translate the participant information sheets, in order to allow flexibility, as the most commonly-used local languages vary between clinics. (Protocol section 8.3, page 26).
12. For the pilot study we propose to recruit a systematic sample of around 100 patients per study clinic, to allow a slightly larger number if necessary (Protocol section 4, page 21)
13. We have updated contact details for Investigators and added Ms Nicola Foster (junior health economist) as co-investigator (pages 6 , 26 and all participant information sheets and consent forms)
14. We have added the protocol reference numbers for Aurum Institute and all the research ethics committees which have approved the study (page 1)

The following documents which have been modified as detailed above are enclosed. Version numbers and dates have been revised, and all changes have been highlighted. For each document, one clean copy and one copy which clearly identifies changes are enclosed. .

| *Document* | *Version* | *Date* |
|---|---|---|
| Protocol | V3.0 | 15/11/2012 |
| Main Study Information Sheet and Consent Form | V3.0 | 15/11/2012 |
| Pilot Study Information Sheet and Consent Form | V3.0 | 15/11/2012 |
| Pre-ART with CD4<200 Information Sheet and Consent Form | V1.0 | 15/11/2012 |

## 10.2. XPHACTOR participant information sheet and consent form

**XPHACTOR STUDY - MAIN STUDY -AUR2-6-112**

### PARTICIPANT INFORMATION SHEET AND CONSENT FORM: MAIN STUDY

**STUDY TITLE: XPHACTOR: Xpert MTB/RIF for People Attending HIV Care – An Interventional Cohort Study to Guide Rational Implementation**

**The investigators doing this study are:**

- *Aurum Institute, South Africa:* Prof. Gavin Churchyard, Dr Violet Chihota, Dr Salome Charalambous, Dr Kerrigan McCarthy
- *National Health Laboratory Service/University of Witwatersrand, South Africa:* Prof Wendy Stevens, Dr Linda Erasmus
- *University of Cape Town, South Africa:* Professor Mark Nicol, Dr Edina Sinanovic, Ms Nicola Foster
- *Chris Hani Baragwanath Hospital:* Dr Alan Karstaedt
- *Mamelodi Hospital: Dr Lungiswa Adonis*
- *Foundation for Professional Development:* Dr Hans Kinkel
- *London School of Hygiene & Tropical Medicine, UK:* Dr Yasmeen Hanifa, Dr Katherine Fielding, Dr Anna Vassall, Prof Alison Grant

**Collaborators:**

- *National Department of Health:* Dr David Mametja, Dr Lindiwe Mvusi, Dr Norbert Ndjeka, Dr Kgomotso Vilakazi

**INTRODUCTION**

Good day, my name is [*name of researcher*] _____, and I am a researcher with the XPHACTOR study team. We would like to invite you to take part in a research study about a new laboratory test for tuberculosis (TB). Research is the process to learn the answer to a question and this information sheet explains our study. You are free to decide whether you wish to participate, and before you decide, it is important that you understand why the research is being done and what it will involve. Please ask me if there is anything which is not clear. If you decide to take part, to show that you understand the study and agree to take part, we will ask you to sign or make your mark or thumbprint on a consent form. It is your right to withdraw from the study at any time. Your decision to take part or not will not affect your health care in any way.

**WHY ARE WE DOING THIS STUDY?**

TB is a major health problem in South Africa, especially among people with HIV infection, in whom it can be more difficult to diagnose: but TB can be cured. One reason it has been difficult to control TB in countries like South Africa is that the traditional test, which looks for the TB germ in sputum (spit from the chest) with a microscope, does not detect every case of TB first time. A new sputum test called Xpert MTB/RIF is being introduced across labs in South Africa. This test picks up more cases of TB, although it is not perfect, and it is more expensive than the traditional test.

Experts recommend that patients with HIV are checked for TB every time they attend a clinic, and a spit test sent if they have any TB symptoms (cough, fever, night sweats or weight loss). We know that although many patients will have these symptoms, most will not have TB.

In our study we want to find out:

1. The best way of using the new Xpert TB test in patients with HIV, so those with TB can start correct treatment faster, and those without TB do not have unnecessary tests.
2. The best way to diagnose TB if it is not picked up in the first spit test.
3. Among patients who have symptoms suggesting TB, but tests do not find TB, what illness they have; and if no other illness is found, how long the symptoms last.
4. How much it costs the health department to do all these tests.

This study will include about 3750 people in total, from about 3 clinics across South Africa, and will take about two years to complete. It is funded by the Bill and Melinda Gates Foundation.

**IF I TAKE PART IN THIS STUDY, WHAT WILL HAPPEN?**

If you agree to take part in this study, with your permission and in a private space we will:

***Today***:

- Ask you some questions about yourself (such as your age, address, your education, the sort of house you live in, and how much it costs you to come to clinic).
- Ask about your health, including symptoms you have at the moment (in particular symptoms which might indicate that you are sick with TB) and when they started.
- Check your clinic records to find the most recent CD4 count result.
- Measure your height and weight.
- Ask about any treatments you are taking, and whether you have been treated for TB in the past.
- Ask you to give us a sputum sample (spit from the chest).
    - If our assessment suggests it is likely that you might have TB, we will send your sputum for testing today, using the new TB test.
    - Otherwise we will freeze your sputum and check it for TB with the new test at the end of the study.
- If we check your sputum for TB today, we will ask you to come back after *[time period appropriate to the clinic]* for the result.
    - If TB is found, you will be started on TB treatment by the clinic in the normal way, according to South African guidelines. TB can be cured by the correct treatment.
    - If TB is not found on this first test, we may arrange further tests (sputum and chest X-ray) as recommended by South African guidelines; and we may phone you to see how you are. We may also ask you for another sputum sample to freeze. At the end of the study, we will check the sample using the new TB test. If you need a chest x-ray and this clinic does not provide x-rays, we will reimburse you for travel to a clinic that does [*cost of return travel to x-ray facility closest to clinic*].
- [*FOR PARTICIPANTS NOT ON ART, OR WITH CD4<200 ONLY:* Ask you to give us a urine sample which we may test for TB. These tests would be only for our study, and we will not give the results back to the clinic]
- *[FOR PARTICIPANTS SELECTED FOR HEALTH ECONOMICS STUDY ONLY:* To help us understand how much it costs you to come to clinic when you are sick, and the effect on your family, we would like to ask you some more detailed questions about your household income, and things you pay for or have in your home; how much it has cost you in money and time to attend pharmacies, clinics and healers about this illness, and how much any tests and treatment have cost you so far; also about any income you have lost if you needed to take time off work because of this illness, or to seek care; or if family members have needed to take time off to look after you or others because of this illness. These questions will take about 20 minutes. We would be very grateful if you are able to take the time to help us by answering these questions.]

***For the next 3 months (all participants)***:

- *[ON-ART, "LOW PRIORITY" AT ENROLMENT:* If today we assess it is highly unlikely you have TB, we will see you at the clinic for your 3-month visit, and phone you around once a month before this to keep in touch with you and check your contact details. Each time we confirm any changes to your contact details we will give you cell phone airtime, around ZAR12.50 (depending on your network).*]*
- *[ALL OTHER PARTICIPANTS* we will see you once a month at the clinic (3 more visits), to check if you have TB symptoms. If you are unable to come to the clinic, we may phone you, and ask you the same questions by phone. If, at any visit, our assessment suggests it is likely that you might have TB, we will send your sputum for testing, using the new TB test. We will ask you to come back for the results to start treatment if TB is found. If TB is not found, we will review you, which may be by phone, and we may arrange further tests if necessary, as above.*]*
- At your 3-month visit we will ask you for a sputum sample to test for TB using the traditional test, and a blood sample to test for TB as sometimes TB is only found in the blood. If you are not able to produce a sputum sample, we will ask you to breathe in some mist through a mask (called a nebuliser) to help you cough up sputum. If you cannot attend the clinic for the 3-month visit, we may ask your permission to visit you at home to do all of these procedures, except the nebuliser.
- If by the 3-month visit we have found that you have TB, to help us understand how much it costs you to come to clinic for tests and treatment, and the effect on your family, we would like to ask you some more detailed questions about your household income, and things you pay for or have in your home; how much it has cost you in money and time to attend pharmacies, clinics and healers about this illness, and how much any tests and treatment have cost you so far; also about any income you have lost if you needed to take time off work because

of this illness, or to seek care; or if family members have needed to take time off to look after you or others because of this illness. These questions will take about 45 minutes. We would be very grateful if you are able to take the time to help us by answering these questions.

- [FOR PARTICIPANTS SELECTED FOR HEALTH ECONOMICS STUDY ONLY: At each visit, we would like to ask you more questions about how often you have come to the clinic and any money that you have to pay to get to the clinic. These questions will take about 20 minutes.]

If possible, we will arrange these visits which are part of the study to be at the same time as your routine appointments at the clinic. We will give you ZAR50 at the visits after one and two months, or if we ask you to come back for further tests or results, and ZAR100 at the 3-month visit, so for most people who pay about ZAR20 for travel, you will get about ZAR30 for your time at the one- and two-month visits, and ZAR70 at the 3-month visit.

***After 3 months if you still have TB symptoms but we have not picked up TB on any tests:***

We will ask a smaller group of patients (around 250 in total) who still have TB symptoms at 3 months to continue in the study for three more months, up to six months in total. If you are chosen for this group:

- We may discuss with your clinic doctor to arrange further tests to try to find out what is causing your symptoms.
- We will ask you to continue to come for study visits every month for 3 more months, to see if you are diagnosed with any other illness, or if your symptoms get better. If you are unable to come to the clinic, we may phone you, and ask you the same questions by phone. If, at any visit, our assessment suggests it is likely that you might have TB, we will send your sputum for testing, using the new TB test. We will ask you to come back for the results to start treatment if TB is found, or to check you again (in person or by phone) if TB is not found.
- At the 6-month visit, we would like to ask you some questions about how much it costs you to get health care, how often you have come to clinic, and other costs such as transport. These questions will take about 20 minutes.

These further visits (after months 4, 5 and 6) may coincide with your routine appointments at the clinic. We will give you ZAR50 at the visits after four and five months, or if we ask you to come back for further tests or results, and ZAR100 at the 6-month visit, to cover your travel and time as detailed above.

We will also check your medical records from time to time over the next 12 months to check your health, the results of any further tests your doctor requests at the clinic as part of your HIV care, and treatment given.

We would also like your permission to use the sputum and urine sample(s) you give us and the information we collect from this study for other research studies to help us understand HIV and TB better. We would only do this if the ethics committees, who are there to protect the interests of people taking part in our studies, first approved these further research studies.

It is very important for this study that we have a reliable way to contact you. This is, first, because if we get a positive test result, we need to be sure you know about it and are on the correct treatment. Second, it is very important that we find out how you are at the end of the study. So we can do this, we will ask you to give us the best phone number to reach you, and the phone numbers of two close friends or family members, as well as details about where you live. If we need to contact you, we will first try to contact you directly, using the phone number you give us, taking care to be sure we are talking to you in person before we ask any questions about your health. If you do not have a phone we will discuss with you today and agree how best to contact you. If we cannot contact you directly, we would then contact your friend or relative, using the numbers you give us, to ask your friend / relative if they know where you are and can help us contact you, taking care not to give away any information about your health. If you would prefer not to give contact details of a friend or relative, it is still ok for you to take part in the study. If we cannot trace you at the end of the study, we may approach the Department of Home Affairs to check their registers so we can be sure you did not pass away.

This study will take about 40 minutes of your time today [60 minutes if participating in the health economics study]. The visits after one and two months should take about 20 minutes of your time [35 minutes if participating in the health economics study]. The visit after 3 months may take about 45 minutes [an hour if participating in the health economics study].
For people asked to continue in the study to 6 months, the visits after four and five months should take about 35 minutes each, and the visit after 6 months may take about an hour.

**WHAT ARE THE RISKS AND BENEFITS OF TAKING PART IN THIS STUDY?**

Although it is recommended that people with HIV who have any symptom of TB are tested for TB straight away, in most clinics (including this one) this is not done, because it would mean doing very many TB tests when most people do not actually have TB. In this study, we aim to identify those people at highest risk from TB and make sure they get tested straight away. People at lower risk will not be tested straight away, but will be reviewed after a month and checked to see if they need a TB test at that point. If you are selected for telephone contact only, but you report feeling unwell during this contact, we will ask you to see clinic staff as soon as possible, so you can be tested for TB and other illnesses.As we are only testing patients who are most likely to have TB with new TB test, it is important that you return to the clinic as soon as possible if you feel unwell in between your clinic appointments, so you can be tested for TB and other illnesses.

If the questions about your health, or the results of the tests, suggest you might have TB, and this is confirmed by further tests, this will benefit your health, because you will start on TB treatment quickly, which will reduce any risk that your body will be damaged by TB. However, the results of the study will help us know how best to use this new test for TB, and so it will help people like you in the future.

**WHAT HAPPENS IF I DO NOT AGREE TO TAKE PART IN THIS STUDY?**

You do not have to take part in this study: if you do not take part, this will not affect the medical care that you receive. You can stop taking part in the study at any time, without giving a reason.

**HOW WILL THE INFORMATION COLLECTED DURING THIS STUDY BE KEPT CONFIDENTIAL?**

All information collected on paper during the course of this study will be kept securely and confidentially in a locked cabinet: Dr Hanifa is responsible for this. The only exception to this is if we find TB in any of your samples: we are required by law to inform the health service of positive TB results, so that you can receive correct treatment. In order to access your medical records we need to record your name and identifying details. This information will only be available to study staff and will be stored securely, separately from the other information about your health. The rest of the information we collect will be identified on forms and computer files only by a study number, not your name. When we enter your information into a computer, we will keep your identifying details separately, protected by a password, and only restricted study staff will have access. The rest of the information will be entered into another database, identified only by your study number, and we will use only this database to find the answers to our study questions. Only restricted study staff can link your identifying details with the rest of your information on the computer databases, ensuring that your information remains confidential.

Study information may be reviewed by the Ethics Committee, and independent monitors, to check that the study procedures were done correctly and the information is correct. Your information will remain confidential, unless we are required by law to release information. Reports about the study and results that may be published in scientific journals will not include any information which allows you to be identified.

**WHAT IF I HAVE QUESTIONS ABOUT THIS STUDY?**

If you have any questions about this study, please feel free to ask me now. If you have questions later you can ask study staff here at the clinic, or telephone Dr Hanifa on 010 590 1300.

The committees reviewing this study are the University of the Witwatersrand Human Research Ethics Committee, and the Research Ethics Committees of the University of Cape Town and the London School of Hygiene & Tropical Medicine, UK. If you have any questions or concerns about your rights as a person taking part in a research study, or if you wish to make a complaint about the study, you may contact Prof Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee, an independent committee established to help protect the rights of research participants, at 011 717 2301.

We will give you a copy of this sheet which explains the study to take away with you.

If you would like a copy of a report on this study, and you give us an email or postal address, we will send you a report. The final results may not be available until 2-3 years from now.

**PARTICIPANT CONSENT FORM: XPHACTOR STUDY** **Study ID No:** || |-| || || |-|

**STUDY TITLE: XPHACTOR: Xpert MTB/RIF for People Attending HIV Care – An Interventional Cohort Study to Guide Rational Implementation**

**Investigators:**

- *Aurum Institute, South Africa:* Prof. Gavin Churchyard, Dr Violet Chihota, Dr Salome Charalambous, Dr Kerrigan McCarthy
- *National Health Laboratory Service/University of Witwatersrand, South Africa:* Prof Wendy Stevens, Dr Linda Erasmus
- *University of Cape Town, South Africa:* Professor Mark Nicol, Dr Edina Sinanovic, Ms Nicola Foster
- *Chris Hani Baragwanath Hospital:* Dr Alan Karstaedt
- *Mamelodi Hospital:* Dr Lungiswa Adonis
- *Foundation for Professional Development:* Dr Hans Kinkel
- *London School of Hygiene & Tropical Medicine, UK:* Dr Yasmeen Hanifa, Dr Katherine Fielding, Dr Anna Vassall, Prof Alison Grant

***Collaborators:***

- *National Department of Health:* Dr David Mametja, Dr Lindiwe Mvusi, Dr Norbert Ndjeka, Dr Kgomotso Vilakazi

- I have read the information sheet about this study (or the information sheet about this study has been read to me) and I understand what will be required of me and what will happen if I take part in the study.
- My questions concerning this study have been answered by:

| Research staff name (printed) | Signature | Date |
|---|---|---|

- I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.
- I agree for my sputum sample to be stored and used for related research | *( Y= yes, N= no)*
- I agree for my urine sample to be stored and used for related research ⌜ ( Y= yes, N= no)
- I agree to take part in the study

| Study participant name (printed) | Signature/mark/thumbprint | Date |
|---|---|---|

*If the information sheet and consent form were translated or explained to the participant, enter the name of the translator here and their signature:*

| Translator name (printed) | Signature/mark/thumbprint | Date |
|---|---|---|

*If the participant gave verbal consent, enter the name of the person who witnessed the consent here and their signature:*

| Witness name (printed) | Signature/mark/thumbprint | Date |
|---|---|---|

## 10.3. XPHACTOR enrolment questionnaire

Study Identifier: AUR2-6-112-___-___-_ (Protocol - Site code - Participant ID)

Date of Visit: __/___/20__ (dd/MMM/yyyy)

Visit Code: 00.0

### DM001: BASELINE DEMOGRAPHICS

*Instructions: Complete this CRF for all patients enrolled.*

1. Participant country of origin/birth? ..... __

   01 = South Africa
   02 = Lesotho
   03 = Swaziland
   04 = Mozambique
   05 = Botswana
   06 = Namibia
   07 = Zimbabwe
   08 = Malawi
   96 = Other, Specify: _____

2. What is your ethnic group? ..... _

   1 = Black/African
   2 = Coloured/Mixed race
   3 = Indian/Asian
   4 = White/European
   6 = Other, Specify: _____

3. What is the highest level of education you have completed? ..... __

   01 = No School/Pre-school
   02 = Grade 1-3
   03 = Grade 4-7
   04 = Grade 8-11
   05 = Grade 12
   06 = Matric with Technical Qualification or Diploma
   07 = Bachelor's
   08 = Master's or Doctoral
   96 = Other, specify: _____

4. What is your marital status? ..... _

   1 = Single, never married
   2 = Married
   3 = Married and currently separated
   4 = Cohabitating
   5 = Divorced
   6 = Widowed

5. How many people live in your household? ..... __

*(A household is defined as people living in the same house and sharing the same income for food and other essentials for living.)*

6. What is the **main** source of income? *(Record the one providing greatest income.)* ..... __

   01 = Formal employment
   02= Self-employment
   03 = Odd jobs
   04= Government grant (childhood/disability, etc.)
   05 = Income from investments
   06 = Maintenance
   07 = Student/pupil/learner
   08 = Pension
   09 = No income/unemployed
   96 = Other, specify: _____

7. On average, what is the ***combined monthly income*** of your household? *(Include all sources.)* ..... _

   1 = ≤ R 600
   2 = R 601-1000
   3 = R 1001-2000
   4 = R 2001-4000
   5 = More than R 4000
   9 = Don't know

Completed By: | | Verified By: | | Date Verified *(dd/MMM/yyyy)*: | | /| | /20 | |

Study Identifier: AUR2-6-112-[ ][ ][ ]-[ ][ ][ ][ ]-[ ] Date of Visit: [ ][ ]/[ ][ ]/20[ ][ ] Visit Code: [ ][ ][ ].[ ]

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

8. What type of dwelling do you live in? .....

01 = House or brick/concrete block structure on separate stand or yard or on a farm
02 = Traditional dwelling/hut/structure made of traditional materials
03 = Flat or apartment in a block of flats
04 = Cluster house in complex
05 = Townhouse (semi-detached house in a complex)
06 = Semi-detached house
07 = House/flat/room in backyard
08 = Informal dwelling (shack in backyard)
09 = Informal dwelling e.g. in an informal squatter settlement or on a farm
10 = Room/flatlet on a property or a larger dwelling, servant's quarters, or granny flat
11 = Caravan/tent
12 = Homeless
96 = Other, specify: _____

9. What is the occupational status of your household? .....

1 = Owned, fully paid off
2 = Owned, not fully paid off
3 = Rented
4 = Occupied rent free
6 = Other, specify: _____

10. What is the main material of your floor? .....

1 = Natural floor (earth/sand/dung)
2 = Rudimentary floor (bare wood planks)
3 = Finished floor (parquet/polished/ceramic tiles/cement/carpet)

11. What is the main material of your walls? .....

01 = Plastic or cardboard
02 = Mud
03 = Mud and cement
04 = Corrugated iron or zinc
05 = Prefab or wood
06 = Bare brick or cement blocks
07 = Plaster or finished
96 = Other, specify: _____

12. What is the main source of drinking water for members in your household? .....

01 = Piped (tap) water inside dwelling
02 = Piped (tap) water inside the yard
03 = Piped (tap) water on community stand
04 = No access to piped water
05 = Borehole
06 = Open source (river or stream)

96 = Other, specify: _____

Completed By: [ ][ ] Verified By: [ ][ ] Date Verified *(dd/MMM/yyyy)*: [ ][ ]/[ ][ ]/20[ ][ ]

Study Identifier: AUR2-6-112-[ ][ ][ ]-[ ][ ][ ][ ]-[ ] Date of Visit: [ ][ ]/[ ][ ]/20[ ][ ] Visit Code: 00.0

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

13. What kind of toilet facilities does your household have? ..... ||

01 = Flush toilet connected to sewage
02 = Flush toilet connected to septic tank
03 = Chemical toilet
04 = Pit toilet/latrine with ventilation (VIP)
05 = Pit toilet without ventilation
06 = Bucket toilet
07 = None
96 = Other, specify: _____

14. Does your household have any of the following in working condition?

14a. Electric/gas stove: ..... *0=No, 1=Yes* |
14b. Vacuum cleaner: ..... *0=No, 1=Yes* |
14c. Washing machine: ..... *0=No, 1=Yes* |
14d. Satellite television: ..... *0=No, 1=Yes* |
14e. DVD player: ..... *0=No, 1=Yes* |
14f. Motorcar: ..... *0=No, 1=Yes* |
14g. Mail Post box/bag: ..... *0=No, 1=Yes* |
14h. Mail delivery at home: ..... *0=No, 1=Yes* |
14i. Radio: ..... *0=No, 1=Yes* |
14j. TV: ..... *0=No, 1=Yes* |
14k. Computer: ..... *0=No, 1=Yes* |
14l. Refrigerator: ..... *0=No, 1=Yes* |
14m. Landline telephone: ..... *0=No, 1=Yes* |
14n. Cell phone: ..... *0=No, 1=Yes* |
14o. Bicycle: ..... *0=No, 1=Yes* |
14p. Motorcycle or scooter: ..... *0=No, 1=Yes* |

Completed By: | | Verified By: || Date Verified *(dd/MMM/yyyy)*: | |/| ||/20 ||

Study Identifier: AUR2-6-112-___-___-_ (Protocol - Site code - Participant ID)

Date of Visit: __/___/20__ (dd/MMM/yyyy)

Visit Code: 00.0

## RK003: RISK FACTORS/MEDICAL HISTORY

***Instructions**: Complete this CRF for all Participants at enrolment.*

***WORKING CONDITIONS***

1. Have you ever worked for the mines? ..... *0=No, 1=Yes* ☐
   - 1a. If yes, for how many years? ..... *97=Not applicable; 99=Don't know* ☐☐
   - 1b. If yes, did you ever work underground? ..... *0=No, 1=Yes, 7= Not applicable* ☐
2. Have you ever been a health care worker? ..... *0=No, 1=Yes* ☐
   - 2a. If yes, have you ever been close enough to talk to /be coughed upon by patients? ..... *0=No, 1=Yes, 7=NA* ☐
   - 2b. If yes, are you currently a health care worker? ..... *0=No, 1=Yes, 7=NA* ☐
3. Have you ever worked in a medical laboratory? ..... *0=No, 1=Yes* ☐
4. Have you ever been **incarcerated** in a correctional facility, jail or prison? ..... *0=No, 1=Yes* ☐
5. Have you ever **worked** in a correctional facility, jail or prison? ..... *0=No, 1=Yes* ☐

***HEALTH CONDITIONS***

6. Have you smoked at least 100 cigarettes in your entire life? ..... *0=No, 1=Yes* ☐

   *(If no, score out, initial and date questions 6a to 6d*
   - 6a. At what age did you start smoking? ..... ☐☐
   - 6b. Have you smoked any cigarettes in the last year? ..... *0=No, 1=Yes* ☐
   - 6c. At what age did you stop smoking? ..... *97=Participant is still smoking* ☐☐
   - 6d. When you smoked OR currently, how many cigarettes on average did/do you smoke per day? ..... ☐☐☐

Completed By: ☐☐ Verified By: ☐☐ Date Verified *(dd/MMM/yyyy)*: ☐☐/☐☐☐/20☐☐

Study Identifier: AUR2-6-112-[ ][ ][ ]-[ ][ ][ ][ ]-[ ]

Protocol - Site code - Participant ID

Date of Visit: [ ][ ]/[ ][ ][ ]/20[ ][ ]

dd/MMM/yyyy

Visit Code: 0 0 .0

*'Now I am going to ask you some questions about whether you have had any alcohol in the **PAST YEAR**.'*

7. How often do you have a drink containing alcohol? [ ]

0 = Never
1 = Monthly or less
2 = 2 to 4 times a month
3 = 2 to 3 times a week
4 = 4 or more times a week

***If never, score out, initial and date questions 7a to 7n***

7a. **MEN**: How often do you have EIGHT or more drinks on one occasion? [ ]

**WOMEN**: How often do you have SIX or more drinks on one occasion?

*1 drink = 1 small bottle of beer or 1 glass of wine or 1 single measure spirits.*

0 = Never
1 = Less than monthly
2 = Monthly
3 = Weekly
4 = Daily/almost daily

*On average*, how many of each do you consume ***per week***: (***Enter 000 if none***)
***ROUND UP, i.e. answers CANNOT be 000 for all items***

7b. **Beer, Lager or Cider**: **Small bottle** (275mL): [ ][ ][ ]

7c. Beer, Lager or Cider: **Can** (440mL): [ ][ ][ ]

7d. Beer, Lager or Cider: **Sakiya** (carton): [ ][ ][ ]

7e. Beer, Lager or Cider: **Pint** (568mL): [ ][ ][ ]

7f. Beer, Lager or Cider: **Quart/large bottle** (750mL): [ ][ ][ ]

7g. **Wine**: Standard glass (175mL): [ ][ ][ ]

7h. Wine: Large glass (250mL): [ ][ ]

7i. Wine: **Bottle** (750mL): [ ][ ][ ]

7j. **Spirits** (whisky, brandy, gin, vodka, etc.): **Tot/single pub measure** (25mL): [ ][ ][ ]

7k. Spirits (whisky, brandy, gin, vodka, etc.): **Nip** (200mL): [ ][ ][ ]

7l. Spirits (whisky, brandy, gin, vodka, etc.): **Half-jack** (375mL): [ ][ ][ ]

7m. Spirits (whisky, brandy, gin, vodka, etc.): **Bottle** (700mL): [ ][ ][ ]

7n. **Alcopop** (Smirnoff Ice/Spin, Bacardi Breezer, Brutal Fruit): **Standard bottle** (330mL): [ ][ ]

Completed By: [ ][ ] Verified By: [ ][ ] Date Verified *(dd/MMM/yyyy)*: [ ][ ]/[ ][ ][ ]/20[ ][ ]

Study Identifier: AUR2-6-112-___-___-_ Date of Visit: __/___/20__ Visit Code: 00.0

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

***MEDICAL CONDITIONS***

***'Have you ever been told by a medical doctor that you have...'***

8. Asthma? ..... *0=No, 1=Yes* |

9. Chronic bronchitis/emphysema (COPD)? ..... *0=No, 1=Yes* |

10. Silicosis/phytisis or other occupational lung disease? ..... *0=No, 1=Yes* |

11. Any other chronic 'lung damage', e.g. bronchiectasis? ..... *0=No, 1=Yes* |

12. Do you take any medications for your chest (lungs)? ..... *0=No, 1=Yes* |

*Record all medications using coding below, use 0 for empty boxes.* ..... |

1 = Bronchodilator inhaler / pump / spray (e.g. ventolin / salbutamol)
2 = Steroid inhaler / pump / spray (becotide / beclomethasone / budesonide) ..... |
3 = Steroid tablet (prednisolone / predisone – small white tablets)
4 = Aminophylline or theophylline tablets
6 = Other, specify below ..... |
***7 = Not applicable (does not take medications for chest)***
9 = Does not know name of medication taken for this ..... |

12a. Specify other medications for chest (lungs):_____

13. Sugar diabetes? ..... *0=No, 1=Yes* |

13a. Do you take any medicines for diabetes? ..... *0=No, 1=Yes* |

*Record all medications using coding below, use 0 for empty boxes.* ..... |

1 = Tablets (metformin, gliclazide, glibenclamide)
2 = Insulin injection ..... |
***7 = Not applicable (does not take medicines for diabetes)***

14. Heart problem? ..... *0=No, 1=Yes* |

15. BP (high blood pressure)? ..... *0=No, 1=Yes* |

Completed By: __ Verified By: __ Date Verified *(dd/MMM/yyyy)*: __/___/20__

Study Identifier: AUR2-6-112-___-___-_ Date of Visit: __/___/20__ Visit Code: 00.0

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

16. Do you take any medications for your heart or BP? ..... *0=No, 1=Yes*

*Record all medications, using coding below, use 0 for empty boxes.*
1 = Water tablet (diuretic) Furosemide / frusemide / hydrochlorthiazide / spironolactone
2 = ACE or ARB (enalapril, perindopril, ramipril, losartan)
3 = Digoxin
4 = Beta blocker (carvedilol, bisoprolol, atenolol)
5 = Isosorbide mono/di- nitrate
6 = Amlodipine / nifedipine
***7 = Not applicable (does not take medications for heart or BP)***
8 = Aspirin
9 = Does not know name of medication taken for this

17. Hay fever or allergies (itchy eyes, blocked nose, clear discharge from nose, sneezing, cough)? ..... *0=No, 1=Yes*

17a. Do you take any medications for hay fever/allergies? ..... *0=No, 1=Yes*

*Record all medications, using coding below, use 0 for empty boxes.*
1 = Antihistamine tablet e.g. cetirizine, chlorpheniramine
2 = Steroid nasal spray e.g. beclomethasone
***7 = Not applicable (does not take medication for hayfever/allergies)***

18. Acid reflux (heartburn/indigestion)? ..... *0=No, 1=Yes*

18a. Do you take any medicines for acid reflux?. ..... *0=No, 1=Yes*

*Record all medications, using coding below, use 0 for empty boxes.*
1 = Antacid (gaviscon, aluminium hydroxide, magnesium trisilicate)
2 = PPI (omeprazole, lansoprazole)
3 = Cimetidine/ranitidine
***7 = Not applicable (does not take any medicines for acid reflux)***
9 = Does not know name of medication taken for this

19. Hyperthyroidism (overactive thyroid)? ..... *0=No, 1=Yes*

19a. Do you take any medicines for your thyroid? ..... *0=No, 1=Yes*

*Record all medications, using coding below.*
1 = carbimazole
2 = thyroxine
***7 = Not applicable (does not take any medicines for thyroid)***
9 = Does not know name of medication taken for this

20. Hot flushes and sweats due to menopause? ..... *0=No, 1=Yes, 7 = Male*

21. Mental health disorder (e.g. anxiety, depression, schizophrenia, drug misuse)? ..... *0=No, 1=Yes*

22. Do you have any other serious illnesses? ..... *0=No, 1=Yes*

22a. If yes, record: _____

Completed By: __ Verified By: __ Date Verified *(dd/MMM/yyyy)*: __/__/20__

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Date of Visit: □□/□□□/20□□ (dd/MMM/yyyy)

Visit Code: 0 0 .0

## SS001: TODAY'S CLINIC VISIT (Enrolment)

***Instructions**: Complete this CRF at the enrolment visit for all participants.*

### *SYMPTOM SCREEN*

1. Do you have cough currently (in the past 24 hours)? ***(If no: score out, initial and date 1a*** ..... *0=No, 1=Yes* |

*Weeks* *Days*

1a. How long have you had a cough? ..... | || |.....|

2. Do you have a fever? ***(If no: score out, initial and date 2a*** ..... *0=No, 1=Yes* |

*Weeks* *Days*

2a. How long have you had a fever? ..... □□□.....□

3. Do you have drenching night sweats *(sweat so much that your clothes or pillows are soaking wet)*?

***(If no: score out, initial and date 3a*** ..... *0=No, 1=Yes* □

*Weeks* *Days*

3a. How long have you had drenching night sweats? ..... | || |.....|

4. Have you unintentionally lost weight or has your clothing become looser in the last 6 months? . *0=No, 1=Yes* □

***(If no: score out, initial and date 4a to 4b***

*Weeks* *Days*

4a. How long have you had unintentional weight loss? ..... □□□.....□

4b. Have you lost **more than** a dress/trouser size ***unintentionally*** in the last 6 months? ..... *0=No, 1=Yes* □

5. What was your weight about 6 months ago? ..... *(in kgs) 999=Don't know* □□□

6. Have you ***deliberately*** lost weight **since then** (e.g.. dieting or childbirth)? ..... *0=No, 1=Yes* □

7. Do you currently have any other symptoms? ***DO NOT PROMPT*** ..... *0=No, 1=Yes* □

***(If no: score out, initial and date 7a to 7d***

7a. Additional symptom 1: ..... □□

01 = Indigestion
02 = Cold / Flu
03 = Chest pain
04 = Coughing blood
05 = Difficulty breathing
06 = Loss of appetite
07 = Tired most of the time
08 = Swollen lymph node (gland)
96 = Other, specify: _____

Completed By: | | Verified By: | || Date Verified *(dd/MMM/yyyy)*: |/| ||/20 ||

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Visit Code: 0 0 .0

| | Weeks | Days |
|---|---|---|
| 7b. How long have you had this symptom? | ☐☐☐ | ☐ |

7c. Additional symptom 2: *(If NO "additional symptom 2" score out, initial and date 7c to7d)* ☐☐

01 = Indigestion
02 = Cold / Flu
03 = Chest pain
04 = Coughing blood
05 = Difficulty breathing
06 = Loss of appetite
07 = Tired most of the time
08 = Swollen lymph node (gland)
96 = Other, specify: _____

| | Weeks | Days |
|---|---|---|
| 7d. How long have you had this symptom? | ☐☐☐ | ☐ |

***'IN THE LAST 1 MONTH HAVE YOU HAD...'***

8. Any allergy symptoms (itchy eyes, blocked nose, clear discharge from nose)? ... *0=No, 1=Yes* ☐

8a. Do you currently have any allergy symptoms? ... *0=No, 1=Yes* ☐

9. A cold or flu? ... *0=No, 1=Yes* ☐

9a. Do you currently have a cold or flu? ... *0=No, 1=Yes* ☐

***(If no to both 8 and 9: score out, initial and date 10 to 18a***

***'If you had allergy symptoms / cold / flu in the last month, did you have'***

10. Fever or chills (feeling cold with shivering)? ... *0=No, 1=Yes* ☐

10a. Do you currently have fever or chills? ... *0=No, 1=Yes* ☐

11. Headache? ... *0=No, 1=Yes* ☐

11a. Do you currently have a headache? ... *0=No, 1=Yes* ☐

12. Muscle aches or pains? ... *0=No, 1=Yes* ☐

12a. Do you currently have muscle aches or pains? ... *0=No, 1=Yes* ☐

13. Sore throat? ... *0=No, 1=Yes* ☐

13a. Do you currently have a sore throat? ... *0=No, 1=Yes* ☐

Completed By: ☐☐ Verified By: ☐☐ Date Verified *(dd/MMM/yyyy)*: ☐☐/☐☐/20☐☐

Study Identifier: AUR2-6-112-□□□-□□□□-□ Date of Visit: □□/□□□/20□□ Visit Code: 0 0 .0

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***'If you had allergy symptoms / cold / flu in the last month, did you have'***

14. Sneezing? ..... *0=No, 1=Yes* |

14a. Are you currently sneezing? ..... *0=No, 1=Yes* |

15. A runny or blocked nose? ..... *0=No, 1=Yes* |

15a. Do you currently have a runny or blocked nose? ..... *0=No, 1=Yes* |

16. Cough? ..... *0=No, 1=Yes* □

17. Coughing up sputum (spit from chest)? ..... *0=No, 1=Yes* □

17a. Are you currently coughing up sputum? ..... *0=No, 1=Yes* □

18. Feeling generally unwell? ..... *0=No, 1=Yes* |

18a. Are you currently feeling generally unwell? ..... *0=No, 1=Yes* |

******Pregnancy questions:*** ***(If MALE: score out, initial and date pregnancy questions)***

19. When was the first day of your last menstrual period? ..... *dd/MMM/yyyy* | | / || |/| ||

11/NOV/1119 = Don't know

20. Are you pregnant? ..... *0=No, 1=Yes, 9=Don't know* □

a. If yes, when is the estimated due date for your baby? *dd/MMM/yyyy* □□/□□□/□□□□

11/NOV/1117=Not known to be pregnant
11/NOV/1119 = Don't know

********REASON FOR CLINIC VISIT********

21. What reason best describes why you visited the clinic today? ..... □

1 = For symptoms described above
2 = Routine follow-up visit
3 = Routine medicine collection from pharmacy

6 = Other, specify: _____

Completed By: | | Verified By: || Date Verified *(dd/MMM/yyyy)*: |/| ||/20 ||

Study Identifier: AUR2-6-112-□□□-□□□□-□ (Protocol - Site code - Participant ID)

Date of Visit: □□/□□□/20□□ (dd/MMM/yyyy)

Visit Code: 0 0 .0

## CARE FOR CURRENT SYMPTOMS

****REFERS to SS001: cough (Qn 1), fever (Qn 2), night sweats (Qn 3), unintentional weight loss (Qn 4)****

***If participant does not have any of these symptoms: score out, initial and date 22 to 26h***

22. Where was the first place you went to ask for help for these symptoms? ..... □

***(If 0=has not asked for help: score out, initial and date Qn 22a) to 26h)***

| | |
|---|---|
| 0 = Has not asked for help | 4 = Public Hospital |
| 1 = Pharmacy | 5 = Traditional Healer |
| 2 = Public clinic | 6 = Other, specify: _____ |
| 3 = Private Doctor | 7 = Private hospital |

22a. When did you first go to this place? .....*(dd/MMM/yyyy)* /| || || |/

*(If this is the first visit, enter today's date.) Use the following conventions:*

**If day not known, but week is known:** Use Wednesday of week
**If day + week not known:** Use 15th of Month
**If day + month not known, but season and year known:**
01/MAR/YYYY = Autumn
01/JUN/YYYY = Winter
01/SEP/YYYY = Spring
01/DEC/YYYY = Summer
**Only year known =01/ JUL/YYYY**

*Score out, initial and date if "Not applicable"*

23. When did you first attend a health facility (public clinic, private doctor, hospital) for these symptoms? .....

*(If this is the first visit, enter today's date.) Use coding as per Qn 22a (dd/MMM/yyyy)* /| || || |/

24. Before today, have you given a sputum (spit) specimen for these symptoms? ..... *0=No, 1=Yes*

24a. When did you provide this sputum specimen? .....*(dd/MMM/yyyy)* □□/□□□/□□□□

*Use coding as per Qn 22a*

25. Before today, have you had a chest x-ray for these symptoms? ..... *0=No, 1=Yes*

25a. If yes, when? *Use coding as per Qn 22a* ..... *dd/MMM/yyyy,* □□/□□□/□□□□

26. From the time you first had these symptoms, how many times did you visit the following for any of these symptoms:

26a. Pharmacy? .....*(number of visits, enter 00 if not visited)* □□

26b. Public clinic? .....*(number of visits, enter 00 if not visited)* |

26c. Public hospital (outpatient)? .....*(number of visits, enter 00 if not visited)* □□

26d. Public hospital (inpatient)? .....*(number of visits, enter 00 if not visited)* |

26e. Private doctor?.....*(number of visits, enter 00 if not visited)* □□

26f. Private hospital (outpatient)? .....*(number of visits, enter 00 if not visited)* □□

26g. Private hospital (inpatient)?.....*(number of visits, enter 00 if not visited)* □□

26h. Traditional healer? .....*(number of visits, enter 00 if not visited)* |

Completed By: | | Verified By: | || Date Verified *(dd/MMM/yyyy)*: | |/| || |/20 ||

Study Identifier: AUR2-6-112-□□□-□□□□-□ Date of Visit: □□/□□□/20□□ Visit Code: 0 0 .0

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

## *CLINIC INFORMATION*

27. How did you get to the clinic today? ..... ||

01 = Walked
02 = Bicycle
03 = Bus/taxi
04 = Metered taxi
05 = Own car
06 = Lift in a car
07 = Train
96 = Other, specify: _____

28. How long did it take to get from your home to this clinic today (**one way**)?.....*(in minutes)* □□□

*(Enter number of **minutes** [1 hour = 60 mins, 2 hours = 120 mins]*

29. In total, how much did you pay for transport to the clinic today (**one way**)? ..... R | |. ||

*(Enter 000.00 if no cost.)*

30. How often do you attend this clinic? ..... □□

*(Exclude visits only for medicine collection from pharmacy.)*

01 = Monthly
02 = Every 2 months
03 = Every 3 months
04 = Every 4 months
05 = Every 5 months
06 = Every 6 months
96 = Other, specify: _____

31. How often do you attend this clinic pharmacy to pick up your medicines for HIV (ARVs / CPT / IPT)?..... ||

01 = Monthly
02 = Every 2 months
03 = Every 3 months
04 = Every 4 months
05 = Every 5 months
06 = Every 6 months
07 = Never
96 = Other, specify: _____

Completed By: | | Verified By: || Date Verified *(dd/MMM/yyyy)*: |/|| |/20 ||

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*Protocol - Site code - Participant ID*  *dd/MMM/yyyy*

## SS002: BASELINE TB and HIV HISTORY (Enrolment)

***Instructions**: Complete this CRF for all participants at enrolment.*

### *HIV CARE HISTORY*

1. When did you first test positive for HIV: ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

*If unknown use the following conventions:*
**If day not known, but week is known**: Use Wednesday of week
**If day + week not known**: Use 15th of Month
**If day + month not known, but season and year known:**
**01/MAR/YYYY = Autumn** **01/JUN/YYYY = Winter**
**01/SEP/YYYY = Spring** **01/DEC/YYYY = Summer**
**Only year known =01/ JUL/YYYY**

2. Have you ever taken Antiretroviral Therapy (ARVs/treatment for HIV)? ..... *0=No, 1=Yes* [ ]

***(If no: score out, initial and date questions 2a to 2g***

2a. If **MALE,** when did you **first** start ART? ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

*Use coding as per question 1. Score out, initial and date if female.*

***(If male: score out, initial and date questions 2b to 2d***

2b. If **FEMALE,** have you ever taken ARVs for a short time to protect baby during pregnancy? .... *0=No, 1=Yes* [ ]

2c. If **FEMALE,** have you ever taken ARVs for your own health? ..... *0=No, 1=Yes* [ ]

2d. If **FEMALE,** when did you **first** start ART **for your own health**? *Score out, initial and date if not applicable*.....

*Use coding as per question 1.* ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

2e. Are you currently taking ARVs?..... *0=No, 1=Yes* [ ]

***If no: score out, initial and date questions 2ei and 2eii.***

2ei. If yes, record ARVs: *Enter 000 for (d) if participant takes only 3 ARVs*.....a. [ ][ ][ ]

| | | |
|---|---|---|
| TDF = TENOFOVIR | 3TC = LAMIVUDINE | EFV = EFAVIRENZ |
| AZT = ZIDOVUDINE | FTC = EMTRICITABINE | NVP = NEVIRAPINE |
| D4T = STAVUDINE | DDI = DIDANOSINE | LPR = LOPINAVIR/RITONAVIR (ALUVIA / KALETRA) |
| ABC = ABACAVIR | | |
| OTH = OTHER, RECORD | | |

.....b. [ ][ ][ ]

.....c. [ ][ ][ ]

.....d. [ ][ ][ ]

2eii. Specify other ARVs: _____
*Score out if not applicable.*

2f. If you have stopped ARVs, when did you stop? ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

*Most recent time if more than once. Score out, initial and date if not applicable. Use coding as per question 1.*

Completed By: [ ][ ]  Verified By: [ ]  Date Verified *(dd/MMM/yyyy)*: [ ][ ]/[ ][ ][ ]/20[ ][ ]

Study Identifier: AUR2-6-112-[ ][ ][ ]-[ ][ ][ ]-[ ] Date of Visit: [ ][ ]/[ ][ ]/20[ ][ ] Visit Code: [0][0].[0]

*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

2g. Since you first took ART have you noticed any change in the amount of fat in your:

***(Score out, initial and date questions i) to vii) if participant has never taken ART)***

i. Face? ..... *0=No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

ii. Arms? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

iii. Legs? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

iv. Buttocks? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

v. Abdomen? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

vi. Neck? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

vii. Breasts? ..... *0= No change, 1=Decreased, 2=Increased, 9=Don't know* [ ]

3. Have you ever received isoniazid (INH) preventive therapy IPT)? ..... *0=No, 1=Yes, 9=Don't know* [ ]

***If no or don't know: score out, initial and date questions 3a to 3c***

3a. Are you currently taking IPT? ..... *0=No, 1=Yes* [ ]

3b. When did you start IPT? ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

*Use coding as per question 1.*

3c. When did you stop IPT? ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ][ ]

*Use coding as per question 1. Score out, initial and date if not applicable=currently on IPT.*

4. Have you ever received cotrimoxazole preventive therapy (Bactrim/Dapsone)? . *0=No, 1=Yes, 9=Don't know* [ ]

***If no or don't know: score out, initial and date questions 4a to 4b***

4a. Are you currently taking CPT (Bactrim/Dapsone)? ..... *0=No, 1=Yes* [ ]

4b. When did you start taking CPT? ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ][ ]

*Use coding as per question 1*

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*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

## *TB HISTORY*

5. Have you ever been treated for TB? ..... *0=No, 1=Yes* [ ]

*If no: score out, initial and date questions 5a to 5d.*

5a. How many times have you been treated for TB? ..... [ ]

5b. When did you start treatment for ***the most recent episode*** of TB? .....

*Use coding as per question 1.* ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

5c. When did you stop treatment for ***the most recent episode*** of TB? .....

*Use coding as per question 1.* ..... *dd/MMM/yyyy* [ ][ ]/[ ][ ][ ]/[ ][ ][ ][ ]

5d. In total, how many months of TB treatment did you take for ***the most recent episode*** of TB? ..... [ ][ ]

Completed By: [ ][ ] Verified By: [ ] Date Verified *(dd/MMM/yyyy)*: [ ][ ]/[ ][ ]/20[ ][ ]

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## SS003: STUDY PRIORITY (Enrolment)

***Instructions**: Complete this CRF for all participants at enrolment.*

***INFORMATION FROM CLINIC RECORDS***

1. CLINIC weight 6 months ago (or closest) from CLINIC RECORDS: .....*in kgs, 999.9=Not available* .

   1a. Date for weight: .....*(dd/MMM/yyyy)* / /

   *Score out, initial and date if weight not available*

2. Most recent CD4 cell count from CLINIC RECORDS:..... *cells/µL, 9999= Not available*

   2a. Date for CD4 count: .....*(dd/MMM/yyyy)* __ __/__ __ __/__ __ __ __

   *Score out, initial and date if CD4 count not available*

***TODAY'S HEIGHT, WEIGHT AND MUAC MEASURED BY RESEARCHER***

3. MUAC left arm:.....*(in centimetres, 99.9 if cannot measure MUAC)* | |.

4. Weight today *(remove shoes)*: ..... *(in kgs, 999.9 if cannot stand on scales)* __ __ __.__

5. Height *(remove shoes)*: .....*(in meters, 9.99 if cannot stand for height measurement)* __.__ __

6. BMI = weight today ÷ height $^2$: .....*(in meters, 99.9= no height or weight measurement)* □□.□

   *Record to 1 decimal point.*

   Round down decimals XX.X0 to XX.X4. Round up if XX.X5 to XX.X9.

   e.g. 18.40 to 18.44=18.4 vs. 18.45 to 18.49=18.5

***CALCULATE WEIGHT LOSS: USING CLINIC WEIGHT 6 MONTHS AGO (see question 1)***

***Instructions**: Score out questions 7 and 8 if:*

- DELIBERATE weight loss: **SS001 question 6 = Yes, *OR***
- Weight missing = questions 1 OR 4 above are not available.

*For DM*

7. CLINIC weight 6 months ago (Q1) _└└.└ - today's weight (Q4) _└└.└ = *(in kgs)* __.□

   *Enter 00.0 if weight steady OR has gained weight.*

8. % weight loss: {Weight lost Q7 | . ÷ CLINIC weight 6 months ago Q1 | |.|} x 100= *(%)* .| |

   *Enter 00.0 if weight steady OR has gained weight.*

***CALCULATE WEIGHT LOSS: USING PARTICIPANT REPORTED WEIGHT 6 MONTHS AGO (see SS001 Q5)***

***Instructions**: Score out questions 9 and 10 if:*

- DELIBERATE weight loss: **SS001 question 6 = Yes, *OR***
- Weight missing = SS001 question 5 OR question 4 above are not available.

*For DM*

9. REPORTED weight 6 months ago SS01 Q5 | | – Today's weight Q4 | |.| = *(in kgs)* __.□

   *Enter 00.0 if weight steady OR has gained weight.*

10. % weight loss: {Weight lost Q9 | |.| ÷ REPORTED weight 6 months ago SS01 Q5 | |} x100= % .| |

    *Enter 00.0 if weight steady OR has gained weight.*

Completed By: | | Verified By: | Date Verified *(dd/MMM/yyyy)*: | |/| ||/20 ||

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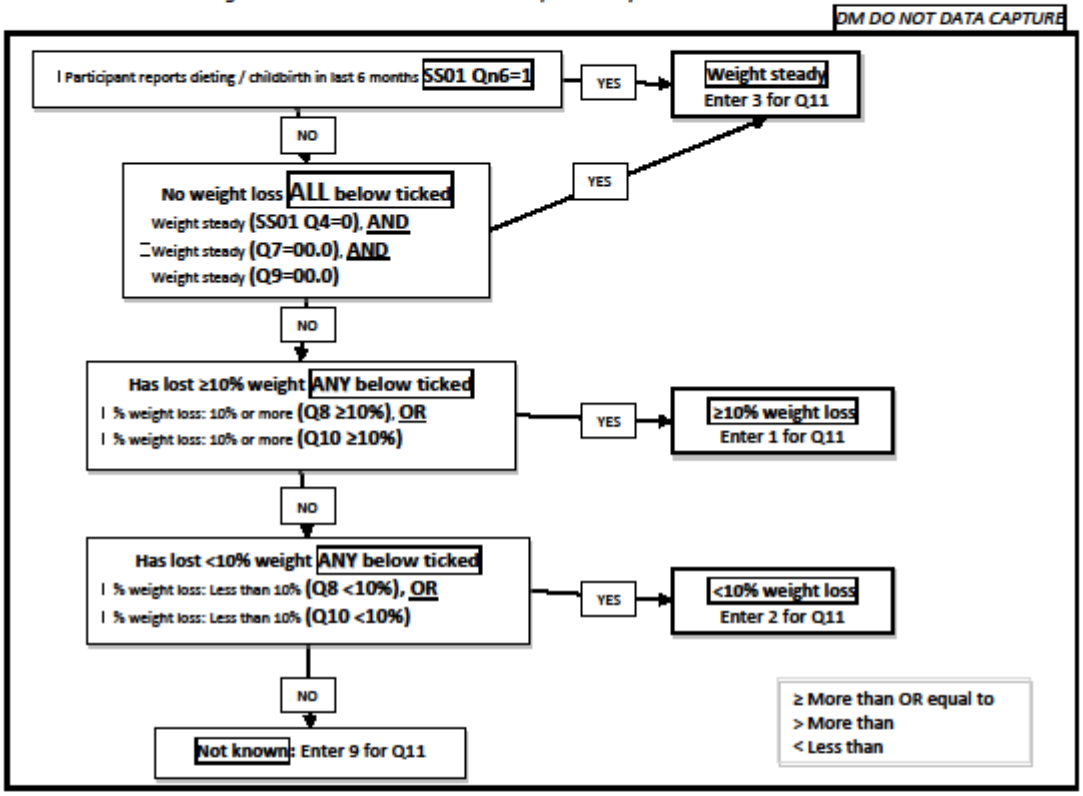
*Protocol - Site code - Participant ID* *dd/MMM/yyyy*

***WEIGHT LOSS CATEGORY***

***Instructions:** Use the algorithm below to determine the response to question 10.*

*DM DO NOT DATA CAPTURE*




11. Record weight loss category: ..... ⌐¬

1 = ≥ 10% weight loss
2 = < 10% weight loss
3 = Weight steady
9 = Not known

***ANY OTHER FEATURE HIGHLY SUGGESTIVE OF TB?***

12. Does patient have any other feature of concern? ..... a. |

*Refer to SS001 question 7a and 7c. If none, enter 0.* ..... b. |

0=No
1=Swollen lymph node (gland)
6=Other, specify

12c. If other, specify: _____

Completed By: | | Verified By: | Date Verified *(dd/MMM/yyyy)*: | |/| ||/20 ||

Study Identifier: AUR2-6-112-⌐¯¬-⌐¬⌐¬⌐¬-¯ Date of Visit: ⌐¬/⌐¬⌐¬/20⌐¬ Visit Code: 0 0 .0

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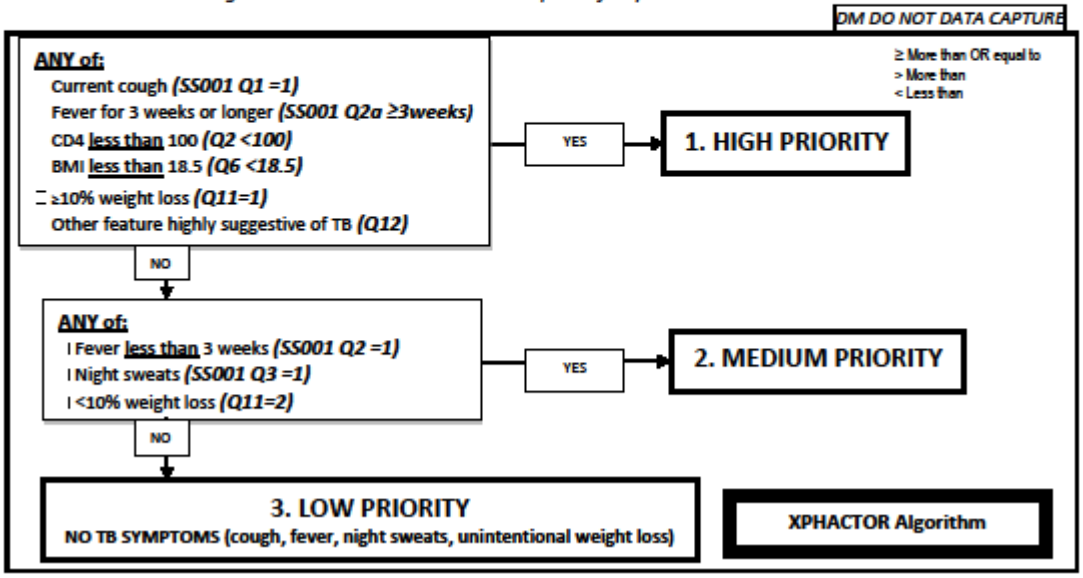
***PRIORITY FOR TB INVESTIGATION***

***Instructions:** Use the algorithm below to determine the response for question 13.*

*DM DO NOT DATA CAPTURE*




13. What priority is participant for immediate investigation? ..... ⌐¬

1 = High priority
2 = Medium priority
3 = Low priority

***DM: Do not data capture 13a and 13b***

13a. Priority has been checked by second member of staff? .....*1=Yes* ⌐¬

***CHECK Page 1 of SS001 "Today's Clinic Visit", pages 1 to 3 of this form SS003 "Study Priority" AND repeat CALCULATIONS.***

13b. Initials of staff member who has checked priority: ..... ⌐¬⌐¬

***INVESTIGATIONS (**Sputum must be sent for immediate Xpert in all "pre-ART with CD4<200" / HCT / ANC**)***

14. **HIGH priority (Qn13=1) *OR* PRE-ART WITH CD4 <200 *OR* HCT *OR* ANC:** Did you collect sputum for immediate Xpert? .....*0=Unable to produce; 1=Yes, 7= Not applicable* ⌐¬

14a. Stick sputum barcode here:

Completed By: ⌐¬⌐¬ Verified By: ⌐¬ Date Verified *(dd/MMM/yyyy)*: ⌐¬⌐¬/⌐¬⌐¬/20⌐¬⌐¬

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15. **MEDIUM** (2) or **LOW** (3) priority: Did you collect sputum to **STORE** at **research laboratory**? .....

.....*0=Unable to produce; 1=Yes, 7= Not applicable (high priority / pre-ART with CD4 < 200 / HCT / ANC)* ⌐

15a. If **MEDIUM** or **LOW** priority– stick sputum barcode here:

16. **'Pre-ART' OR "on ART with CD4 <200":** did you collect urine to store at **research laboratory**? .....

.....*0=Unable to produce; 1=Yes, 7= Not applicable* ⌐

16a. Stick URINE barcode here:

Completed By: | | Verified By: | Date Verified *(dd/MMM/yyyy)*: | |/| || |/20 ||

## 10.4. Standard operating procedures for clinician assessment for "Causes of TB symptoms" study

### A. Purpose:

To outline the following

1) Clinical evaluation performed by research clinician at initial assessment of participants enrolled to Aim 3 including:
   a) Assessment of all participants
   b) Assessment specific to those reporting cough
   c) Assessment specific to those with ≥5% measured unintentional weight loss since enrolment
   d) Assessment specific to those reporting fever / night sweats
2) On-going evaluation by research clinician of participants enrolled to Aim 3
3) Assignment of final diagnosis

### B. Scope:

Applies to all staff involved in the follow-up of participants at XPHACTOR study sites.

### C. Responsibilities:

The Project Manager is responsible for:

- Ensuring that sites are appropriately resourced to perform XPHACTOR study procedures

The Research Clinicians are responsible for:

- Ensuring adherence to this SOP

**Laboratories:**

**Centre for Tuberculosis (CTB)**, National Institute for Communicable Diseases

1) TB culture (sputum, urine, stool) and may undertake TB microscopy and culture from FNA if requested.

**Centre for Respiratory Diseases and Meningitis (CRDM)**, National Institute for Communicable Diseases

- PCR and culture for pertussis and atypical bacteria, and sputum for MC&S.

**Clinical Lab Services** (CLS), Spencer Lister Building, NHLS Complex

2) All other Aim 3 samples, unless the responsible clinic physician requests these are sent via the routine clinic system

| D. SOP Text | | |
|---|---|---|
| **Step** | **Responsibility** | **Activity** |
| **1.** | Research Clinician | **Overview of initial assessment by research clinician**<br><br>The research clinician (RC) will assess ALL participants enrolled in study Aim 3 at an appointment arranged by the research team at the 3-month visit (see SOP XPH-015). The assessment should occur within 2 weeks of the 3-month visit.<br><br>This assessment will include:<br><br>1) Performing a clinical assessment using case report form (CRF) AIM3_002.<br>2) Reviewing the participant's study file and clinic notes and completing CRF AIM3_File Review.<br>3) Arranging a standard set of investigations according to the participant´s symptomatology, medical history and previous investigations.<br>4) Prescribing treatment and providing lifestyle advice, appropriate to the suspected diagnosis, in collaboration with the responsible clinic physician.<br>5) Organising referral for a specialist opinion, when clinically appropriate, in collaboration with the responsible clinic physician.<br>6) Facilitating admission to hospital if participant is acutely unwell. |
| **2.** | Research Clinician | **Initial evaluation of ALL participants enrolled in Aim 3**<br><br>The RC will<br><br>1) Review the participant's file and clinic records, for symptom frequency and duration, major diagnoses, investigations and results (including all TB investigations sent as part of XPHACTOR study or by clinic physicians), last cervical smear (if applicable), and treatment to date including detailed ART history; and complete CRF AIM3_File Review.<br><br>2) Take a history which will include detailed assessment for cough, fever, night sweats and unintentional weight loss, review of current medications, systems review, and past medical history, date and result of last cervical smear (if applicable).<br>a) Cough assessment will include: duration, nature and frequency of cough; history of preceding respiratory infection; associated symptoms and trigger factors; diurnal variation, smoking status and environmental exposures; and use of ACE inhibitors.<br>b) Assessment for unintentional weight loss will include: direct enquiry regarding symptoms suggestive of acute or chronic infection, endocrine disease, malignancy, systemic disease, loss of appetite or difficulty eating (odynophagia), malabsorption, drug or alcohol misuse, psychological illness, loss of body fat since starting ART and ART history.<br>c) Assessment for fever and/or night sweats will include: duration and pattern, assessment for possible focus of infection, malignancy, |

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| | | connective tissue / endocrine / blood disorder, travel history, alcohol and drug history, and if female assess for peri-menopause / menopause.<br><br>3) Screen / evaluate all participants for the following using validated tools:<br>a) Anxiety and depression<br>b) Household food insecurity<br><br>4) Perform a physical examination, including:<br>a) Assessment for lymphadenopathy.<br>b) Measurement of temperature (see SOP XPH-015).<br>c) Respiratory, cardiovascular and abdominal examination.<br>d) Ear, nose and throat examination.<br>e) Skin, oropharynx, and palate (e.g. for Kaposi sarcoma, candidiasis, and other lesions).<br>i) If clinically indicated, refer for biopsy of skin lesion(s).<br>f) Urine dipstick for protein, glucose, blood, nitrites, leucocytes; send for microscopy, and culture if abnormal and cytology if clinically indicated.<br>g) Further examination as indicated by history, e.g. for focus of infection / neurological / rectal examination / breast examination / vaginal examination.<br><br>5) If applicable, review of temperature chart that participant has completed (thermometers are given at 3-month visit for those who report fever or night sweats, for twice daily temperature and temperature at time feels feverish / night sweats).<br><br>6) Review most recent chest radiograph (CXR) and report, and facilitate further management of abnormal CXR findings. All Aim 3 participants without CXR in preceding 6 weeks will have had CXR arranged at the 3-month visit.<br><br>7) Arrange the following investigations:<br>a) *If measured temperature (see 3b) is >38.3*: aerobic and anaerobic blood cultures will be taken. Blood culture for TB will have already been taken at the 3-month visit.<br>b) *If axillary or cervical lymph nodes display features requiring further investigation* (see below), RC will facilitate fine needle aspiration (FNA). The aspirate will be sent for TB microscopy and cytology and, if feasible, also for TB culture.<br>If lymph node is exuding caseous material via a fistula, then material will be sent for TB microscopy and, if feasible, also for TB culture. |

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| | | Features indicating need for FNA are:<br><br>i) large (> 2 cm diameter) or rapidly growing lymph nodes<br>ii) asymmetrical lymphadenopathy<br>iii) tender/painful lymph nodes not associated with local infection<br>iv) matted/fluctuant lymph nodes<br>c) *If pleural effusion is present on CXR,* RC will facilitate diagnostic pleural aspiration. The aspirate will be inspected and sent for ADA (adenosine deaminase), protein, TB microscopy and culture, MC&S (microscopy, culture and sensitivity), cytology and LDH.<br>d) Send sputum, stool and urine samples collected by the participant on the day of the assessment for TB culture (CTB) |
| **3.** | Research Clinician | **Initial assessment of COUGH:**<br><br>CXR will have already been requested for all Aim 3 participants at 3-month visit, if no CXR from the preceding 6 weeks (see SOP XPH-015). The participant will attend the assessment with this CXR.<br><br>1) **FOR ALL** participants reporting cough at 3-month visit:<br>a) *Sputum for TB culture*. One sample is sent for all Aim 3 participants (early morning sample collected by participant on day of appointment with clinician) on the day of Clinician Assessment (see above), - this should be induced if participant is unable to expectorate spontaneously and there are no contraindications to sputum induction. At the three-month visit PN will have also sent a sputum sample for TB culture.<br>b) *Sputum for bacterial culture (MC&S and atypical bacteria)*. This sample was sent at 3-month visit (see SOP XPH-015).<br>c) *Nasopharyngeal swab and oropharyngeal swabs for PCR* (pertussis and atypical bacteria). These samples were sent at 3-month visit (see SOP XPH-015).<br><br>2) **For participants reporting acute cough (≤3 weeks)**:<br>a) Sputum samples for MC&S, atypical bacteria, and TB investigation already sent (see above).<br>b) If pneumonia suspected (cough and at least one of new focal chest signs, fever > 4 days or dyspnoea / tachypnoea, pulse>100, and without other obvious cause):<br>i) Send blood for FBC and differential, CRP.<br>ii) Arrange CXR if no CXR since onset of cough.<br>iii) Take aerobic and anaerobic blood cultures.<br>iv) If feasible send blood for serology for atypical bacteria (*Mycoplasma pneumonia*e, *Chlamydophila pneumonia, Legionella* spp, and *Coxiella).*<br><br>3) **For participants reporting subacute cough (>3 to <8 weeks)**<br>a) Ensure that two spontaneous and one induced (if feasible and there are no contraindications to induction [see SOP XPH-008]) sputum sample have been sent for TB culture within the last 2 weeks. |

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| | | b) If cough has not followed an obvious preceding respiratory tract infection, then investigate further as per chronic cough.<br><br>4) **For participants reporting chronic cough (≥8 weeks)**:<br>a) Ensure that two spontaneous and one induced (if feasible and there are no contraindications to induction – [see SOP XPH-008]) sputum samples have been sent for TB culture within the last 2 weeks.<br>b) Send blood for FBC, differential and CRP, if no recent result (within last 1 month).<br>c) Refer all for spirometry pre- and 20 minutes post- 400mcg inhaled salbutamol via large volume spacer / 5mg nebulised salbutamol (or 10mg nebulised terbutaline / 500mcg terbutaline via large volume spacer), unless contraindicated. A referral letter is required to the respiratory clinic / spirometry provider. Spirometry should not be requested for patients who have had any of the following within last 3 months:<br>i) Unstable cardiovascular status:<br>(1) Myocardial infarction / unstable angina<br>(2) Pulmonary embolism<br>(3) Uncontrolled hypertension<br>ii) Surgery:<br>(1) Hernia repair<br>(2) Eye surgery<br>(3) Thoracic / abdominal / other major surgery<br>iii) Pneumothorax.<br>iv) Ear Infection<br>v) Haemorrhagic cerebrovascular event<br>vi) 3rd Trimester pregnancy<br>vii) Haemoptysis of unknown origin<br><br>Preparation instructions for spirometry should be given to participant, i.e. ***avoid***<br><br>i) a large meal 2 hours pre-testing<br>ii) smoking for 24 hours pre-testing<br>iii) drinking alcohol 2 hours pre-testing<br>iv) taking short acting bronchodilators 6 hours pre-testing<br>v) taking long acting bronchodilators for 12 hours pre-testing<br>vi) taking sustained release theophyllines 24 hours pre-testing<br>d) Assess cough severity using visual analogue scale (VAS, 0-100mm).<br><br>5) **For participants with features suggestive of cardiac failure** (orthopnoea / paroxysmal nocturnal dyspnoea / exertional dyspnoea / peripheral oedema): measure serum natriuretic peptides. |

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| | | 6) **If *Pneumocystis jiroveci* pneumonia is likely**, i.e. participant has the following:<br>a) CD4 <200 cells/µl *and*<br>b) fever / exertional dyspnoea / tachypnoea, *with or without*<br>c) characteristic chest radiograph features (bilateral diffuse/mid-zone symmetrical ground-glass or interstitial shadowing)<br>Discuss referral to hospital / for admission with responsible clinic physician and if feasible arrange the following investigations:<br>i) Bronchoalveolar lavage (BAL) fluid (induced sputum if BAL not available) for cytology for *Pneumocystis jirovecii* cysts.<br>ii) Serum for 1,3-β-D-glucan<br>iii) Exercise oximetry<br><br>**Initial management plan for cough:**<br><br>1) **TB likely:** Facilitate TB treatment if positive sputum results (Xpert or AFB/TB culture). If negative or pending microbiology and CXR features of active TB, facilitate TB treatment (discuss first with responsible clinic physician).<br><br>2) **If any red flags (see Appendix 1):** Persistent haemoptysis in smokers or ex-smokers who are ≥40 years or a chest X-ray suggestive of lung cancer: refer urgently to chest physician for assessment (including CT scan of chest or bronchoscopy as deemed clinically appropriate). Consider FBC and differential at same time as arranging referral.<br><br>3) **Pneumocystis jiroveci pneumonia likely:** Participant should be managed by responsible clinic physician, as admission is likely to be required, although mild cases might be managed on an outpatient basis with high dose trimethoprim-sulfamethoxazole for 21 days and prednisolone.<br><br>4) **Acute cough (≤ 3 weeks)**<br>a) **Suspect common cold** if: nasal congestion / discharge, postnasal drip, sneezing and sore throat. Advise symptomatic treatment.<br>b) **Suspect influenza** if: fever with ≥1 of headache, myalgia, cough and sore throat. Advise symptomatic treatment.<br>c) **Suspect community acquired pneumonia (CAP)** if: cough and at least one of new focal chest signs, fever > 4 days or dyspnoea / tachypnoea, pulse>100, and without other obvious cause. ***Definite CAP*** *if above supported by CXR findings of lung shadowing that is likely to be new.*<br>i) Prescribe antibiotics (amoxicillin first-line, and tetracycline or macrolide in case of hypersensitivity), and review response to treatment. Advise patient to return if no clinical improvement in 3 days or any deterioration, or if symptoms take longer than |

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| | | 3 weeks to resolve. Discuss admission with responsible clinic physician if CRB-65 severity score>0 (1 point for each of the following).<br>(1) Confusion<br>(2) Respiratory rate ≥30/min<br>(3) Systolic BP<90 or diastolic BP≤60 mm Hg<br>(4) Age ≥ 65 years<br><br>d) **Suspect acute bronchitis** if:<br>i) Cough associated with at least one of sputum production / dyspnoea / wheeze / chest discomfort or pain, and<br>ii) No evidence of pneumonia (clinical or radiographic), and<br>iii) Common cold, acute asthma, and exacerbation of COPD have been ruled out.<br>Usually viral in origin, and hence antibiotics are not routinely indicated. Bronchodilators may be useful if wheezing present.<br><br>e) **Suspect exacerbation of pre-existing condition** if:<br>i) History of bronchiectasis and acute deterioration, with worsening cough (with increased sputum volume, viscosity, or purulence; with or without increasing wheeze, breathlessness, or haemoptysis) and/or systemic upset.<br>(1) Ensure sputum has been sent for culture and sensitivity, and discuss with responsible clinic physician regarding appropriate antibiotic prescription and other recommended treatment / admission<br>ii) History of asthma and acute dyspnoea / wheeze / chest tightness<br>(1) If admission is not required arrange bronchodilator and prednisolone prescription.<br>iii) History of COPD and increasing dyspnoea / purulent sputum / clinical signs of pneumonia<br>(1) advise increased frequency of bronchodilator use<br>(2) prescribe oral steroids if significant increase in breathlessness<br>(3) prescribe antibiotics if purulent sputum or signs of pneumonia<br><br>f) **Suspect pertussis if:**<br>i) **Suspected case:** acute cough lasting for ≥14 days, without an apparent cause plus one or more of the following<br>(1) Paroxysms of coughing<br>(2) Post-tussive vomiting<br>(3) Inspiratory whoop and<br>(4) Absence of laboratory confirmation |

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| | | ii) **Confirmed case:** signs and symptoms of pertussis with B pertussis isolated from respiratory sample or confirmed B. pertussis PCR positive in a respiratory clinical specimen<br>• Provide antibiotic therapy if within 3 weeks of onset of illness: azithromycin 500mg od for 3 days / clarithromycin 500mg for 7 days / if pregnant erythromycin 500mg qds for 7 days / if macrolide contraindicated cotrimoxazole 960mg bd for 7 days (not in pregnancy).<br><br>5) **<u>For chronic cough</u>**<br>a) **Suspect bronchiectasis** if chronic cough with copious sputum production and/or suggestive chest x-ray, discuss with responsible clinic physician regarding referral to chest physician for CT scan of chest and further management.<br>b) **Suspected heart failure:**<br>i) If serum natriuretic peptides raised or high facilitate echocardiogram<br>(1) High levels: BNP > 400 pg/ml (116 pmol/litre) or NTproBNP > 2000 pg/ml (236 pmol/litre)<br>(2) Raised levels: BNP 100–400 pg/ml (29–116 pmol/litre) or NTproBNP 400–2000 pg/ml (47–236 pmol/litre)<br>ii) Arrange further management according to clinical symptoms and echocardiogram findings, in collaboration with clinic physician. If no access to echocardiogram, arrange electrocardiogram.<br>c) **If spirometry performed:**<br>i) Ensure quality control (*see Reference 4. Levy et al*):<br>(1) Within-manoeuvre criteria - individual spirograms are acceptable if:<br>(a) They are free from artefacts i.e. free from:<br>(i) Cough during first second of exhalation<br>(ii) Glottis closure that influences measurement<br>(iii) Early termination or cut-off<br>(iv) Effort that is not maximal throughout<br>(v) Leak or obstructed mouthpiece<br>(b) They have good starts:<br>(i) Extrapolated volume <5% of FVC or 0.150 L, whichever is greater<br>(c) They show satisfactory exhalation<br>(i) Duration ≥6s for adults or plateau in volume time curve or<br>(ii) If patient cannot or should not continue to exhale<br><br>(2) Between-manoeuvre criteria, -apply the following tests after three acceptable individual spirograms have been obtained:<br>(i) Two largest values of FVC must be within 0.150 L of each other, **<u>&</u>** |

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| | | (ii) Two largest values of FEV1 must be within 0.150 L of each other<br><br>If the above criteria are not met then testing should be continued until both criteria are met, or total of eight tests have been performed, or patient cannot / should not continue.<br><br>Three satisfactory manoeuvres should be saved.<br><br>ii) If FEV1/FVC <0.7 and >400ml improvement in FEV1 after bronchodilator, treatment trial for asthma (200mcg inhaled beclomethasone twice daily for 6 weeks, or oral prednisolone 30mg daily for 2 weeks) and review clinical response.<br>iii) If FEV1/FVC <0.7 and FEV1< 80% predicted, age>35yr and smoking history (or history of exposure to air pollution, e.g. wood burning stove), without >400ml improvement in FEV1 after bronchodilator, treat for COPD for 6-8 weeks and review clinical response.<br><br>d) **If spirometry is not available and high probability of asthma** (see features listed below), start treatment trial for asthma, and review clinical response.<br>(1) >1 of cough, wheeze, breathlessness, chest tightness especially if symptoms are:<br>(a) worse at night and in early morning<br>(b) in response to exercise, allergen exposure and cold air<br>(c) after taking aspirin or beta blockers<br>(2) History of atopy<br>(3) Family history of atopy or asthma<br>(4) Widespread wheeze<br>(5) Otherwise unexplained eosinophilia<br><br>e) **If participant complains of frequent gastrointestinal symptoms suspect gastro-oesophageal reflux disease (GORD):** Symptoms include daily heartburn (sensation of discomfort or burning behind the sternum rising up to the neck) or regurgitation (effortless return of stomach contents into the pharynx), cough worse with or after meals/stooping, cough on phonation, dysphonia, and abatement of cough during sleep.<br>**If any red flag symptoms (Appendix 1) facilitate urgent referral for endoscopy:**<br><br>i) Dyspepsia in patient aged ≥55 years with onset of dyspepsia <1yr previously / continuous symptoms since onset<br>ii) Dysphagia at any age |

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| | | iii) Dyspepsia at any age with any of: anaemia, persistent vomiting or weight loss<br>iv) Dyspepsia with any of: family history upper GI cancer in >2 first-degree relatives, Barrett's oesophagitis, pernicious anaemia, peptic ulcer surgery >20 years previously, known dysplasia, atrophic gastritis, intestinal metaplasia, jaundice, or upper abdominal mass<br><br>**If no red flags treat as GORD:**<br><br>• Provide diet and lifestyle advice: Reduce weight if overweight, stop smoking, reduce alcohol and aggravating foods (fat, chocolate, citrus), raise the head of the bed, and avoid eating during the three hours before going to bed.<br>• Provide trial of PPI (e.g. omeprazole 20mg twice daily) for 8 weeks (30 minutes before food) and review response. If no improvement discuss with responsible clinic physician regarding referral for upper gastrointestinal endoscopy.<br><br>f) **If upper airways symptoms predominate suspect upper airways disease:** Symptoms include post nasal drip (sensation of having something drip down into throat), persistent nasal discharge / congestion, and recurrent need to clear throat).<br><br>Differential diagnoses include:<br><br>i) Allergic rhinitis: suggested by sneezing and itching eyes / ears, and may be seasonal or perennial. Provide steroid nasal spray / antihistamine and review response at 8 weeks.<br>ii) Post-viral: suggested by preceding upper respiratory tract infection (URTI). Provide antihistamine and review response at 8 weeks.<br>iii) Secondary to chronic sinusitis: suggested by symptoms > 12 weeks of facial discomfort (often unilateral and worse when bending forwards) or pain; nasal obstruction or (purulent) nasal discharge or postnasal drip; and decreased or absent sense of smell. Provide steroid nasal spray for 12 weeks and review response. Consider ENT referral.<br>iv) Anatomic abnormality e.g. deviated nasal septum / nasal polyp. Refer for ENT opinion (unilateral polyp may be a sign of malignancy).<br>v) Rhinitis medicamentosa most commonly due to long-term use of topical decongestant. Withdraw offending agent one nostril at a time. |

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| | | g) **If on ACE inhibitor**, discuss replacing this (e.g. with angiotensin receptor blocker or other alternative) with responsible clinic physician. If replaced, review response at 4 weeks and 12 weeks as ACE inhibitor-induced cough may linger for up to 3 months in a subgroup of individuals.<br><br>6) **If current smoker:** Provide smoking cessation advice, and review response at 8 weeks post-cessation:<br>a) Ask about smoking.<br>b) Advise participant to quit smoking unless there are exceptional circumstances.<br>c) If participant is interested in quitting provide advice, which may include attending a smoking cessation service if available, or discussing pharmacotherapy (not available within public sector in South Africa).<br>d) If participant is not ready to quit ask him/her to consider the possibility and encourage seeking help in the future.<br><br>**SECOND LINE if no clear diagnosis for COUGH identified**<br><br>1) Suspected TB: after discussion with responsible clinic physician treat for TB and review response to treatment.<br><br>2) Referral to respiratory physician for further respiratory investigation e.g. bronchoscopy if deemed appropriate |
| **4.** | Research Clinician | **Initial assessment of UNINTENTIONAL WEIGHT LOSS:**<br><br>Defined as: ≥ 5% measured weight loss since enrolment.<br><br>**For ALL**:<br><br>1) Complete lipodystrophy assessment CRF<br>2) Blood tests:<br>a) FBC and differential count, renal function, liver function, HbAlc, thyroid function.<br>3) Repeat CXR if no CXR in last 4 weeks.<br>4) Abdominal ultrasound scan<br>5) If diarrhoea (loose or liquid stools more than three times daily) reported:<br>a) Stool for microscopy, bacterial culture and parasitology.<br>b) Test for clostridium difficile toxin if history of antibiotic prescription in last 12 weeks.<br>c) Arrange further management if any of the above tests are positive.<br><br>6) If any red flags are present discuss with responsible clinic physician and refer for as appropriate urgent gastroscopy, gastroenterology / surgical / gynaecological opinion and pap smear (review last result if available): |

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| | | a) Change in bowel habit, rectal bleeding, dyspepsia, dysphagia, melaena, persistent vomiting, unexplained iron deficiency anaemia, abdominal or rectal mass, jaundice, see above for suspected GORD red flags)<br>i) Ensure digital rectal examination if:<br>(1) Unexplained lower gastrointestinal tract symptoms<br>(2) Male patient with any features suggestive of prostate cancer<br>ii) Ensure breast examination if patient complains of breast symptoms<br>b) Intermenstrual bleeding, postmenopausal bleeding, postcoital bleeding, alteration in vaginal discharge. Patient requires full pelvic examination.<br><br>**Second line investigations for unintentional weight loss**<br><br>1. Blood tests: lactate (if other symptoms of hyperlactataemia or on D4T), and discuss with responsible clinic physician. |
| **5.** | Research Clinician | **Initial assessment of FEVER / NIGHT SWEAT**<br><br>**For ALL**<br><br>1) Assess for likely focus of infection and travel history (as detailed above in "Initial assessment for all") and arrange further investigation as indicated history and examination.<br>2) Review participant's temperature record.<br>3) If likely focus of infection identified then:<br>a) Respiratory tract infection: evaluate as per section above for cough.<br>b) Urinary symptoms / abnormal urine dipstick: MSU for microscopy and culture.<br>c) Diarrhoea reported (loose or liquid stools more than three times daily): stool for microscopy, bacterial culture, and parasitology, test for clostridium difficile toxin if history of antibiotic prescription in last 12 weeks.<br>d) Malaria film if indicated by travel history.<br>e) Cellulitis / skin lesions (sores / ulcers / oozing lesions) or discharging ear: swab if possible and send for microscopy and culture.<br>f) Abscess identified: refer for incision and drainage, and if feasible send sample for MC&S.<br>g) Tonsillitis likely, consider throat swab.<br>h) Pelvic infection likely (lower abdominal pain, deep dyspareunia, abnormal vaginal bleeding, purulent vaginal discharge): facilitate cervical swabs for chlamydia and gonorrhoea.<br>i) Sexually transmitted infection likely: facilitate appropriate investigation.<br>j) Acute abdomen: refer to hospital for further investigation and management. |

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| | | k) Meningitis likely (headache, stiff neck, altered mental state, shock, focal neurological deficit): refer to hospital for further investigation (CT brain, lumbar puncture) and management<br>l) Endocarditis suspected ([Risk factors: valvular heart disease, valve replacement, structural congenital heart disease, hypertrophic cardiomyopathy, previous endocarditis, recreational drug abuse, invasive vascular procedures] and [Features: non-specific symptoms, murmur, petechiae, splinter haemorrhages, clubbing, arthritis, Osler's nodes, Janeway's lesions, congestive cardiac failure]): discuss referral to hospital with responsible clinic physician for further investigation and management.<br>m) Osteomyelitis / septic arthritis suspected: refer to hospital for investigation and further management.<br>n) If appropriate, arrange further imaging (e.g. CT scan or sinus radiography) after discussion with responsible clinic physician<br><br>4) No likely focus of infection identified:<br>a) If measured temperature is >38.3: aerobic and anaerobic blood cultures.<br>b) FBC + differential, CRP, renal and liver function, HbAlc, glucose and thyroid function.<br>i) Discuss with responsible clinic physician if blood film should be arranged through routine system for suspected haematological cancer.<br>c) Send urine for microscopy and culture.<br>d) Repeat CXR if no CXR since onset of symptoms, or no CXR in last 4 weeks.<br>e) Abdominal ultrasound scan<br>f) If measured fever: consider CT scan abdomen.<br>g) If measured fever: consider sinus radiograph.<br>5) If female < 45 years of age and symptoms suggestive of early menopause (hot flushes, night sweats, and irregular menstrual cycle): consider measurement of follicle-stimulating hormone (FSH) levels.<br><br>**Initial management plan for fever / night sweats:**<br><br>1) If focus of infection identified and participant does not require referral to hospital (mild (37.2-38) or moderate pyrexia (38.1-40) and not acutely unwell) then facilitate prescription for appropriate treatment for infection.<br>2) Facilitate referral to hospital (discuss with responsible clinic physician) for further evaluation if:<br>a) Acutely unwell<br>b) High fever (>40) at assessment<br>c) Hospital management required (e.g. surgery or further assessment) |

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| | | 3) If no measured or recorded fever discuss with responsible clinic physician.<br><br>**SECOND LINE if no clear diagnosis for fever / night sweats identified**<br><br>Discuss with responsible clinic physician regarding whether the following investigations are deemed clinically appropriate and can be arranged through clinic or by referral to specialist, for patients with measured fever or reported night sweats and no focus of infection identified.<br><br>1) Autoimmune screen (antinuclear antibody [ANA], Rheumatoid factor)<br>2) Abdominal CT scan<br>3) Bone marrow aspiration |
| **6.** | Research Clinician | Participants will be reviewed at scheduled monthly follow up with the research team (or more frequently if clinically indicated).<br><br>Follow up will include:<br><br>1. Sputum to be sent for TB culture if cough reported<br>2. If persistent cough, repeat CXR and ensure 3 sputa for TB culture (of which one induced, if feasible, and no contraindication to sputum induction [see SOP XPH-008])<br>3. Follow up of results of investigations<br>4. Follow up of evolution of symptoms<br>5. Follow up of outcome of specialist referrals<br>6. Follow up of response to any treatments prescribed |
| **7.** | Research Clinician | **Assignment of final diagnosis**:<br><br>Will be based assigned at 6 months based on results of investigations and response to any trials of treatment. |

## 10.5. Evaluation of WHO 4-Symptom Tool to Rule Out TB: Data from the XPHACTOR Study (Poster)

# Evaluation of WHO 4-symptom tool to rule out TB: data from the XPHACTOR study

Yasmeen Hanifa[1], Katherine Fielding[1], Violet Chihota[2], Nontobeko Ndlovu[2], Alan Karstaedt[3], Faieza Sahid[3], Lungiswa Adonis[4], Linda Erasmus[5], Mark Nicol[5], Gavin Churchyard[2], Alison Grant[1]

Yasmeen.Hanifa@lshtm.ac.uk

[1]London School of Hygiene & Tropical Medicine, London, UK; [2]Aurum Institute for Health Research, Johannesburg, South Africa; [3]Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa; [4]Mamelodi Hospital, Pretoria, South Africa; [5]National Health Laboratory Service, Johannesburg, South Africa.

## Introduction

**Background**

- WHO recommends screening HIV+ individuals for TB using tool comprising any cough, weight loss, fever or night sweats ("WHO tool")
- Tool developed from meta analysis of individual participant data, largely not on ART; designed to maximise sensitivity and negative predictive value (NPV) to exclude TB so IPT and ART can be initiated
- XPHACTOR is an interventional cohort study evaluating a risk-based algorithm to prioritise testing with Xpert MTB/RIF amongst adults attending for HIV care in South Africa

**Aim**

- To evaluate performance of the WHO tool as a test to rule out TB among patients in the XPHACTOR study

## Methods

**Population**

- Systematic sample of adults attending 4 HIV clinics in Gauteng were screened for TB, Sept 2012 – Feb 2014
- Enrolled in three groups, on ART vs. pre-ART vs. newly diagnosed HIV + from HIV Counselling and Testing (HCT)
- Excluded if any TB treatment in last 3 months

**Procedures**

- Structured questionnaire incorporating WHO tool
- Xpert MTB/RIF requested if high priority for TB investigation according to XPHACTOR algorithm (any of: cough, BMI<18.5, CD4<100, weight loss ≥10%) and from all HCT
- Monthly review with reinvestigation if indicated, to 3 months, when sputum and blood were taken for TB culture
- Clinic file review to identify additional TB diagnoses

**Definitions**

- Definite TB: Xpert+ or culture+ for *M. tuberculosis*
- Clinical TB: Started on TB treatment by health care provider
- Not TB: No positive microbiology for *M. tuberculosis* (at least one specimen)

**Excluded from analysis**

- On isoniazid preventive therapy (IPT) at enrolment
- Died within 3 months of enrolment without TB diagnosis
- TB diagnosed >6m from enrolment
- Did not meet criteria for TB or Not TB based on definitions

## Results

**Table 1: Characteristics of study population at enrolment (N=3229)**

| Characteristic | On ART: N=2439 | Pre-ART: N=693 | New HIV + HCT: N=97 |
|---|---|---|---|
| Age, yrs | 41 (35-48) | 35 (29-43) | 36 (30-41) |
| Female | 70.5 (1719) | 67.2 (466) | 50.5 (49) |
| House owner | 47.3 (1154) | 33.5 (232) | 20.6 (20) |
| Previous TB treatment | 39.7 (967) | 9.0 (62) | 7.2 (7) |
| Previous IPT | 2.5 (61) | 22.5 (156) | N/A |
| History of CPT | 78.2 (1906) | 47.6 (330) | N/A |
| BMI, kg/m² (N=3225) | 25.0 (21.6-29.4) | 24.5 (20.8-29.0) | 23.2 (20.1-28.4) |
| Duration since HIV diagnosis, months (N=3107) | 67 (39-100) | 9 (1-33) | N/A |
| Duration on ART, months (N=2429) | 49 (28-79) | N/A | N/A |
| CD4, cells/mm³ | 439 (283-621) | 360 (184-528) | 248 (106-412) |
| Positive WHO symptom screen | 30.2 (736) | 42.6 (295) | 62.9 (61) |

Data are median (IQR) or % (n); IPT=Isoniazid preventive therapy; CPT=Cotrimoxazole preventive therapy HCT=HIV counselling and testing

**Figure 1: Prevalence of TB and basis for diagnosis % (95% CI)**

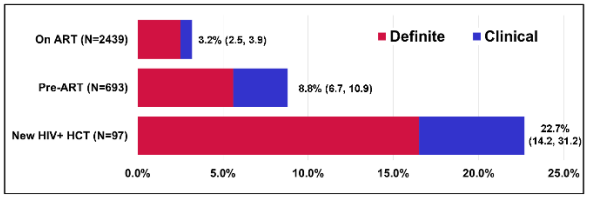


**Table 2: Performance of WHO tool**

| | Sensitivity n/N % (95% CI) | Specificity n/N % (95% CI) | NPV n/N % (95% CI) | PPV n/N % (95% CI) |
|---|---|---|---|---|
| All TB[1]: On ART (79/2439) | 53/79 67.1% (55.6,77.3) | 1677/2360 71.1% (69.2,72.9) | 1677/1703 98.5% (97.8,99) | 53/736 7.2% (5.4,9.3) |
| All TB[1]: Pre-ART (61/693) | 58/61 95.1% (86.3,99) | 395/632 62.5% (58.6,66.3) | 395/398 99.2% (97.8,99.8) | 58/295 19.7% (15.3,24.7) |
| All TB[1]: New HIV+ HCT (22/97) | 20/22 90.9% (70.8,98.9) | 34/75 45.3% (33.8,57.3) | 34/36 94.4% (81.3,99.3) | 20/61 32.8% (21.3,46) |
| Definite TB[2]: On ART (61/2421) | 37/61 60.7% (47.3,72.9) | 1677/2360 71.1% (69.2,72.9) | 1677/1701 98.6% (97.9,99.1) | 37/720 5.1% (3.6,7.0) |
| Definite TB[2]: Pre-ART (39/671) | 37/39 94.9% (82.7,99.4) | 395/632 62.5% (58.6,66.3) | 395/397 99.5% (98.2,99.9) | 37/274 13.5% (9.7,18.1) |
| Definite TB[2]: New HIV+ HCT (16/91 ) | 14/16 87.5% (61.7,98.4) | 34/75 45.3% (33.8,57.3) | 34/36 94.4% (81.3,99.3) | 14/55 25.5% (14.7,39.0) |

[1] Gold standard=definite and clinical TB combined; [2] Gold standard=definite TB (clinical TB excluded from analysis)
NPV=Negative predictive value; PPV=Positive predictive value

## Results

Prevalence of TB is high, particularly among those not on ART (figure 1)

**Performance of WHO tool (table 2):**

- NPV (proportion without TB of those WHO -ve)
  - >98% in pre-ART and on ART groups
  - Lower in new HIV+ HCT among whom TB prevalence is very high (23%)
- PPV (proportion with TB of those WHO +ve) is low, particularly among those on ART (7.2%)

## Conclusions

- NPV of WHO 4-symptom tool is very high among on ART and pre-ART groups
  – supporting use of WHO tool to rule out TB in people established in HIV care
- NPV lower in HCT attendees newly testing HIV+ where TB prevalence very high
  – this group requires systematic investigation rather than screening
- Relatively low PPV among on-ART and pre-ART groups necessitates guidance on prioritisation of further investigation to avoid burdening health care systems in resource-limited settings

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**XPHACTOR Team:** Nontobeko Ndlovu, Jessie Witkoei, Soneni Maphosa, Kutlwano Mmine, Khethekile Ntsontso, Mphonyana Motsapi, Mateboho Rantho, Simphiwe Ntshuntshe, Ndumiso Sithole, Johanna Masanabo, Crawford Maesela, Sanah Mutau, Mokgadi Letsatsi, Nontobeko Mokone, Lebogang Masia, Snenhlanhla Zondi, Nondumiso Masango, Matimba Chauke, Keolebogile Ntshamane, Mapaseka Pooe, Sandra Toro Silva, Gertrude Monkoe, Heather Mogola, Minty van der Meulen
**Clinic Staff:** Sisters Fikile Mavuso, Mary Mothupi, Bowa Matlala, Sana Daba, Phumla Mnyamde; and all medical and nursing staff

## 10.6. Frequency and seasonal variation of TB symptoms amongst people taking antiretroviral therapy in South Africa (Poster)

# Frequency and seasonal variation of "TB symptoms" amongst people taking antiretroviral therapy in South Africa

Yasmeen Hanifa[1], Violet Chihota[2], Nontobeko Ndlovu[2], Faieza Sahid[3], Lungiswa Adonis[4], Katherine Fielding[1], Alison Grant[1]
[1]London School of Hygiene & Tropical Medicine, [2]The Aurum Institute, [3]Chris Hani Baragwanath Hospital, [4]Mamelodi Hospital

44th Union Conference PC-940-03

Alison Grant Alison.grant@lshtm.ac.uk

## Background

- WHO recommends screening HIV+ individuals for TB at every clinical encounter using a tool comprising any cough, weight loss, fever or night sweats ("WHO tool")
- Xpert MTB/RIF recommended as initial diagnostic test for evaluation of those who screen positive
- The XPHACTOR study evaluates a novel algorithm, amongst adults attending for HIV care, which:
  i. prioritises immediate Xpert MTB/RIF testing for those at highest risk of TB mortality/transmission
  ii. allows deferral of investigation for those at low risk

## Aim

- To assess the prevalence of TB symptoms among adults attending for antiretroviral therapy (ART) in South Africa in the context of the XPHACTOR study

## Methods

- Systematic sample of adults attending 2 clinics in Gauteng for ART were screened for TB, Sept 2012 - Aug 2013
- Excluded those currently on or completed TB treatment in last 3 months
- Structured questionnaire incorporating WHO tool & questions about conditions with similar symptomatology
- Xpert MTB/RIF requested if high priority for TB investigation according to XPHACTOR algorithm (Figure 1)
- Prevalence of cough plotted vs. SA influenza surveillance data

**Figure 1: XPHACTOR algorithm**

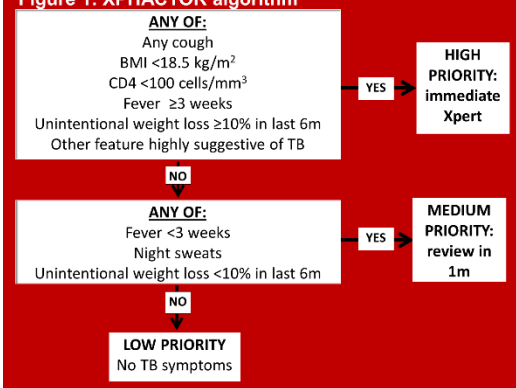



**Table 1: Characteristics of study population**

| Characteristic | Site 1: N=674 n (%) | Site 2: N=751 n (%) |
|---|---|---|
| Age, yrs (median [IQR]) | 40 (34-47) | 43 (37-50) |
| Female | 469 (69.6) | 505 (67.2) |
| Ethnic origin: Black African | 662 (98.2) | 732 (97.5) |
| Employed | 419 (62.2) | 300 (39.9) |
| CD4, cells/mm³ (median [IQR]) | 417 (270-584) | 413 (262-595) |
| Duration on ART, yrs (median [IQR]) | 3 (2-5) | 4 (2-7) |
| Previous TB treatment | 235 (34.9) | 356 (47.4) |
| Xpert MTB/RIF positive at enrolment | 4 (0.6) | 6 (0.8) |

**Figure 1. Frequency of TB symptoms at enrolment**

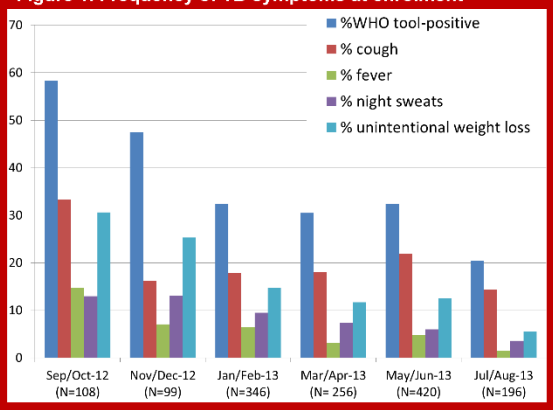
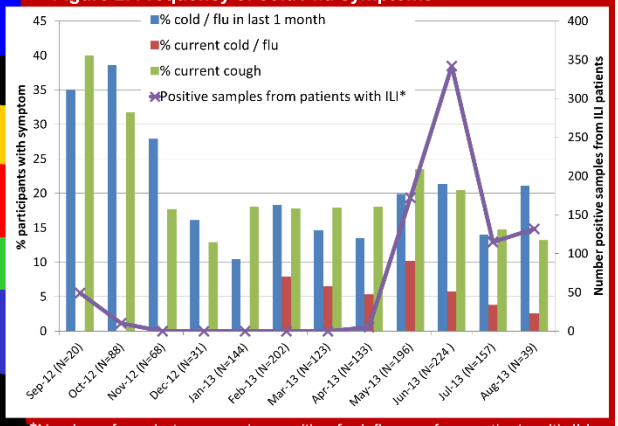



**Figure 2. Frequency of cold / flu symptoms**




*Number of respiratory samples positive for influenza from patients with ILI (influenza-like illness), NICD sentinel influenza surveillance programme

## Limitations

- % of individuals with TB diagnosed reflects those with positive Xpert MTB-RIF at enrolment, based on testing only those assigned high priority by the XPHACTOR algorithm
- In the XPHACTOR study, all participants are followed to 3 months when blood and sputum samples for TB culture are taken
  Additional data on true TB prevalence will be available at end of the study

## Conclusions

- The reported prevalence of TB symptoms is high, but number diagnosed with TB (representing a minimum estimate) is low
- This suggests that most TB symptoms reported are not due to TB
- ILI appears unlikely to be a major contributor to reported cough

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**Clinic Staff:** Sisters Gertrude Monkoe, Mary Mothupi, Bowa Matlala, Sana Daba, Phumla Mnyamde; and all medical and nursing staff

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